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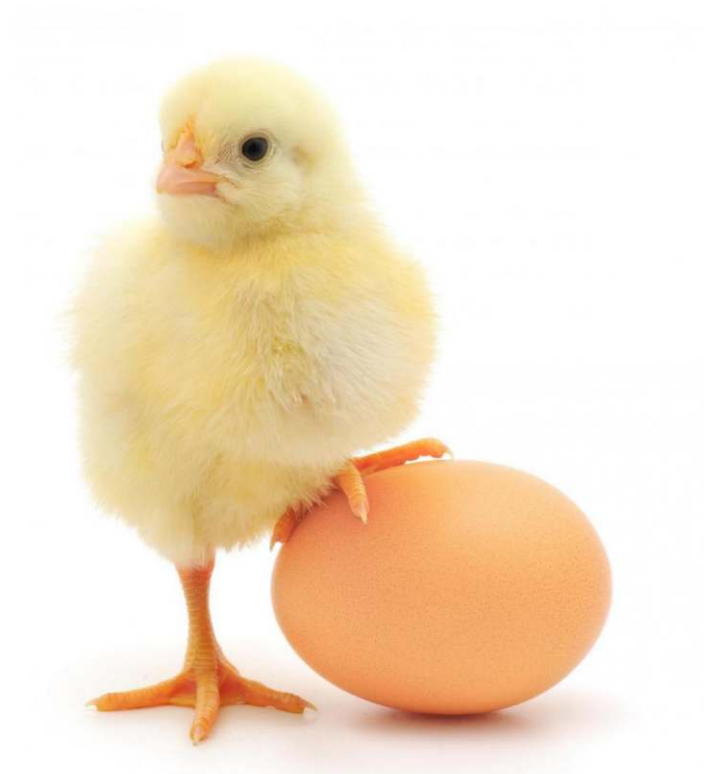

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Effects of Feeding Organic Zinc, Manganese and Copper on Broiler Growth, Carcass Characteristics, Bone Quality and Mineral Content in Bone, Liver and Excreta

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Abstract: The objective of the study was to investigate the effects of dietary organic Zinc (Zn), Manganese (Mn) and Copper (Cu) supplements at 50% or 100% combinations of the broiler strain recommendations for these essential micronutrients on growth performance, carcass characteristics, tibia bone morphometric parameters and strength and mineral deposition in tibia, liver and content in excreta. A total of 648 one-day-old Arbor Acres female broiler chicks were randomly allotted to 9-groups with 4-replicates of 18-chicks each. A corn-soybean meal basal diet supplemented with 100% Zn + 100% Mn + 100% Cu as inorganic salts (Control) and other 8-combinations of 50% or 100% of Zn, Mn and Cu as organic minerals were added to form 9 dietary treatments. Results showed that birds fed diet supplemented with 50% Zn + 50% Mn + 50% Cu, added as organic minerals, improved growth performance, carcass characteristics, tibia quality accompanied with decreasing trace mineral deposition in tibia, liver and excreta. The increased bioavailability of these micronutrients, when presented in organic forms, may be due to improved absorption because of their binding to organic moieties, or to the protective effect due to their association with organic matrices.

Key words: Broiler chickens, organic minerals, growth, carcass characteristic, tibia, liver, excreta

INTRODUCTION

Trace minerals, such as Zn, Mn and Cu, are involved in a wide variety of physiological processes, making them essential for optimal bird growth and health (Richards *et al*., 2010). They act as catalysts in many enzyme and hormone systems (Suttle, 2010) and, as a result, influence growth, bone development, feathering, enzyme structure and function, and appetite (Nollet *et al*., 2007). Traditionally, inorganic mineral salts, e.g. sulphates, oxides and carbonates, have been used in feed formulations (Saripinar Aksu *et al*., 2010). The availability of minerals from these sources varies, but in general, sulfates and oxides are thought to have the highest bioavailability (Pesti and Bakallli, 1996). Nowadays, livestock is generally fed highly concentrated diets that are formulated to provide an excess of nutrients to maximize performance (Leeson, 2003). Esenbuga *et al*. (2008) reported that in commercial practices, Inorganic Trace Minerals (ITM) are used to supply between 2 and 10 times more minerals than the amounts recommended by the National Research Council for poultry diets (NRC, 1994) in order to avoid trace mineral deficiencies and allowing birds to reach their genetic growth potential (Zhao *et al*., 2010). Increased levels of ITM in the feed can interfere with the bioavailability of other minerals (Suttle, 2010). Because of the wide safety margins of ITM and their low retention rates (Mohanna and Nys, 1998), they are excreted in high levels (Aksu *et al*., 2011). Therefore, high level of ITM supplementation is not only wasteful addition to the cost of operation, but may also be harmful to the environment (Bao *et al*., 2007). High mineral concentrations in manure, when used as fertilizer, can lead to high soil concentrations that reduce crop yield (Nollet *et al*., 2007). These excess minerals can leach through soils, potentially contaminating surface and underground water supplies (Jackson *et al*., 2003). Increased awareness of potential mineral pollution has stimulated discussions on how to reduce mineral supplementation levels in livestock nutrition without jeopardizing bird health and negatively affecting growth performance (Aksu *et al*., 2011).

Based on the hypothesis that Organic Trace Minerals (OTM) may have a higher bioavailability than inorganic salts (Abdallah *et al*., 2009), use of OTM in premixes has been suggested as a potential solution to these problems. This implies that OTM may be added at a much lower concentration in the diet than ITM, without causing any negative effect on production performance and potentially reducing mineral excretion (Bao *et al*., 2009).

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The term "organic mineral" refers to a variety of compounds including metal-amino acid complexes, metal amino chelates, metal proteinates, metal-polysaccharide complexes, metal-yeast complexes and metal-organic acid complexes (Patton, 1990). The use of OTM can improve intestinal absorption of trace elements as they reduce interference from agents that form insoluble complexes with the ionic trace elements (Van Der Klis and Kemme, 2002).

