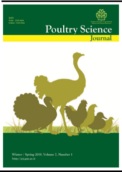

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Effects of dietary organic and inorganic zinc and copper supplements on performance, footpad dermatitis, carcass characteristics, and blood profile of broiler chickens

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Abstract
The aims of this study was to determine the effect of organic and inorganic Zn and Cu supplements on performance, footpad dermatitis (FPD), carcass characteristics and blood profile in broiler chickens. A total of 336 one-day-old Ross 308 broiler chicks were distributed into 24 floor pen and reared for 42 days. A basal mineral premix (without Zn and Cu) was supplemented with organic or inorganic Zn and Cu to meet 100% (i.e., 110 and 16 mg/kg of the mineral premix, respectively) or 50% (i.e., 55 and 8 mg/kg of the mineral premix, respectively) of the recommended levels. Six dietary trace mineral premix were formed with inclusion of 1) inorganic Zn and Cu at the level 100%, 2) organic Zn and Cu at the level 100%, 3) inorganic Zn and Cu at the level 50%, 4) organic Zn and Cu at the level 50%, 5) organic Zn at the level 50% and inorganic Cu at the level 100%, and 6) inorganic Zn at the level 100% and organic Cu at the level 50%. Results indicated that organic Zn and Cu supplementation improved ($P < 0.05$) body weight gain and feed conversion ratio than inorganic form. However, the incidence and severity of FPD were significantly lower in broilers received only organic Zn and Cu than those of birds under inorganic Zn and Cu at the level 50%. Broilers fed lower level of inorganic Zn and Cu supplement showed the lowest breast yield compared to the other treatments. In contrast to the alkaline phosphatase enzyme, serum cholesterol concentration decreased ($P < 0.05$) in broiler chickens received organic Zn when compared to the supplementing of inorganic Zn and Cu at the level 50%. The results showed that organic Zn and Cu, as a proper alternative to inorganic forms, are useful to improve performance and health of broilers.

Introduction
Mineral Supplementation is essential in poultry farming to avoid several disorders causing by their deficiency in the diet. Minerals participate in the biochemical processes necessary for normal growth and to achieve optimum performance, embryos and bone development, higher hatchability, and formation of eggshell in poultry (Favero *et al*., 2013). A large portion of minerals applied for diet formulation are as oxides, sulfates, carbonates, and phosphates which drived from inorganic sources. Recently, organic trace minerals are more considered because of higher bio-availability and easily absorption and storage within body tissues than inorganic sources (Stefanello *et al*., 2014). Organic trace minerals provided as metal-amino acid chelate, metal-proteinate, metal-polysaccharide, and metal-organic acid (Wedekind *et al*., 1992).

Zinc (Zn) and copper (Cu) are two of the microelements that act as a cofactor in many enzymes involved in protein synthesis and energy metabolism. Zinc deficiency could decrease the growth and immunity, and also may lead to skin lesions and poor feathering (Star *et al*., 2012). Copper deficiency able to disturb the synthesis of plasma proteins (e.g., hemoglobin, erythrocuprein, ceruloplasmin), and eggshell membrane formation (Leeson, 2009). Studies indicated higher bioavailability for organic Zn and Cu compared with inorganic forms (Nollet *et al*., 2007; ShyamSunder *et al*., 2013; Das *et al*., 2014a). It is reported that the use of organic Zn and Cu could be reduced by about 50 percent of the

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inorganic form in diets, without any adverse effect on performance (El-Husseiny *et al.*, 2012). However, there is an antagonism between Zn and Cu availability as excess supplementing of each one in diet will reduce intestinal absorption of the other (Ao *et al.*, 2009). Also, after application of poultry manure when fertilizing farm land, reduction of crop yield and ground water pollution could be occur because of Zn and Cu accumulation in the soil (Yang *et al.*, 2011).

Hence, the use of organic Zn and Cu is a way to avoid the antagonistic effect and reduce their excretion into the environment (Bao *et al.*, 2010). This study was carried out to determine the effect of dietary Zn and Cu supplementation from organic and inorganic sources on performance, litter characteristics, footpad lesion score, and blood parameters in broiler chickens.

