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NANO COPPER VERSUS COPPER SULPHATE SUPPLEMENTATION IN BROILER DIETS – EFFECT ON GROWTH RESPONSE, CARCASS TRAITS AND COPPER RETENTION

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Abstract

A 42-day trial was conducted with 144 (arbor acre) day-old broiler chicks to evaluate the growth response and carcass yield of broiler chickens to dietary supplementation of copper sulphate and nano copper and consequently copper retention. Birds were divided into four groups and assigned to the dietary treatments; 250 ppm $CuSO_4$, 225 ppm Cu-NP, 275 ppm Cu-NP supplementation and control (antibiotics). Data collected were subjected to one - way Analysis of Variance. Results indicated that birds fed dietary nano copper had similar ($P < 0.05$) growth and dress yield [%] compared to other groups. Supplemented birds at higher dosages (250 mg/kg and 275 mg/kg of $CuSO_4$ and Cu-NP, respectively) exhibit high faecal copper excretion and retention in liver compared to the control group. The study therefore concludes that copper residue in faeces and liver increased with dietary copper supplementation, however, nano copper is better available to the birds than $CuSO_4$. Hence, supplementation of 225 ppm of nano copper is recommended for less liver copper load and excretion.

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Introduction

For over a decade now, the edict in European nations on the use of antibiotics in livestock production arising from several challenges associated with its use both in animals and human as final consumers continuously increased researches on alternatives to it. Several studies (OBERDORSTER et al. 2005, RICHARDS et al. 2010) have evaluated the use of trace element especially copper as an effective growth promoter in poultry with encouraging results.

RICHARDS et al. (2010) and SAMANTA et al. (2011) reported that copper is efficient in promoting growth and health as well as reducing meat cholesterol in poultry. Various studies have recommended copper inclusion at high levels (CHORI and PARK 1989, BAKER et al. 1991, SAMANTA et al. 2011) while some other authors obtained excellent result with lower dosages (GONZALES-EGUIA et al. 2009, LEESON 2009, ZHAO et al. 2010, KARIMI et al. 2011). These conflicting results necessitate more studies to have the knowledge of the best level(s) that would yield optimum growth with less adverse effect on the health of the animals. Copper (Cu) is mostly supplied in the diet of broiler chickens in form of copper sulphate (RICHARDS et al. 2010, SCOTT et al. 2018), however there have been reports that an average of 250 mg/kg recommended (CHORI and PARK 1989, BAKER et al. 1991) are not readily absorbed and are thus excreted causing environmental pollution while some are retained in the muscle and organs of the birds (GONZALES-EGUIA et al. 2009, LEESON 2009, ZHAO et al. 2010, KARIMI et al. 2011). Hence, the reason for increased investigation on the availability and absorption of its nano particles in poultry which have vast socio-economic and environmental benefits (*National Nanotechnology Initiative* 2011). According to HILL and LI (2017), the choice of nano copper (Cu-NP) as an alternative growth and health promoter in animal feed is to reduce the population of harmful bacteria and enhance the growth of beneficial bacteria for improved animal performance. The catalytic or biological roles of nano minerals are greatly dependent on their particle size (DICKSON and LYON 2000, LEWIS and KLOIBANOV 2005).

Nano materials are easily absorbed in the small intestine and further distributed into the blood, brain, lung, heart, kidney, spleen, liver, intestine, and stomach (HILLYER et al. 2001). Hence, it becomes imperative not to focus only on growth response of the birds to dietary copper inclusion ($CuSO_4$ or Cu-NP) but also investigate the residue in these organs at the various levels of dietary inclusion to deduce the possible influence on their integrity. This study therefore focused on the comparison between these two forms of copper (CuSO4 and Cu-NP) as regards their influence on the growth, carcass yield and their retention in organs of broiler chickens.

Materials and Methods

Site of study

The study was carried out at the poultry facility of A-cube Farms, Apakila, Camp, Abeokuta, Ogun State, Nigeria. It is located in climate with the derived savannah zone of South-Western Nigeria. It has a humid climate with mean annual rainfall of 1037 mm and temperature of about 34.7°C.

