

# Comparative antioxidative and hypocholesteremic properties of copper sulphate and copper nanoparticles supplemented in diet of broiler chickens

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Submission: 11 August 2021

Revised: 11 January 2022

Accepted: 8 February 2022

## ABSTRACT

One hundred and forty-four, one-day-old broiler (Arbor Acres strain) chicks were used in a 49-day trial to compare the antioxidative and hypocholesteremic properties of dietary supplemented copper sulphate (CuSO<sub>4</sub>) and copper nanoparticles (Cu-NP). The birds were divided into four groups of three replicates containing 12 birds each and randomly assigned to copper supplementation groups which were the control group (control diet), and the diet supplemented with 250 ppm CuSO<sub>4</sub>, 225 ppm Cu-NP and 275 ppm Cu-NP. Data collected were subjected to a one-way analysis of variance. The results showed that the oxidative stress biomarkers were significantly influenced by copper supplementation except for the glutathione. Albumin level was lowered by copper supplementation with the least values noted in birds supplemented 250 ppm CuSO<sub>4</sub> and 275 ppm Cu-NP. Uric acid was also reduced markedly in birds supplemented with either CuSO<sub>4</sub> or Cu-NP. Total bilirubin increased significantly ( $P < 0.05$ ) in birds supplemented 225 ppm Cu-NP (0.700 mg/dL) with statistically similar ( $P > 0.05$ ) values recorded among other groups. Dietary supplementation of 275 ppm Cu-NP (0.73U/L) increased ( $P < 0.05$ ) blood level of superoxide dismutase (SOD). Supplementation of CuSO<sub>4</sub> increased glutathione peroxidase (GSH-Px). Low-density lipoprotein (LDL) was reduced in the blood of copper supplemented groups, however, only 225 ppm supplementation reduced meat LDL significantly. The study concluded that supplementation of Cu-NP and CuSO<sub>4</sub> can help to reduce oxidative stress in broiler chickens. Also, copper supplementation in excess of the requirement reduced LDL level in the blood of broiler chickens. However, nanocopper at the rate of 225 ppm further reduced LDL concentration in the broiler meat.

**Keywords:** Cholesterol, oxidative stress, nanocopper, copper sulphate, antibiotics, broiler chickens

Thai J. Agric. Sci. (2021) Vol. 54(4): 272–279

## INTRODUCTION

Copper (Cu) is an essential micronutrient required for the healthy growth, development, and functioning of living organisms (Kozłowski *et al.*, 2018). It is needed for the development of antibodies and white blood cells, in addition to antioxidant enzyme production (Sharma *et al.*, 2005). According to the NRC (1994), the demand for copper in growing chickens is 4 mg/kg of feed mixture, 8 mg/kg in broiler chickens, and 2.5 mg/kg in laying hens.

Leeson (2009) reported that a 20-fold increase in the amount of copper supplied with respect to requirements can have a health-promoting effect, and only an amount 100 times greater than the requirement for this element may be toxic.

Samanta *et al.* (2011) reported that copper is efficient in promoting growth and health as well as reducing meat cholesterol in poultry. Rahman *et al.* (2001) reported increased haemoglobin and plasma triglyceride with a reduction in plasma cholesterol in broiler chickens supplemented copper above

nutritional requirement. Cholesterol reduction activity by copper (Zahedi *et al.*, 2013) can be attributed to its ability to regulate cholesterol biosynthesis indirectly by decreasing the reduced form of glutathione and increasing the oxidized form of glutathione (Bakalli *et al.*, 1995). Pesti and Bakalli (1996) reported that the addition of 250 mg/kg of supplemental copper to the diet reduces total plasma cholesterol and increases growth performance. Copper has been introduced into the diet in various forms e.g., CuO, CuSO<sub>4</sub>, and organic copper (copper plus amino acid) with varying results in terms of availability, absorption, and consequently efficacy. Copper is mostly supplied in the diet of broiler chickens in form of copper sulphate (Scott *et al.*, 2018), however, some authors have reported that copper nanoparticles (Cu-NP) are more available and better absorbed in the intestine of chickens hence reducing the quantity needed to be supplemented. Gatoo *et al.* (2014) stated that the nano size and larger surface area of Cu-NP might help the cellular uptake and translocation of the particles in the animal's body.

