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Influence of Feeding Diets Supplemented with Different Levels and Sources of Zinc, Copper and Manganese on the Mineral Concentrations in Tibia and Performance of Broiler Chickens

A.A. Gheisari, A. Rahimi-Fathkoohi, M. Toghyani and M.M. Gheisari
Department of Animal Science, Islamic Azad University, Khorasgan (Isfahan) Branch, 81595-158 Isfahan, Iran

Corresponding Author: Abbas Ali Gheisari, Department of Animal Science, Islamic Azad University, Khorasgan (Isfahan) Branch, 81595-158 Isfahan, Iran

ABSTRACT

This experiment was conducted to evaluate the effect of dietary supplementation of organic chelates of zinc, manganese and copper compare to their inorganic sources on performance and concentration of these minerals in the tibia of broiler chicks. Three hundred and twelve day-old broiler chicks (Ross 308) were randomly distributed into 6 treatments with four replicates of 13 birds each based on a completely randomized design. Treatments and mineral levels included: A: control (commercial diet) containing 100, 100 and 10 mg kg^{-1} Zn, Mn and Cu from inorganic sources; B: containing 140, 140 and 17 mg kg^{-1} Zn, Mn and Cu from inorganic and organic sources; C: containing 40, 60 and 8 mg kg^{-1} Zn, Mn and Cu from inorganic sources; D: 40, 40 and 7 mg kg^{-1} Zn, Mn and Cu from organic sources; E: 40, 40 and 7 mg kg^{-1} Zn, Mn and Cu from inorganic (sulfate) sources; F: 60, 60 and 10.5 mg kg^{-1} Zn, Mn and Cu from organic source, respectively. Feed intake of chicks fed B diet from 0 to 21 days of age was significantly ($p<0.05$) higher than birds receiving control diet, but from 21 to 42 days of age the E treatment significantly ($p<0.05$) consumed more feed compared to control group. Feed conversion ratio of control group improved significantly ($p<0.05$) in comparison with C treatment. According to obtained results, corn-soybean diets supplemented with 40-40-7 mg kg^{-1} Zn-Mn-Cu from their sulfate or organic sources instead of application of 100-100-10 mg kg^{-1} Zn-Mn-Cu from their oxide sources can support performance of broiler chickens properly.

Key words: Broiler, zinc, copper, manganese, organic mineral, productive performance

INTRODUCTION

Trace minerals such as copper, zinc and manganese are essential elements for normal growth and development in broiler chicks. They play a key role in the maintenance and development of the skeleton and are essential components of a number of enzymes, vitamins and hormones. It seems that NRC (1994) gives the minimum levels that are necessary for optimum productivity. In practice, feed manufacturers use much higher concentrations than those specified by NRC (1994) to achieve maximized performance. Hence, mineral deficiencies are not commonly observed (Sayadi *et al.*, 2005).

On The other hand, large-scale commercial livestock production systems, have given rise to many environmental concerns, since the excess mineral concentrations in manure can lead to mineral depositions that exceed crop nutrient requirements (Payne *et al.*, 1988; Leeson and Caston, 2008). Therefore, there is currently an increasing interest in studying factors that improve absorption and metabolization of these trace elements (Fernandes *et al.*, 2008). There is a wealth of information describing the mineral bioavailability from various inorganic sources (Wedekind and Baker, 1990; Sandoval *et al.*, 1997; Ledoux *et al.*, 1991). In general, sulfates are thought to have higher bioavailability than do their oxides (Ledoux *et al.*, 1991; Smith *et al.*, 1995; Pesti and Bakalli, 1996; Sandoval *et al.*, 1997). Organic sources or metal chelates have also been used with aim of enhancing trace mineral bioavailability than inorganic salt analogues by binding minerals to organic molecules, allowing the formation of structure with unique characteristics and high bioavailability (AAFCO, 1997). This implies that chelated minerals can be utilized at a much lower concentration in the diet than inorganic minerals, without a negative impact on production performance. Therefore, organic mineral sources, such as proteinate and amino acid chelates, have been used increasingly in recent years due to their higher bioavailability (Wedekind and Baker, 1990; Wedekind *et al.*, 1992; Cao *et al.*, 2000) and lower manure loading (Manon *et al.*, 2005; Pierce *et al.*, 2005). Although, Pimentel *et al.* (1991) did not observed any differences in relative bioavailability between oxide and organic zinc sources in broilers. Baker *et al.* (1991) also evidenced similar utilization of organic copper (copper-lysin) and copper sulfate, but higher zinc oxide availability in broilers. On the other hand, Kienholz (1992) showed that feeding zinc chelates to layers submitted to heat stress, associated with low calcium intake, maintained size egg, whereas inorganic zinc supplementation reduced egg size.

