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Original Article

Comparative Effect of Zinc Concentration and Sources on Growth Performance, Accumulation in Tissues, Tibia Status, Mineral Excretion and Immunity of Broiler Chickens

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ABSTRACT

This experiment was conducted to investigate the effect of feeding different concentrations and sources of zinc (Zn) on the growth performance, tissue mineral status, bone morphology and immunity responses in 0–4-week broiler chickens. Four hundred and forty 1-d-old broiler chickens were assigned randomly to 11 dietary treatments with 4 cages per treatment and 10 broiler chickens per cage in a completely randomized design. Dietary treatments were: corn–soybean meal basal diet (negative control), basal diet supplemented with 5 g yeast/kg (yeast), and basal diet supplemented with 20, 50, or 80 mg of added Zn/kg as ZnSO4, Zn-Met, or Zn-yeast in a 3 x 3 factorial arrangement of treatments. The results showed that broilers fed Zn supplemented diets had greater average weight gain and average feed intake than chickens fed the negative control diet (p<0.05). The Zn deposition in tibia, meat (thigh and breast) and excreta increased (p<0.01), regardless of source, in response to increasing dietary Zn concentrations. Zinc level increased dry weight of tibia bone and its large diameter. The strength of tibia bone as judged by Seedor index and breaking strength was improved (p<0.01) with Zn concentration in increased diets. Furthermore, supplemental Zn up to 50 mg/kg improved immunity responses of broiler chickens (p<0.01). It is concluded that supplementation with 50 mg Zn may be sufficient for normal broiler growth up to 28 d of age and the dietary inclusion of organic Zn could be utilized more effectively when compared to inorganic sources.

INTRODUCTION

Zinc is an essential trace element that acts as a cofactor in many metabolic pathways including cell proliferation, growth, skeletal development, immune system, reproduction, hormone secretion, and antioxidant defense system, as well as many biochemical processes Swiatkiewicz *et al.*, 2001; Ao *et al.*, 2011; Tomaszewska *et al.*, 2017; Muszyński *et al.*, 2018). Thus, it is critical to use an optimal supplementation inclusion rate of Zn to allow poultry to reach their genetic potential and performance. The National Research Council (NRC 1994) recommended the minimum levels of 35 mg/kg in the diet that are necessary for optimum productivity for young broiler chickens. It has been reported that 71±13 mg Zn/g in a maize-soybean meal basal diet was necessary to maximize growth in broilers from hatch to 21 d of age (Huang *et al.*, 2007). However, natural Zn concentrations in common feedstuffs are generally lower than the daily Zn requirement for poultry, leading to the necessity of dietary Zn supplementation. In practice, food manufacturers and producers formulate diets to contain 100–120 mg supplemental Zn/kg (Shyam Sunder *et al.*, 2008; Feng *et al.,* 2010). On the other hand high dietary Zn supplementation in diet may affect the balance of other trace elements (Abedini *et al.*, 2017), and

causes toxicity (Carl *et al.,*2003). High Zn consumption could also lead to increased excretion of Zn in feces, which causes environmental contamination (Pierce *et al.*, 2005).

Research shows that the bioavailability of trace minerals in inorganic forms is low in poultry carcass (Cao *et al.,* 2002). Therefore, enhancement of Zn bioavailability using more available sources can help to solve such problems. As such, organic mineral sources such as proteinate and amino acid chelates have been increasingly used in recent years because of their greater bioavailability and less excretion (Pierce *et al.*, 2005). Previous studies have shown that the effect of different mineral sources, organic or inorganic, varies on production performance (Schlegel *et al.,* 2013; Sahoo *et al.,* 2014; Badawi *et al.,* 2017). While in many studies, organically bound Zn has been demonstrated to have greater relative bioavailability than that of inorganic forms (Wedekind *et al.,* 1992; Cao *et al.,* 2000), others have seen no differences in bioavailability among organic and inorganic Zn sources (Hudson *et al.,* 2004; Zakaria *et al.,* 2017).

To our knowledge, the bioavailability of Zn-enriched yeast has not been previously fully elucidated and no comparative study (between this form and other organic and/or inorganic forms) has been carried out in broilers. Thus, the other objectives of the present study were to examine the effects of dietary supplementing different concentrations of Zn from various sources on growth performance, Zn excretion, leg development and immune responses in broiler chickens.