Materials and Methods

Birds and diets

A total of 336 one-d-old Ross 308 male broiler chickens were randomly assigned to 6 experimental treatments with 4 replicates of 14 birds per floor pen (120 × 120 × 70 cm) and reared for 42 days in a temperature-controlled house. The initial temperature of 33°C was gradually reduced according to the age of birds (2-3°C every week) until reaching 22°C, and was then kept constant. The lighting program was 24 h per day for the entire experiment. A basal diet was formulated to meet the requirements of broiler chickens, according to the recommendation from Ross Broiler Nutrition Specification (2014) for starter (days 1 to10), grower (days 11 to 24) and finisher (days 25 to 42) periods (Table 1).

Table 1. Composition (% of as-fed) and calculated analysis of the basal diet

Ingredients	1 to10 d	11 to 24 d	25 to 42 d
Corn, 7.6% CP	53.71	56.73	61.12
Soybean meal, 45.1% CP	39.46	35.83	30.89
Soybean oil	2.28	3.33	4.15
Dicalcium phosphate	2.03	1.81	1.64
Limestone	1.00	0.92	0.85
Sodium bicarbonate	0.18	0.16	0.16
Salt (NaCl)	0.23	0.25	0.25
Vitamin premix†	0.25	0.25	0.25
Mineral premix‡	0.25	0.25	0.25
L-Lysine HCl (78%)	0.19	0.14	0.14
DL-Methionine (98%)	0.33	0.28	0.26
L-Threonine (98.5%)	0.09	0.05	0.04
Calculated values			
ME (kcal/kg)	2900	3000	3100
CP (%)	22.23	20.80	18.89
Lysine (%)	1.24	1.11	1.00
Methionine (%)	0.62	0.56	0.51
Methionine+Cystine (%)	0.92	0.84	0.78
Calcium (%)	0.93	0.84	0.77
Available phosphorus (%)	0.46	0.42	0.38
Analysed values[2]			
Zinc (mg/kg)	30.76	29.21	27.73
Copper (mg/kg)	7.25	6.84	6.04

†Contained per kilogram of diet: vitamin A (trans - retinyl acetate), 10,000 IU; vitamin D_3 (cholecalciferol), 2,000 IU; vitamin E (DL - α - tocopherol acetate), 10 mg; vitamin K (bisulfate menadione complex), 1 mg; vitamin B_1 (thiamin mononitrate), 1 mg; vitamin B_2 (riboflavin), 5 mg; vitamine B_3 (Niacin), 30 mg; vitamine B_6 (pyridoxine - hydrochloride), 1.5 mg; vitamine B_8 (biotin), 0.05 mg; vitamin B_5 (D - calcium pantothenate), 10 mg; vitamine B_9 (folic acid), 1 mg; and antioxidant (butylated hydroxytoluene), 10 mg.

‡Contained per kilogram of diet: Mn (manganese sulfate), 60 mg; Fe (ferrous sulfate), 30 mg; I (potassium iodide), 3 mg; Se (sodium selenite), 0.1 mg; and Co (cobalt carbonate), 0.1 mg.

The amount of Zn and Cu was analyzed in the basal diet according to AoAC method 990.10 (AOAC, 1990). The basal diet supplemented with a mineral premix containing inorganic Zn (110 mg/kg of the premix) and Cu (16 mg/kg of the premix) or that the inorganic Zn and Cu were replaced with 50 or 100%organic source (Table 2). The inorganic supplementations were as zinc sulfate ($ZnSO_4$) and copper sulfate ($CuSO_4$. $5H_2O$) and also the organic supplementation consisted of a combination of complex proteinates metals (Bioplex®). All the birds received feed and water (containing no analytically detectable levels of Zn and Cu (< 0.001 mg/L)) *ad - libitum* throughout the experiment.

Table 2. Contents (as % of recommended value)† and forms of Zn and Cu supplemented in the mineral premix

Treatment	Inorganic form		Organic form‡	
	Zn contents	Cu contents	Zn contents	Cu contents
Ino-Zn_{100} + Ino-Cu_{100}	100	100	-	-
Org-Zn_{100} + Org-Cu_{100}	-	-	100	100
Ino-Zn_{50} + Ino-Cu_{50}	50	50	-	-
Org-Zn_{50} + Org-Cu_{50}	-	-	50	50
Org-Zn_{50} + Ino-Cu_{100}	-	100	50	-
Ino-Zn_{100} + Org-Cu_{50}	100	-	-	50

† Recommended value of Zn and Cu is respectively 110 and 16 mg/kg of mineral supplement (Ross Broiler Nutrition Specification, 2014).
‡ Organic Zn is BioplexZn and organic Cu is BioplexCu (both supplied by Alltech Inc., Nicholasville, KY).