Paik (2001) evaluated the utilization of organic zinc, copper and manganese, either individually or in combination and observed improvement in the production performance of birds fed organic copper and the combination of these three metals in an organic complex. However, no performance improvement was observed with use of either organic zinc or combination of copper and zinc chelates.

Organic mineral sources exist in the forms of metal amino acid chelate, metal proteinate and metal specific amino acid complexes. Metal amino acid chelate and metal proteinate are the chelation of a soluble salt with amino acids or hydrolysed protein. The molar ratio is 1 mole of a soluble salt with 2 or 3 moles of an amino acid. Whereas, metal specific amino acid complexes consists of a specific amino acid complexed with a soluble metal salt in a molar ratio of 1:1 (Dozier *et al.*, 2003). The combination of one metal ion complexed with one single amino acid may account for the increased utilization of the mineral by the bird compared with metal proteinates and inorganic mineral sources (Burrell *et al.*, 2004). The review of the literature suggests that it may be possible to improve chick's performance and immune system through unconventional methods of diet manipulation. As a result, to ensure the normal levels of such minerals, premixes are added to the ration.

The objective of this study was to evaluate the effect of feeding diets supplemented with the combination of three metal specific amino acid (Zn, Mn and Cu-Methionn complexes) named Aviala ZMC (Zinpro Animal Nutrition Inc., Eden Prairie, Mn 55344) compared to their inorganic (oxide and sulfate) sources on the performance and concentration of the minerals in tibia ash of broiler chicks.

MATERIALS AND METHODS

Birds and diets: This study was performed from January 1 to February 20, 2009 at the Isfahan Research Center for Agriculture and Natural Resources, Isfahan, Iran. Three hundred and twelve, day-old mixed sex broiler chicks (Ross 308) were individually weighed and randomly divided into 6 main groups of 52 chicks. To limit differences due to position, these groups were each divided into 4 subgroups of 13 chicks each. The chicks were randomly allocated to floor pens and exposed to continuous light for the first 2 days, then to 23 h light and 1 h darkness until 49 days of age. Diets (in mash form) and water were provided for *ad libitum* consumption. Corn-soybean experimental diets (Table 1) were formulated to meet broiler chickens nutritional requirements, as recommended by NRC (1994).

These diets consisted of the basal diet supplemented with added Zn, Mn and Cu in combination, from either inorganic or organic sources. The mineral premix used in this experiment was a commercial premix without Zn, Mn and Cu. The inorganic supplementations were zinc sulfate ($ZnSO_4$), zinc oxide (ZnO), manganese sulfate ($MnSO_4$), manganese oxide (MnO) and copper sulfate ($CuSO_4$. $5H_2O$). Organic supplementation (Aviala ZMC, Zinpro Animal Nutrition Inc., Eden Prairie, Mn 55344) consisted of a combination of complex amino-acid-metals that contained 40 mg kg^{-1} Zn, 40 mg kg^{-1} Mn and 7 mg kg^{-1} Cu, respectively.