MATERIALS AND METHODS

Birds, diets, and experimental design

The experimental protocol was approved by the animal-welfare committee of Tarbiat Modares University, and the animals were handled and treated in a humane manner.

Four hundred and forty 1-day-old broiler chickens (Ross 308) were assigned randomly to 1 dietary treatment group consisting of 4 replicates of 10 broilers in a completely randomized design arrangement of treatments. The house for the broilers was provided with programmed lighting and ventilation. Ambient temperature was gradually decreased from 32°C on day 1 to 22°C by the end of the experiment. Broilers were allowed ad libitum access to experimental diets and tap water containing no detectable Zn (<0.001 mg/L). Also, feed and water were provided using plastic instruments to minimize environmental Zn contamination.

The basal corn–soybean meal diets (Table 1), which was fed in mash form, were formulated to meet or exceed the NRC (1994) requirements for starter and grower broilers except for Zn. Dietary treatments included the un-supplemented basal diet with Zn (control), and supplemented with 20, 50, or 80 mg of Zn/kg as feed-grade Zn sulfate ($ZnSO_4 \cdot 7H_2O$), Zn Methionine (Bioplex Zinc; Alltech, Inc) and Zn-enriched yeast (that was produced on a laboratory scale as described by Kamran Azad et al 2014). All of the diets were calculated to contain equal concentrations of methionine. The background Zn concentration of the control diets were 24.4 and 21.6 mg/kg, in the starter and grower diets, respectively (Table 1).

Table 1 – Ingredient and nutrient composition of the basal diets.

Ingredients (%) (Basal Diet)	Composition Starter phase (1—14 d)	Grower phase (15—28 d)
Maize	57.5	62.1
Soy-bean meal	32.3	26.7
Maize gluten meal	4.4	4.5
Vegetable oil	2.1	3.5
Dicalcium phosphate	1.7	1.3
Oyster shell	1.1	1.1
Sodium chloride	0.3	0.2
Vitamin-mineral premix[1]	0.5	0.5
L-Lysine.H	0.1	0.1
Calculated values[2] (as fed basis)		
AME (kcal/kg)	3000	3150
Protein (g/Kg)	220	200
Ca (g/Kg)	8.9	8.0
Nonphytate P (g/Kg)	4.8	4.0
Zn (mg/kg)	24.4	21.6

Vitamin premix provided per kilogram of diet: vitamin A, 7,040 IU; vitamin D3, 2,000 IU; vitamin E, 8.8 IU; vitamin K3, 1.76 mg; biotin, 0.12 mg; thiamine, 1.2 mg; riboflavin, 3.2 mg; pantothenic acid, 6.4 mg; pyridoxine, 1.97 mg; niacin, 28 mg; vitamin B12, 0.008 mg; choline, 320 mg; folic acid, 0.38 mg. Mineral premix provided per kilogram of diet: Mn, 60 mg (MnO); Fe, 60 mg ($FeSO_4 \cdot 7H_2O$; Cu, 4.8 mg ($CuSO_4 \cdot 5H_2O$); I, 0.69 mg; Se, 0.16 mg (Na_2SeO_3); Zn, 0 mg.

The values were calculated from NRC (1994).

The yeast contained 39.9% of protein, almost 6897 KJ/kg of metabolizable energy and 11.3 mg Zn/g. Moreover, the amount of accumulated organically bound Zn in yeast biomass was 87 % of the total Zn in the cell. The dietary concentration of Zn for Zn sulfate (42.1, 69.3 and 103.6) , Zn methionine (46.2, 71.0, 104.5) and Zn-enriched yeast (48.3, 70,8, 101.4) were respectively at inclusion levels of 20, 50 and 80 (mg/kg)

The experiment lasted 28 days. Body weight of all broilers and feed consumption of each group were

recorded at weekly intervals, starting from one day of age. Growth performance was evaluated in terms of average weight gain (AWG), average feed intake (AFI) and feed conversion ratio (FCR) at the end of each feeding period.

Sample collection and measurements

On d 28 of the experiment, 2 broilers from each replicate cage (8 broilers per treatment) were randomly selected within the cage following a 6-h fast, weighed individually, and were then killed.