The objective of the study was to investigate the effect of dietary Zn, Mn and Cu supplementation from organic sources on growth performance, carcass characteristics, tibia bone quality and trace mineral status in tibia, liver and excreta of 35-day-old female broiler chickens reared under floor pen conditions.

MATERIALS AND METHODS

A total of 648 one-day-old Arbor Acres female broiler chickens were randomly assigned to 9-groups with 4-replicates of 18-chicks each. All birds were housed in floor pens of an open house. All birds were kept under similar condition of management throughout the experimental period.

The 1-day-old chicks were offered electrolyte solution upon arrival. The brooding temperature was maintained close to their requirement, first by heating device for 3-days following arrival of chicks. No additional heating was required as the summer room temperature was found to be appropriate for up to 2-weeks of age. Cooler fans were used during day time for the last 3-weeks of rearing period to circulate and cool the air. Birds were allowed *ad-libitum* access to the diets and tap water containing no analytically detectable levels of Zn, Mn, Cu and Fe (<0.001 mg/L). Lighting was continuous throughout the 5-week feeding period.

Corn-soybean meal basal diets (Table 1) were formulated to either meet or exceed all nutrient requirements of broilers as recommended by the Arbor Acres Broiler Nutrition Specification (2009) except for Zn, Mn and Cu which were added separately to form the different experimental diets. Mineral premix free of Zn, Mn and Cu, purchased from a local feed additives company, Misr Feed Additives Company, Heliopolis, Cairo, Egypt, was used in formulating the diets. Starter basal diet, without Zn, Mn and Cu supplementation, was given for the first 7-days of age to all birds to deplete the supply of those micronutrients in the yolk sacs, followed by introducing the starter diet, supplemented with Zn, Mn and Cu, for 3-days, from 8- to 10-days of age, to complete the starter phase. The grower diets were given from day 11 to day 35 of age. Thus, the trial lasted for 4-weeks, from the 2nd to the 5th week of age.

The Control diet was formulated using inorganic Zn, Mn and Cu salts by providing each kg of the basal diet with 100 mg Zn as ZnO (75%), 120 mg Mn as MnO (60%)

Table 1: Composition of the basal diets for broilers

Ingredient (%)[1]	SP	GP
Corn yellow	48.54	53.65
Soybean meal, 44 % CP	42.43	36.97
Sunflower oil	4.89	5.80
Limestone	1.18	0.96
Dicalcium phosphate	1.85	1.62
Sodium chloride	0.30	0.30
L-Methionine 99%	0.35	0.29
L-lysine 98%	0.16	0.11
Zn-, Mn- and Cu-free Mineral mixture[2]	0.15	0.15
Vitamin mixture[3]	0.15	0.15
Total	100.00	100.00
Nutrient content[4]		
ME (kcal/kg)	3026.00	3150.00
CP (%)	23.00	21.00
Ca (%)	1.05	0.90
Available P (%)	0.50	0.45
Lysine (%)	1.43	1.24
Methionine (%)	0.70	0.61
Methionine + cysteine (%)	1.07	0.95
Zn, mg/kg (unsupplemented)	26.00	24.00
Mn, mg/kg (unsupplemented)	16.00	14.00
Cu, mg/kg (unsupplemented)	10.80	9.70

[1]Ingredient and nutrient composition are reported on an as-fed basis.

[2]Zn-, Mn- and Cu-free mineral mixture supplied per kg of diet: Iron 45 mg; Iodine 1 mg; Selenium 0.2 mg; Cobalt 0.1 mg; Choline chloride 1000 mg.

[3]Vitamin mixture supplied per kilogram of diet: Vit. A 12,000 IU; Vit. D_3 4000IU; Vit. E 60 mg; Vit K_3 3 mg; Vit. B_1 2 mg; Vit. B_2 6.5 mg; Vit. B_6 5 mg; Vit. B_{12} 0.02 mg; Pantothenic 12 mg; Nicotenic 45 mg; Follic acid 2 mg; Biotein 0.08 mg.

[4]Calculated based on NRC (1994). SP = Starter phase (1 to 10 days of age); GP = Grower phase (11 to 35 days of age)

and 16 mg Cu as $CuSO_4$ (25%) to meet the recommended supplementary levels of Zn, Mn and Cu for Arbor Acres broiler feeds. These standard levels reflect the normal supplementary levels and sources of trace minerals for commercial broiler.

Organic Zn, Mn and Cu were separately added to the basal diet in eight combinations calculated to provide 50% or 100% of the inorganic levels used in formulating the control diet. The organic mineral sources used were Bioplex® Zn, Mintrex® Mn and Mintrex® Cu, to form eight experimental diets (Table 2). Bioplex® Zn is a Zn-proteinate (Bioplex® Zn, Alltech Inc., Nicholasville, KY, USA), contains 15% Zn. Mintrex® Mn is a Mn-methionine hydroxy analogue chelate (Mintrex® Mn, Novus Inc., St. Charles, MO, USA), contains 13% Mn. Mintrex® Cu is a Cu-methionine hydroxy analogue chelate (Mintrex® Cu, Novus Inc., St. Charles, MO, USA), contains 15% Cu.

