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Effects of diet supplementation with copper sulphate on growth performance and heamatological parameters of broiler chickens

[1*]Unigwe, C.R., [2]Njoku, C. and [3]Orakwue, O.

[1]*Department of Veterinary Biochemistry and Animal Production, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria*
[2]*Federal College of Agriculture, Ishiagu, Ebonyi State, Nigeria*
[3]*Federal College of Animal Health and Production Technology, Ibadan, Oyo State, Nigeria*

***Corresponding Author:** robinsonunigwe@gmail.com **Phone Number:** +2348037707965*

***Target Audience**: Animal scientists, Poultry farmers, Researchers and Veterinarians.*

Abstract

Diets of ninety-six day-old Abor-acre chicks were supplemented with different levels of copper sulphate ($CuSO_4$) to assess the growth performance and haematological parameters. The birds were conventionally brooded for two weeks after which they got allotted to; T_1 (control), T_2 (100 mg $CuSO_4$ kg^{-1}), T_3 (200 mg $CuSO_4$ kg^{-1}), and T_4 (300 mg $CuSO_4$ kg^{-1} of diet), in a completely randomized design. Each treatment had three replicates (n = 8). The supplements were given for 49 days during which feed intake and weekly weight gain were recorded. At the 49^{th} day (63 day old), blood was aseptically collected via the wing vein using sterile syringe and needle for haematological studies. All data were subjected to analysis of variance and means separated using Duncan's New Multiple Range Test. The results showed that T_3 had superior ($p<0.05$) weight gain and best FCR with enhanced feed intake similar ($p>0.05$) to T_1 and T_2 but differed ($p<0.05$) from T_4 that had the worst growth performance. PCV, Hb and RBC of treated groups differed ($p<0.05$) from T_1 that recorded the least haematological values. The WBC and its differentials did not statistically differ ($p>0.05$) except eosinophils where T_3 spiked above others though statistically similar to T_1 and T_4 whereas T_2 was the least. It could be concluded that $CuSO_4$ supplementations at 100 and 200 mg kg^{-1} diets had beneficial effects on growth performance whereas there was no detrimental effect of $CuSO_4$ supplementations on all the haematological parameters of the broilers.

***Keywords**: Broiler chickens, Copper sulphate, growth, haematology*

Description of Problem

Food production in Nigeria has not kept pace with its population growth, because the population is growing at about 3.2% per annum while food production is at about 2.0% (1). The differences between the rate of food production and population growth has led to a food demand supply gap resulting in increased food importation and high rate of increase in food prices and ultimately wide spread hunger and malnutrition are evident in the country (2).

For many years, antibiotic growth promoters (AGP) have been incorporated into poultry diets because of their favorable effects on growth rate, and feed efficiency (3). However, there are increasing concerns that the frequent feeding of AGP is leading to the rise of antibiotic resistance in many pathogenic and opportunistic bacteria isolated from production animals as well as from humans (4). With the growing public concern of bacterial resistance to traditional antibiotics, poultry production industries need alternatives to orthodox antibiotics that could maintain intestinal health, have antimicrobial properties, and allow for optimal growth. Copper (Cu) has received great attention because of its antimicrobial properties that improve animal growth performance when fed over the minimum requirement (5; 6).

(7) recommended 8 mg Cu/kg of broiler diet as the minimum requirement and it has

been reported that its sulphate form ($CuSO_4$) is more effective than other forms (8). Copper sulphate is the main Cu source in the diet of chickens and other animals; however, the inorganic salt shows poor bioavailability caused by the presence of ingredients that can inhibit absorption (9). However, the feed industry still prefers $CuSO_4$ for economic reasons. Cu is a key element required for animal growth and development of bones, connective tissue, the heart and several other organs (10). An excess of Cu may also have adverse effects on chicken performance (11). Others (11; 12) have also shown that supplemental copper has little or no effect on growth performance and feed utilization of boiler chickens. Copper is usually fed commercially at much higher pharmacological levels (100 to 300 mg/kg) because of its growth promoting properties, which is caused by its antibacterial properties (13).

Copper is needed for the development of antibodies and white blood cells, in addition to antioxidant enzyme production (14). Copper is also needed by animals to prevent microcytic hypochromic anaemia, thus, emphasizing its role in iron metabolism (15). In particular, feeding $CuSO_4$ at 200 mg/kg in white leghorn hens has shown a positive response, while levels of 400 mg/kg and above showed a progressively negative response (8). According to (16) broiler performance and intestinal physiology can be positively influenced by dietary Cu level (188 mg of Cu/kg of diet from $CuSO_4$ vs. no supplemental copper).

