HEAMATOLOGICAL AND SERUM BIOCHEMICAL INDICES OF BROILERS FED INORGANIC AND CHELATED TRACE MINERALS

Ayoola A.A. [+], Fafiolu A.O[+] [*], Oluwatosin O.O[+] [*], Osinowo O.A[+] [×], Ariyo O.W[*]

[+]Centre of Excellence in Agricultural Development and Sustainable Environment, Federal University of Agriculture, Abeokuta, Ogun State Nigeria.
[*]Animal Nutrition Department, Federal University of Agriculture, Abeokuta, Ogun State Nigeria.
[×]Agricultural Education Department, Federal College of Education, Osiele, Abeokuta Ogun State Nigeria.
Corresponding Email: remyportable@yahoo.com, Phone Number: +2348034935823

Abstract

This study was carried out to evaluate the effect of supplemental inorganic and chelated Cu, Zn and Mn on haematology and serum biochemistry of broiler chickens. A 42-day feeding trial was conducted using a total of three hundred Arbor Acre broiler chickens. The birds were allocated to five dietary groups. Each group had six replicates with ten birds each. The treatments were control (basal diet), 100% inorganic trace minerals (ITMs) supplemental level (15, 100, 100 mg/kg for Cu, Zn, Mn respectively), 50% ITMs supplemental level (7.5, 50, 50 mg/kg of Cu, Zn, Mn), 50% Chelated trace minerals (CTMs) supplemental level (7.5, 50, 50 mg/kg of Cu, Zn, Mn) and 25% CTM supplemental level (3.75, 25, 25 mg/kg of Cu, Zn, Mn, in that order). Data collected were subjected to one–way Analysis of Variance using a Completely Randomised Design. A lower ($p<0.05$) white blood cell count ($11.07x10^6/mm^3$) and an improved ($p<0.05$) red blood cell count ($13x10^6/mm^3$) was observed for the group fed 50% CTM supplemental level. The group fed 50% supplemental level of CTM had the highest ($p<0.05$) total protein (73.2 g/L), albumin (41.20 g/L) and globulin (32 g/L) in serum as compared to the other groups. The serum Cu, Zn and Mn were significantly higher ($p<0.05$) for the group fed 25% supplemental diet of CTM. The study concluded that trace minerals supplementation increased serum trace minerals of broiler chicken.

Key words: chelates, inorganic salts, haematology, serum biochemistry, broilers

Introduction

Evaluating haematological and serum biochemical parameters is a means of assessing the health status of animals and effect of treatments can be deduced from these analyses. Trace minerals are essential nutrients in broiler chicken's diets and they play very important roles in birds and these include cell proliferation and growth, tissue and bone development and integrity, immune development and response, reproduction, enzymes formation, gene regulation, and protection against oxidative stress and damage (Richards *et al*., 2010). Inorganic trace minerals (ITM) of Cu, Zn and Mn are often used in broiler chicken diets at the higher inclusion level than the recommended quantity by NRC (1994), but the absorption is limited because of the antagonistic effect in diet and gastrointestinal tract (Manangi *et al*., 2012). This results to increasing concerns over the excretion of minerals as pollutant in the environment (Manangi *et al*., 2012). A chelated mineral means the bonding or combination of metal ions with organic ligand or ligand complex such as amino acids, proteinate, polysaccharides or organic yeast (Bao, *et al*., 2006). Therefore, this study was carried out to investigate the effect of supplemental inorganic and chelated Cu, Zn and Mn on haematology and serum biochemistry of broiler chickens.

Materials and Methods

This study was carried out according to the research ethics and guidelines of the College of Animal Science and Livestock Production of the Federal University of Agriculture, Abeokuta, Nigeria. It was carried out at the broiler section (unit) of the Directorate of University Farm (DUFARM), Federal University of Agriculture, Abeokuta, Ogun State. A total of 300 unsexed day old Arbor Acre (AA) broiler chicks used for the study. The broilers were allocated to five (5) treatments; each treatment was divided into six (6) replicates with ten (10) birds each, making a total of sixty (60) birds in a treatment. The treatments were control (basal diet), 100% inorganic trace minerals (ITMs) supplemental level (15, 100, 100 mg/kg for Cu, Zn, Mn respectively), 50% ITMs supplemental level (7.5, 50, 50 mg/kg of Cu, Zn, Mn), 50% Chelated trace minerals (CTMs) supplemental level (7.5, 50, 50 mg/kg of Cu, Zn, Mn) and 25% CTM supplemental level (3.75, 25, 25 mg/kg of Cu, Zn, Mn, respectively). Feed and water were given *ad*