Body Weight (BW) and Feed Intake (FI) were recorded weekly. Body Weight Gain (BWG) and Feed Conversion Ratio (FCR) were calculated weekly. Mortality was recorded daily and calculated weekly as a percentage for each pen. Production Efficiency Factor was calculated according to the Arbor Acres Broiler Management Guide (2009) as follows: PEF = [(livability (%) x live

Table 2: Sources and supplementary levels of trace minerals fed to broilers

Diet	Mineral source	Added Zn (mg/kg)	Added Mn (mg/kg)	Added Cu (mg/kg)
Control (100%Zn+100%Mn+100%Cu (INORG))	Inorganic	100	120	16
100%Zn+100%Mn+100%Cu (ORG)	Organic	100	120	16
50%Zn+50%Mn+50%Cu (ORG)	Organic	50	60	8
100%Zn+50%Mn+50%Cu (ORG)	Organic	100	60	8
50%Zn+100%Mn+50%Cu (ORG)	Organic	50	120	8
50%Zn+50%Mn+100%Cu (ORG)	Organic	50	60	16
100%Zn+100%Mn+50%Cu (ORG)	Organic	100	120	8
100%Zn+50%Mn+100%Cu (ORG)	Organic	100	60	16
50%Zn+100%Mn+100%Cu (ORG)	Organic	50	120	16

[1]Arbor Acres Broiler Nutrition Specification (2009)

weight (kg)) / (age in days x FCR)] x 100. Where: Livability = 100 - mortality (%) and live weight (kg) = BW (kg). Relative Economic Efficiency was calculated for the entire experimental period considering the cost of the consumed diets according to prices in the local market at the time of preparing the study. Relative Economic Efficiency was calculated for the entire experimental period as the cost of feed needed to get one unit of BWG relative to the control diet.

At 35-days of age, 4-birds from each treatment (1-bird from each replicate) were randomly selected, fasted for 12 hrs, weighed individually, sacrificed by severing the jugular vein, bled for 120 sec and semi-scalded for 30 sec before plucking as described by Yalcin *et al.* (1999). Carcass characteristics were measured according to the European Communities Marketing Standards for Poultry Meat Commission Regulation 543/08 (2008) and Poultry Meat Quality Guide (2011) recommendations. Dressing, eviscerated yield, giblets, inedible parts, breast muscles, leg muscles and wings percentages were calculated relative to live BW. Right tibias and livers were weighed individually, placed in plastic bags and stored in deep freezer.

Tibia bone morphometric parameters, i.e. tibia weight, tibia length, diaphysis diameter, tibia weight/length index and tibia robusticity index were determined as described by Mutus *et al.* (2006). After collecting tibias, they were cleaned manually to clear off residual muscles and cartilages. Tibia weight and length were determined using a digital caliper. The bone weight/length index was obtained by dividing the tibia weight by its length (Seedor *et al.*, 1991). The robusticity index was determined using the following formula as described by Reisenfeld (1972): Robusticity index = bone length/cube root of bone weight. Prior to breaking tibias for determining tibias strength, diaphysis diameters were measured perpendicular and parallel to the direction of the applied force using a digital caliper. Tibia strength was measured using Digital Force Gauge apparatus and expressed in kilograms force necessary for breaking bones (Masic *et al.*, 1985). Broken tibias were placed in plastic bags and stored in deep freezer for dry matter, ash and mineral content determination.

Zinc, Mn, Cu and iron (Fe) concentrations were determined in the collected tap water, tibia, liver and excreta samples. Furthermore, Ca and P were determined in the collected tibias. Frozen bones were thawed at room temperature. Tibias trace mineral content, i.e. Zn, Mn, Cu and Fe, were determined as described by Gajula *et al.* (2011). Tibial bone samples were oven dried at 100°C for 24 hrs, then weighed and ashed in muffle furnace at 600±5°C for 4 hrs. Total ash was calculated on percent dry weight basis. From each replicate, 0.2 g of bone ash was dissolved in 5 ml of 50% HCl. Digested samples were filtered and diluted with deionized water to the required volume and analyzed for Zn, Mn, Cu, Fe and Ca using atomic absorption spectrophotometer (Varian, SpectrAA-220FS, Mulgrave, Australia) at wavelength 213.9, 279.5, 324.7, 248.3 and 239.7 nm, respectively. Tibia P content was determined using commercial colorimetric kit (Quimica Clinica Aplicada S.A., Amposta, Spain) according to Fiske and Subbarow (1925) and Goodwin (1970).

Liver trace mineral content, i.e. Zn, Mn, Cu and Fe, were determined as described by Gajula *et al.* (2011). Frozen livers were thawed at room temperature and oven-dried at 100°C for 24 hrs, weighed and finely ground for mineral analysis. Approximately 0.5 g of ground sample was predigested with 5 ml of concentrated HNO_3 for 1 hr at 120°C. The contents were further digested using 30% H_2O_2 at 200°C for 45 min. The digested samples were filtered, diluted to the required volume using deionized water and analyzed for Zn, Mn, Cu and Fe using atomic absorption spectrophotometer (Varian, SpectrAA-220FS, Mulgrave, Australia) at wavelength 213.9, 279.5, 324.7 and 248.3 nm, respectively.

At the end of the experimental period, a digestion trial was carried out, from 33- to 35-days of age, to estimate excreta trace mineral content for each group. A total of 36-birds (4-birds/treatment) were individually used. Feed and fresh water were offered *ad-libitum* during the three day collection period. Excreta, that fell on polyethylene sheets, were collected quantitatively every 24 hrs. Feathers and scattered feed were removed from the excreta. The collected excreta samples were dried in an air-draft oven at 60°C for 24 hrs till each reached a constant weight then weighed, well mixed and stored in screw-top glass jars for determining excreta trace mineral content, i.e. Zn, Mn, Cu and Fe, as described by Dozier *et al.* (2003). 1 g of ground excreta was oven-dried at 120°C for 1 hr then weighed and ashed in muffle furnace at 600±5°C for 4 hrs. The ash was then dissolved by adding 10 ml of 3M HCl to the sample, covering it with a watch glass and boiling for 10 min. The sample was allowed to cool then it was diluted to the required volume using deionized water and analyzed for Zn, Mn, Cu and Fe using atomic absorption spectrophotometer (Varian, SpectrAA-220FS, Mulgrave, Australia) at wavelength 213.9, 279.5, 324.7 and 248.3 nm, respectively.