Domestic birds like broilers are believed to be susceptible to oxidative stress which may be as a result of the genetic selection toward larger breast muscles, increased total weight, and faster growth rates (Sihvo *et al.*, 2014). Scott *et al.* (2018) reported that the copper nanoparticles with longer chain ligands have surfaces that are better protected from oxidation and a corresponding lower reactive oxygen species (ROS)-generating capacity than the particles with shorter chain ligands. On the other hand, copper nanoparticles with greater surface oxidation also have a higher ROS-generating capacity (Shi *et al.*, 2012). Despite all these findings, Scott *et al.* (2018) argued that little work has been done to explore the ability of copper nanoparticles as feed additives in animal feed and particularly in poultry to serve as a good antioxidant and lipid-lowering agent. Hence, this study was carried out to compare the antioxidative and hypocholesteremic properties of copper sulphate and copper nanoparticles supplemented in the diet of broiler chickens.

## MATERIALS AND METHODS

### Experimental Site

The experiment was carried out at the poultry unit of the Teaching and Research Farms of the Federal University of Agriculture, Abeokuta, South West, Ogun State, Nigeria with a climate of derived savannah zone of South-Western Nigeria. The annual rainfall and temperature are about 1,037 mm and 34.7°C, respectively.

### Source of Test Ingredient

The copper sulphate (CuSO<sub>4</sub>) and copper nanoparticles (Cu-NP) used for this study were procured from a reputable store in Lagos, Nigeria.

### Management of Birds and Experimental Design

One hundred and forty-four (144), one-day-old Arbor Acres strain of broiler chicks were sourced from a reputable hatchery in Ibadan, Nigeria. Thorough washing and disinfection of the pen and necessary equipment were carried out prior to the arrival of the birds. On arrival, birds were divided into four groups of 36 birds each. Each group was randomly assigned to the four dietary treatments as follows; 250 ppm CuSO<sub>4</sub>, 225 ppm Cu-NP, 275 ppm Cu-NP supplementation, and control (without dietary copper supplementation but antibiotics). The groups were replicated thrice to consist of 12 birds each. Brooding was carried out for two weeks. Feed and water were provided *ad libitum* while birds were raised on deep litter for 6 weeks. Commercial broiler starter (22% crude protein and metabolizable energy 2,900 kcal/kg) and finisher (20% crude protein and metabolizable energy 2,800 kcal/kg) diet 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. All necessary vaccinations and medications were done except that the birds on dietary copper supplementation were not administered antibiotics. The composition of commercial feed that was fed to the birds is shown in Table 1.

**Table 1** Nutrient composition of commercial feed

Parameter	Starter diet	Finisher diet
Crude protein (%)	21.00	18.00
Fat (%)	6.00	6.00
Crude fiber (%)	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
Copper (ppm)	16.00	16.00
Metabolizable energy (kcal/kg)	2,900	2,800

### Collection of Blood and Meat Samples

On the 42<sup>nd</sup> day, two birds were randomly selected from each replicate for the collection of blood samples via the jugular vein. The bleeding (5 mL) was done using a 5 mL syringe firmly fitted with a 24-gauge sterile hypodermic needle. The aspirated blood was divided into two (2.5 mL each) and transferred into two sterilized plain bottles for the determination of oxidative stress biomarkers and blood lipid profile. For meat samples, two birds per replicate were sacrificed and 50 g of breast meat was collected from each which was used for the determination of meat lipid profile.

### Determination of Oxidative Stress Biomarkers

The concentration of the following oxidative stress biomarkers was determined in the blood of the experimental birds: albumin, total bilirubin, uric acid, superoxide dismutase (SOD), glutathione (GSH), and glutathione peroxidase (GPx) activities. Albumin concentration of the blood plasma was measured via the Bromcresol green (BCG) assay method as described by Toghyani *et al.* (2012). The concentration of uric acid and total bilirubin were also determined by the examination of the blood plasma as described by Ognik and Wertelecki (2012). In the erythrocytes, glutathione peroxidase activity was measured with the Ransel kit (Randox). The concentrations of glutathione and the activities of superoxide dismutase were determined in blood

plasma as described by Toghyani *et al.* (2012).