Source of test materials

Management of birds and experimental design

Nanocopper (Cu-NP) and copper sulphate ($CuSO_4$) were procured from Acrochem store in Lagos, Nigeria.

One hundred and forty-four (144) day old arbor acre strain of broiler chicks were sourced from a reputable hatchery in Ibadan, Nigeria. The pen and all necessary equipment were thoroughly washed and disinfected prior to the arrival of the birds. On arrival, birds were divided into four groups of 36 birds each. Each group was assigned to one of the four dietary treatments as follows; 250 ppm $CuSO_{4,}$ 225 ppm Cu-NP, 275 ppm Cu-NP supplementation and control (without dietary copper supplementation but antibiotics). The groups were replicated three times (housed in separate pens) to consist 12 birds each. Brooding was carried out for two weeks. Feed and water were provided *ad libitum* and birds were raised on deep litter for 6 weeks. Commercial broiler starter (22% CP and 12.72 MJ/kg) and finisher (20% CP and 11.72 MJ/kg) diets were supplemented with the various copper types and levels, mixed evenly and fed to the birds at the starter (0–4 weeks) and finisher (4–6 weeks) phases, respectively (Table 1). All necessary vaccinations and medications were done except that the birds on dietary copper supplementation were not administered antibiotics.

Table 1

Nutrient composition of commercial feed

Parameter	Starter diet	Finisher diet
Crude protein [%]	22.00	20.00
Fat [%]	6.00	6.00
Crude fiber [5%]	5.00	5.00
Calcium [%]	1.00	1.00
Available phosphorus [%]	0.45	0.40
Lysine [%]	1.00	0.85
Methionine [%]	0.50	0.35
Salt [%]	0.30	0.30
Metabolizable energy [MJ/kg]	12.72	11.72

Data collection

Growth performance evaluation

Feed intake. The amount of feed given to the birds and the leftover were measured weekly to determine the feed intake.

Feed intake [kg] = feed given [kg] - feed leftover [kg]

Body weight and weight gain. The birds were weighed on replicate basis at the commencement of the experiment and subsequently on weekly basis.

Body weight [g] = total weight of birds [g] / total no of birds

Weight gain [g] = final weight [g] - initial weight [g]

Average daily weight gain. Average daily weight gain = (final weight - initial weight) / number of birds.

Feed conversation ratio. This is the proportion of feed that was converted into flesh in the birds. It was calculated as daily feed intake divided by weight gain.

FCR = total feed intake [g] / total weight gain [g]

Mortality rate. This is the measure of the number of deaths recorded in birds. It was calculated as the total number of dead birds divided by the total number of birds and expressed in percentage.

Mortality [%] = (total number of dead birds / total number of birds) · 100

Carcass yield evaluation

At the end of the 6^{th} week, two (2) birds with body weight equal to average of the birds in each replicate were selected to evaluate carcass traits. Feed was removed 4 hours before slaughtering. Each bird was slaughtered via the jugular vein and allowed to bleed for 2 minutes followed by scalding at 60°C, and then removal of feathers.

The head and shank were removed and weighed. The visceral were removed and the dressed weight was determined. The weight of the cut parts (thigh, breast, neck, back, wings, and drumstick), organs (kidney, lungs, liver, and gizzards), offal (intestine) and the abdominal fat were determined and expressed as a percentage of the live weight according to ODUNSI et al. (1999).

Samples collection

Blood collection

At the 42^{nd} day of the experiment, two birds were randomly selected from each replicate for bleeding with a 5 ml syringe fitted with a 24-gauge sterile hypodermic needle, 3 ml of blood was carefully drawn from the left wing at the point of bifurcation of the vein and put into a sterilized plain bottle for copper content analysis in the laboratory.

Muscle and organs collection

At the 42^{nd} day of the experiment, two (2) birds were randomly selected from each replicate, fasted overnight, culled and sacrificed. The birds were opened and meat sample was taken from the breast muscle. Manual evisceration was done carefully to avoid rupturing of the organs and the liver, spleen and heart were removed for copper content analysis in the laboratory.

Faecal collection

Two birds per replicate were chosen at random, housed in a different pen and their droppings were collected into a plain bottle. The collected droppings were taken to the laboratory for assessment of the copper content.