Chemical analysis

Zinc concentrations in Zn sources, diets, tap water and tissues were determined by flame atomic absorption spectrophotometry (Model Avanta S, Atomic Absorption, GBC, Sydney, NSW, Australia) after wet digestions, as described by Sandoval *et al.* (1998). Briefly, samples of tissues and diets were dried at 105 ºC for 12 h. Tissues were predigested in HNO_3, till charring was completed, then all samples were ashed dry at 550 ºC for 12 h, solubilized in HCl, and filtered through 42 Whatman paper.

The right tibia was removed and cleaned of the soft tissue before the bone was dried at 105 ºC for a minimum of 24 h. After 72 h-extraction in diethyl ether, the bone was dried for 12 h at 105 ºC and ashed at 550 ºC overnight in a muffle furnace (Yuan *et al.* 2011). Charred bones were digested as described before. The bone outer dimensions were measured by a digital caliper (Mitutoyo, Mizonokuchi Co, Utsonomiya, Japan). These bone parameters were measured on un-dried tibia samples. For determination of ash, the bones were placed in the oven for 24 h at 105 °C to dry completely and weighed. After that, the bones were ashed (650 °C for 14 h) and the ash weight was recorded. The material obtained from 2 duplicate was pooled.

For bone mechanical and geometric properties character, the following measurements were conducted. The Seedor index is the value obtained when the bone weight is divided by its length, as proposed by Seedor *et al.* (1991). It is used as a bone density indicator, the higher the value the denser the bone. Bone breaking strength was determined by material testing machine (Model H50KS; Hounsfield Co, London, England). The robusticity indexes were determined using the following formulas (Reisenfeld, 1972).

Robusticity index = bone length / cube root of bone weight

To collect the excreta, 2 birds from each replicate were placed in a cage. A polythene sheet was attached under the cages of the birds. Feed and feathers were carefully removed. The excreta were homogeneously mixed replicate-wise, representative samples of excreta were collected in a moisture cup and oven-dried at 105°C for 24 hr, and finely ground for mineral analysis as described previously.

Immune response

On the 14th and 21th day of the experimental period, 2 birds were selected from each group and intramuscularly injected with 2 ml of 0.5% sheep red blood cell (SRBC). On the 21st and 28th day of the experiment, blood samples were collected and serum samples were used to measure humoral immunity. Antibody titre produced against haemagglutination was measured according to Peterson *et al.* (1999).

Statistical analysis

To test the effect of supplemented Zn, data were analysed using single degree of freedom contrast to compare all supplemental Zn treatment with the control treatment. Data were further analyzed by 2-way ANOVA (excluding control treatment) using the General Linear Model (GLM) procedure of SAS institute (SAS 2003). Replicate was considered as the experimental unit for all data. The model included main effects of supplemental Zn level, Zn source and their interaction. Influences regarding one (Zn level) of the main effects were based on irthohnal comparsion for linear response of dependent variables to independent variables. Duncan's multiple range tests was used to assess any significant differences at the probability level of $p \leq 0.05$ among the experimental treatments.

RESULTS

Growth performance

The broiler chickens fed diet without Zn supplementation had lower ($p<0.01$) AWG and AFI than those fed zinc supplemented diets (Table 2). Similarly, in the entire 4-week period, AWG increased with the dietary Zn content ($p<0{\cdot}05$), up to dietary concentration of 50 mg Zn/kg. No additional response was observed at higher Zn concentrations. The inadequacy of Zn in the control diet depressed feed consumption ($p<0.05$); so that the lowest feed intakes were attributed to un-supplemented groups and the highest FI were shown in the 50 and 80 mg/kg of Zn. Moreover, the main effect of Zn level was not

Table 2 – Effects of Zn source and level on growth performance of broiler chicks (1 to 28 d).

Item [1]	Number of pens	Added Zn (mg/kg)	Weight gain (g)	Feed consumption (g)	FCR
Control	4	0	1165	2033	1.75
Main effect					
Zn level (mg/Kg)					
	12	20	1265[b]	2164[b]	1.71
	12	50	1337[a]	2216[a]	1.66
	12	80	1328[a]	2220[a]	1.67
Zn source					
Zn sulfate	12		1261	2148	1.70
Zn- Methionine	12		1283	2169	1.69
Zn-enriched Yeast	12		1277	2157	1.69
Pooled SEM			7.42	9.82	0.01
Source of variation					
Zn level			<0.001	0.050	0.220
Zn source			0.056	0.527	0.787
Zn level × source			0.881	0.942	0.993
Control vs all supplemental Zn groups			0.018	<0.001	0.228

[a-b] Means in the same column with no superscript letters after them or with a common superscript letter following them are not significantly different (p<0.05).

