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Effects of Organic Minerals Supplementation on Growth, Bioavailability and Immunity in Grower Birds

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Abstract: Effect of replacement of inorganic minerals viz., zinc (Zn), copper (Cu) and manganese (Mn) with their corresponding organic minerals (methionine) on growth, bioavailability and immunity was studied in grower birds (9th to 20th week). At 20th week, the body weight of grower birds were significantly ($p<0.05$) higher in 100% organic Zn group, 100 and 50% organic Zn, Cu and Mn supplemented groups. The cumulative feed consumption and FCR of all the treated groups showed no significant ($p>0.05$) difference. The serum glucose, after cholesterol SGOT, SGPT and ALP levels differ significantly ($p<0.05$) of all the treated groups of differed significantly ($p<0.05$). The CBH response and the antibody titers against SRBC were found to be significantly higher in 100% organic Zn group and 100% Zn, Cu and Mn supplemented groups. Tibia bone weight (g), tibia calcium (%) and tibia phosphorus (%) varied insignificantly ($p>0.05$). But significant ($p<0.05$) difference was observed as regard to the tibia ash content. Faecal excretions of Zn, Cu and Mn were significantly lower in organic mineral fed groups. Replacement of inorganic Zn, Cu and Mn with their corresponding organic minerals improved the body weight and immunity, with lower faecal excretion of minerals in grower birds.

Key words: Organic minerals, growth, biochemical, bioavailability, immunity, grower birds

INTRODUCTION

Trace minerals, such as Zn, Cu and Mn are essential for growth of the poultry because of their involvement in many digestive, physiological and biosynthetic processes within the body. They function primarily as catalysts in enzyme systems within cells or as parts of different enzymes. They are constituents of hundreds of proteins involved in intermediary metabolism, hormone secretion pathways and immune defence systems. To meet the requirement, these minerals have been supplemented in poultry diets using inorganic salts such as oxides and sulphates. The trace minerals tend to dissociate in the low pH environment of upper gastrointestinal tract, leaving the minerals susceptible to various nutrients and ingredient antagonisms that impair absorption (Underwood and Suttle, 1999). Chelated minerals can be utilized at a much lower concentration in the poultry diet than inorganic minerals, due to higher bioavailability (Wedekind and Baker, 1990; Wedekind *et al*., 1992; Cao *et al*., 2000). Abdallah *et al*. (2009) observed that chicks fed diets containing 100% organic minerals (Zn, Cu, Mn and Fe) had significantly higher body weight and better feed conversion on comparison with those of inorganic minerals. The effects of organic minerals are not limited to the growth and production. Also they have some beneficial impact on immune system as well as in reduction of stress in the birds (Wei *et al*., 2001; Aksu *et al*., 2010). The variability of the results observed by the researchers posted questions on the validity of

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chelated mineral used in poultry production. Also most of the studies, as reported, were carried out in either broilers or layer birds particularly during laying phase and the output of those reported research work cannot be taken as a guideline for feeding of organic minerals in growing period of layer birds. Hence, the present study was planned with the objective of studying the effect of replacement of inorganic minerals with their corresponding organic minerals (methionine) on growth, bioavailability and immunity of grower birds.

MATERIALS AND METHODS

Animals' diet and experimental procedure: Commercial (BV 300) layer chicks (n = 120) were randomly allocated to six dietary treatments. Each treatment group had 2 replicates containing 10 chicks in each replicate. Earlier in the first phase, the experiment continued till 8th week of age (starter phase) (Das *et al*., 2014). This was a continuous study of the same treatment groups from 9th-20th weeks. The grower birds were provided 24 h free access to clean drinking water. The quantity of feed offered to the birds in every week was same in all the groups as per the BV 300 guideline. The formulation of experimental diet is presented in Table 1. Samples of the experimental feed were analyzed for proximate principles as per AOAC (1995). Calcium and phosphorus was measured according to the method modified by Talapatra *et al*. (1940). The micro minerals content of the feed samples were estimated by using atomic absorption spectrophotometer (Model: SL 243, ELECO Limited, Hyderabad, India).