libitum throughout the experimental period. Chelated trace minerals of Cu, Zn and Mn (Mintrex®) containing 15% Cu from Cu (2-hydroxyl- 4-methylthiobutanoic acid as $HMTBa)_2$, 16% Zn from $Zn(HMTBa)_2$ and 13% Mn from $Mn(HMTBa)_2$ were obtained from Novus Inc., USA. The inorganic sources of Cu, Zn and Mn were purchased from an inorganic manufacturer in Lagos. The composition of the inorganic sources used are described as follow; copper sulphate pentahydrate ($CuSO_4.7H_2O$: containing 25% Cu), zinc oxide (ZnO: containing 72% Zn) and manganese dioxide (MnO_2: containing 64% Mn) were used as inorganic supplemental Cu, Zn and Mn respectively. The five (5) treatments diets with varying supplemental level of inorganic trace minerals (ITM) and chelated trace minerals (CTM) is shown in Table 1.

Table 1: Gross Composition of Experimental Basal diets

Ingredients (Kg)	(0 – 3 weeks)	(4 – 6 weeks)
Maize	54.00	65.00
Soya meal	40.00	30.70
Wheat Bran	2.00	1.00
Lime stone	1.50	1.00
Bone meal	1.20	1.00
Fish meal (72% CP)	0.50	0.50
Methionine Hydroxyl Analog	0.25	0.20
Lysine	0.20	0.20
Salt	0.15	0.15
Vitamin-Mineral Premix (Cu, Zn & Mn free)[1]	0.25	0.00
Vitamin-Mineral Premix (Cu, Zn & Mn free)[2]	0.00	0.25
Determined analysis		
Dry matter (%)	88.90	94.00
Crude protein (%)	23.70	19.20
ME (KCal/kg)	2946.23	3054.90
Nitrogen free extract (%)	47.02	55.6
Crude Fibres (%)	4.11	4.48
Ether extract (%)	5.23	4.92
Ash content (%)	8.84	9.80

Blood Sample Collection

At day 42, 30 birds (a bird from each replicate) were bled via wing vein puncture. 2 mL of blood was collected from each bird using sterile syringe and needle and deposited into tubes containing ethylenediamine-tetra-acetate (EDTA) and another 2 mL was collected into plain sterile bottles. The blood in the EDTA bottles were used to determine haematological parameters while that in the plain bottles were used to determine serum parameters The PCV was determined by microhaematocrit method and Hb and RBC were determined using colorimetry cyanomethaemoglobin and improved Neubauer haemocytometer methods respectively. The serum total protein, albumin and globulin were analysed colorimetricaly using a diagnostic reagent kit.

Statistical Analysis

Data collected were subjected to a Completely Randomized Design using SAS (2007). Comparisons among means were done using Tukey's test of the software.

Results and Discussion

The effect of supplemental trace minerals on haematology are presented in Table 2. Groups fed 50% and 25% supplemental level of CTM had higher ($p<0.05$) packed cell volumes (34.67% and 33.67%) and haemoglobin (11.33 g/dL and 11.20 g/dL respectively) relative to other dietary groups. Higher haemoglobin and PCV concentration in blood of broiler birds fed diets supplemented with chelated trace minerals compared to BD reflects the importance of Cu in haemoglobin synthesis. This indicates that Cu was more bioavailable in the CTM groups since Cu and Fe are known to play vital roles in the

synthesis of haemoglobin and for the synthesis of enzyme needed for normal metabolism (Close, 1999). The white blood cell of the group fed 50% CTM level was significantly lower ($p<0.05$) compared to the other groups and it also had a significantly higher ($p<0.05$) red blood cell while the groups fed 100% ITM, 50% ITM level and BD had lower RBC. The increased WBC of birds given BD relative to those receiving CTM suggests a stimulation of the immune system by the control diet. This showed that the birds fed CTM supplemented diets with the lower WBC had immune stability and the birds were not challenged. This is in line with the report of Jegede *et al*., (2011) who reported higher haemoglobin and PCV with lower WBC in broilers fed Cu-proteinates.

