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Effects of organic chelates of zinc, manganese and copper in comparison to their inorganic sources on performance of broiler chickens

[redacted]

1 SUMMARY

This experiment was conducted to compare organic chelates of Zinc (Zn), Manganese (Mn) and Copper (Cu) 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 10mg/kg Zn, Mn, and Cu from inorganic sources; B: containing 140, 140, and 17 mg/kg Zn, Mn, and Cu from inorganic and organic sources; C: containing 40, 60, and 8 mg/kg Zn, Mn, and Cu from inorganic sources; D: 40, 40, and 7 mg/kg Zn, Mn, and Cu from organic sources; E: 40, 40, and 7 mg/kg Zn, Mn, and Cu from inorganic (sulfate) sources; F: 60, 60 and 10.5 mg/kg 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 Zn-Mn-Cu from their sulfate or organic sources instead of application of 100-100-10 mg/kg Zn-Mn-Cu from their oxide sources can support performance of broiler chickens properly.

2 INTRODUCTION

Trace minerals such as copper, zinc and manganese are essential elements for development and growth 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, etc. National Research Council (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 (Jafari sayadi et al., 2005).

Today, 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. There is a wealth of information describing the mineral bioavailability from various salts. In general, sulphates are thought to have higher bioavailability than do their oxides (Ledoux et al., 1991; Smith et al., 1995;

Pesti and Bakalli, 1996). The use of organically complexed or chelated minerals in premixes for livestock diets has been suggested based on the hypothesis that such mineral complexes have a higher bioavailability than inorganic salt analogues. 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. 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, 1989; Wedekind et al., 1992; Cao et al., 2000) and lower manure loading (Manon et al., 2005; Pierce et al., 2005).

Organic mineral sources exist in the form of metal amino acid chelates, metal proteinate, and metal specific amino acid complexes. Metal amino acid chelates and metal proteinate are the chelating of a soluble salt with amino acids or hydrolyzed protein. The molar ratio is 1 mole of a soluble salt with 2 or 3 moles of an amino acid. However, metal specific amino acid complexes and metal amino acid complexes consist of a specific amino acid or free amino acids complexed with a soluble metal salt in a molar ratio of 1:1. 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 supplementing Zn-Mn-Cu in organic form (Avila ZMC) compared to their inorganic sources on performance and concentration of these minerals in the tibia of broiler chicks.

3 MATERIALS AND METHODS

3.1 Birds and diets: 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. The composition of the starter (1-21 days), grower (21-42 days) and finisher (42-49 days) diets are given in Table 1. 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 consisted of a combination of complex amino-acid-metals that contained 40 mg/kg Zn, 40 mg/kg Mn, and 7 mg/kg Cu, respectively.

Six dietary treatments based on corn and soybean meal with different mineral premix levels were formulated as follows. A: basal diet supplemented with common mineral premix containing 100, 100, and 10 mg/kg 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 Zn, Mn, and Cu from inorganic (ZnO, MnO, and $CuSO_4$) and organic (40, 40, 7 mg/kg Zn-Mn-Cu); C: basal diet supplemented with premix providing NRC (1994) requirements for Zn, Mn, and Cu supplied by sulfates and oxide (40, 60, 8 mg/kg); D: basal diet supplemented with 40, 40, and 7 mg/kg Zn, Mn, and Cu from Zn-Mn-Cu-amino acid complex; E: basal diet supplemented with 40, 40, and 7 mg/kg Zn, Mn, and Cu supplied by sulfates; F: basal diet supplemented with 60, 60, and 10.5 mg/kg Zn, Mn, and Cu from Zn-Mn-Cu-amino acid complex.