The dietary treatments of the experiment were: T_1: Basal diet (no Cu, Zn and Mn minerals) + 80 ppm Zn, 15 ppm Cu and 80 ppm Mn supplementation from inorganic source in form of $ZnSO_4$, $CuSO_4$ and $MnSO_4$, respectively, T_2: T_1 with replacement of 80 ppm inorganic Mn with 80 ppm Mn-methionate T_3: T_1 with replacement of 15 ppm inorganic Cu with 15 ppm Cu-methionate, T_4: T_1 with replacement of 80 ppm inorganic Zn with 80 ppm Zn methionate, T_5: Basal diet+ 50% Zn (40 ppm), Cu (7.5 ppm) and Mn (40 ppm) supplementation from organic source (Zn-methionate, Cu-methionate and Mn-methionate) and T_6: Basal diet +100% Zn (80 ppm), Cu (15 ppm) and Mn (80 ppm) supplementation from organic source (Zn-methionate, Cu-methionate and Mn-methionate).

Organic minerals source: The organic Zn, organic Cu and organic Mn minerals were obtained from S.A. Pharmaceuticals Pvt. Limited, Vadodara, Gujurat, India. The analytical report revealed the methionine activity in organic Cu, ooganic Zn and Organic Mn were 67.04, 73.13 and 73.60%, respectively. Similarly, Zn, Cu and Mn activity were 12.67, 18.20 and 14.85%, respectively.

Growth performance: Weights of all the individual grower birds in each group were determined at initial and at the end of experiment. At the same time, survival was also determined by counting the individuals in each group:

$$\text{Relative gain} = \frac{\text{Final weight-Initial weight}}{\text{Initial weight}} \times 100$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Feed offered-Feed residue}}{\text{Final weight-Initial weight+Mortality weight}}$$

Biochemical analysis: Blood and serum samples were collected at 20th weeks of post feeding for biochemical analysis. The serum biochemical indices determined were serum glucose, cholesterol, urea, Alkaline phosphate (ALP), Aspirate amino transferase (AST), Alanine amino transferase (ALT) total protein, albumin, globulin, Calcium (Ca) and Phosphorus (P) by using Crest biosystems (Goa, India) Kit.

Processing of organs: After 20th weeks of post feeding, 4 number of birds were randomly chosen from each treatment and slaughtered for collection of liver, tibia bone, spleen and thymus. The birds were kept off fed

Table 1: Formulation of experimental grower bird diet

Formulation of experimental diet				Proximate analysis	
Ingredients	%	Additives	%	Parameters	Dry weight (%)
Maize	56	Biocholine	0.50	Crude protein	17.17
Soyabean meal	21	Biobantox	0.50	Ether extract	2.52
Deoiled rice bran	20	Layvit	0.50	Crude fibre	6.95
Mineral mixture (premix)#	2.7	Livoline	0.25	Total ash	10.94
Common salt	0.3	E-sel-powder	0.10	Nitrogen free extract*	62.42
L-lysine	0.03	K-zyme	0.50	Calcium	0.92
DL-methionine	0.05			Available phosphorus	0.57
				Metabolisable energy*(kcal kg^{-1})	2500.00
				Zinc (ppm)	24.38
				Copper (ppm)	4.12
				Manganese (ppm)	17.94

#Mineral mixture-without Zn, Cu and Mn minerals, *Calculated value overnight before bleeding and only water was provided. The live weight of the birds was recorded as per slaughter weight. The birds were bled by modified Kosher'smethod (Panda and Mohapatra, 1989). Spleen, liver and thymus were weighed in a top pan electronic balance.

Tibia bone parameters: The tibia length (mm), weight (g), ash (%), Ca (%) and phosphorus (%) were determined to study the effect of dietary treatments on bone mineralization. The weight of the dried tibia was recorded with the help of electronic balance. Length and width of tibia was determined using electronic slide calliper. The tibia bones were ashed in a muffle furnace at 600°C for 4 h. The total ash was determined on percent weight basis. The tibia bone calcium was determined according to the method modified by Talapatra *et al.* (1940) and available phosphorus was determined as per IS: 1374-1968.

Micro mineral content in liver and tibia: The collected liver samples were oven dried at 100°C for 24 h and finely ground. The micro mineral content in the liver samples were determined by collecting 0.5 g samples and digesting at 120°C with 5 mL concentrate HNO_3 for 1 h using KEL plus digestion system. The digested samples were cooled and further digested with 30% H_2O_2 at 200°C. The process continued until the content appeared clear and colourless. The digested samples were filtered into a volumetric flask. The contents of digestion tubes were repeatedly washed with triple distilled water to obtain complete extract of the mineral. For determination of tibia micro mineral content, 0.2 g of ash samples were solubilized in 5 mL of 50% HCl and the mineral extract was filtered into a volumetric flask. The extract was then diluted using triple distilled water to the required volume and the micro mineral concentration was determined by using atomic absorption spectrophotometer.