Six dietary treatments based on corn and soybean meal with different mineral premix levels were formulated as follow: A: basal diet supplemented with common mineral premix containing 100, 100 and 10 mg kg^{-1} Zn, Mn and Cu from ZnO, MnO and $CuSO_4$ sources, respectively (control diet); B: basal diet supplemented with 140, 140 and 17 mg kg^{-1} Zn, Mn and Cu from inorganic (ZnO, MnO and $CuSO_4$) and organic (40, 40, 7 mg kg^{-1} Zn-Mn-Cu); C: basal diet supplemented

Table 1: Composition of the basal diets

Items	Starter (0-21 day)	Grower (21-42 day)	Finisher (42-49 day)
Ingredients (%)			
Corn	57.60	58.60	64.11
Soybean meal	37.21	33.76	28.60
Soybean oil	1.09	4.00	4.00
Di-Calcium phosphate	1.82	1.24	0.99
Oyster shell	1.32	1.42	1.34
Vitamin-mineral premix[1]	0.50	0.50	0.50
DL-methionine	0.13	0.06	0.01
L-lysine	-	0.16	0.36
Salt	0.33	0.26	0.18
Calculated composition			
Metabolizable energy (Kcal kg^{-1})	2865.00	3100.00	3175.00
Crude protein (%)	20.60	19.40	17.80
Calcium (%)	1.00	0.90	0.80
Available phosphorus (%)	0.45	0.35	0.30
Methionine + Cystein (%)	0.89	0.71	0.60
Lysine (%)	1.00	1.00	0.80
Zinc (mg kg^{-1})	25.00	24.00	23.00
Manganese (mg kg^{-1})	14.80	13.80	12.80
Copper (mg kg^{-1})	9.90	9.20	8.20

[1]Provided per kg of diet: vitamin A (as all-trans retinol acetate): 15,000 IU; cholecalciferol: 3900 IU; vitamin E (as all-rac--tocopherol acetate): 30 IU; vitamin K (as menadione sodium bisulfate): 3.0 mg; thiamin (as thiamin mononitrate): 2.4 mg; riboflavin: 9.0 mg; vitamin B6: 4.5 mg; vitamin B12: 0.021 mg; calcium pantothenate: 30 mg; niacin: 45 mg; folic acid: 1.2 mg; biotin: 0.18 mg; choline (as choline chloride): 700 mg; iron (from iron sulphate): 80 mg; iodine (from potassium iodide): 0.35 mg; selenium (from sodium selenite): 0.15 mg

with premix providing NRC (1994) requirements for Zn, Mn and Cu supplied by sulfates and oxide (40, 60, 8 mg kg^{-1}); D: basal diet supplemented with 40, 40 and 7 mg kg^{-1} Zn, Mn and Cu from Zn-Mn-Cu-amino acid complex; E: basal diet supplemented with 40, 40 and 7 mg kg^{-1} Zn, Mn and Cu supplied by sulfates; F: basal diet supplemented with 60, 60 and 10.5 mg kg^{-1} Zn, Mn and Cu from Zn-Mn-Cu-amino acid complex.

Performance: Body weight was determined at 1, 21, 42 and 49 days of age. Feed intake, weight gain and feed conversion ratio (feed intake: weight gain) were recorded in different periods and overall growth period.

Carcass components: At the end of the experiment (49 days of age), two birds were c randomly chosen from each replicate, slaughtered and the abdominal fat, liver, bursa of Fabercius and spleen were collected, weighed and calculated as a percentage of live body weight.

Zn, Mn and Cu concentrations in Tibia Ash: At 49 days of age, right tibia was removed from two birds per replicate for determination of bone ash percentage and Zn, Mn and Cu concentration according to the method described by Kurtoglu *et al.* (2005).

Statistical analysis: The data were subjected to analysis of variance procedures appropriate for a completely randomized design using the General Linear Model procedures of SAS Institute (1997). Means were compared using Duncan multiple range test. Statements of statistical significance are based on ($p<0.05$).

RESULTS

Neither dietary Zn, Mn and Cu concentrations nor Zn, Mn and Cu sources significantly altered body weight at days 21, 42 and 49 (Table 2). The effects of Zn, Mn and Cu levels and sources on feed consumption were significant ($p<0.05$) only when trace minerals were greater in the diets (B treatment) in starter period, but in grower period E treatment had a higher feed intake. Dietary supplementation of Zn, Mn and Cu did not affect daily feed intake in other periods.