Many nutritionists and feed manufacturers are concerned about the contradictory reports on the growth promoting effect of different sources of supplemental copper. Therefore, the present study was carried out to evaluate the effects of supplemental $CuSO_4$ on the growth performance and haematology of broiler chickens.

Materials and Methods

Site of the study and ethical consideration

The experiment was carried out at the Federal College of Animal Health and Production Technology, Moor Plantation, Ibadan, Oyo State, Nigeria. Ibadan is located approximately on longitude 3° 5' to 4° 36' E and latitude 7° 23' to 7° 55' N (17). Ibadan has a tropical wet and dry climate, with a lengthy wet season. It has mean total rainfall of 9,233.60 mm, mean maximum and minimum temperatures of 39.82 °C and 22.5 °C respectively (18) and relative humidity of 74.55%. Ethical conditions governing the conduct of experiments with live animals were strictly observed as stipulated by (19). The experimental protocol was approved by the institutions ethical committee for the use of animals for experiment.

Animals, feed, test ingredient, design and duration

Ninety-six (96) day-old Abor Acre broiler chicks were purchased from CHIMERO FARMS in Ibadan, for the experiment. Commercial Starter Top Feed® (3,200 ME/Kcal/Kg, CP = 22%), Finisher Top Feed® (2800 ME/Kcal/Kg, CP = 18%) and copper sulphate were also purchased from open market and used for the experiment. The treatments included T_1 (control), T_2 (100 mg $CuSO_4$/kg of diet), T_3 (200 mg $CuSO_4$/kg of diet) and T_4 (300 mg $CuSO_4$/kg of diet). Each treatment was replicated into three with 8 birds each. Birds were allotted to treatments using completely randomized design. The experiment lasted for 49 days.

Management of the birds

On arrival, the day-old-chicks were provided with clean cool water mixed with multivitamins (Vitalyte®) and glucose to serve as anti-stress. Conventional brooding commenced during which they were served Starter plain feed (no $CuSO_4$) and clean water *ad libitum*. Routine vaccinations (Newcastle disease [intra-ocular and lasota] and Infectious bursal disease vaccines) as prescribed by the Veterinarian were strictly followed. After two weeks of acclimatization, they were weighed individually with sensitive scale (CAMRY, Model: EK5055, Max. 5kg, Min. 1g) and recorded and allotted to treatments and replicates as stated above. The Starter treatment diets (with $CuSO_4$) commenced for another 2 weeks after which Finisher treatment diets (with $CuSO_4$) were used for another 5 weeks. Therefore, they were on treatment diets for 49 days. The treatment diet and clean water *ad libitum* continued till the end of the study (at 63-day old). Standard management practices were followed throughout the experimental period.

Data collection

Feed intake and weekly weight gain were got by weigh-back mechanism. At the end of the study, blood was aspirated aseptically via the jugular vein into EDTA sample bottle for haematological analysis as described by (20).

Statistical analysis

All data obtained were subjected to analysis of variance (ANOVA) using a Statistical Package for Social Sciences *(*SPSS*)* version 20.0. Significantly different means were separated using Duncan's New Multiple Range Test (DNMRT) as described by (21).

Table 1: Growth performance of broilers fed diets supplemented with copper sulphate

Parameters	$T_{1\ (control)}$	$T_{2\ (100mg)}$	$T_{3\ (200mg)}$	$T_{4\ (300mg)}$	±SEM
Initial weight (g)	233.33	231.20	232.40	232.77	0.67
Final weight (g)	2138.75^{b}	2222.58^{ab}	2309.17^{a}	1875.10^{c}	52.30
Total weight gain (g)	1905.42^{b}	1991.38^{ab}	2076.77^{a}	1642.33^{c}	49.53
Daily weight gain (g)	38.89^{b}	40.64^{ab}	42.38^{a}	33.52^{c}	0.86
Feed intake (g)	5344.91^{a}	5533.83^{a}	5476.13^{a}	$4675{\cdot}07^{b}$	165.63
Daily feed intake (g)	109.08^{a}	112.94^{a}	111.76^{a}	95.41^{b}	2.25
Feed conversion ratio	2.81	2.78	2.64	2.85	0.05