Measure of cellular immunity: At 20th week of age, two birds from each replicate in each dietary treatment were injected intra-dermally in the comb with 100 μg of Phytohaemaglutinin-P (PHAP) in 0.1 mL of normal saline to measure the cellular immune response by Cutaneous Basophillc Hyper sensitivity (CBH) test (Edelman *et al.*, 1986). The thickness of comb was measured using digital calliper before inoculation and 24 h post inoculation and CBH response was calculated using the following equation:

$$\text{CBH response (\%)} = \frac{\text{Post injection skin thickness}}{\text{Pre-injection thickness}} \times 100$$

Measure of humoral immunity: The measure of humoral immunity was carried out as per the method described by Abdallah *et al.* (2009). Sheep Red Blood Cells (SRBC) were used as test antigens to quantitatively analyse specific antibody response as measure of humoral immunity. At 20th weeks of age, two birds from each replicate in each dietary treatment were immunized intravenously via a wing vein with 0.07 mL packed RBC mixed with 0.93 mL physiological saline (0.9% NaCl) for measurement of primary response. The SRBC were obtained in heparin solution from local sheep (reared at Instructional Livestock Farm, Bhubaneswar, Odisha) and washed three times in physiological saline. Seven days following the antigen challenge, blood samples were collected and serum samples were used to measure humoral immunity. Antibody production to SRBC was measured using micro titration haemagglutination technique with microtitre plate U shape of 96 wells (8 rows×12 column) according to Bachman and Mashaly (1986) and Kai *et al.* (1988). All SRBC antibody titers were expressed as $\log_2$ of the reciprocal of the highest serum dilution causing agglutination of SRBC.

Collection of faeces: The faeces of the experimental grower birds were collected at 20th week of age. Three birds of each group were taken for individual collection of faeces. A polythene sheet was attached under the cages of the birds and light was turn off for 1 h. The collected faeces were homogeneously mixed replicate wise and representative samples of the faeces were oven dried at 105°C for 24 h. For determination of micro mineral content in faeces, 2 g faecal samples were taken in a digestion tube and to it 12 mL of tri-acid mixture (7 mL HNO_3, 3 mL H_2SO_4 and 2 mL Perchloric acid) were added and digested at 200°C. The process continued until the content appeared clear and colorless. The digested samples were filtered into a volumetric flask.

Statistical analysis: Data retrieved from the experiment was subjected to statistical analysis wherever required. The statistical analysis of the data was done according to Snedecor and Cochran (1998).

RESULTS

Body weight: Growth performance and feed utilization of grower birds with different mineral sources is presented in the Table 2. The average body weights (g) of layer birds at the end of 20th week were 1161.53±19.01, 1152.28±24.91,138.94±20.76,1219.33±20.09, 1218.84±17.80 and 1232.50±18.20 in the treatments T_1, T_2, T_3, T_4, T_5 and T_6, respectively. The 20th week body weight of all the dietary treatments differ significantly ($p<0.05$). The 20th week body weight of T_6 was observed to be highest with significant difference ($p<0.05$) from T_1, T_2 and T_3 groups. The cumulative feed intake were 6833.75, 6747.86, 6763.78, 6925.76, 6795.38 and 6918.20 g per grower bird up to the end of 20th week of experimental period in the treatments T_1, T_2, T_3, T_4, T_5 and T_6, respectively. The FCR values (kg feed consumed/kg weight gain) at the end of 20th week were 6.08±0.35, 6.01±0.05, 6.10±0.12, 5.83±0.21, 5.72±0.18 and 5.76±0.09 in the treatments T_1, T_2, T_3, T_4, T_5 and T_6, respectively. The cumulative feed consumption and FCR of all the treated groups did not differ significantly (p>0.05).

Biochemical profile: Serum biochemical parameters viz., glucose, cholesterol, triglyceric acid, total protein, albumin, globulin, urea, BUN, SGPT, SGOT, ALP, calcium and phosphorus of 20th week BV 300 grower birds are presented in the Table 3. The levels of triglyceric acid, total protein, albumin, globulin, urea, Ca and P in serum of the grower birds varied insignificantly (p>0.05) between the treatments whereas, glucose, cholesterol, SGPT, SGOT and ALP levels in the blood serum showed significant (p<0.05) differences.