[1] Mean values are based on data obtained from all (n=10) chicks from each of the 4 replicate pens per treatment (n=40 individual birds per treatment). Through analysis, a dummy variable was considered for the control and the three Zn sources so that the variable set to be zero for the birds not in the relevant group and one when they were in the relevant group.

significant for feed conversion ratio (p<0.05). On the other hand the FCR was not affected with the dietary Zn sources but decreased with increasing Zn levels in both organic and inorganic groups.

Tibia Zn concentration

Zinc concentration in tibia was low in the broilers fed diets with no Zn supplementation (Table 3) but it increased in proportion to the dose of Zn supplementation to the basal diet and reached plateau at 50mg/kg (p<0.01). Tibia Zn concentrations were also strongly related to the Zn source origin, as organically bound Zn significantly increased the Zn content compared to inorganic supplementation (p<0.05). There were small changes in tibia Zn when broiler chickens were fed on either Zn-enriched yeast or commercial Zn methionine sources. The interaction between Zn level and its source was not significant for tibia Zn status.

Zinc content of meat and excretion

The Zn deposited in breast and thigh muscles reflected the level of dietary Zn (Table 3), the higher the inclusion level, the higher Zn content of muscles

Table 3 – Effects of dietary Zn source and level on tissue Zn content in 28-d-old broiler chicks.

Item [1]	Number of pens	Added Zn (mg/kg)	Breast Zn content (µg/g DM)	Thigh Zn content (µg/g DM)	Breast Zn content (µg/g DM)	Excreta Zn content (µg/g DM)
Control	4	0	162.1	22.5	3.91	175. 6
Main effect						
Zn level (mg/Kg)						
	12	20	234.9 [b]	24.9 [b]	4.90 [b]	265.7 [c]
	12	50	276.7 [a]	27.7 [a]	5.15 [a]	377.9 [b]
	12	80	276.3 [a]	28.3 [a]	5.23 [a]	515.9 [a]
Zn source						
Zn sulfate	12		228.9 [b]	24.9 [b]	4.79	339.4
Zn- Methionine	12		241.2 [a]	26.2 [a]	4.82	332.5
Zn-enriched Yeast	12		242.3 [a]	26.3 [a]	4.83	332.5
Pooled SEM			4.93	0.46	0.93	22.18
Source of variation						
Zn level			<0.001	<0.001	<0.001	<0.001
Zn source			0.031	0.040	0.047	0.367
Zn level × source			0.810	0.720	0.740	0.980
Control vs all supplemental Zn groups			<0.001	<0.001	<0.001	<0.001

[a-b] Means in the same column with no superscript letters after them or with a common superscript letter following them are not significantly different (p<0.05).

1 There were 8 replicate consisting of samples taken from 2 birds per replicate (cage).

(p<0.01). Zn accumulation was more substantial in birds fed organically bound Zn supplemented diets relative to those fed inorganic Zn counterpart (p<0.01). Although the source of zinc affected the Zn content of thigh meat, it did not influence the Zn content of breast. Zinc excretion increased nearly three folds (p<0.01) from 175 ug/g (DM) control group to 515 ug/g (DM) for 80 mg/kg zinc level (p<0.01). There was no effect of zinc source on zinc excretion.

Mechanical and geometric character of bone

Mechanical and geometric characters of bones are presented in Table 4. Dry weight, Sidoor index, and breaking strength of tibia bone were affected by zinc content (p<0.05). However, there was no further increase in these parameters beyond 50 mg/kg zinc levels. The zinc source had no significant effect on mechanical and geometric characters of bone measured. There was no interaction between level and zinc source effect on any parameters measured.

Immunity

The dietary treatment had no effect on weight of bursa Fabricius while the spleen weight was increased as compared to the control group (p<0.05). There was neither effect of zinc source nor interaction effect between zinc source and zinc level (p<0.05) on the weights of bursa Fabricius and spleen (Table 5). Although there was no significant difference in preliminary antibody titer among all birds, the chicks that had received zinc supplementation had a higher secondary antibody titer as compared to the control (p<0.05).