^{abc}Means with different superscripts in the same row are significantly different ($p<0.05$)

Results

Growth performance and haematological parameters

Table 1 shows the growth performance of broilers fed diets supplemented with copper sulphate. There were significant effects ($p<0.05$) of $CuSO_4$ on growth performance indices (Final weight [FW], Daily weight gain [DWG], and Feed intake [FI]) except feed conversion ratio (FCR) of the chickens. The DWG of T_3 was superior and significantly different ($p<0.05$) from T_1 and T_4 but similar to T_2. However, T_1 and T_2 equally outperformed ($p<0.05$) T_4. With respect to FI, though T_2 consumed more than others, it did not statistically differ ($p>0.05$) from T_1 and T_3 but T_4 ($p<0.05$). However, though there was no significant ($p>0.05$) effect of $CuSO_4$ on FCR of the chickens, numerically, T_3 was the most efficient in converting feed to meat whereas T_4 was the least.

Table 2 shows the haematological parameters of broilers fed diets supplemented with copper sulphate. Copper sulphate supplementation improved PCV, Hb and RBC significantly ($p<0.05$) when compared with the control group. There were no statistical differences ($p>0.05$) among the $CuSO_4$ treated groups in this regard. The WBC and the differentials had no significant difference (p>0.05) among the treatments except for eosinophils that had T_3 as the highest and statistically differed (p<0.05) from other groups.

Table 2: Heamatological parameters of broilers fed diets supplemented with copper sulphate

Parameters	T_1	T_2	T_3	T_4	±SEM
PCV (%)	22.67^b	26.67^a	24.33^{ab}	26.00^a	0.56
Hb (g/dL)	7.23^b	8.63^a	8.27^a	8.67^a	0.19
RBC ($x10^3/mm^3$)	1.84^b	2.87^a	2.11^{ab}	2.76^a	0.16
WBC ($x10^3/mm^3$)	12.50	13.00	14.45	14.18	3.67
Platelet ($x10^5/mm^3$)	967.00	1750.33	2010.00	1084.00	213.95
Lympho ($x10^3/mm^3$)	67.67	63.67	62.00	67.67	1.54
Hetero ($x10^3/mm^3$)	26.33	33.33	31.33	27.00	1.41
Mono ($x10^3/mm^3$)	2.67	2.67	3.33	3.67	0.34
Eosino ($x10^3/mm^3$)	2.67^{ab}	2.00^b	4.00^a	2.67^{ab}	0.32
Basophil ($x10^3/mm^3$)	0.33	0.67	0.33	0.33	0.15

^{ab}Means with different superscripts in the same row are significantly different (p<0.05)

Legend: Lympho = lymphocyte, Hetero = heterocyte, Mono = monocyte, Eosino = eosinophil

Discussion

Different sources such as Cu chloride, Cu oxide, Cu citrate, Cu sulphate and tribasic Cu chloride at different concentrations have been applied in poultry feed (22). However, the feed industry still prefers $CuSO_4$ for economic reasons. Cu is a key element required for animal growth and development of bones, connective tissue, the heart and several other organs (10). There was gradual improvement in growth indices as the $CuSO_4$ supplementation increased from 100 to 200 mg kg^{-1} diet, but not with higher level (300 mg kg^{-1} of diet). This result is in conformity with the reports of some authors (9; 23; 24; 25; 37) but contradicted the result of (26) probably because copper oxide nanoparticles (CuO-NPs) was used in lieu of $CuSO_4$. (27) reported that Cu improved nitrogen retention whereas (9; 28) stated that the mechanism is by stimulating hormone and growth factors as well as increasing the availability of amino acids for absorption in the intestines. (7) recommended 8 mg/kg of Cu as the minimum requirement for broiler diets and it has been reported that its sulphate form is more effective than other forms (8). Copper (Cu) has antimicrobial properties that improve animal growth performance and feed efficiency in broilers when provided at much higher pharmacological levels (5; 6; 29). One reason for this is that Cu plays an antioxidant role in the system of animals (11; 30) and has antibacterial properties (13; 31). In particular, feeding $CuSO_4$ at 200 mg/kg in white leghorn hens has shown a positive response, while levels of 400 mg/kg and above showed a progressively negative response (8). Addition of $CuSO_4$ could improve intestinal mucosal morphology, which may contribute in improving nutrient availability and is associated with increasing goblet cell numbers, total goblet cell area, goblet cell mean size, mucosal thickness and a greater number of segmented filamentous bacteria compared to control (32). (33) demonstrated that Cu might be involved in pituitary growth hormone gene expression and secretion of several neuro-peptides in the hypothalamus. Supplementation of $CuSO_4$ to pig diets reduces the number of ureolytic bacteria as a group, of which *Streptococci* make up approximately 75%, inhibits the coliforms in the caecum and the colon, and reduces the number of *Streptococci* in fecal samples (22). The present results agree with the findings of (34), (35), (36) and (31) that reported that copper supplementation at the levels of 125 to 250 ppm improved growth and feed efficiency in broilers. However, contrary to the results of the present work, some authors (11; 32; 38; 39) have reported no positive effect of dietary copper