Immunity: Antibody titer against SRBC and CBH response against PHA-P and weight of lymphoid organs, were used as measures to study the immunity status of the grower birds under different dietary treatments. The antibody titer against SRBC and CBH response against PHA-P and weight of lymphoid organs are presented in Table 4 and 5, respectively. At 20 weeks of age, the CBH response was found to be significantly (p<0.05) higher in T_4 (199.37±12.40) than T_1 (159.22±13.24), T_2 (143.55±10.89)

Table 2: Growth performance and feed utilization of grower birds with different mineral sources (inorganic and organic)

	Groups					
Parameters	T_1	T_2	T_3	T_4	T_5	T_6
Body weight (g) at 9th week (initial body weight)	542.10±11.45^{ab}	509.65±14.90^{b}	510.15±17.73^{b}	585.50±22.27^{a}	556.15±16.82^{a}	587.85±14.44^{a}
Final weight (g) at 20th week post feeding	1161.53±19.01^{b}	1152.28±24.91^{b}	1138.94±20.76^{b}	1219.33±20.09^{a}	1218.84±17.80^{a}	1232.50±18.20^{a}
Cumulative feed consumption (g) till the end of 8th week	1751.75±33.75	1665.86±37.35	1681.78±31.38	1843.76±47.86	1713.38±48.39	1836.20±30.76
Cumulative feed consumption (g) till the end of 20th week	6833.75±33.75	6747.86±37.35	6763.78±21.38	6925.76±37.86	6795.38±68.39	6918.20±30.77
FCR at 20th week	6.08±0.35	6.01±0.05	6.10±0.12	5.83±0.21	5.72±0.18	5.76±0.09

FCR: Feed conversion ratio. Results were presented as Mean±SE of triplicate observations, Values bearing different superscripts in a row differ significantly (p<0.05)

Table 3: Biochemical profile of grower birds with different mineral sources (inorganic and organic)

	Groups					
Parameters	T_1	T_2	T_3	T_4	T_5	T_6
Glucose (mg dL^{-1})	194.23±7.23^{ab}	211.83±10.22^{a}	206.33±9.73^{a}	168.60±8.14^{c}	200.17±4.85^{a}	175.58±9.78^{bc}
Cholesterol (mg dL^{-1})	112.53±5.35^{a}	110.41±4.45^{a}	109.58±3.59^{a}	95.93±2.93^{b}	102.51±2.62^{ab}	97.19±2.69^{b}
Triglyceride (mg dL^{-1})	36.20±1.87	38.94±1.47	37.26±1.62	38.09±1.01	36.45±1.27	35.47±1.77
Total protein (g dL^{-1})	3.60±0.15	3.54±0.14	3.86±0.18	4.05±0.12	3.53±0.15	4.02±0.10
Albumin (g dL^{-1})	1.94±0.03	1.91±0.05	1.92±0.01	1.97±0.06	2.00±0.07	2.05±0.05
Globulin (g dL^{-1})*	1.66±0.13	1.63±0.15	1.94±0.19	2.08±0.12	1.53±0.13	1.97±0.10
A/G ratio*	1.21±0.10	1.23±0.12	1.06±0.13	0.97±0.07	1.36±0.12	1.06±0.07
Urea (mg%)	3.53±0.18	3.70±0.18	3.35±0.20	4.04±0.14	3.42±0.28	3.91±0.08
SGPT (U L^{-1})	22.48±1.06^{bc}	21.36±0.70^{c}	21.80±0.98^{c}	23.64±0.70^{bc}	25.57±1.34^{ab}	27.45±1.01^{a}
SGOT (U L^{-1})	102.860±2.69^{b}	100.88±2.28^{b}	100.30±2.62^{b}	99.91±1.75^{b}	105.92±3.59^{ab}	111.17±1.93^{a}
ALP (U L^{-1})	94.98±1.60^{b}	95.30±2.33^{b}	92.77±1.99^{b}	111.04±2.53^{a}	100.99±1.68^{b}	110.99±4.96^{a}
Ca (mg dL^{-1})	9.93±0.22	10.84±0.39	10.02±0.38	10.11±0.34	10.40±0.36	10.98±0.17
P (mg dL^{-1})	4.58±0.10	4.74±0.14	4.54±0.16	4.48±0.10	4.47±0.14	4.78±0.17