Data from the experiment were subjected to one-way ANOVA as a completely randomized design using the General Linear Model procedure of the Statistical Analysis System (SAS Institute, 2003). Pen means were used as the experimental units for all evaluated variables. When significant ($p<0.05$), treatment means were compared using Duncan's new multiple range test procedure. The results of the statistical analyses are shown in corresponding tables as mean values.

RESULTS AND DISCUSSION

Growth performance of birds fed the different diets is presented in Table 3. In the 2nd week of age, OTM supplementations significantly ($p<0.001$) increased FI, BWG and worsened FCR. During the 3rd week of age, OTM supplementations decreased FI and improved FCR. In the 4th and 5th week of age, 50%Zn+50%Mn+50%Cu (ORG) diet significantly ($p<0.001$) improved BWG and FCR.

Growth performance of the entire experimental period indicated that 50%Zn+50%Mn+50%Cu (ORG) diet, contained 50 mg Zn + 60 mg Mn + 8 mg Cu added as OTM/kg diet to bring the supplemented Zn, Mn and Cu closer to NRC (1994) requirements for broilers (Fig. 1), significantly ($p<0.001$) decreased FI, increased BWG, improved FCR and Production Efficiency Factor. Bao and Choct (2009) suggested that trace mineral supplementations recommended by NRC (1994) were determined by maximal weight gain, which was far below the current broiler weight gain. Thus, it is reasonable in the modern, rapidly growing broiler strains that much higher supplementations than those recommended by NRC are used. However, these trace minerals may not act as growth promoters and supplementing organic Zn, Mn and Cu closer to NRC recommendations may support optimal growth of broiler chicks due to their inherent high bioavailability. These results are in agreement with Gheisari *et al.* (2010) who reported that BWG and FCR were improved by supplementing broilers diet with 50%Zn + 50% Mn + 50%Cu of their recommendations as OTM compared with adding 100% of those mineral recommendations

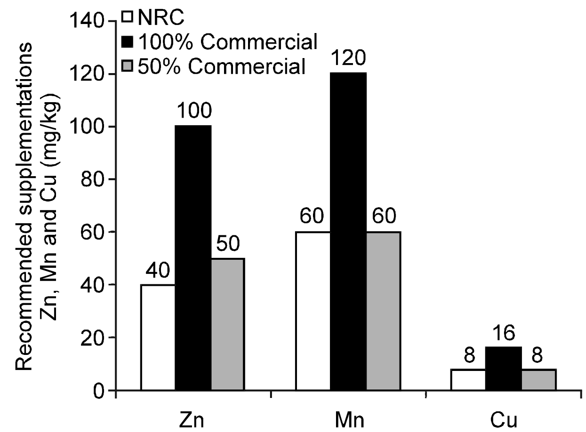

Fig. 1: Recommended supplementation levels for Zn, Mn and Cu to broilers diets according to NRC (1994) (open bars), 100% of Arbor Acres Broiler Nutrition Specification (2009) (solid bars) and 50% of Arbor Acres Broiler Nutrition Specification (2009) (gray bars)

as ITM. Birds fed 100%Zn+50%Mn+50%Cu (ORG) diet followed those fed 50%Zn+50%Mn+50%Cu (ORG) diet in increasing BWG, decreasing FI, improving FCR and Production Efficiency Factor significantly ($p<0.001$). Bao *et al.* (2010) suggested that Zn level is the first limiting element among Zn, Mn, Cu and Fe because supplemental organic Zn alone or combined with other elements, Mn, Cu and Fe, significantly ($p<0.01$) improved BWG and FCR. The noted drop in growth performance in the current study, compared with the Arbor Acres Broiler Performance Objectives (2012), was due to the variation in the ambient temperature of the open housing system during the 4th and 5th week of age. No significant differences were observed in weekly mortality rates between the 9-groups. Overall mortality was within the acceptable ranges, therefore mortality was not related to the dietary treatments or housing temperature.

Although Production Efficiency Factor allows comparing individual flocks and understanding the impact of changes in livability, live body weight, age and FCR on the flock efficiency, Production Efficiency Factor does not link cost with production. Therefore, Relative Economic Efficiency was calculated to consider the cost of production. Relative Economic Efficiency decreased by supplementing OTM to the basal diet, except 100%Zn+100%Mn+50%Cu (ORG) and 100%Zn+50%Mn+100%Cu (ORG) diets, indicating increasing profitability. The 50%Zn+50%Mn+50%Cu (ORG) and 100%Zn+50%Mn+50%Cu (ORG) diets gave the best Relative Economic Efficiency. This result was in agreement with Abdallah *et al.* (2009) findings who reported that chicks fed diet supplemented with 50% or 100% of Zn or Mn or Cu as OTM of the broiler strain

Table 3: Effect of experimental diets on broilers growth performance[1]