41; 42) reported that body weight gain were reduced significantly by the supplementation of Cu at 500 mg kg^{-1} in the diet of chicken and layer pullets. (43) demonstrated that supranormal level of Cu gave different responses in different species. (44), (45) and (46) reported that Cu has antimicrobial actions thus has growth stimulating action, promotes hypothalamic appetite-regulating genes (47) and acts as a catalyst in enzyme systems within cells (48). It has also been demonstrated that intravenous injection of Cu stimulates growth of weanling pigs (33). Deficiency of Cu will certainly affect chicken growth, while an excess of Cu is not recommended because either it will be excreted or have an adverse effect on performance (9). Statistically, similar FCR in the present study agrees with the results of some authors (39; 49; 50) but contradicts those of others (51; 52). With 300 mg kg^{-1} diet, the body weight gain and feed intake significantly decreased as well as higher FCR which is in tandem with the report of (53).

In supplemented birds, Cu plays a major role as cofactor in haematogenesis. Cu is one of the most critical trace elements in livestock because it is necessary for haemoglobin formation, iron absorption from GI-tract and iron mobilization from tissue stores (54). The birds in the present study did not show any haematological sign of excessiveness of Cu supplementation since even at 300 mg kg^{-1} diet, the PCV, Hb and RBC increased beyond the control group. This is in consonance with the earlier reports of (24; 25; 55) where significant ($p < 0.05$) enhancement of PCV, Hb, and RBC counts were recorded in the $CuSO_4$ group. Excess of dietary copper results in an accumulation of copper in the liver with decreased Hb concentration and packed cell volumes of blood (56). (57) reported similar finding in Wister albino rats. It can be inferred from the present study that Cu was not in excess in the diets with respect to heamatological parameters. Additionally,

of erythrocytes in the peripheral blood of the birds (37). The concentration of haemoglobin was increased, which allowed the birds to keep oxygen transport at an appropriate level (37; 58). The increased level of haemoglobin could be due to its continued synthesis by erythrocytes already circulating in the peripheral blood. It is also likely that the increased level of haemoglobin could be linked with the homeopathic function of copper. Cu has been shown to directly stimulate erythrocyte synthesis, as it determines iron absorption into the body and its incorporation in haemoglobin (59). With respect to WBC count, the marginal increase could be associated with the dietary $CuSO_4$ corroborating the findings of (27; 58) where there were increased counts of WBC after *in-ovo* injection of $CuSO_4$. Monocyte count was enhanced in the present study which contradicted the results of (37) who reported reduced percentage of monocytes probably because they used heat stressed birds in their study. With the numerical enhancement of monocytes, it can be suggested that phagocytic and metabolic activities could be accelerated thereby conferring further protection upon the birds due to $CuSO_4$ supplementation.

Conclusion and Applications

1. The results obtained in the present study revealed that supplementation of $CuSO_4$ at 100 and 200 mg kg^{-1} of diets numerically increased feed intake and significantly enhanced growth performance of broiler chickens.
2. Contrary to the above, at a higher rate of 300 mg kg^{-1} of diet, the performance

indices and feed intake declined significantly.

3. With respect to haematological parameters, even at 300 mg kg^{-1} of diet, there was no deleterious effect on the parameters measured but rather significantly improved haemopoietic parameters of the birds

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copper sulphate supplementation at the rates of 100 to 200 mg kg^{-1} of diet improved the growth performance of broiler chickens and that the haematological parameters measured were enhanced even at 300 mg kg^{-1} diet supplementation. Therefore, the use of $CuSO_4$ at 100 - 200 mg kg^{-1} of diet is advocated.

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