Supplementation of diets with the highest levels of inorganic forms of Zn, Mn and Cu (100, 100 and 10 mg kg^{-1}, respectively) (A treatment) and the lowest levels of their organic forms (40, 40 and 7 mg kg^{-1}) markedly improved feed conversion ratio (1.41 and 1.42 g g^{-1}, respectively) in starter period. Average feed conversation ratio at 21-42 day of age for A treatment and also group fed diet containing 60, 60 and 10.5 mg kg^{-1} Zn, Mn and Cu, respectively (F treatment) were significantly better than chickens fed the diet in which Zn, Mn and Cu concentrations were according to NRC recommendation (C treatment) from their inorganic sources. However, the best overall feed conversion ratio was observed for birds fed diets supplemented with the highest levels of inorganic forms of the elements during different periods of the experimental period (A treatment). On the other hand, as shown in Table 2 the highest average of overall feed conversion rate (2.15 g g^{-1}) was observed for group fed diet containing 40-60-8 mg kg^{-1} Zn-Mn-Cu from their oxide, oxide and sulfate sources, respectively (C treatment).

Dietary treatments had only a significant ($p<0.05$) effect on carcass yield at 49 d of age (Table 3). Birds fed Zn, Mn and Cu, in sulfate forms (E treatment) significantly ($p<0.05$) had a higher carcass yield than those fed oxide forms of Zn, Mn and Cu. Supplementary diets with

Table 2: Effect of different mineral supplementations on the performance of broiler chickens

	Treatment[1]						
Parameters	A (inorganic)	B (mix)	C (inorganic)	D (organic)	E (inorganic)	F (organic)	SEM
Body weight (g)							
21 day	524.00	520.00	524.00	539.00	525.00	501.00	0.035
42 day	1899.00	1898.00	1859.00	1917.00	1961.00	1894.00	0.021
49 day	2448.00	2447.00	2443.00	2470.00	2521.00	2448.00	0.286
Feed intake (g day^{-1})							
0-21 day	31.30^{a}	35.00^{b}	34.30^{ab}	33.80^{ab}	34.50^{ab}	34.40^{ab}	0.200
21-42 day	132.20^{a}	142.51^{ab}	147.90^{ab}	137.50^{ab}	154.1^{0b}	134.80^{a}	2.460
42-49 day	183.50	190.30	179.40	176.60	182.40	179.70	2.970
0-49 day	97.10	103.30	103.80	98.60	106.90	98.20	1.320
Feed conversion ratio (g:g)							
0-21 day	1.41^{a}	1.54^{ab}	1.49^{ab}	1.42^{a}	1.50^{ab}	1.56^{b}	0.019
21-42 day	2.01^{a}	2.20^{ab}	2.34^{b}	2.13^{ab}	2.25^{ab}	2.03^{a}	0.042
42-49 day	2.37	2.37	2.29	2.23	2.30	2.27	0.039
0-49 day	1.97^{a}	2.10^{ab}	2.15^{b}	2.00^{ab}	2.11^{ab}	1.99^{ab}	0.024

[1]A: 100, 100 and 10 mg kg^{-1} Zn, Mn and Cu from ZnO, MnO and $CuSo_4$; B: 100, 100 and 10 mg kg^{-1} Zn, Mn and Cu from ZnO, MnO, $CuSo_4$ and 40, 40, 7 mg kg^{-1} Zn-Mn-Cu-Amino acid; C: 40, 60 ,8 mg kg^{-1} Zn, Mn and Cu from ZnO, MnO and $CuSO_4$; D: 40, 40 and 7 mg kg^{-1} Zn, Mn and Cu from Zn-Mn-Cu-Amino acid; E: 40, 40 and 7 mg kg^{-1} Zn, Mn and Cu supplied by sulfates. F: 60, 60 and 10.5 mg kg^{-1} Zn, Mn and Cu from Zn-Mn-Cu-amino acid. Means with different superscript within a row are significantly different ($p<0.05$)