Sample analysis

The meat, organs and faecal samples collected were dried in an oven at 65°C for 36 hours to remove moistures. Then 2 grams of each dried sample was put in a flask and digested with a mixture of HNO_3 and H_2SO_4 (3:1 v/v). The digestion process was continued until the solution became clearer. The samples were transferred into another flask and diluted to 25 ml with distilled water. Copper residue was estimated by Atomic Absorption Spectrophotometer. Copper residue in blood was also estimated using the Atomic Absorption Spectrophotometer.

Analysis of data

Data collected were subjected to one-way ANOVA in a Completely Randomized Design using SPSS version 23. Significant difference among means was separated at 5% level of significance using Tukey's test.

Results

Effect of dietary copper supplementation on the growth performance of broiler chickens

Effect of dietary copper supplementation on the growth performance of broiler chickens is presented in Table 2. All parameters measured were similar ($P > 0.05$) across the dietary treatments. Final weight across the treatments were 2096.33, 2060.00, 1996.33 and 2100.33 g for control,

Table 2

Effect of copper supplementation on the performance of broiler chickens

Parameters [g]	Copper supplementation					
	antibiotics	$CuSo_4$ (250 ppm)	Cu-NP (225 ppm)	Cu-NP (275 ppm)	SEM	*P*-Value
Initial weight g]	38.72	39.06	38.91	39.00	0.07	0.81
Final weight [g]	2096.33	2060.00	1996.33	2100.33	24.08	0.91
Weight gain [g]	2057	2020.94	1957.41	2061.33	24.02	0.91
Daily weight [g]	48.99	48.11	46.61	49.08	0.57	0.91
Total feed [g]	6056.33	6286.33	5968.00	5933.33	79.45	0.78
Daily feed [g]	144.19	149.68	142.09	141.27	1.89	0.75
FCR	2.95	3.10	3.08	2.87	0.05	0.75
Mortality [%]	0.33	0.67	0.00	0.00	0.16	0.68

250 ppm $CuSO_4$, 225 ppm Cu-NP and 275 ppm Cu-NP, respectively. Feed intake per day ranged from 141.27 g (control) to 149.68g (250 ppm $CuSO_4$). Feed conversion ratio ranged from 2.87 to 3.10 across the treatments.

Effect of dietary copper supplementation on carcass characteristics of broiler chickens

Effect of dietary copper supplementation on the carcass characteristics of broiler chickens is presented in Table 3. All parameters measured for the effect of dietary copper supplementation did not vary significantly ($P > 0.05$) except for back, spleen and intestine weight that were statistically ($P < 0.05$) differed. Back and intestine weight (13.00% and 5.05% respectively) were least in birds given antibiotics and was increased in birds fed diet containing 275 ppm/kg of Cu-NP (16.08% and 6.35%, respectively). Spleen weight of birds fed diet containing 225 ppm/kg of Cu-NP and 275 ppm/kg of Cu-NP (0.09 and 0.11) were the highest across the treatment.

Table 3

Effect of dietary copper supplementation on the carcass characteristics of broiler chickens

Copper supplementation						
Parameters	control	$CuSO_4$ (250 ppm)	Cu-NP (225 ppm)	Cu-NP (275 ppm)	SEM	*P*-value
Live weight [g]	2266.67	2133.00	2032.67	2187.00	49.11	0.25
*Dressed weight [%]	59.15	63.95	65.84	62.50	1.41	0.23
***Cut parts [%]**						
Head	2.34	2.32	2.22	2.34	0.03	0.94
Neck	3.21	3.71	3.63	3.20	0.14	0.58
Breast	22.13	21.85	24.39	20.55	0.80	0.28
Back	13.07^{c}	15.01^{bc}	13.77^{ab}	16.08^{a}	0.67	0.03
Wings	6.56	7.54	7.35	6.75	0.23	0.38
Thigh	9.51	10.33	10.04	9.42	0.22	0.80
Drumstick	8.17	9.03	9.57	9.77	0.36	0.64
Shank	3.13	3.53	3.54	3.60	0.11	0.68
***Internal organs [%]**						
Liver	1.91	1.65	2.25	1.79	0.13	0.25
Lungs	0.51	0.62	0.58	0.56	0.02	0.49
Spleen	0.04^{b}	0.06^{b}	0.09^{a}	0.11^{a}	0.02	0.01
Empty gizzard	1.74	1.80	1.73	0.02	1.77	0.98
Intestine	5.05^{b}	5.73^{ab}	5.92^{ab}	6.35^{a}	0.27	0.01
Heart	0.39	0.57	0.52	0.49	0.04	0.62