Parameter	C (100%Zn+ 100%Mn+ 100%Cu (INORG))	100%Zn+ 100%Mn+ 100%Cu (ORG)	50%Zn+ 50%Mn+ 50%Cu (ORG)	100%Zn+ 50%Mn+ 50%Cu (ORG)	50%Zn+ 100%Mn+ 50%Cu (ORG)	50%Zn+ 50%Mn+ 100%Cu (ORG)	100%Zn+ 100%Mn+ 50%Cu (ORG)	100%Zn+ 50%Mn+ 100%Cu (ORG)	50%Zn+ 100%Mn+ 100%Cu (ORG)	SEM	Sig.
WG (g/bird/day)											
2nd week	33.04^{bc}	31.17^{d}	34.53^{ab}	35.36^{a}	35.36^{a}	36.05^{a}	33.04^{bc}	32.41^{cd}	33.31^{bc}	±0.56	***
3rd week	57.82^{b}	58.40^{ab}	60.40^{a}	54.19^{d}	48.14^{f}	55.32^{cd}	57.34^{bc}	50.28^{e}	56.68^{bc}	±0.71	***
4th week	44.89^{cd}	47.02^{bc}	53.12^{a}	49.39^{b}	44.97^{cd}	50.15^{ab}	42.87^{d}	39.40^{e}	42.95^{d}	±1.09	***
5th week	32.73^{c}	43.88^{ab}	48.28^{a}	48.25^{a}	43.69^{ab}	38.66^{bc}	41.39^{ab}	46.43^{a}	39.30^{bc}	±2.19	***
Overall	1179.32^{d}	1263.28^{bc}	1374.39^{a}	1310.31^{b}	1205.09^{cd}	1261.16^{bc}	1222.44^{cd}	1179.62^{d}	1205.59^{cd}	±19.43	***
FI (g/bird/day)											
2nd week	60.77^{e}	65.25^{d}	68.28^{c}	79.20^{a}	64.08^{d}	74.37^{b}	68.66^{c}	80.04^{a}	60.50^{e}	±0.49	***
3rd week	123.67^{a}	115.14^{c}	118.70^{b}	109.24^{e}	96.64^{g}	109.24^{e}	112.55^{d}	103.41^{f}	102.35^{f}	±0.58	***
4th week	130.95^{f}	133.26^{e}	120.47^{g}	135.39^{d}	146.21^{a}	139.56^{bc}	145.12^{a}	140.66^{b}	138.39^{c}	±0.62	***
5th week	150.79^{a}	143.92^{c}	151.65^{a}	133.26^{f}	139.46^{d}	146.21^{b}	152.08^{a}	136.16^{e}	152.07^{a}	±0.66	***
Overall	3263.26^{b}	3202.92^{c}	3213.72^{c}	3199.56^{cd}	3124.71^{e}	3285.59^{b}	3348.80^{a}	3221.89^{c}	3173.17^{d}	±9.35	***
FCR (FI/WG)											
2nd week	1.84^{e}	2.10^{c}	1.98^{d}	2.24^{b}	1.82^{e}	2.07^{cd}	2.09^{cd}	2.47^{a}	1.82^{e}	±0.04	***
3rd week	2.14^{a}	1.97^{c}	1.97^{c}	2.01^{bc}	2.01^{bc}	1.98^{c}	1.97^{c}	2.06^{b}	1.81^{d}	±0.03	***
4th week	2.92^{c}	2.84^{c}	2.27^{d}	2.75^{c}	3.27^{b}	2.78^{c}	3.40^{ab}	3.57^{a}	3.23^{b}	±0.08	***
5th week	4.73^{a}	3.29^{bcd}	3.18^{cd}	2.78^{d}	3.20^{cd}	3.85^{bc}	3.68^{bc}	2.94^{d}	3.92^{b}	±0.21	***
Overall	2.77^{a}	2.54^{cd}	2.34^{e}	2.45^{de}	2.60^{c}	2.61^{c}	2.74^{ab}	2.74^{ab}	2.64^{bc}	±0.04	***
Mortality (%)											
2nd week	1.39	1.39	0.00	0.00	0.00	0.00	1.39	0.00	0.00	±0.80	NS
3rd week	0.00	0.00	0.00	0.00	0.00	0.00	1.39	1.39	1.39	±0.80	NS
4th week	1.39	0.00	1.39	1.39	5.56	4.17	4.25	2.78	4.17	±1.26	NS
5th week	4.25	0.00	2.78	1.39	1.47	1.39	1.47	1.39	2.94	±1.40	NS
Overall	6.95^{ab}	1.39^{c}	4.17^{abc}	2.78^{bc}	6.95^{ab}	5.56^{abc}	8.34^{a}	5.56^{abc}	8.34^{a}	±1.31	**
PEF[2] (%)	132.78^{d}	162.46^{bc}	184.53^{a}	172.06^{ab}	144.63^{d}	150.81^{cd}	136.64^{d}	136.65^{d}	139.92^{d}	±5.59	***
REE[3] (%)	100.00	95.30	86.30	90.80	96.10	96.30	102.90	101.60	97.50	-	-

^{abc}Means within a row with different superscripts differ significantly (*$p<0.05$, **$p<0.01$, ***$p<0.001$). NS: Not-Significant. C = Control. Sig. = Significance

[1]Values are means of 4-replicate groups of 18-birds.

[2]PEF (Production Efficiency Factor) = [(livability (%) x live weight (kg))/(age in days x FCR)] x 100

[3]REE (Relative Economic Efficiency), assuming that Relative Economic Efficiency of the control treatment equal 100. Relative Economic Efficiency measured as by feed cost/kg live weight

Table 4: Effect of experimental diets on carcass characteristics of 35-day-old broiler chickens[1]