Growth performance, litter quality, and footpad dermatitis

Body weight gain (BWG) and feed intake (FI) were calculated at the end of the experiment. Mortality was recorded daily manner and used for calculation and correction of feed conversion ratio (FCR). Litter samples were collected on day 42 from 5 different points (including drinking and eating area) of each pen and analyzed for moisture content and pH as described by Lopes *et al* (2013). Also, footpad dermatitis (FPD) score determined on d 42. Right and left chickenpaws (no = 4 birds) in each experimental unit were scored by visual ranking as follow: score 1) if no lesions, score 2) if lesions was ≤ 2 mm, score 3) if lesions was > 2 and ≤ 7 mm, score 4) if lesions was > 7 mm, and score 5) if lesions was larger than 7 mm and accompanied by toe lesions. Finally, occurrence of all scores frequency were explained as percentage (Manangi *et al*., 2012).

Carcass yield and tissue analyzing

At 42 d of age, 4 broiler chickens per pen with body weights close to the pen average were slaughtered and used for weighting of carcass, breast, thigh, liver, heart, spleen, abdominal fat pad (Huyghebaert and Pack, 1996), and tibia characteristics (length, weight, diameter, ash, calcium, phosphorus, and Zn) (Shim *et al*., 2012; Star *et al*., 2012). Furthermore, liver samples from each bird were ashed and Cu content was determined as described by AOAC (1990) using a PerkinElmer 5000AA Atomic Absorption Spectrophotometer.

Blood parameters

Before the slaughter, 4 mL of blood was collected from wing vein of 4 birds in each pen (16 chickens in each treatment). Blood was collected in EDTA tubes and then centrifuged(2000 × *g* for 10 min) and serum samples were decanted and stored frozen at -20°C until used for determination of blood parameters (Khosravi *et al*., 2016). Serum cholesterol, glucose, triglycerides, total protein, alkaline phosphatase (ALP), aspartate transaminase (AST), and alanine transaminase (ALT) enzymes activity were analyzed using an automatic biochemical analyzer (Clima; Ral. Co, Barcelona, Spain), following the instructions of the corresponding reagent kit (Diagnostic Products Corporation, Pars Azmoon, Tehran, Iran).

Statistical analysis

Data were subjected to ANOVA using the GLM procedure of SAS (2001). Significant differences among treatments were identified by Tukey's HSD test. All statements of significance were based on a probability of $P < 0.05$.

Results

Performance

The effect of experimental treatments on BWG, FI, FCR, and mortality of broiler chickens are summarized in Table 3. The BWG of the birds under concomitant use (at 50% of recommended level) of inorganic Zn and Cu treatments was significantly lower than other treatments ($P < 0.05$). Also, the highest ($P < 0.05$) body weight gain was observed in chickens received simultaneously both organic Zn and Cu. However, the highest FI was found in the birds under Ino-Zn_{50}+Ino-Cu_{50} treatment. Complete replacing of inorganic Zn and Cu with the organic source led to decrease of FI ($P < 0.05$). Supplemented with organic Zn and Cu in the diets depressed the FCR compared to inorganic sources ($P < 0.05$). Supplementing of diets with organic Zn and Cu significantly improved FCR compared to the inorganic source. Furthermore, the decrease of inorganic Zn and Cu levels had an adverse effect ($P < 0.05$) on performance. Mortality was not affected by treatments.

Footpad dermatitis

The effects of organic or inorganic Zn and Cu sources on the FPD is presented in Table 4. Concomitant use of inorganic Zn and Cu at half of the recommended level resulted in higher ($P < 0.05$) FPD score mean when compared to each of the treatments containing organic trace minerals. Also, the highest percentage of the worst FPD (i.e., scores 3 to 5) was found in birds under concomitant use (at 50% of recommended level) of inorganic Zn and Cu treatments, which had a significant difference compared to those of the only organic Zn and Cu supplementation.

