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Performance, Haematological, Biochemical Studies and Bacterial Loads of Broiler Chickens Fed Copper Sulphate Supplemented Diets

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ABSTRACT

This study was carried out to assess the performance, haematological, biochemical indices and bacteria load of broiler chickens fed diets supplemented with copper sulphate ($CuSO_4$) at 0%, 100ppm, 200ppm and 300ppm inclusion levels. A total of one hundred and twenty (120) broiler chicks were careful selected and assigned to four (4) dietary treatments. The experiment was performed in three (3) replicates with ten (10) chickens per replicate making a total of thirty (30) chicks per diet. The birds were randomly selected using the Completely Randomized Design (CRD). The birds were fed *ad libitum* and given clean and cool water. At the end of the feeding trial, at four (4) weeks, birds fed diet 1 had the highest total fed intake (g/bird) of (34.64) and at 8 weeks, diet 2 containing 100ppm of copper sulphate has the highest feed intake (g/bird) of (180.67). The highest total weight gain (g/birds/day) in birds fed diet 2 containing 100ppm $CuSO_4$ (1632.96) at eight (8) weeks of age. The highest feed conversion ratio was observed in the birds fed diet 3. The results obtained showed that all the haematological indices measured were not significantly ($p>0.05$) influenced by the treatments as the birds fed the supplemented diets were not different from birds fed the control diet which fall within the normal range. The serum biochemical values showed that cholesterol, albumin, total protein, bilirubin, aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline phosphate (ALP), creatinine and globulin were not significantly affected by the dietary treatment. The blood cholesterol level of the birds ranged between 25.09g/l in the 100ppm $CuSO_4$ inclusion to 40.12g/l in the control diet. A decrease at 100ppm CuSO4 inclusion level was observed followed by an initial increase in blood cholesterol was recorded in 200ppm and 300ppm $CuSO_4$ inclusion level. The fact that values obtained for both alkaline phosphatase and globulin concentration did not differ significantly among treatment means showed that the utilization of $CuSO_4$ in the diets of broiler birds will have no detrimental effects on their physiological functions. The bacterial load of liver, spleen and duodenum in birds fed diets 1, 2 and 3 decreased as the levels of inclusion of CuSO4 increases but got higher in those fed diet 4.

Introduction

The need to provide sufficient animal protein for the growing human population is of paramount importance to animal nutritionist, scientist and agriculturist. Developing the poultry industry has been advocated to be the greatest means of bridging the protein gap prevailing in the tropical countries (F.A.O, 2000). The poultry industry portends a great future for Nigerians if developed. It can provide adequate animal protein for the entire population. Poultry meat has a wide acceptance with little or no limitation in terms of traditional and religious taboos as compared to pork which is rejected by Muslims (Afolabi and Oladimeji, 2003).

Nutrition is one of the major constraints to animal production in the world, Nigeria inclusive. Feed occupies a major component of any livestock enterprise. When the economies of production are considered, feed represent by far the largest segment of the total cost of intensive production from 60-70% (Igene and Arijeniwa, 2004). Its inadequacy in terms of quantity could lead to a situation of low nutritional status with a resultant poor weight gain, reproduction ability and low feed conversion ratio. For about a decade ago, poultry meat, eggs and other product have consistently been very costly and consistently making the production unavailable for human. For Nigerians to conveniently afford poultry products, one of the efforts made by researchers in this regard is the manipulation of the scarce and expensive feed materials in such a way that birds are made to utilize them maximally for egg and meat production. In regard, the expense on the diet is being minimized while at the same time the nutrient requirements of the animals are met. The cheaper the feed source (without altering its quality) the better the return to the farmers. Broilers have relatively short period of production. They have potential for rapid growth and can supply the highly needed protein for man in terms of quality and even

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quantity. The growth process depends largely on the composition of feed (Ou *et al.,* 2000). The diet composition quality is reflected in the health status of an animal.