Results were presented as Mean±SE of triplicate observations. Values bearing different superscripts in a row differ significantly (p<0.05). *Calculated

Table 4: Immunity status of grower birds with different mineral sources (inorganic and organic)

	Groups					
Parameters	T_1	T_2	T_3	T_4	T_5	T_6
CBH	159.22±13.24^{bc}	143.55±10.89^{c}	135.97±7.82^{c}	199.37±12.40^{a}	176.52±11.44^{ab}	189.53±8.01^{a}
SRBC	4.83±0.40^{b}	4.67±0.49^{b}	4.83±0.31^{b}	6.17±0.48^{a}	5.67±0.42^{ab}	6.50±0.34^{a}

Results were presented as Mean±SE of triplicate observations. Values bearing different superscripts in a row differ significantly (p<0.05)

Table 5: Lymphoid organ weight (% of live weight) of grower birds with different mineral sources (inorganic and organic)

	Groups					
Parameters	T_1	T_2	T_3	T_4	T_5	T_6
Liver	2.11±0.06	2.18±0.07	2.26±0.05	2.34±0.06	2.15±0.03	2.46±0.05
Spleen	0.12±0.006	0.11±0.002	0.13±0.004	0.13±0.004	0.11±0.003	0.12±0.004
Thymus	0.22±0.007	0.22±0.003	0.21±0.001	0.22±0.003	0.21±0.007	0.24±0.004

Results were presented as Mean±SE of triplicate observations. Values bearing different superscripts in a row differ significantly (p<0.05) and T_3 (135.97±7.82) but did not differ significantly (p>0.05) than that of T_5 (176.52±11.44) and T_6 (189.53±8.01). The similar trend was also observed as regard to the antibody titers (log_2) against SRBC inoculation. Highest antibody titers (log_2) against SRBC inoculation was found in T_6 (6.50±0.34) and it differed significantly (p<0.05) from T_1 (4.83±0.40), T_2 (4.67±0.49) and T_3 (4.83±0.31). The weight of liver, spleen and thymus (percentage of live weight) ranged from 2.11±0.06 to 2.46±0.05, 0.11±0.002 to 0.13±0.004 and 0.21±0.001 to 0.24±0.004, respectively. No significant (p>0.05) difference was observed on the above parameters in all the treated groups.

Tibia bone: The tibia bone weight (g), tibia ash (%), tibia calcium (%) and tibia phosphorus (%) of grower birds are presented in the Table 6. The tibia weight ranged between 0.41±0.02 and 0.45±0.02 g/100 g. The tibia length ranged between 118.29±2.18 and 121.42±1.01 mm. The average tibia ash (%) ranged from 33.15±1.04 to 35.84±1.04. Tibia calcium (%) and tibia phosphorus (%) ranged from 30.52±0.81 to 32.08±0.46% and 14.15±0.50 to 14.72±0.25%, respectively. Tibia bone weight (g), tibia ash, tibia calcium (%) and tibia phosphorus (%) varied insignificantly (p>0.05) between the treated groups.

Bioavailability: The average minerals concentration (ppm) in different organs, serum and faeces of BV 300 grower birds under different dietary treatments is presented in Table 7. The liver Zn (ppm) was recorded to have a range from 57.15±0.78 to 82.78±3.04, liver Mn (ppm) from 2.71±0.37 to 2.88±0.26 and liver Cu (ppm) from 2.64±0.18-4.14±0.34, with non-significant difference (p>0.05) within the dietary treatments. But the liver Zn level was observed to be significantly (p<0.05) higher in organic Zn supplemented groups except T_4. Similarly, the tibia Zn (ppm) ranged from 136.54±2.08-166.95±3.23, tibia Mn (ppm) from 12.46±0.47 to 15.02±0.80 and tibia Cu (ppm) from 3.08±0.21 to 4.29±0.32. The tibia Zn level of T_5 was observed to be significantly (p<0.05) lower than that of other treated groups except T_2. The serum Zn level ranged from 2.69±0.09 to 3.80±0.20, serum Cu (ppm) from 0.31±0.02 to 0.39±0.02 and serum Mn (ppm) from 2.46±0.47 to 3.35±0.19. At 20 weeks of age, the faecal Zn (ppm) excretion was significantly (p<0.05) higher in T_1, T_2 and T_3 than that of other treated groups. The faecal excretion of Cu was found to be significantly (p<0.05) higher in T_1, T_2 and T_4 than that of other treated groups. Similarly, faecal excretion of Mn was found to be significantly (p<0.05) higher in T_1 and T_3 than that of other treated groups.