Table 3. Effect of organic or inorganic Zn and Cu supplementation on performance of broiler chickens

Treatment	BWG (g)	FI (g)	FCR (g/g)
Ino-Zn_{100} + Ino-Cu_{100}	2541^{b}	4896^{ab}	1.93^{bc}
Org-Zn_{100} + Org-Cu_{100}	2582^{a}	4875^{b}	1.89^{d}
Ino-Zn_{50} + Ino-Cu_{50}	2430^{c}	4957^{a}	2.04^{a}
Org-Zn_{50} + Org-Cu_{50}	2570^{a}	4876^{b}	1.90^{cd}
Org-Zn_{50} + Ino-Cu_{100}	2544^{c}	4908^{ab}	1.93^{b}
Ino-Zn_{100} + Org-Cu_{50}	2551^{b}	4884^{ab}	1.92^{bcd}
SEM‡	10.5	10.1	0.01
P-value	<0.0001	0.1593	<0.0001

a-c Means within a column having different superscripts are significantly different ($P < 0.05$).
†BWG= Body weight gain; FI= Feed intake; FCR= Feed conversion ratio.
‡ *SEM* = standard error of means.

Table 4. Effect of organic or inorganic Zn and Cu supplementation on footpad dermatitis in broiler chickens

Treatment	Footpad dermatitis score†		
	Mean of scores	Score 1 and 2 (%)	Score 3, 4, and 5 (%)
Ino-Zn_{100} + Ino-Cu_{100}	2.78^{ab}	53.12^{bc}	46.88^{ab}
Org-Zn_{100} + Org-Cu_{100}	2.53^{b}	68.75^{ab}	31.25^{bc}
Ino-Zn_{50} + Ino-Cu_{50}	3.06^{a}	37.5^{c}	62.5^{a}
Org-Zn_{50} + Org-Cu_{50}	2.41^{b}	75.0^{a}	25.0^{c}
Org-Zn_{50} + Ino-Cu_{100}	2.47^{b}	65.62^{ab}	34.38^{bc}
Ino-Zn_{100} + Org-Cu_{50}	2.44^{b}	65.62^{ab}	34.38^{bc}
SEM‡	0.06	3.39	3.39
P-value	0.01	0.005	0.005

a-c Means within a column having different superscripts are significantly different ($P < 0.05$).
† 1: no lesions, 2: lesions of 2 mm or less, 3: lesions of 2 to 7 mm, 4: lesions larger than 7 mm, 5: lesions larger than 7 mm, including toe lesions Means within a column having different superscripts are significantly different ($P < 0.05$).
‡ *SEM* = standard error of means.

Carcass yield and tissue analyzing

Dietary treatments had no significant effect on carcass yield, except breast percentage which was significantly lesser in low inorganic Zn and Cu level than other treatments (Table 5). Cncomitant use of lower level of inorganic Zn and Cu decreased the breast percentage than other treatments. Tibia length and weight (Table 6) improved by supplementing diets with mixed organic Zn and Cu when compared to the use of inorganic source of them together ($P < 0.05$). Moreover, only supplementing diets with the lower levels of mixed inorganic Zn and Cu led to significant decrease of tibia diameter. The Zn content of tibia in broilers received both complete (recommended) levels of organic Zn and Cu was higher when compared with the concomitant use of inorganic Zn and Cu and also with the use of lower level of organic Zn plus recommended level of Cu in inorganic form. The Cu content of liver in the treatment consisted of both complete levels of organic Zn and Cu was higher than that of supplementing inorganic Zn and Cu together (Figure 1).

Table 5. Effect of organic or inorganic Zn and Cu supplementation on carcass characteristics of broiler chickens†

	Item ‡						
Treatment	Carcass	Breast	Thigh	Liver	Heart	Spleen	Abdominal fat
Ino-Zn_{100} + Ino-Cu_{100}	67.25	26.51^{a}	22.03	1.924	0.471	0.130	1.737
Org-Zn_{100} + Org-Cu_{100}	67.31	26.42^{a}	22.28	1.936	0.484	0.128	1.856
Ino-Zn_{50} + Ino-Cu_{50}	66.20	26.07^{b}	21.95	1.964	0.490	0.131	1.797
Org-Zn_{50} + Org-Cu_{50}	67.35	26.33^{a}	22.07	1.996	0.472	0.126	1.788
Org-Zn_{50} + Ino-Cu_{100}	67.29	26.47^{a}	21.99	1.930	0.485	0.125	1.746
Ino-Zn_{100} + Org-Cu_{50}	66.74	26.34^{a}	22.11	2.006	0.488	0.125	1.832
SEM#	0.16	0.03	0.05	0.02	0.003	0.0008	0.04
P-value	0.25	<0.001	0.39	0.77	0.50	0.21	0.97

a-b Means within a column having different superscripts are significantly different ($P < 0.05$).
† Means obtained from 4 pens per each dietary treatment.
‡ Relative weight (g/100 g body weight) of the cuts and internal organs of broiler chickens fed diets containing different forms and amount of Zn and Cu.
# *SEM* = standard error of means.