DISCUSSION

Growth performance

Zinc is an essential trace element for the normal function of numerous important structural proteins, enzymatic processes, lipid metabolism, hormone production and ultimately necessary for healthy growth and development of chicken. Inadequacy of Zn dosage in the bird diet reduces feed consumption and consequently body weight gain, but it could be reversed by Zn supplementation (Bao *et al.,* 2007). Hudson *et al.* (2004) reported that different sources of Zn significantly affected body weight gain of broilers. Wedekind *et al.* (1992) studies indicated that the improvement in weight gain in broilers fed Zn-supplemented diets may have resulted, in part, from increased consumption of basal diet, because anorexia

Table 4 – Effects of dietary Zn source and level on tibia bone mechanical and geometric properties character of 28-d-old broiler chicks.

Item [1]	Number of pens	Breaking strength (N/cm²)	Robusticity Index (MP)	Seedor index (mg/mm)	Large/ small	Small outer diameter (mm)	Large outer diameter (mm)	Length (mm)	Relative weight (%)	Dry weight (g)	Added Zn (mg/kg)
Control	4	73.34	4.74	59.56	1.54	6.58	10.01	79.72	0.40	4.74	0
Zn level (mg/Kg)											
	12	75.94 ^{ab}	4.80	53.24 ^{b}	1.59	6.59	10.41^{b}	83.65	0.40	5.27 ^{b}	20
	12	76.93 ^{a}	4.84	65.65 ^{a}	1.61	6.78	11.02 ^{a}	86.30	0.42	5.67 ^{a}	50
	12	75.30 ^{b}	4.82	65.65 ^{a}	1.58	6.80	10.76 ^{b}	85.45	0.41	5.66 ^{a}	80
Zn source											
Zn sulfate	12	75.32	4.76	62.21	1.56	6.68	10.35	82.40	0.40	5.19	
Zn- Methionine	12	75.91	4.78	63.54	1.61	6.64	10.65	83.23	0.41	5.33	
Zn-enriched Yeast	12	75.29	4.86	64.92	1.57	6.81	10.68	85.99	0.42	5.53	
Pooled SEM		0.30	0.02	0.54	0.02	0.81	0.10	0.76	0.02	0.11	
Source of variation											
Zn level		0.043	0.861	0.011	0.252	0.510	0.020	0.210	0.070	<0.001	
Zn source		0.635	0.852	0.490	0.760	0.480	0.451	0.790	0.517	0.118	
Zn level × source		0.912	0.840	0.930	0.660	0.991	0.781	0.890	0.904	0.810	
Control vs all supplemental Zn groups		0.022	0.214	0.048	0.191	0.254	0.046	0.016	0.140	0.017	

[a-b] Means in the same column with no superscript letters after them or with a common superscript letter following them are not significantly different (p<0.05).

[1] There were 8 replicate consisting of samples taken from 2 birds per replicate (cage).

Table 5 – Effects of dietary Zn source and level on immune response of 28-d-old broiler chicks.

Item [1]	Number of pens	Added Zn (mg/kg)	Relative weight (%)		Total Anti – SRBC[1]	
			Bursa	Spleen	1st Response (day 21)	2nd response (day 28)
Control	4	0	0.227	0.109	1.50	2.00
Main effect						
Zn level (mg/ kg)						
	12	20	0.200	0.123	1.83	3.00[b]
	12	50	0.208	0.126	1.92	3.42[a]
	12	80	0.198	0.127	2.00	3.17[b]
Zn source						
Zn sulfate	12		0.211	0.120	1.81	2.75
Zn- Methionine	12		0.206	0.122	1.81	2.87
Zn-enriched Yeast	12		0.209	0.122	1.81	3.06
Pooled SEM			0.009	0.008	0.031	0.050
Source of variation					*p*-value	
Zn level			0.150	0.311	0.146	0.033
Zn source			0.121	0.016	0.415	0.785
Zn level × source			0.862	0.981	0.912	0.818
Control vs all supplemental Zn groups			0.092	0.017	0.011	0.008

[a a-b] Means in the same column with no superscript letters after them or with a common superscript letter following them are not significantly different ($p<0.05$).