Haematology studies are primary in studying the health status of an animal. The health status of an organism especially those in animal kingdom can be assessed by using the physical fluids, (the blood lymph, serum and other fluid present in the system). Haematology is the scientific study of the natural functions and disease of blood. Blood examination provides the opportunity to clinically investigate the present of several metabolites and other constituents in the body of the animal (Onifade, 1993). Blood constituents are subject to a large measure of dietary influence such as the quality of protein, vitamins and mineral as well as the presence of anti-nutrients.

Copper (cu) is an essential trace mineral for poultry which functions in numerous physiological processes primarily involved in mitochondria oxidative phosphorylation, free radical detoxification, neurotransmitter synthesis and iron (Fe) metabolism (Underwood and Suttle 1999, Crisponi *et al.*, 2010). The recommended Cu requirement for broiler is 8mg/kg diet (NRC, 1994). Chen *et al.* (1996) observed an increase in the enzymes level as a result of increase in the level of dietary Cu in country chicken and pullet chickens. No such report is published that deals with the supplement Cu that is higher than 250 ppm and less than 500 ppm to see its effects on the haematochemicals and biochemical properties in broiler chickens. Therefore, an experiment was designed to monitor the effect of CuSO4 supplementation on the performance, haematology and biochemical properties and bacterial loads of broiler chickens.

Materials and Methods

The experimental feeding trial was carried out at the poultry unit of the Teaching and Research Farm, the Federal College of Agriculture, Akure, Ondo state, Nigeria and the laboratory analysis was carried out at the Department of Animal Production and Health Nutrition laboratory of the Federal University of Technology, Akure, Ondo State. One hundred and twenty (120) day old broiler chicks were used for the experiment. The birds were purchased from a reputable farm at Ibadan, Oyo State while copper sulphate was purchased at a reliable chemical shop. The feed ingredients were purchased from a reputable feed mill at Akure, Ondo state. Two basal experimental diets were formulated for (starter and finisher) stages of the production cycle. After formulation, the diets were divided into four (4) equal parts, the first part were used as the control diet without copper sulphate inclusion while the second, third and fourth parts were supplemented with 100, 200 and 300ppm inclusion of copper sulphate respectively.

The basal compositions of the starter as well as the finisher diet are as presented in Tables 1. The experiment has a total number of twelve pens, which comprises four treatments and three replicates. Ten birds were randomly allocated to each pen.

Slaughtering of Birds and Blood Collection

At the end of the field trial the birds were starved for three (3) hours and two (2) birds (1 male and 1 female) per replicate were weighed, blood collected with 5ml syringe and needle through the wing vein before slaughtering by severing the jugular vein with a knife without anaesthetizing. The blood was collected into bottle containing a speck of dried Ethylene Diaminetetracetic acid (EDTA) powder. The bottles were immediately capped and the content mixed gently for about a minute by repeated inversion. The blood collected was used for the haematological studies. Some blood were also collected in test tube without anticoagulant and this was separated by centrifugation to obtain the serum using an angle head refrigerated centrifuge set at 2000 rpm for 4 minutes and the samples were kept in sterile universal bottles and kept frozen prior to its analysis.

Results and Discussion

Tables 2 and 3 presents the results of growth performance (g/kg body weight) of broiler chickens fed varying levels of copper sulphate at four weeks and eight weeks respectively. The data presented showed that there were no significant differences ($p>0.05$) in birds fed copper sulphate based diets at the starter phase, but significant differences were observed in total weight gain, daily weight gain and daily feed intake at the finisher phase of the experiment. Of all the diets, $CuSO_4$ supplemented at 100ppm level (Diet 2) had the highest total weight gain, daily weight gain and daily feed intake.