$^{a–c}$ Means with different superscripts across the rows differs significantly ($P < 0.05$)

* All cut parts, organs and dressed weight were expressed as a percent of live weight

Effects of dietary copper supplementation on copper retention in the blood, meat, organs and droppings of broiler chickens

The effect of dietary copper supplementation on copper retention in blood, meat, organs and droppings of broiler chickens is presented in Table 4. Dietary copper supplementation had significant ($P < 0.05$) effect on the copper retention in faeces, meat and liver, however, no significant ($P > 0.05$) variations were noted in heart, spleen and blood.

Table 4

Effects of dietary copper on copper retention in blood, muscle, organs and droppings of broiler chickens

Copper sources						
Parameters [mg/kg]	control	$CuSO_4$ (250 ppm)	Cu-NP (225 ppm)	Cu-NP (275 ppm)	SEM	P-value
Faeces	202.6^{b}	268.9^{a}	189.5^{b}	290.5^{a}	24.69	0.00
Muscle	20.7^{b}	30.1^{ab}	33.4^{a}	23.8^{ab}	2.89	0.04
Heart	7.6	8.5	6.7	8.8	0.47	0.15
Liver	50.3^{b}	62.6^{a}	58.8^{ab}	67.9^{a}	3.70	0.04
Spleen	3.3	3.9	3.7	3.6	0.13	0.47
Blood	0.5	0.4	0.4	0.4	0.03	0.76

$^{a–b}$Means with different superscripts across the rows differs significantly ($P < 0.05$)

Faecal copper content was similar in birds supplemented with 250 ppm $CuSO_4$ (268.9 mg/kg) and 275 ppm Cu-NP (290.5 mg/kg) but significantly ($P < 0.05$) higher than values obtained in other groups. Comparable higher ($p < 0.05$) muscle copper content was recorded in birds fed 225 ppm Cu-NP (33.4 mg/kg), 250 ppm $CuSO_4$ and 275 ppm Cu-NP while the least was observed in birds fed the control diet (21.8 mg/kg).

The mean copper concentration in the liver was similar in birds supplemented with 250 ppm $CuSO_4$ (62.6 mg/kg) and 275 ppm Cu-NP (67.9 mg/kg) but significantly ($P < 0.05$) higher than values obtained in the control groups. However, birds supplemented with 225 ppm Cu-NP (58.8 mg/kg) had comparable values with other groups.

Discussion

In this study, similar growth performance indices among the treatment groups agrees with the report by SARVESTANI et al. (2016) stating that poultry feed supplemented with nano copper (Cu-NP) had comparable growth performance to other groups. REFAIE et al. (2015) also observed similar effect of Cu-NP on the performance of rabbits. Contrarily, PESTI and BAKALLI (1996) indicated that supplementation of either 125 mg/kg or 250 mg/kg of Cu as copper sulphate improved growth and the feed efficiency, but no further growth promoting effect was elicited at higher levels (375 mg/kg) for broiler chickens. However, numerical better performance (highest final weight, weight gain, least feed intake and best FCR) noted in birds supplemented with 275 ppm Cu-NP indicates its potential to improve broiler growth. PRESCOTT et al. (1993) reported that using Cu-NP as a supplement in chicken diet might have beneficial effects on growth, feed efficiency and chicken health by causing damage to pathogens, with a resultant reduction in the production of bacterial toxins, increased synthesis of vitamins and other growth factors, and improved the absorption of nutrients. Similar effect of Cu-NP (275 ppm) could also be as a result of short duration of the study (6 weeks).