Table 6. Effect of organic or inorganic Zn and Cu supplementation on tibia characteristics of broiler chickens

Treatment	Item						
	Length (mm)	Weight (gr)	Diameter (mm)	Ash (%)	Ca (%)	P (%)	Zn (mg/Kg)
Ino-Zn_{100} + Ino-Cu_{100}	105.06^{b}	11.98^{b}	8.73^{a}	51.62	20.46	9.51	216.73^{c}
Org-Zn_{100} + Org-Cu_{100}	107.35^{a}	12.42^{a}	9.09^{a}	52.87	20.74	9.82	228.49^{a}
Ino-Zn_{50} + Ino-Cu_{50}	103.05^{c}	11.70^{c}	8.38^{b}	50.54	20.25	9.27	205.75^{c}
Org-Zn_{50} + Org-Cu_{50}	106.56^{ab}	12.25^{ab}	8.90^{a}	52.04	20.59	9.65	221.98^{ab}
Org-Zn_{50} + Ino-Cu_{100}	106.03^{ab}	12.34^{a}	9.04^{a}	53.17	20.83	9.91	220.04^{b}
Ino-Zn_{100} + Org-Cu_{50}	105.86^{ab}	12.19^{ab}	8.83^{a}	50.89	20.32	9.36	221.19^{ab}
SEM‡	0.33	0.05	0.05	0.35	0.09	0.1	1.39
P-value	0.002	<0.0001	0.001	0.18	0.41	0.41	<0.0001

a-c Means within a column having different superscripts are significantly different ($P < 0.05$).
‡*SEM* = standard error of means.

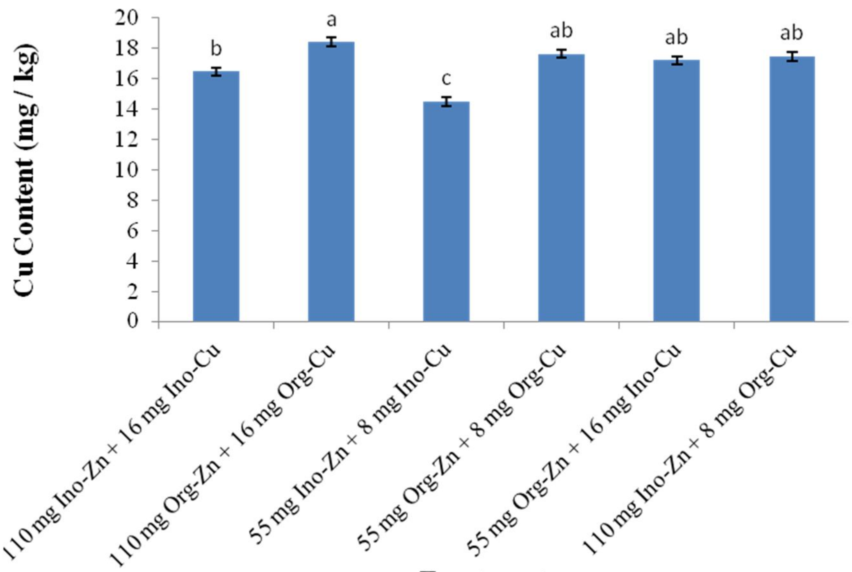

Figure 1. Effects of organic or inorganic Zn and Cu supplementation on Cu content of liver in broiler chickens. a-cColumns with different letters are significantly different ($P < 0.05$). Error bars indicate standard error of means.

Table 7. Effect of organic or inorganic Zn and Cu supplementation on blood profileof broiler chickens