[1] Mean values are based on data obtained from all (n=10) chicks from each of the 4 replicate pens per treatment (n=40 individual birds per treatment). Through analysis, a dummy variable was considered for the control and the three Zn sources so that the variable set to be zero for the birds not in the relevant group and one when they were in the relevant group.

[1]antibody production based on log^{10}

is the most common symptom of Zn deficiency, which led to the depressed growth of broilers. The mechanisms involved in the effects of Zn deficiency on growth are unknown, but a reduction in food consumption may be a protective reaction to allow survival (MacDonald, 2000). An explanation for increased body weight gain may be due to the positive effects of Zn methionine on digestion and absorption of nutrients in the gastrointestinal tract and/or to a higher bioavailability of Zn in the form of Zn methionine. On the other hand, refer to the participation of Zn in protein and carbohydrate metabolism, the reduction in feed intake associated with a lack of Zn (that could reduce feed digestibility), and consequently impaired performance parameters in broilers (Bao *et al.,* 2007). These results are in agreement with Huang *et al.* (2007) who found Zn supplementation induced increase in feed intake and weight gain.

Tibia Zn concentration

Zinc is the most abundant trace element in bone, being present at a concentration of up to 300 mg/kg (Grynpas *et al.,* 1987), and has been considered an important factor in bone metabolism. Not surprisingly, adequate Zn concentration is required for growth, development and mineralization of bone (Bao *et al.,* 2003). Huang *et al.* (2009) demonstrated that the Zn content of the tibia was significantly influenced by dietary levels. Previous studies reported that feeding birds diets with a Zn concentration greater than 85 mg/kg did not influence tibia Zn deposition (Wedekind *et al.,* 1992). However, in this study, tibia Zn concentrations increased with Zn supplementation only up to 50 mg/kg and there was no change at 80 mg/kg. Huang *et al.* (2007) indicated that bone status is commonly used as an indicator of mineral adequacy in poultry diets.

Also in agreement with the present study, other studies indicated that different sources of Zn supplementation affected the levels of Zn in tibia (Ao *et al.,* 2011; Idowu *et al.,* 2011). Moreover, many research studies (Cao *et al.,* 2000; Huang *et al.,* 2009; Ao *et al.,* 2011) indicated that the dietary supplementation of organic Zn can result in greater accumulation of Zn in tibia than the same supplemental concentration of inorganic Zn.

According to Loveridge (1992) bone is a complex heterogeneous tissue that supports the musculature, and thus its growth and development are intimately connected with overall body growth and thus making tibia Zn concentration a good predictor of whole-body growth. This study indicates that using 50 mg/kg Zn might meet the requirement for normal bone mineralization. In the present study, the same birds that obtained optimal body weight had highest tibia Zn.

Zinc content of meat and excretion

Many researchers indicated that dietary Zn level influence Zn content of nearly all types of tissues and organs (Park *et al.,* 2004). The concept of "Functional foods", enriching poultry meat with different nutrients has attracted many researchers in recent decades (Peric *et al.,* 2011). Our results though, indicate that Zn enrichment took place, the magnitude is not too high as compared to the control. The total Zn of thigh and breast meat in birds with no Zn addition is 26.4 (µg/g DM) while the highest level of Zn inclusion (80) resulted to total Zn content of 30 (µg/g DM).

On the other hand the magnitude of Zn excretion is nearly 2.5 folds; 176 (µg/g DM) for no zinc added diet as compared to 515 for 80 mg/kg added Zn. This imbalance between the ability to enrich a product and/ or cause industrial pollution is of great importance to consider. This proves that there is a limit for enriching meat with zinc (like most other minerals) beyond which is depleted through excretion (Keen *et al.,* 2003).

However, Bao *et al.* (2007) had shown organic minerals decreases minerals of excreta. Salim *et al.* (2010) indicated that chelated minerals (such as organic zinc) could resist interferences from dietary anti nutritional factors in the digestive tract and directly reach the intestinal brush border, where it is hydrolyzed and absorbed as an ion into to the blood, resulting in a greater availability.

Mechanical and geometric character of bone

This study suggests that using 50 mg Zn/kg (from yeast Zn) might meet the requirement for normal bone mineralization. Although Zn supplementation at specific levels is essential to optimize bone breaking resistance, it has been reported that higher levels of Zn in the diet appear to interfere with the absorption and utilization of Ca and P, particularly above 80 ppm, and could decrease the bone mineralization (Underwood & Suttle 1981) which was also observed in our study.