Parameter[2]	C (100%Zn+ 100%Mn+ 100%Cu (INORG))	100%Zn+ 100%Mn+ 100%Cu (ORG)	50%Zn+ 50%Mn+ 50%Cu (ORG)	100%Zn+ 50%Mn+ 50%Cu (ORG)	50%Zn+ 100%Mn+ 50%Cu (ORG)	50%Zn+ 50%Mn+ 100%Cu (ORG)	100%Zn+ 100%Mn+ 50%Cu (ORG)	100%Zn+ 50%Mn+ 100%Cu (ORG)	50%Zn+ 100%Mn+ 100%Cu (ORG)	SEM	Sig.
Dressing[3] (%)	93.91^{b}	93.73^{b}	94.52^{a}	92.42^{cd}	92.09^{d}	90.88^{e}	94.83^{a}	92.77^{c}	92.09^{b}	±0.17	***
Heart (%)	0.39^{b}	0.32^{f}	0.38^{bc}	0.36^{cd}	0.32^{f}	0.45^{a}	0.37^{bcd}	0.35^{de}	0.33^{ef}	±0.01	***
Liver (%)	1.76^{c}	1.96^{b}	2.02^{b}	1.91^{b}	1.99^{b}	2.54^{a}	1.75^{c}	2.01^{b}	1.78^{c}	±0.04	***
Gizzard (%)	2.20^{ab}	2.13^{abc}	2.01^{bcd}	1.89^{d}	1.95^{cd}	2.27^{a}	1.89^{d}	2.08^{abcd}	1.95^{cd}	±0.07	**
giblets[4] (%)	7.17^{bc}	7.32^{bc}	7.32^{bc}	7.05^{cd}	7.13^{c}	8.42^{a}	6.76^{de}	7.49^{b}	6.65^{e}	±0.11	***
Inedible parts[5] (%)	27.71^{c}	27.39^{c}	26.92^{c}	30.25^{b}	27.96^{c}	31.52^{a}	25.42^{d}	29.96^{b}	26.96^{c}	±0.39	***
Eviscerated yield[6] (%)	64.60^{c}	65.08^{bc}	65.36^{bc}	62.96^{d}	65.20^{bc}	60.90^{e}	67.45^{a}	63.00^{d}	65.20^{ab}	±0.44	***
Leg muscles[7] (%)	19.59^{b}	19.39^{b}	20.04^{b}	17.66^{cd}	18.63^{bc}	21.60^{a}	19.67^{b}	17.13^{d}	18.63^{b}	±0.44	***
Breast muscles (%)	19.11^{c}	21.02^{a}	20.38^{ab}	19.72^{bc}	18.21^{d}	18.16^{d}	19.02^{c}	19.16^{c}	18.21^{bc}	±0.28	***
Wings (%)	8.14^{a}	7.33^{bc}	6.15^{d}	7.67^{abc}	7.57^{abc}	7.87^{ab}	7.70^{abc}	6.59^{d}	7.57^{c}	±0.19	***

^{abc}Means within a row with different superscripts differ significantly ($^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$). NS: Not-Significant. C = Control. Sig. = Significance

[1]Values are means of 4-sacrificed birds/treatment.

[2]Parameters calculated as a percentage of live BW.

[3]Dressing (%) = (carcass weight (g)/live BW (g)) x 100; where carcass weight (g) = live BW (g) - (blood weight (g) + feathers weight (g))

[4]Giblets including the sum of neck, heart, liver and gizzard as a percentage of live body weight

[5]Inedible parts (%) are the sum of the blood, feathers, head, feet and alimentary canal as a percentage of live BW

[6]Neck is attached to the eviscerated carcass

[7]Leg muscles (%) are the sum of the deboned thigh and the deboned drumstick as a percentage of live BW

Table 5: Effect of experimental diets on tibial bone morphometric parameters and strength of 35-day-old broiler chickens[1]

Parameter	C (100%Zn+ 100%Mn+ 100%Cu (INORG))	100%Zn+ 100%Mn+ 100%Cu (ORG)	50%Zn+ 50%Mn+ 50%Cu (ORG)	100%Zn+ 50%Mn+ 50%Cu (ORG)	50%Zn+ 100%Mn+ 50%Cu (ORG)	50%Zn+ 50%Mn+ 100%Cu (ORG)	100%Zn+ 100%Mn+ 50%Cu (ORG)	100%Zn+ 50%Mn+ 100%Cu (ORG)	50%Zn+ 100%Mn+ 100%Cu (ORG)	SEM	Sig.
Tibia weight (g)	11.47^{a}	10.06^{c}	11.68^{a}	11.41^{ab}	10.40^{bc}	11.29^{ab}	11.41^{ab}	10.39^{bc}	10.17^{c}	±0.33	**
Tibia length (cm)	9.25	9.14	9.28	8.96	9.21	9.09	9.12	8.95	9.22	±0.10	NS
Diaphysis diameter (cm)	0.65	0.67	0.74	0.66	0.68	0.69	0.67	0.68	0.72	±0.03	NS
Tibia weight/length index (g/cm)	123.98^{ab}	110.07^{c}	125.82^{a}	127.39^{a}	112.80^{c}	124.15^{ab}	125.09^{ab}	116.01^{bc}	110.24^{c}	±2.94	***
Tibia robusticity index	4.11^{b}	4.24^{a}	4.09^{bc}	3.98^{c}	4.23^{a}	4.05^{bc}	4.06^{bc}	4.11^{b}	4.26^{a}	±0.04	***
Tibia strength[2] (kgf/cm^2)	38.64^{c}	33.71^{d}	43.40^{b}	36.92^{cd}	32.36^{d}	44.20^{b}	37.02^{cd}	49.78^{a}	44.69^{b}	±1.57	***

^{abc}Means within a row with different superscripts differ significantly ($^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$). NS: Not-Significant. C = Control. Sig. = Significance

[1]Values are means of 4-sacrificed birds/treatment.