Treatment	Blood profile†						
	GLU (mg/dl)	CHOL (mg/dl)	TG (mg/dl)	TP (g/dl)	ALT (IU/L)	AST (IU/L)	ALP (IU/L)
Ino-Zn_{100} + Ino-Cu_{100}	270.71	105.29^{ab}	74.45	3.72	23.51	96.88	120.63^{b}
Org-Zn_{100} + Org-Cu_{100}	266.81	96.03^{c}	66.10	3.57	21.73	103.13	124.38^{a}
Ino-Zn_{50} + Ino-Cu_{50}	267.65	110.45^{a}	59.28	3.82	20.13	96.75	115.88^{c}
Org-Zn_{50} + Org-Cu_{50}	263.31	100.68^{bc}	77.28	3.73	22.19	104.13	125.75^{a}
Org-Zn_{50} + Ino-Cu_{100}	273.33	96.83^{c}	66.36	3.64	20.85	98.63	122.25^{ab}
Ino-Zn_{100} + Org-Cu_{50}	272.26	107.84^{a}	69.44	3.97	20.08	99.38	120.13^{b}
SEM‡	2.42	1.13	2.67	0.07	0.48	1.05	0.65
P-value	0.86	<0.0001	0.44	0.72	0.27	0.19	<0.0001

a-cMeans within a column having different superscripts are significantly different ($P < 0.05$).
† GLU: Glucose; CHOL: Cholesterol; TG: Triglycerides; TP: Total protein; ALT: Alanine transaminase; AST: Aspartate transaminase; ALP: Alkaline phosphatase.
‡ *SEM* = standard error of means.

Blood profile

The effect of organic or inorganic Zn and Cu sources on blood profile of broiler chickens are presented in Table 7. Serum cholesterol concentration significantly decreased with supplementing organic Zn when compared with the only 50% supplying of the trace mineral requirements from inorganic source and also with completely meet the Zn from inorganic source plus 50% supplying of the recommended Cu level from organic source in the mineral premix. Serum cholesterol concentration significantly decreased ($P < 0.05$) with supplementing organic Zn and also with completely meet the Zn and Cu requirements from inorganic source than only 50% supplying of the trace mineral requirements from inorganic source. Broiler chickens fed organic Zn and Cu had significantly ($P < 0.05$) higher ALP activity than those fed diet supplemented with the inorganic forms ($P < 0.05$). None of the treatments had significant effect on serum concentration of glucose, triglycerides, total protein, ALT, and AST.

Discussion

In this study, in contrast to the organic Zn and Cu treatments, the decreased level of inorganic trace minerals was not enough to support optimum growth response and so a higher feed intake strategy was applied to compensate their deficiency in the diets by broiler chickens. However, Rossi *et al*. (2007) reported that zinc deficiency decrease the appetite. Also, in agreement with the results of the present study, it is reported that the use of organic trace mineral improves the performance of broiler chickens (Ao *et al*., 2009; Bao *et al*., 2010; ShyamSunder *et al*., 2013). However, increasing levels of organic trace minerals (i.e., 0, 17, 33, 50, 67, and 100% of replacement) had no significant influence on mortality (Nollet *et al*., 2008). It also noted that the use of organic trace minerals at 50% or 67% during the first 3 weeks more improved growth performance of broiler chickens. On the other hand, some studies found no significant effect on growth performance and mortality when used organic trace minerals instead of the inorganic forms (Nollet *et al*., 2007; Zhao *et al*., 2010; Manangi, 2012). Although the reason of the differences among the studies is not clear for authors, improved performance of the birds under both organic forms of Zn and Cu treatments could be due to the better absorbance of the minerals. Moreover, lower performance of broilers undercomplete (recommended) levels of inorganic Zn and Cu could be because of the antagonism between them (Ao *et al*., 2009).

The FDP is a condition that is commonly seen in commercial broilers (Kjaer *et al*., 2006). Footpad health helps to minimize the welfare issue (Manangi *et al*., 2012). Factors including diet, litter condition (e.g., moisture and type of litter material), sex, drinker design, biotin deficiency, body weight, and genetics could lead to dermatitis (Mayne, 2005; Kjaer *et al*., 2006; Shepherd and Fairchild 2010). Pale skin is the early symptom of FPD, then hyperkeratosis and epidermis necrosis occurs which finally lead to tolesoinsand accompanied by subcutaneous tissue inflammatoin (Ekstrand *et al*., 1997). Lesions on the footpads may serve as a pass for bacterial infections, and finally may negatively affect the health and performance of the broiler chickens (Dibner *et al*., 2007). Although Zhao *et al*., (2010) and Manangi *et al* (2012) reported that supplementing of diet with organic Zn and Cu improved the footpad health; ShyamSunder *et al* (2013) reported that the footpad scores was not affected by the source of the trace minerals. A part of the lower severity and incidence of FPD in treatments containing organic Zn could be because of the role of Zn in collagen (i.e., a major fibrous element of the skin) formation and cross-linking process of the collagen, which hold the cells more tightly together (Saenmahayak *et al*., 2010).