Table 2: Effect of inorganic and chelated trace mineral on the haematological parameters of the experimental broiler chickens (42 days)

PARAMETERS	Control	ITM		CTM			
	(BD)	100%	50%	50%	25%	SEM	P-Value
PCV (%)	28.50^{b}	31.00^{ab}	32.33^{ab}	34.67^{a}	33.67^{a}	0.66	0.00
Hb (g/dL)	9.57^{b}	10.33^{ab}	10.67^{ab}	11.33^{a}	11.20^{a}	0.21	0.02
WBC (10^6/mm^3)	17.97^{a}	17.43^{a}	18.67^{a}	11.07^{b}	13.90^{ab}	0.92	0.01
RBC (10^6/mm^3)	2.42^{c}	2.80^{b}	2.89^{ab}	3.12^{a}	2.98^{ab}	0.07	0.00
Neutrophil (%)	51.00^{a}	26.00^{b}	27.33^{b}	15.67^{b}	14.00^{b}	3.73	0.00
Lymphocyte(%)	46.00^{b}	70.67^{a}	69.00^{a}	80.00^{a}	81.33^{a}	3.71	0.00
Basophil (%)	0.00	0.33	0.00	0.33	0.67	0.15	0.68

^{abc} Means in the same row having different superscripts are significantly different at ($P<0.05$)

The effect of supplemental trace minerals on serum biochemistry of the experimental birds is shown in Table 3. Birds fed 50% CTM recorded the highest ($p<0.05$) total protein value (73.2g/L), albumin (41.20 g/L) and globulin (32 g/L) in serum as compared to other groups with the group fed 100% ITM level having the lowest ($p<0.05$) serum protein of 62.53g/L. Also, the group fed BD had a significantly lower ($p<0.05$) albumin (35.77g/L) compared with 41.2g/L of the group fed 50% supplemental diet of CTM. The serum Cu (16.5ug/dL), Zn (14.13ug/dL) and Mn (1.88ug/dL) were significantly higher ($p<0.05$) for the group fed 25% CTM compared to the group fed 100% supplemental ITM level with 15.08, 11.03, and 1.61ug/dL of serum Cu, Zn and Mn respectively while the group fed BD had the lowest ($p<0.05$) Cu, Zn and Mn in the serum. The improvement in total protein, serum albumin and globulin in groups fed diet supplemented with CTM as a result of the supplemented methionine hydroxyl analog (MHA) chelated with trace minerals compared to the groups fed diet supplemented with ITM. The higher serum protein, albumin and globulin show that the MHA or 2- hydroxyl -4- methyl thiobutanoic acid (HMTBa) which is regarded as precursor of DL- methionine (first limiting amino acid) (Richards *et al*., 2007) were released from the ligand. Copper is also involved in blood proteins and has capacity to influence serum protein (Uany., *et al*, 1998) This study is supported by Corzo *et al*., (2009) who reported that supplemental amino acid (valine and isoleucine) in broiler chicken diet increased the serum protein, albumin and globulin. The increased serum Cu, Zn and Mn of the groups fed supplemental diet of CTM compared with the groups fed ITM level and BD with lower serum minerals indicates that the CTM were more bioavailable to the broiler chickens and this also shows that there is less antagonism with other dietary minerals and among themselves. This study agrees with the report of Salami *et al.*, (2016) who reported higher bioavailability of trace mineral from mineral chelates and less antagonistic interaction of chelates with other dietary constituents in the digestive tract compared with inorganic salts.

Table 3: Effect of inorganic and chelated trace minerals of serum biochemistry of the experimental broiler chickens (42 days)

PARAMETERS	Control	ITM		CTM			
	(BD)	100%	50%	50%	25%	SEM	P-Value
TP (g/L)	65.90^{c}	62.53^{d}	67.00^{bc}	73.20^{a}	69.87^{b}	0.99	0.00
Albumin (g/L)	35.77^{c}	36.67^{bc}	38.30^{bc}	41.20^{a}	39.73^{ab}	0.60	0.00
Globulin (g/L)	30.27^{ab}	25.87^{c}	28.70^{b}	32.00^{a}	30.13^{ab}	0.58	0.00
Creatinine (mg/dL)	0.77	1.40	1.27	0.87	1.43	0.11	0.17
Uric (mg/dL)	5.80	8.57	8.33	7.93	7.60	0.41	0.21
Serum Cu (ug/dL)	6.77^{c}	15.08^{b}	15.53^{b}	16.17^{a}	16.50^{a}	0.97	0.00
Serum Zn (ug/dL)	4.05^{e}	11.03^{d}	12.80^{c}	13.53^{b}	14.13^{a}	0.98	0.00

[abc] Means in the same row having different superscripts are significantly different at (P<0.05)

Conclusion

Trace mineral supplementation increased serum trace mineral concentration. Supplementation of CTM (Cu, Zn and Mn) at 50 % improved haemoglobin and packed cell volume of broiler chickens.

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