DISCUSSION

The earlier studies on organic mineral feeding in poultry are mostly reported in broiler birds and during the laying stage of layer birds and not much reference were available for grower birds. So the discussion of this experimental finding is mainly based on the available literature on broiler and layer birds. This experimented was

Table 6: Tibia bone parameters of grower birds with different mineral sources (inorganic and organic)

	Groups					
Parameters	T_1	T_2	T_3	T_4	T_5	T_6
Weight (g/100 g body weight)	0.45±0.02	0.41±0.02	0.41±0.02	0.41±0.01	0.43±0.02	0.43±0.02
Tibia length (mm)	119.65±1.38	121.42±1.01	118.29±2.18	118.76±1.40	121.35±2.70	118.31±1.88
Tibia ash (%)	34.64±0.90	35.84±1.04	33.15±1.04	35.60±0.44	34.74±0.81	35.61±0.48
Tibia calcium (%)	30.70±0.57	30.52±0.81	31.20±0.62	32.08±0.46	31.93±0.53	31.67±0.99
Tibia phosphorus (%)	14.29±0.73	14.21±0.24	14.15±0.50	14.72±0.25	14.54±0.32	14.18±0.17

Results were presented as Mean±SE of triplicate observations, Values bearing different superscripts in a row differ significantly (p<0.05)

Table 7: Average minerals concentration (ppm) in different organs, serum and faeces of grower birds with different mineral sources (inorganic and organic)

	Groups					
Organs and minerals	T_1	T_2	T_3	T_4	T_5	T_6
Serum						
Zn	2.96±0.06^{bc}	3.57±0.12^{a}	2.69±0.09^{c}	3.80±0.20^{a}	3.34±0.22^{ab}	3.67±0.07^{a}
Cu	0.32±0.02^{b}	0.33±0.08^{b}	0.38±0.03^{a}	0.31±0.01^{b}	0.31±0.02^{b}	0.39±0.02^{a}
Mn	2.46±0.47	3.35±0.19	2.84±0.20	2.95±0.17	3.17±0.25	2.76±0.26
Liver						
Zn	65.68±2.67^{cd}	68.27±1.29^{bc}	57.15±0.78^{e}	74.81±1.63^{ab}	60.24±1.16^{de}	82.78±3.04^{a}
Cu	3.18±0.43	3.12±0.60	4.14±0.34	3.47±0.44	2.64±0.18	3.21±0.14
Mn	2.72±0.40	2.87±0.42	2.81±0.44	2.87±0.09	2.71±0.37	2.88±0.26
Tibia bone						
Zn	160.97±3.21^{a}	145.49±2.44^{bc}	148.47±3.49^{b}	166.95±3.23^{a}	136.54±2.08^{c}	161.76±1.17^{a}
Cu	3.26±0.41	3.08±0.21	4.29±0.32	3.40±0.07	4.02±0.09	3.59±0.20
Mn	12.46±0.47	15.02±0.80	13.17±0.37	13.62±0.39	13.51±0.47	13.09±0.40
Faeces						
Zn	221.47±7.64^{a}	210.84±8.61^{ab}	220.28±5.38^{a}	189.54±4.77^{b}	158.75±4.55^{c}	188.48±8.58^{b}
Cu	54.58±2.09^{a}	59.73±2.16^{a}	34.90±2.99^{b}	56.62±2.40^{a}	26.18±0.88^{c}	41.42±2.63^{b}
Mn	302.23±6.30^{a}	248.48±5.19^{b}	299.87±7.59^{a}	285.01±5.29^{a}	173.21±10.62^{d}	215.59±7.25^{c}