Some studies showed that use of organic trace mineral had no significant influence on carcass characteristics (Virden *et al*., 2003; Manangi *et al*., 2012). However, De Marco *et al* (2017) found that supplementing metal chelates of glycine improve breast yield of broiler chickens. In the present study, insufficient supplementation of Zn and Cu had an adverse effect on breast development (about 40% of the carcass weight) due to the lower growth rate of the broiler chickens. It's reported that under severe Zn deficiency, the activities of some enzymesinvolved insynthesis of DNA, RNA, and proteins may be greatly depresses and impairs cellular division and growth rate (Sarvari *et al*., 2015).

Although, some research showed that use of the organic trace minerals had no significant effect on tibia characteristics, and Zn and Cu content of tissues (Bao *et al*., 2010; Zhao *et al*., 2010; Das *et al*., 2014a), others (Ao *et al*., 2009; El-Husseiny *et al*., 2012; ShyamSunder *et al*., 2013) showed that treatment containing organic trace mineral had higher tibia weight and Zn. Zinc has a stimulating effect onformation, mineralization and preservation of bone tissue throught modification of hydroxyl apatite crystallization, improvement of collagen synthesis (Dibner *et al*., 2007), and prevention of osteoclastic bone resorption by inhibiting the change of marrow cells to osteoclast-like cell (Yamaguchi, 1998). On the other hand, Cuis essential for activity of lysyl oxidase enzyme to form lysine- derived cross-link in collagen, which gives bone its tensile strength and elasticity (Dibner *et al*., 2007). Hence, occurrence of the lowest value of length, weight, diameter, and Zn content of tibia in the treatment containing 50% organic trace mineral may be related to deficiency of Zn and Cu in the diets.

Ao *et al* (2009) reported that high level of organic Znin diets decrease the concentration of Cu in liver due to the sequestration of dietary Cu by intestinal metallothionein; so that, more Zn absorption leads to more intestinal metallothionein formation. According to the lower levels of liver Cu in treatment consisted of both inorganic Zn and Cu supplement, it could be concluded that the supplementing levels of inorganic Cu were not sufficient to compensate the Cu sequestration.

Zinc and Copper are related to lipid metabolism. Some studies (Idowu *et al*., 2011; Das *et al*., 2014b) indicated that the use of organic Zn supplement decrease serum total cholesterol compared to the inorganic Zn (e.g., zinc oxide, zinc sulphate, and zinc carbonate). In contrast, Al-Daraji and Amen (2011) reported that supplementing of different levels of pure Zn to diet increased serum cholesterol concentration. The Cu deficiency and high ratios of Zn/Cu often increases serum cholesterol (Johnson, 1990). Hence, higher cholesterol concentration of serum in birds received half of the recommended level of organic Zn and Cu may be related to deficiency of Cu and antagonistic effect of Zn supplement on Cu absorption.

Furthermore, Zn is a cofactor for hepatic enzymes such as ALP, AST, and ALT (Bennett *et al*., 2001) and interferes in many metabolic and enzymatic functions (Prasad *et al*., 2009). Das *et al* (2014a) reported that these hepatic enzymes activity were affected by organic Zn in diets. It is reported that supplementing of organic Zn increased ALP activity, but not affected AST and ALT, when compared to the inorganic Zn (Idowu *et al*., 2011). On the other hand, Das *et al* (2014a) reported that the organic minerals had no effect on ALP, AST, and ALT. ALP is an important protein marker in bone for osteoblast differentiation (Graneli *et al*., 2014). High ALP activity usually is symptoms of either the liver damage or increased-activity of cell bone (Sarac and Saygili, 2007). However, according to the results of ALT, AST, and growth performance of the broiler chickens, it could be concluded that higher ALP level in the birds received the diet supplemented with organic Zn and Cu occurred because of higher bones growth and development.

Conclusion

The results of this study showed that organic Zn and Cu are noticeably effective on improvement of growth performance of young broiler chickens. Also, supplementation levels of organic Zn and Cu in diet could be lowered to 50% of the recommended levels for broiler chickens, without any adverse effect on performance. Furthermore, results of footpad lesion score and blood profile confirmed that organic Zn and Cu are an adequate alternative to inorganic forms of the minerals and antagonistic effect between inorganic Zn and Cu could be overcome by using at least organic Zn or Cu in diets.

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