Blood examinations provide the opportunity to clinically investigate the presence of several metabolites and other constituents in the body of an animal. It is also a good way of assessing the health status of animals (Onifade, 1993). Changes at the haematological levels usually present or signal the onset to outward signs such as weight changes, loss of hair/feather, changes in milk or egg production e.t.c following dietary treatments (Aletor and Egberongbe, 1992). The value obtained in this study as presented in Table 4 for haematological indices falls within the normal range as reported by Mitruka and Rawnsley, (1977), Ross *et al*., (1978). Reports by Aletor and Egberongbe, (1992) indicated those blood variables mostly consistently affected by dietary treatment are Red Blood cell count (RBC), Packed Cell Volume (PCV) and plasma protein. In this present study, however, the PCV, RBC, MHC and differential cell counts were not significantly ($P>0.05$) influenced by the dietary treatment. The measurement of Mean Cell Volume (MCV), Mean Corpuscular Hemoglobin Concentration (MCHC) and Mean corpuscular Hemoglobin (MCH) lies in their use in the diagnosis of anaemia and an index of capacity of bone marrow to produce Red Blood Cell (Aletor and Egberongbe, 1992), and with regards to the blood parameters, the erythrocytes sedimentation rate (ESR) were similar in all treatments. However, the values obtained in this study are consistent with those reported for chickens by Mitruka and Rawnsley, (1977) and Ross *et al*., (1978) i.e. PCV (%) ranges from 25.0 – 45.0, this implies that inclusion of $Cuso_4$ in diets of broilers at the present supplemented levels has no effect on the relative quantity of blood cells as compared with the total volume of blood (Smith, 1974). High amount of copper sulphate lead to reduction in the number of erythrocytes and consequently to macrocytic anaemia, in particular in young animals naturally deprived of iron (Lillie *et al*., 1977). Since none of the haematological indices were not significantly affected ($P>0.05$) by the dietary treatments, this mean that feeding of diets with $CuSO_4$ to broilers has no effect on their health status. This is consistent with previous report by Xia, (2000).

The serum biochemical values (Table 6) showed that cholesterol, albumin, total protein, bilirubin, aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline phosphate (ALP), Creatinine and globulin were not significantly affected by the dietary treatment. The blood cholesterol level of the birds ranged between 25.09g/l in the 100ppm CuSO4 inclusion to 40.12g/l in the control diet. A decrease at 100ppm CuSO4 inclusion level was observed

followed by an initial increase in blood cholesterol was recorded in 200ppm and 300ppm $CuSO_4$ inclusion level. The birds on 100ppm and 300ppm inclusion level had significantly high levels of albumin in their blood (12.71g/dl and 11.68g/dl respectively) while the control diet was lower (8.91g/dl). The total protein content of the serum was statistically similar among dietary treatments ranging from 19.91g/dl in the 100ppm CuSO4 to 25.86g/dl in the 300ppm group. The general trend of observation was a linear increase in total protein with increase in the level of CuSO4 in the diets. The level of serum creatinine statistically significant among the treatment means were all comparable to that of the control and did not follow a particular trend.

The levels of aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) vary significantly among the dietary treatments. These two enzymes are biomarkers for tissue damage and hepatic degeneration (Agbede *et al*., 2011). A general increase in the level of the two enzymes was observed with the inclusion of $CuSO_4$ in the diets while a slight increase observed with 300ppm inclusion of $CuSO_4$ was still within the ambit of statistical insignificance relative to the control. Since significant elevation of these enzymes in blood serum is indicative of serious tissue and hepatic damage, $CuSO_4$ could therefore be adjudged safe at the levels at which it is incorporated into the diets. Even the highest level of ALT (24.33u/l) obtained under this study was still within the normal blood range of 1-37u/l reported by Ker *et al*., (1982) for chickens. The AST and ALT values obtained under this study were within the range reported by Agbede *et al*., (2011) for broiler finishers fed graded level of fermented cassava tuber waste.