[2]Tibia strength: tibia breaking strength

Table 6: Effect of experimental diets on tibia, liver and excreta mineral concentration of 35-day-old broiler chickens[1]

Parameter	C (100%Zn+ 100%Mn+ 100%Cu (INORG))	100%Zn+ 100%Mn+ 100%Cu (ORG)	50%Zn+ 50%Mn+ 50%Cu (ORG)	100%Zn+ 50%Mn+ 50%Cu (ORG)	50%Zn+ 100%Mn+ 50%Cu (ORG)	50%Zn+ 50%Mn+ 100%Cu (ORG)	100%Zn+ 100%Mn+ 50%Cu (ORG)	100%Zn+ 50%Mn+ 100%Cu (ORG)	50%Zn+ 100%Mn+ 100%Cu (ORG)	SEM	Sig.
Tibia											
Dry matter (%)	46.11^{c}	49.35^{abc}	52.14^{ab}	52.51^{a}	47.99^{bc}	49.20^{abc}	51.17^{ab}	51.01^{ab}	50.77^{ab}	±1.36	*
Ash (%)	46.13	43.85	43.52	44.41	43.78	45.06	44.90	49.31	44.68	±1.23	NS
Zn (mg/kg)	187.59^{cd}	201.07^{bc}	170.75^{e}	215.84^{a}	187.48^{cd}	167.00^{e}	185.01^{d}	200.90^{bc}	202.84^{b}	±4.48	***
Mn (mg/kg)	13.21^{c}	21.17^{a}	13.18^{c}	14.66^{bc}	16.60^{b}	13.61^{c}	21.83^{a}	15.47^{bc}	16.63^{b}	±0.78	***
Cu (mg/kg)	13.77^{a}	9.01^{bc}	9.32^{bc}	7.76^{cd}	8.47^{bcd}	7.24^{d}	9.87^{b}	9.32^{bc}	10.10^{b}	±0.54	***
Fe (mg/kg)	542.13^{b}	374.60^{e}	477.96^{c}	271.18^{f}	414.36^{d}	422.20^{d}	607.89^{a}	443.80^{d}	357.03^{e}	±11.58	***
Ca (%)	15.98^{c}	20.55^{a}	20.66^{a}	14.17^{d}	18.90^{b}	18.67^{b}	18.65^{b}	17.64^{b}	21.28^{a}	±0.54	***
P (%)	6.68^{e}	7.66^{a}	7.00^{d}	7.33^{bc}	7.38^{abc}	7.14^{cd}	7.64^{a}	7.47^{ab}	7.64^{a}	±0.10	***
Liver											
Zn (mg/kg)	57.81^{a}	42.36^{bc}	47.43^{bc}	46.66^{bc}	43.50^{bc}	48.19^{b}	45.37^{bc}	45.66^{bc}	40.11^{c}	±2.40	**
Mn (mg/kg)	8.31^{ab}	8.65^{ab}	5.74^{d}	9.12^{a}	7.92^{ab}	7.24^{bc}	6.48^{cd}	8.56^{ab}	8.86^{a}	±0.45	***
Cu (mg/kg)	13.22^{a}	13.28^{a}	12.92^{a}	11.56^{a}	9.74^{b}	12.45^{a}	11.64^{a}	11.80^{a}	12.13^{a}	±0.55	**
Fe (mg/kg)	418.71^{a}	226.48^{cd}	252.09^{c}	227.23^{cd}	217.74^{d}	235.78^{cd}	286.71^{b}	300.83^{b}	301.15^{b}	±10.39	***
Excreta											
Zn (mg/kg)	177.14^{c}	229.27^{b}	148.84^{d}	150.11^{d}	172.06^{c}	213.93^{b}	260.16^{a}	177.58^{c}	174.02^{c}	±5.80	***
Mn (mg/kg)	225.88^{e}	342.72^{a}	150.15^{g}	260.95^{d}	287.73^{c}	215.11^{ef}	251.65^{d}	213.19^{f}	320.88^{b}	±3.81	***
Cu (mg/kg)	40.32^{d}	48.21^{b}	24.03^{e}	24.48^{e}	43.03^{cd}	50.87^{b}	43.40^{cd}	55.10^{a}	46.70^{bc}	±1.46	***
Fe (mg/kg)	156.74^{ab}	134.57^{bc}	137.69^{bc}	157.44^{ab}	179.09^{a}	136.50^{bc}	155.42^{abc}	124.15^{c}	130.10^{bc}	±9.86	*

^{abc}Means within a row with different superscripts differ significantly (*p<0.05, **p<0.01, ***p<0.001). NS:Not-Significant. C=Control. Sig.=Significance. [1]Values are means of 4-sacrificed birds/treatment

recommendations for these essential micronutrients recorded better Relative Economic Efficiency than those fed diet supplemented with 100% of the commercial recommendations for these 4-minerals as ITM through 35-days of age.

Carcass characteristics are recorded in Table 4. 50%Zn+50%Mn+50%Cu (ORG) diet significantly (p<0.001) improved dressing (%), liver (%) and breast muscles (%), while significantly (p<0.001) decreased wings (%) and had no effect on the heart (%), gizzard (%), giblets (%), inedible parts (%), eviscerated yield (%) and leg muscles(%). Contradictory to these results, Zhao *et al.* (2010) reported that Arbor Acres male broilers fed diets supplemented with 40 mg Zn + 60 mg Mn + 8 mg Cu as OTM/kg diet, bring the supplemented Zn, Mn and Cu closer to those in 50%Zn+50%Mn+50%Cu (ORG) diet in the current study, had no effect on breast muscles (%) and wings (%) compared with those fed diet supplemented with 80 mg Zn + 120 mg Mn + 8 mg Cu as ITM/kg diet on 52-days of age. This disagreement may be due to that Zhao *et al.* (2010) sacrificed elder birds than those in the current study leading to less OTM benefits. Bao and Choct (2009) observed that the efficiency of OTM decreases as the bird's age increases, suggesting that there may be less benefit in using OTM in feeds for adult birds (Smith *et al.*, 1995). There were no significant differences between 50%Zn+50%Mn+50%Cu (ORG) and 100%Zn+50%Mn+50%Cu (ORG) diet in heart (%), liver (%), gizzard (%), giblets (%) and breast muscles (%), but the latter one showed significantly (p<0.001) lower dressing (%), eviscerated yield (%) and leg muscles (%).