Results were presented as Mean±SE of triplicate observations, Values bearing different superscripts in a row differ significantly (p<0.05) continued in two phases i.e. starter (0-8 week) and grower (9-20 week) in a continuous manner and the performance of the layer chicks was published earlier (Das *et al*., 2014). The body weight of grower birds of 100% organic mineral supplemented group (T_6) was found to be significantly ($p<0.05$) higher than T_2 and T_3 from 9th to 20th weeks of age. But the body weight of growers of T_6 did not differ significantly ($p>0.05$) from T_5 and T_4 throughout the experimental feeding period. The 20th week's body weight of growers implied that supplementation of either organic minerals in combination at 50 or 100% level or organic zinc supplementation at 100% level resulted in higher growth rate. The body weight of growers of T_3 did not differ significantly from T_1. This implied that organic Cu supplementation had no significant effect on the body weight of growers. This corroborated with the findings of Paik *et al*. (1999). Since the birds of all the groups were maintained on a fixed quantity of feed, the higher body weight gain in groups T_4, T_5 and T_6 indicated better utilization of consumed feed which might be due to higher bioavailability of these minerals. This corroborated with the findings of Abdallah *et al.* (2009) who reported that the body weight of chicks at 35th day fed with 100% organic zinc were significantly higher than the group fed with 50% organic and 50% inorganic zinc and group fed with 100% inorganic zinc. Similar findings were also reported by Swiatkiewicz *et al*. (2001). Also the higher body weight of T_6 might be explained as trace minerals, such as Cu, Mn and Zn, are essential for grower birds growth and are involved in many digestive, physiological and biosynthetic processes within the body. They function primarily as catalysts in enzyme systems within cells or as parts of enzymes (Abdallah *et al*., 2009).

The cumulative feed consumption of grower birds did not differ significantly ($p>0.05$). Since the offered feed quantity to the growers was fixed for each week, not much difference was expected as far as feed consumption concerned. The FCR of all the weeks of all the treatments did not show any significance between the groups. The insignificant differences in feed efficiency between 50% organic Zn, Cu and Mn (T_5) and 100% organic Zn, Cu and Mn (T_6) groups indicated that the levels used for chelated minerals were already sufficient to meet the requirement for normal feed efficiency. Wang *et al*. (2008) stated that chelated trace minerals can be added at a lower level without negative effect on the feed efficiency of broilers.

The serum Triglyceride, total protein, albumin, globulin, albumin and globulin ratio, urea, calcium and phosphorus levels of all the treated groups did not differ significantly ($p>0.05$). Feng *et al*. (2010) in their experiments in broiler on feeding of organic Zn reported no-significant difference in serum albumin level. Non-significant diffrences in serum calcium and phosphorus was reported by Parak and Strakova (2011) while comparing feeding of inorganic with organic Zn in breeding cocks which corroborated with the present finding. In contrast to this, Al-Daraji and Amen (2011) reported significantly higher levels of serum calcium and phosphorus on increasing Zn concentration in the diet by addition of 100 mg pure Zn/kg of diet than that of control (basal diet) in broiler breeders from 58-66 weeks of age. The significant increase of serum calcium and phosphorus as reported by Al-Daraji and Amen (2011) might be due to higher level of Zn in the diet. The serum glucose, cholesterol SGOT, SGPT and ALP levels of all the treated groups of grower birds differed significantly ($p<0.05$). Osman *et al*. (2007) also observed significant ($p<0.05$) effects of Zn methionine supplemented at 30, 40 and 50 ppm on serum SGPT level. The serum glucose and serum cholesterol levels were significantly ($p<0.05$) lower in T_4 (organic Zn fed group) than T_1 (inorganic mineral fed group) and T_5 (50% organic mineral fed group). This observed cholesterol level is in agreement with the finding of Aksu *et al*. (2010). In the present study, a significant variation in the levels of serum cholesterol of grower birds was found in case of Zn supplemented groups than the control. Herzig *et al.* (2009) reported significant decrease of plasma cholesterol when broilers were fed with high amounts of Zn in diet. It was also observed by Parak and Strakova (2011) while comparing feeding of inorganic with organic Zn in breeding cocks. The ALP level in the serum of 100% organic Zn supplemented groups (T_4 and T_6) was found to be significantly ($p<0.05$) higher from the inorganic Zn supplemented group. Idowu *et al*. (2011) also observed significant difference in the levels of serum ALP and serum Zn concentrations between control and Zn proteinate groups with higher levels in Zn proteinate and opined that due to Zn binding capacity of serum, alkaline phosphatase acts as good indicator of Zn status. Al-Daraji and Amen (2011) reported significantly higher levels of serum ALP level on increasing Zn concentration in the diet by addition of 100 mg pure Zn/kg of diet than that of control (basal diet) in broiler breeders from 58-66 weeks of age. The increase in ALP level on Zn supplementation might be due to increase in corticosteroid hormone secretion, epinephrine and nor-epinephrine (Al-Daraji and Amen, 2011). In contrast to this, no significant difference in serum ALP in organic Zn fed groups was reported by Aksu *et al*. (2010) on feeding different levels of organic Zn, Cu and Mn in broiler ration. In the experimental birds, the CBH response and the antibody titers against SRBC were found to be significantly higher in T_4, T_5 and T_6 than that of other treated groups. This implied that in organic Zn supplemented groups, the immune response was observed to be better than that of other treated groups. Hudson *et al.* (2005) reported that immune response to PHA-P injection was enhanced when dietary zinc supplementation was solely from Zn Amino Acid (ZnAA). The grower birds provided diets supplemented with zinc from zinc amino acid might have increased thymulin activity; therefore, enhancing immune response through increased maturation of T-lymphocytes and activation of B lymphocytes by T-helper cells (Hudson *et al*., 2005). The non-significant effect on CBH and antibody against SRBC of T_5 and T_6 implied that supplementation with reduced doses of trace elements (Cu, Zn and Mn) in the organic forms had the same effect on the haematological and indices as the feeding of diets with recommended doses of their inorganic salts (Petrovic *et al*., 2009).