### Determination of Lipid Profile Parameters

Cholesterol (CHOL) was measured using a colorimetric reflectance spectrophotometric method. Direct high-density lipoprotein (HDL) cholesterol was assayed by colorimetric reflectance spectrophotometry after samples were treated with phosphotungstic acid/magnesium chloride to precipitate HDLs and non-HDLs. Triglyceride (TRIG) was measured by colorimetric reflectance spectrophotometry. Low-density lipoprotein (LDL) cholesterol was calculated: Total CHOL – [HDL + (TRIG / 5)]. Very low-density lipoprotein (VLDL) was estimated by dividing the triglyceride value by 5 (in mg/dL).

### Statistical Analysis

Data collected were subjected to a one-way analysis of variance using a general linear model in SPSS (IBM Corporation, 2012). The significant means were separated using Duncan's new multiple range test at a 5% level of significance.

### Ethical Approval

All procedures guiding animal welfare were strictly adhered to following the rules and regulations of the Animal Welfare Committee of College of Animal Science and Livestock Production, Federal University of Agriculture, Abeokuta.

## RESULTS AND DISCUSSION

The comparative effect of dietary supplementation of copper sulphate and nanocopper on oxidative stress in broiler chickens is shown in Table 2. All the oxidative stress biomarkers were significantly ( $P < 0.05$ ) influenced by copper supplementation except the glutathione. Albumin level was lowered by copper supplementation with the least values noted in birds supplemented 250 ppm  $\text{CuSO}_4$  and 275 ppm Cu-NP. This negates the findings of Kumar *et al.* (2013) in which there was an increase in albumin levels in chickens fed 100, 200, and 400 mg/kg of  $\text{CuSO}_4$ . The decrease in albumin level may be as a result of albumin being a hard corona protein and

easily reacting with nanocopper. Hard corona proteins interact directly with the surface of nanoparticles, whereas soft corona proteins interact with hard corona proteins via weak protein-protein interactions (Walkey and Chan, 2012). According to Kozłowski *et al.* (2018), hard corona proteins have an affinity for nanoparticles, and albumins interact most readily with the surface of this nano-metal. The interactions between albumins and the nano-metal or other proteins can decrease plasma levels of free albumin. The result obtained in this study shows that uric acid also reduced markedly in birds supplemented with either  $\text{CuSO}_4$  or Cu-NP, however, the effect was similar ( $P > 0.05$ ) between the copper sources and among the levels of supplementation.

**Table 2** Comparative effect of dietary supplementation of copper sulphate ( $\text{CuSO}_4$ ) and copper nanoparticles (Cu-NP) on oxidative stress in broiler chickens

Parameters	Copper sources				SEM	P-value
	Control	$\text{CuSO}_4$ (250 ppm)	Cu-NP (225 ppm)	Cu-NP (275 ppm)		
Albumin (g/L)	43.050 <sup>a</sup>	35.600 <sup>c</sup>	41.400 <sup>b</sup>	35.600 <sup>c</sup>	0.789	0.001
Uric acid (mg/dL)	7.740 <sup>a</sup>	5.910 <sup>b</sup>	5.800 <sup>b</sup>	4.825 <sup>b</sup>	0.321	0.003
T. bilirubin (mg/dL)	0.550 <sup>b</sup>	0.550 <sup>b</sup>	0.700 <sup>a</sup>	0.550 <sup>b</sup>	0.016	0.000
SOD (U/L)	0.017 <sup>b</sup>	0.015 <sup>b</sup>	0.014 <sup>b</sup>	0.730 <sup>a</sup>	0.008	0.007
GSH (U/L)	290.160	260.035	284.635	274.095	6.138	0.340
GSH-Px (U/L)	5.600 <sup>c</sup>	8.250 <sup>a</sup>	6.800 <sup>b</sup>	6.900 <sup>b</sup>	0.225	0.000