Tibia bone morphometric parameters and strength of the 35-day age female broilers are tabulated in Table 5. Onyango *et al.* (2003) stated that a number of invasive methods (bone weight, length, width, breaking strength and ash) exist to determine the bone mineralization in poultry. Birds fed 50%Zn+50%Mn+50%Cu (ORG) diet showed improved tibia weight, length, diaphysis diameter, weight/length index and tibia robusticity index. Seedor *et al.* (1991) and Reisenfeld (1972) used the bone weight/bone length index and the robusticity index, respectively, to describe bone mineralization. The bone weight/bone length index is a simple index of bone density (Seedor *et al.*, 1991), the higher the bone weight/bone length index, the denser is the bone (Monteagudo *et al.*, 1997). On the contrary, low robusticity index indicates a strong bone structure (Reisenfeld, 1972). Feeding 50%Zn+50%Mn+50%Cu (ORG) diet significantly (p<0.001) improved tibia breaking strength by 11%. Tibia strength results in the reported study herein were in disagreement with Zhao *et al.* (2010) who reported that broilers fed diets supplemented with 40 mg Zn + 60 mg Mn + 8 mg Cu as

OTM/kg diet had no effect on tibia strength compared with those fed diet supplemented with 80 mg Zn + 120 mg Mn + 8 mg Cu as ITM/kg diet on 52-days of age. This disagreement may be due to that Zhao *et al.* (2010) sacrificed elder birds than those in the current study leading to less OTM benefits (Smith *et al.*, 1995). No significant differences were observed in tibia morphometric parameters between 50%Zn+50% Mn+50%Cu (ORG) and 100%Zn+50%Mn+50%Cu (ORG) diet, but the latter one showed significantly ($p<0.001$) lower tibia strength.

Mineral contents of tibia, liver and excreta are figured out in Table 6. Mineral homeostasis is precisely maintained in the body and is predominantly achieved by balancing tissue storage and excretion (Suttle, 2010). Tissue mineral concentrations are indicators of body storage and mineral status and have been used as biomarkers in bioavailability studies (Wang *et al.*, 2007). Current study showed that 50%Zn+50%Mn+50%Cu (ORG) diet significantly ($p<0.001$) decreased tibia Zn, Mn, Cu and Fe concentrations, while significantly ($p<0.001$) increased tibia dry mater, Ca and P concentrations and had no effect on tibia ash concentration. Tibia trace mineral results are similar to Gheisari *et al.* (2010) who found that feeding broiler chickens diet supplemented with 60 mg Zn + 60 mg Mn +10.5 mg Cu or 40 mg Zn + 40 mg Mn + 7 mg Cu as amino acid complex/kg diet had lower tibia Zn, Mn and Cu content than those in birds fed diet supplemented with 100 mg Zn as oxide + 100 mg Mn as oxide + 10 mg Cu as sulfate/kg diet. There were no significant differences between 50%Zn+50% Mn+50%Cu (ORG) and 100%Zn+50%Mn+50%Cu (ORG) diet in tibia mineral content, except the former diet had higher ($p<0.001$) tibia Fe and Ca content where the latter one had higher ($p<0.001$) tibia Zn and P content.

Diet supplemented with 50%Zn+50%Mn+50%Cu (ORG) significantly ($p<0.01$) decreased liver Zn, and Fe concentrations. Although 50%Zn+50%Mn+50%Cu (ORG) decreased liver Cu content, this decrease was insignificant. This result was in agreement with Zhao *et al.* (2010) who observed that broilers fed diet supplemented with 40 mg Zn + 60 mg Mn + 8 mg Cu as methionine/kg diet had no effect on liver Cu content compared to those fed diet supplemented with 80 mg Zn + 120 mg Mn + 8 mg Cu as sulfates/kg diet. 50%Zn+50%Mn+50%Cu (ORG) diet significantly ($p<0.001$) decreased liver Mn. These results were in agreement with Aksu *et al.* (2011) findings who reported that lowering dietary supplemented organic Zn, Mn and Cu by 50% significantly ($p<0.01$) decreased liver trace mineral content (Zn, Mn, Cu and Fe) compared with those fed diet supplemented with 100% inorganic Zn, Mn and Cu. There were no significant differences between 50%Zn+50%Mn+50%Cu (ORG) and 100%Zn+50%Mn+50%Cu (ORG) diet in liver mineral content, except the latter one had higher ($p<0.001$) liver Mn content.

50%Zn+50%Mn+50%Cu (ORG) diet significantly ($p<0.001$) decreased excreta content of Zn, Mn, Cu and Fe by 16, 34, 40 and 12%, respectively. In agreement with findings reported by Zhao *et al.* (2010) who observed that broilers fed diet supplemented with 40 mg Zn + 60 mg Mn + 8 mg Cu as methionine/kg diet reduced Zn and Cu excretion compared to those fed diet supplemented with 80 mg Zn + 120 mg Mn + 8 mg Cu as sulfates/kg diet. Suttle (2010) reported that a reduction in the dietary supply of minerals is usually overcome by a combination of responses, including reduced excretion. There were no significant differences between 50%Zn+50%Mn+50%Cu (ORG) and 100%Zn+50%Mn+50%Cu (ORG) diet in excreta mineral content, except the latter one showed significantly ($p<0.001$) higher Mn.

It is concluded that using organic trace mineral sources in broiler chicken diets can allow for a reduction in dietary Zn, Mn and Cu supplementation by 50% of the strain recommendations accompanied with improved growth, carcass characteristics, tibia quality with decreasing tibia and liver trace mineral content and environmental pollution. Organic sources of the selected trace minerals improved tibia quality and tibia breaking strength due to their positive effects in increasing Ca and P deposition in tibia. The increased bioavailability of these micronutrients, when presented in OTM forms, may be due to the improved absorption because of the protective effect of the organic matrices.

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