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Table 1: Composition of the basal diets

Ingredients (%)	Starter (0-21 d)	Grower (21-42 d)	Finisher (42-49 d)
Corn	57.6	58.6	64.11
Soybean meal	37.21	33.76	28.6
Soybean oil	1.09	4	4
Di-Calcium phosphate	1.82	1.24	0.99
Oyster shell	1.32	1.42	1.34
Vitamin- mineral premix[1]	0.5	0.5	0.5
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)	2865	3100	3175
Crude protein (%)	20.6	19.4	17.8
Calcium (%)	1	0.9	0.8
Available Phosphorus (%)	0.45	0.35	0.3
Methionine + Cystein (%)	0.89	0.71	0.6
Lysine (%)	1	1	0.8
Zinc (mg/kg)	25	24	23
Manganese (mg/kg)	14.8	13.8	12.8
Copper (mg/kg)	9.9	9.2	8.2

[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.

3.2 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.

3.3 Carcass components: At the end of the experiment (49 days of age), two birds were c randomly hosen 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.

3.4 Determination of Zn, Mn and Cu concentration in tibia: 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).

3.5 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 ($P < 0.05$).

4 RESULTS

The influence of dietary treatments on performance traits are listed in Table 2. Neither dietary Zn, Mn, and Cu concentrations nor Zn, Mn, and Cu sources significantly altered body weight at days 21, 42 and 49. 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.

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

	Treatment1 Sources	A Inorganic	B Mix	C Inorganic	D Organic	E Inorganic	F Organic	SEM	Significance
Body weight (g)	21d	524	520	524	539	525	501	0.035	NS
	42d	1899	1898	1859	1917	1961	1894	0.021	NS
	49d	2448	2447	2443	2470	2521	2448	0.286	NS
Feed intake (g/d)	0-21 d	31.3^{a}	35^{b}	$34.\ 3^{ab}$	33.8^{ab}	34.5^{ab}	34.4^{ab}	0.20	*
	21-42 d	132.20^{a}	142.51^{ab}	147.9^{ab}	137.5^{ab}	154.1^{b}	134.8^{a}	2.46	*
	42-49d	183.5	190.3	179.4	176.6	182.4	179.7	2.97	NS
	0-49 d	97.1	103.3	103.8	98.6	106.9	98.2	1.32	NS
Feed conversion ratio (g:g)	0-21 d	1.41^{a}	1.54^{ab}	1.49^{ab}	1.42^{a}	1.5^{ab}	1.56^{b}	0.019	*
	21-42 d	2.01^{a}	2.2^{ab}	2.34^{b}	2.13^{ab}	2.25^{ab}	2.03^{a}	0.042	*
	42-49 d	2.37	2.37	2.29	2.23	2.30	2.27	0.039	NS
	0-49 d	1.97^{a}	2.10^{ab}	2.15^{b}	2.0^{ab}	2.11^{ab}	1.99^{ab}	0.024	*

[1] A: 100, 100, and 10 mg/kg Zn, Mn, and Cu from ZnO, MnO, and $CuSo_4$; B: 100, 100, and 10 mg/kg Zn, Mn, and Cu from ZnO, MnO, $CuSo_4$ and 40, 40, 7 mg/kg Zn-Mn-Cu-Amino acid; C: 40, 60 ,8 mg/kg Zn, Mn, and Cu from ZnO, MnO, and $CuSo_4$; D: 40, 40, and 7 mg/kg Zn, Mn, and Cu from Zn-Mn-Cu-Amino acid; E: 40, 40, and 7 mg/kg Zn, Mn, and Cu supplied by sulfates. F: 60, 60, and 10.5 mg/kg Zn, Mn, and Cu from Zn-Mn-Cu-amino acid. * $p<0.05$

Supplementation of diets with the highest levels of inorganic forms of Zn, Mn, and Cu (100, 100 and 10 mg/kg, respectively) (A treatment) and the lowest levels of their organic forms (40, 40 and 7 mg/kg) markedly improved feed conversion ratio (1.41 and 1.42g/g, respectively) in starter period. Average feed conversation ratio at 21-42 d of age for A treatment and also group fed diet containing 60, 60 and 10.5 mg/kg 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 (2.15g/g) was observed for group fed diet containing 40-60-8 mg/kg Zn-Mn-Cu from their oxide, oxide and sulfate sources, respectively (C treatment).