The weight of lymphoid organs (spleen and thymus) of experimental growers at 20th week of age of all treatments did not differ significantly. The results for spleen percentage as observed in the present experiment corroborated with the findings of Moghaddam and Jahanian (2009) and Feng *et al*. (2010). Supplementation of zinc did not improve the weight of the lymphoid organs as more nutrients being repatriated to develop body weight and production where as immune system needed a small amount of nutrient in relation to that needed for growth and production (Bartlett and Smith, 2003).

Tibia bone weight (g), tibia ash, tibia calcium (%) and tibia phosphorus (%) varied insignificantly ($p>0.05$) between the treated groups. Similar results were also reported by Abdallah *et al.* (2009) who reported that supplementation of organic Zn, Cu and Mn at 50 and 100% levels in the diet of broilers did not have any significant effect on the tibia wt (g), tibia length (mm), tibia Ca (%) and tibia P (%) levels. Swiatkiewicz and Koreleski (2008) in their experiment reported that organic zinc supplementation had no significant effect on tibia length, relative weight of tibia and tibia ash content which corroborated with present findings.

The Mn level of serum and other studied organs did not differ significantly ($p>0.05$). Similar results were also reported by Aksu *et al.* (2010). Contradictory to this finding, Britanico *et al.* (2012) reported significantly ($p<0.05$) higher level of Mn in liver and tibia bone. At 20 weeks of age, except T_3 and T_1, Zn levels of serum did not differ significantly ($p>0.05$). Lower level of tibia Zn was observed in T_5 group than that of other groups. Liver Zn was significantly lower in organic Cu fed group and higher in 100% organic Zn fed groups. This implied that the inclusion of organic copper in the diet at 100% level exhibited lower serum zinc level in T_3. The lower serum zinc level as observed in T_3 might be due to the existence of an antagonism between Zn and Cu occurred (Ao *et al*., 2009). This corroborated with the finding of Ao *et al.* (2009). The tibias from birds fed the diet containing organic Zn had significantly higher Zn content than those from birds fed the diet containing inorganic Zn. These results are consistent with published data by Ledoux *et al.* (1991), Miles *et al.* (1998), Ao *et al.* (2006), Lim and Paik (2006) and Shelton and Southern (2006). Moreover, zinc competes with copper for binding to metallothionein and consequently at higher dietary zinc levels, less copper is absorbed when inorganic forms of these minerals were included in the diet. Similarly, the serum Cu level was found to be significantly higher in 100% organic Cu fed groups. This corroborated with the finding of Bao *et al.* (2007). As regard to excretion of trace minerals concerned, the excretion of Zn, Cu and Mn were significantly higher in inorganic mineral fed groups than organic mineral fed groups even at same level of feeding. This was because organically complexed trace minerals provide alternative pathways for absorption, thus leading to a reduction in the excretion of minerals (Leeson, 2003). Burrell *et al.* (2004) reported that the cumulative zinc excretion of organic zinc fed groups were significantly lower than inorganic zinc fed groups.

CONCLUSION

Replacement of inorganic Zn with organic Zn at 100% level or replacement inorganic Zn, Cu and Mn with organic Zn, Cu and Mn either at 50 or 100% level improved the body weight and immunity of grower birds. Also faecal excretions of organic minerals were lower than inorganic minerals.

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