Similar feed intake among the birds on various treatment in this study is in consonance with the result reported by HUANG et al. (2015) using weanless piglets. HUANG et al. (2015) stated that average daily gain and feed intake were not affected by concentration of Cu, however, CROMWELL et al. (1998) reported that Cu supplementation at 100 to 250 mg/kg diet increases growth and feed intake in swine. In a related study, SKRIVAN et al. (2002) reported that broilers fed on diets containing greater concentrations of copper had reduced feed intake. Though not significant, no occurrence of mortality among birds supplemented with Cu-NP is an indication that Cu-NP has the potential to support survivability of broiler chickens.

Similar carcass trait among birds supplemented with Cu-NP and $CuSO_4$ corroborated the report of AKINSANMI and IGBASAN (2011) stating that similar carcass trait was observed among broiler chickens fed diet supplemented with copper from different sources ($CuSO_{4,}$ CuO and Copper acetate).

Excess copper in animal diet could result in low digestibility and absorption in poultry and cause more copper to be excreted in the faeces as reported by GONZALES-EGUIA et al. (2009), LEESON (2009), ZHAO et al. (2010) and KARIMI et al. (2011). DOZIER et al. (2003) asserts that a minimum of 95% of pharmacological levels of Cu are excreted as against 85% at the normal levels.

This is in tandem with the high level of copper excreted by the birds in this study. Feeding 240 ppm rather than 9 ppm Cu increased the excreta content from 25 to 400 ppm Cu (SKRIVAN et al. 2006). The report of this study agrees as posited by the authors as there was increase in faecal copper with increase in dietary copper level irrespective of the source. However, similar faecal copper content observed in this study; Cu-NP

(275 ppm) vs $CuSO_4$ (250 ppm) despite the higher dosage of the former indicates that nano copper is better absorbed in the body of broiler chickens than $CuSO_4$.

Low copper residue found in the liver of the control birds reflects that no additional copper ($CuSO_4$ or Cu-NP) was included in their diet. Higher residues noted in other copper supplemented groups is in harmony with the report of UNDERWOOD and SUTTLE (1999) who posited that the main target organ for copper is the liver and its concentration in the liver is based on dietary intake. KARIMI et al. (2011) also reported that high level inclusion in diets increases its concentration because it is readily retained and form residues in the liver. This result is also consistent with the report of GUO et al. (2001) and JEGEDE et al. (2011). Similar concentration in liver of birds supplemented with 250 ppm $CuSO_4$ and 275 ppm nanocopper is an indication that nanoparticles are better absorbed in birds than their salts.

High doses of dietary supplementation of copper lead to copper toxicity in the body because when the liver reaches its storage limit, it releases copper which is then accumulated in different organs. Non-significance in the copper residue in the heart and spleen depicts that the sources as well as levels of dietary copper adopted in this study did not reach threshold levels in the liver. Variation in copper content in meat contradicts the report of HAMDI et al. (2018) that copper content of the breast muscle increases with increase in dietary copper level. JEGEDE et al. (2011) reported that supplementation levels of copper did not vary the copper content in blood, heart, lung, thigh, breast and bone except the liver. MROCZEK et al. (2014) reported that copper was highest in liver and the lowest in breast muscle which confirms the result in this study as copper content in meat is lower than in liver. OGNIK et al. (2019) reported that increase in the addition of Cu, by 54 or 96% in relation to the amount recommended in NRC (1994), did not cause a linear increase in the content of this element in the liver or breast muscle. The differences in residue in meat could neither be adduced to copper types (copper sulphate and nanocopper) nor inclusion levels as no definite trend was observed, hence could not be explained. UNDERWOOD and SUTTLE (1999) reported that copper dietary intake influences its concentration in the liver and kidney but no relationship was found in muscles.

Conclusion

The use of copper sulphate and varying levels of nanocopper in this study yielded similar effect on the growth performance and dressing percentage of broiler chickens though administration of nanocopper at the rate of 275 ppm demonstrated a slightly promising result in terms of weight gain, feed conversion ratio and survivability. It was also deduced from this study that the liver is a major organ for copper retention and increased with increasing levels of supplementation regardless of the copper type. Copper excretion was also relatively better in birds supplemented with dietary nanocopper. Hence, nanocopper can be adopted as a viable alternative growth promoter without posing environmental challenges arising from excretion.

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