Table 3: Effects of dietary Zn, Mn and Cu source and levels on carcass traits and lymphoid organs (percentage of live weight)

	Supplement (mg kg^{-1})				Carcass	Abdominal fat	Bursa of fabricius	Spleen	Liver
Treatment	Zn	Mn	Cu	Source	(%)				
A	100	100	10	Inorganic	74.680^{ab}	2.160	0.055	0.103	1.970
B	100+40	100+40	10+7	Mix	74.950^{ab}	1.940	0.121	0.118	2.030
C	40	60	8	Inorganic	73.590^{a}	2.240	0.042	0.095	1.830
D	40	40	7	Organic	74.070^{a}	2.040	0.060	0.102	1.950
E	40	40	7	Inorganic	75.590^{b}	1.670	0.050	0.096	2.000
F	60	60	10.5	Organic	74.530^{ab}	2.150	0.053	0.114	1.920
SEM					0.192	0.092	0.012	0.003	0.027

Means with different superscript within a column are significantly different ($p<0.05$)

different sources and levels of Zn, Mn and Cu did not have any significant effects on abdominal fat, bursa of Fabricius and spleen percentage (Table 3). However, increasing Zn, Mn and Cu levels improved bursa of Fabricius and spleen percentages (treatment B).

Tibia ash percentage increased in chicks fed diets containing the highest level of Zn, Mn and Cu (B treatment), but this increase was not significant (Table 4). Tibia Mn and particularly Zn and Cu concentrations increased in chickens fed diets containing high levels of Zn, Mn and Cu from combination of both their organic and inorganic sources (B treatment). On the other hand, among the groups fed diet supplemented with the same concentrations of Zn, Mn and Cu (C, D and E treatments) birds supplemented with organic sources of the minerals tended to have higher levels of tibia ash and also Mn and Cu concentrations in tibia ash at 49 day of age. During the experimental period mortality was within acceptable ranges (less than 3%) and was not related to dietary treatments.

Table 4: Effects of dietary Zn, Mn and Cu sources and levels on tibia ash and concentration of Zn, Mn and Cu in tibia ash of broiler chickens

	Supplement (mg kg^{-1})							
					Tibia ash	Zn	Mn	Cu
Treatment	Zn	Mn	Cu	Source	(%)	(mg kg^{-1})		
A	100	100	10	Inorganic	51.150	126^{ab}	7.120	1.250^{ab}
B	100+40	100+40	10+7	Mix	58.590	131^{b}	8.000	1.440^{b}
C	40	60	8	Inorganic	54.500	115^{a}	6.500	0.540^{a}
D	40	40	7	Organic	56.560	120^{ab}	7.180	0.880^{ab}
E	40	40	7	Inorganic	55.960	123^{ab}	6.870	0.680^{ab}
F	60	60	10.5	Organic	57.980	125^{ab}	7.250	0.980^{ab}
SEM					0.559	2.024	0.239	0.105

Means with different superscript within a column are significantly different ($p<0.05$)

DISCUSSION

In the present experiment broiler chicks responses to different dietary Zn, Mn and Cu sources and levels were studied. According to the results showed in Table 1 application of only high levels of the experimental minerals (Zn, Mn and Cu) from their inorganic sources (ZnO, MnO and $CuSO_4.5H_2O$) (B treatment) supported an optimal growth performance of broiler chicks. Whereas, dietary application of Zn, Mn and Cu at equal amounts of NRC recommendation (40, 60 and 8 mg kg^{-1}, respectively) (C treatment) from the sources same as B treatment caused remarkable negative effects on body weight, feed intake and feed conversion rate during different stages of rearing period. It means that birds fed oxide form of the manganese and zinc faced with their bioavailability, since increasing their dietary concentrations from 40 to 100 mg kg^{-1} markedly improved most of the growth performance indices, particularly feed conversion ratio and their concentrations in tibia ash (Table 4). The results are in contradictory with findings of Pimentel *et al*. (1991) who observed no differences in relative bioavailability between oxide and organic zinc sources in broilers. In this relation, availability of minerals within inorganic sources has been shown to vary from 60 to 96% (Henry *et al*., 1989; Zanetti *et al*., 1991) although for a specific ingredient such as Mn oxide, bioavailability has been reported to vary by 60% depending on its source (Wong-Valle *et al*., 1989).