The blood alkaline phosphatase level of the birds ranged between 128.76ui/l in the 300ppm CuSO4 diet to 132.85ui/l in the control diet. An initial increase in blood alkaline phosphatase was observed in 100ppm and 200ppm $CuSO_4$ inclusion levels, followed by a decline at 300ppm CuSO4 inclusion level, also there was an inverse relationship between blood alkaline phosphatase and globulin concentration in birds fed $CuSO_4$ diets. The fact that values obtained for both alkaline phosphatase and globulin concentration did not differ significantly among treatment means showed that the utilization of $CuSO_4$ in the diets of broiler birds will have no detrimental effects on their physiological functions.

Table 4.6 showed the bacterial load of some selected organs (liver, spleen and duodenum) in broiler chickens fed diets containing graded levels of copper sulphate. It was observed that the bacterial load of liver, spleen and duodenum in birds fed diets 1, 2 and 3 decreased as the levels of inclusion of CuSO4 increases but got higher in those fed diet 4.

Conclusion

It was concluded that $CuSO_4$ incorporated into broiler finisher diet at 100ppm increased the body weight of the birds.

*Combined vitamin A(4,000,000iu); D(800,000iu); E(14,000iu); K(760mg); B12(7.6mg); Riboflavin(2800mg); Pyridoxine(1250mg); Thiamine(880mg); D Pantothenic acid(4400mg); Nicotinic acid(18,000mg); Folic acid(560mg); Biotin(45.2mg); and Trace elements such as Cu(3200mg); Mn (25600mg); Zn(16,000mg); Fe(12800mg); Se(64mg) and other items such as Co(160mg); Chlorine(19,000mg); Methionine(20,000); BHT(2,000mg) and Spiramicin (2,000mg) per 1.0kg.

Table 1. Gross composition of the two basal diets (%)

Ingredient	Diet I Starter)	Diet II (Finisher)
Maize	51.8	57.28
Soybean meal (42%)	15.00	11.00
Groundnut cake	16.00	14.00
Brewery dry grain	7.13	9.58
Fish meal (72%)	4.00	2.00
Bone meal	2.50	2.50
Oyster shell	1.0	1.0
Premix*	0.50	0.50
Methionine	0.15	0.17
Lysine	0.12	0.17
Salt	0.30	0.30
Vegetable oil	1.50	1.50
Total	**100**	**100**

Calculated chemical composition

Crude Protein (%)	23.20	20.21
M.E (Kcal/K)	2998.49	3019.63
Phosphorus (%)	0.62	0.56
Calcium (%)	1.56	1.46
Lysine (%)	1.17	1.02
Methionine (%)	0.53	0.50

KEY

M.E: Metabolisable Energy

Table 2. Growth performance (g/kg body weight) of broiler chickens fed different levels of copper sulphate at four (4) weeks old.

Age at 4 weeks	1 Control	DIETS 2 $CuSO_4$ 100ppm	3 $CuSO_4$ 200ppm	4 $CuSO_4$ 300ppm	±SEM
Initial weight (g/bird)	53.09	53.42	53.27	52.68	1.69
Final weight (g/bird)	933.33	896.67	916.67	880.00	32.79
Total weight (g/bird)	880.24	843.24	863.40	827.32	32.10
Total feed intake (g/bird)	986.4	950.09	969.94	932.68	33.54
Feed conversion ratio (FCR)	1.12	1.13	1.12	1.13	0.00
Daily weight gain(g/bird)	31.44	30.12	30.84	29.55	1.15
Daily feed intake (g/bird)	32.33	33.93	34.64	33.31	1.20

Table 3. Growth performance (g/body weight) of broiler chickens fed varying levels of copper sulphate at eight (8) weeks old.