Many previously conducted studies show the negative impact of Zn deficiency on bone growth, and disorders that are associated with reduced activity of the growth plate (Brown *et al.,* 1978). Scrimgeour *et al.* (2007) indicated that when Zn is not supplied sufficiently, proliferation, differentiation and survival of the bone cells are compromised. It seems that Zn, by increasing the number and activity of osteoblasts, leads to the deposition of calcium in the diaphysis of bones and increase mineralization in the tibia. Masayoshi and Hidetoshi (1989) demonstrated that Zn enhanced the effects of vitamin D on bone metabolism by stimulating the synthesis of DNA in bone cells. On the other hand, Zn by stimulating bone metabolism and bone protein synthesis by increasing the activity of enzymes such as alkaline phosphatase, is involved in increasing bone mineralization and strength (Yamaguchi *et al.,* 1988). It has also been suggested that Zn is involved in insulin-like growth factor I-(IGF-I) production that increases the collagen, DNA and bone matrix syntheses (Hock *et al.,* 1988). Therefore, many effects of Zn on bone metabolism may be related to nucleic acid and protein metabolism.

Immunity

Since the spleen is the organ that is directly related to antibody production, it is expected that its weight be directly related to antibody production (Steiniger and Barth, 2000). While there are no concrete evidences as to the effect of zinc sources on weight of the brusa Fabricius and the spleen, there is not much dispute in regard to the effect of zinc levels on these organs. Yu *et al.* (2005) showed that diet lacking in zinc results to lower weight of the spleen. Increase of the weight of bursa (and not the spleen) in this study was similar with the result of Bartlett and Smith (2003), who showed a slight increase in the weight of lymphoid organs. Also, in their experiments on broilers reported that thymus, spleen, and bursa of Fabricius increased linearly with increasing dietary Zn (from 35mg/kg to 68mg/kg). These findings could be due to the role of Zn in the growth and function of lymphocytes.

The results of this study indicated that Zn supplementation improves immune responses, as compared to the control. The immune system is dependent on the functions of cellular metabolism. Zn is ubiquitous in cellular metabolism and functions both structurally and catalytically in metalloenzymes (Bartlett & Smith, 2003).

In this study the birds with higher spleen weight had higher SRBC secondary titer. However, the maximum secondary SRBC immune response was seen in birds receiving 50 mg/kg zinc ($p<0.05$) but there was no differences with the 80mg/kg level. In the other hand according to Sunder *et al.* (2008), humoral and cell-mediated immune responses were significantly higher in broilers supplemented with 80 mg/kg or greater amounts of Zn than those supplemented with less than 80 mg/kg of Zn. Hudson *et al.* (2004) observed a higher cellular immune response to PHA and antibody titres against the Newcastle disease in broiler breeders fed with diets supplemented with organic sources of Zn, as compared to inorganic sources. Zinc is essential

for thymulin, a thymic hormone that regulates T lymphocyte maturation. Thus, birds fed with diets supplemented with a more available Zn source might have more thymulin activity and, therefore, promote immune responses through the increased maturation of T-lymphocytes and activation of B-lymphocytes by T-helper cells.

CONCLUSION

This experiment was conducted to examine the effect of different levels of zinc from different sources on broiler chickens performances. The levels were chosen to reflect the earlier proposed zinc requirement (NRC), recommended levels to present faster grower birds, and higher level than commonly practiced. As many earlier experiments to determine zinc requirement were done with inorganic zinc, it was presumed that organic zinc being more bioavailable will enhance the broiler performances at lower levels as compared to inorganic Zn. The main objective of our study apart from general performances criteria was to evaluate the effect of zinc on tissue and excretion zinc level and tibia morphology. According to our results, the optimal dietary Zn requirements for 0–4-wk-old broilers were 50 mg/kg diet. Zinc level above 50 mg/kg not only did not improved performances criteria further, it adversely increased zinc excretion and ultimately environment pollution. Unlike many other studies, our results did not indicate that zinc sources (sulfate vs organic) within the range tested in this experiment had much influence on broiler performances.

DISCLOSURE STATEMENT

No potential conflict of interest was reported by authors.

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