On the other hand, substitution of oxide sources with their organic (D treatment) or sulfate forms (E treatment) to supply Zn, Mn and Cu requirements suggested by NRC (40, 60 and 8 mg kg^{-1}, respectively) supported growth performance rate similar to that of A treatment as and more better than C treatment. This result is in agreement with Ledoux *et al*. (1991), Smith *et al*. (1995), Pesti and Bakalli, (1996) and Sandoval *et al*. (1997) who reported that sulfates forms of the trace minerals have higher bioavailability than their oxides. Thereby, some researchers (Wong-Valle *et al*., 1989; Wedekind and Baker, 1990) have shown that using minerals in sulfate form at 40% of current oxide form inclusions, supports bird performance. Regarding bioavailability of organic sources of minerals, Mn proteinates have been shown to be 50-70% more available than oxides (Smith *et al*., 1995), while Henry *et al*. (1989) suggested 30 to 40% greater bioavailability of a manganese-methionine chelate compared to an oxide source. While, Miles *et al*. (2003) showed reduced potency of amino acid chelates of Cu and Mn compared to sulfates.

On the other hand, some studies indicated small or no differences in bioavailability of Zn between inorganic and organic Zn sources (Pimentel *et al*., 1991; Baker and Ammerman, 1995). However, variability in availability of organic minerals may relate to their ability to remain chelated during solubilization (Guo *et al.*, 2001). Interestingly, use of additional dosages of even organic forms of the minerals more than 100, 100 and 10 mg kg^{-1} Zn, Mn and Cu existing in A treatment (B treatment) or 40, 40 and 7 mg kg^{-1} existing in D treatment (F treatment) did not any significant improvement in growth performance parameters (Table 2) or their concentrations in tibia ash (Table 4). It means that with inclusion of additional dosage of trace elements into diets improvement of performance would not be expected. Meanwhile, according to current researches on trace minerals it is suggested that broilers fed diets supplemented with trace minerals far in excess of their actual requirements.

In current experiment, dietary supplementation of the trace minerals did not significantly affect lymphoid organ weights. Nevertheless, the relative weights of bursa of Fabricius and spleen in group fed diet containing high levels of zinc, manganese and copper (B treatment) were higher than the other groups. Virden *et al.* (2004) also showed that dietary supplementation of Zn and Mn increased relative weight of spleen and bursa Fabricius in progeny chicks.

The increase in tibia zinc observed when broiler diets were supplemented with a combination of Zn-AA+$ZnSO_4$ (B treatment) may be due in part to increased zinc absorption, which by supplying both organic and inorganic sources of zinc could involve more absorption sites or transporters in the intestine, hence increasing zinc retention (Burrell *et al.*, 2004). Irrespective of dietary concentration impact of the minerals on their deposition rate in tibia, our results showed that with feeding different diets containing same concentrations of Zn, Mn and Cu (C, D and E treatments) birds will be able to deposit organic form of minerals more efficient than their inorganic forms. Henry *et al.* (1989) also reported that the estimated bioavailability based on bone Mn accumulation was 108% for Mn-Met relative to Mn sulfate in chicks fed corn-soybean meal diets.

In conclusion, present study shows that NRC (1994) recommendation for supplementation of corn-soybean diet with 40-60-8 mg kg^{-1} Zn-Mn-Cu cannot support maximum broiler chicken's performance. In addition, corn-soybean diets supplied with 40-40-7 mg kg^{-1} Zn-Mn-Cu from their sulfate or organic (metal amino acid complexes) sources instead of application of 100-100-10 mg kg^{-1} Zn-Mn-Cu from their oxide sources can support performance of broiler chickens properly.

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