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)					Carcass	Abdominal fat	Bursa of Fabricius	Spleen	Liver
Treatment	Zn	Mn	Cu	Source	(%)	(%)	(%)	(%)	(%)
A	100	100	10	Inorganic	74.68^{ab}	2.16	0.055	0.103	1.97
B	100+40	100+40	10+7	Mix	74.95^{ab}	1.94	0.121	0.118	2.03
C	40	60	8	Inorganic	73.59^{a}	2.24	0.042	0.095	1.83
D	40	40	7	Organic	74.07^{a}	2.04	0.060	0.102	1.95
E	40	40	7	Inorganic	75.59^{b}	1.67	0.050	0.096	2.00
F	60	60	10.5	Organic	74.53^{ab}	2.15	0.053	0.114	1.92
SEM					0.192	0.092	0.012	0.003	0.027
Significance					*	NS	NS	NS	NS

*$p<0.05$

The effects of diets containing different Zn, Mn, and Cu sources and levels on carcass, abdominal fat, bursa of Fabricius, and spleen percentage are shown in Table 3.

Dietary treatments had only a significant ($p<0.05$) effect on carcass yield at 49 d of age. 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 different sources and levels of Zn, Mn, and Cu did not have any significant effects on abdominal fat, bursa of Fabricius, and spleen percentage (Table3). However, increasing Zn, Mn and Cu levels improved bursa of Fabricius and spleen percentages (treatment B).

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)					**Tibia ash**	**Zn**	**Mn**	**Cu**
Treatment	**Zn**	**Mn**	**Cu**	**Source**	**(%)**	**(mg/kg)**	**(mg/kg)**	**(mg/kg)**
A	100	100	10	Inorganic	51.15	126^{ab}	7.12	1.25^{ab}
B	100+40	100+40	10+7	Mix	58.59	131^{b}	8.00	1.44^{b}
C	40	60	8	Inorganic	54.50	115^{a}	6.50	0.54^{a}
D	40	40	7	Organic	56.56	120^{ab}	7.18	0.88^{ab}
E	40	40	7	Inorganic	55.96	123^{ab}	6.87	0.68^{ab}
F	60	60	10.5	Organic	57.98	125^{ab}	7.25	0.98^{ab}
SEM					0.559	2.024	0.239	0.105
Significance					NS	*	NS	*

* $p<0.05$

Table 4 summarizes the data on tibia ash percentage. 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. 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 d of age.

During the experimental period, mortality was within acceptable ranges (less than 3%) and was not related to dietary treatments.

5 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, 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 mg/kg to 100 mg/kg markedly improved most of the growth performance indices, particularly feed conversion ratio, and their concentrations in tibia ash (Table 4). 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 40, 60 and 8 mg/kg Zn, Mn and Cu, respectively supported growth performance rate similar to that of A treatment as and more better than C treatment. Some researchers (Wong-Valle et al., 1989; Wedekind and Baker, 1989) 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 chelates 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). The use of additional dosages of even organic forms of the minerals more than 100, 100 and 10 mg/kg Zn, Mn and Cu existing in A treatment (B treatment) or 40, 40 and 7 mg/kg existing in D treatment (F treatment) did not show 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 weights of spleen and bursa of Fabricius in progeny chicks.

The increase in tibia zinc observed when broiler diets were supplemented with a combination of Zn-AA+ZnSO4 (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 Zn-Mn-Cu cannot support maximum broiler chicken's performance. In addition, corn-soybean diets supplied with 40-40-7 mg/kg Zn-Mn-Cu from their sulfate or organic (metal amino acid complexes) sources instead of application of 100-100-10 mg/kg Zn-Mn-Cu from their oxide sources can support performance of broiler chickens properly.

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