Ages at 8 weeks	1 Control	2 $CuSO_4$ 100ppm	DIETS 3 $CuSO_4$ 200ppm	4 $CuSO_4$ 300ppm	±SEM
Initial weight (g/birds)	933.33	896.67	916.67	880.00	32.79
Final weight (g/birds)	2500.00	2529.63	2433.33	2444.44	68.38
Total weight (g/birds)	1566.67	1632.96	1516.67	1564.44	81.75
Feed conversion ratio (FCR)	3.12	3.10	3.21	3.13	0.09
Daily weight gain (g/birds)	55.95	58.32	54.17	55.87	2.91
Daily feed intake (g/birds)	178.57	180.67	173.81	174.60	4.88

Table 4. Haematological indices of Broilers chickens fed diet containing graded levels of copper sulphate ($CuSO_4$)

Parameters	1 Control	2 $CuSO_4$ 100ppm	DIETS 3 $CuSO_4$ 200ppm	4 $CuSO_4$ 300ppm	±SEM	SS
ESR (mm/hr)	2.33	2.17	2.33	2.67	0.58	NS
PCV (%)	30.00	30.17	31.17	28.00	2.49	NS
RBC($\times 10^6/mm^3$)	3.03	3.04	3.04	2.72	0.40	NS
Hb(g/100ml)	10.00	10.05	10.40	9.33	0.83	NS
MCHC (%)	33.34	33.29	33.37	33.1	0.09	NS
MCH(pg)	33.07	33.33	34.83	34.66	2.54	NS
MCV(μm^3)	99.21	99.55	104.37	103.96	7.55	NS
Lymphocytes(%)	68.17	62.67	66.17	65.83	4.50	NS
Neutrophil (%)	17.33	20.33	17.17	17.50	4.71	NS
Monocytes (%)	10.67	13.17	12.83	12.67	2.37	NS
Basophil (%)	3.00	2.50	2.17	2.67	0.70	NS
Eosinophil (%)	0.83	1.50	1.67	1.33	0.74	NS

ESR= Erythrocyte Sedimentation Rate, PCV= Packed Cell Volume, RBC= Red Blood Cell Count, Hb = Haemoglobin, MCHC= Mean Corpuscular Haemoglobin Concentration, MCV= Mean Corpuscular Volume, ± SEM= Statistical Error Mean, SS= Standard Significance, NS= Not Significant.

Table 5. Serum enzyme and metabolite of birds fed diets containing graded levels of copper sulphate ($CuSO_4$)

PARAMETERS	1 Control	2 $CuSO_4$ 100ppm	DIETS 3 $CuSO_4$ 200pm	4 $CuSO_4$ 300pm	±SEM	SS
Cholesterol (mg/dl)	40.12	25.09	30.30	29.63	17.17	NS
Albumin (g/dl)	8.91	12.71	11.46	11.68	4.47	NS
Total Protein (g/dl)	20.81	19.91	21.74	25.86	6.12	NS
Creatinine (mg/kg)	0.27	0.20	0.18	0.22	0.08	NS
Bilirubin (g/dl)	3.90	3.61	2.48	3.31	1.78	NS
AST (U/L)	55.50	37.83	45.17	45.33	24.77	NS
ALT (U/L)	18.00	10.83	12.50	24.33	13.42	NS
ALP (IU/L)	132.85	129.61	131.84	128.76	3.92	NS
Globulin (iu/ml)	11.91	7.20	10.28	12.42	5.07	NS

KEY

±SEM = Statistical Error Mean,
ALP = Alkaline Phosphate
SS = Standard Significance,
AST = Aspartate amino transaminase
NS = Not Significant,
ALT = Alanine Amino Transferase

Table 4.6. Bacteria load (X 10cfu/ml) of some selected organs of Broiler chickens fed diets containing graded levels of copper sulphate.

Parameter	1 Control	2 CuS04 100ppm	DIETS 3 CuS04 200ppm	4 CuS04 300ppm	± SEM
Liver	86.83	63.83	64.17	66.33	13.87
Spleen	69.00	66.83	47.00	72.83	23.66
Duodenum	113.17	110.50	111.00	110.67	11.62

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