**Note:** <sup>a,b,c</sup> Means within a row with different superscripts differ significantly at  $P < 0.05$

T. bilirubin = total bilirubin, SOD = superoxide dismutase, GSH = glutathione, GSH-Px = glutathione peroxidase, SEM = standard error of mean

The reduction obtained in plasma uric acid concentration in groups fed diets supplemented with Cu-NP suggests that amino acid was more efficiently utilized than other groups. In a related study, Yang *et al.* (2009) reported that birds supplemented with Cu-NP diets better-utilized amino acids for growth. The result of this experiment was corroborated by the findings of Akbarian *et al.* (2016) where Cu-NP

(50 ppm and 20 ppm) and  $\text{CuSO}_4$  (50 ppm and 20 ppm) were administered in the drinking water of chickens and injected into the chicken as well. Another implication of reduced plasma uric acid is that there will be increased oxidative activity. Simoyi *et al.* (2002) stated that plasma uric acid (PUA) concentration is inversely correlated with oxidative activity in birds and that as PUA levels are

lowered, oxidative stress increases dramatically.

Total bilirubin increased significantly ( $P < 0.05$ ) in birds supplemented 225 ppm Cu-NP (0.700 mg/dL). Several lines of evidence suggest that high serum bilirubin concentrations are associated with increased total antioxidant capacity and confer protection against oxidative stress-induced diseases (Vitek, 2012). Though the group with an inclusion level of 225 ppm of Cu-NP had a higher level of bilirubin than 275 ppm Cu-NP, this does not prove that the latter inclusion level is toxic. This cannot be fully explained as other factors could have contributed to this. Increased SOD level in birds supplemented with Cu-NP (275 ppm) is in consonance with the findings of Kozłowski *et al.* (2018) where the addition of the highest copper dose to turkey diets increased the activities of SOD concentrations in blood plasma. Furthermore, some studies demonstrated that the activity of SOD was enhanced with Cu-NP supplementation in animal diets (Refaie *et al.*, 2015).

Increased SOD is inversely correlated to oxidative stress as the enzyme aids in the removal of the damage caused by ROS by catalyzing the dismutation of two superoxide radicals to hydrogen peroxide and oxygen (Lin *et al.*, 2008). Similar glutathione (GSH) level recorded in this research is in consonance with the results of Ognik *et al.* (2019) where copper (Cu) and copper nanoparticles (Cu-NP) were administered into the water of broilers though at a lower dose (5, 10, and 15 mg/bodyweight/day). The numerical reduction in GSH in copper supplemented diets birds as compared to the control in this experiment also corroborated the results of Ajuwon *et al.* (2011) on the effects of dietary copper supplementation on oxidative and antioxidant systems in broiler chickens. The GSH numerical values reduction in relation to the control experiment noted in this research might be due to the reaction of GSH with copper or with free radicals incited by copper supplementation (Deleve and Kaplowitz, 1991). Also, an impairment of GSH synthesis could be

responsible for the observed reduction of GSH (Hultberg *et al.*, 1997). Increased values were observed in the GSH-px level among the copper supplemented groups in this study. Surai *et al.* (2018) listed copper deficiency as one of the nutritional means of decreasing GSH-Px activities in various tissues. Also, ascorbic acid, a dietary antioxidant with lower absorption (240–280 nm) than Cu-NP (280–360 nm) has been reported to increase the activity of the GSH-Px in chicken plasma (Oztürk-urek *et al.*, 2001) and this indicates the potential of Cu-NP as an antioxidant. This may be the reason for the increase in GSH-px activity in the plasma of the birds as compared to the control. This implies birds with Cu-NP dietary inclusion experienced minimal oxidative stress in comparison to the control group as indicated by Surai *et al.* (2018) that increased expression of GSH-Px is associated with better protection against oxidative stress.

Table 3 presents the blood lipid and meat profile of broiler chickens as varied by copper sulphate and nanocopper dietary supplementation. Results obtained showed that all parameters were not significantly ( $P < 0.05$ ) affected by copper supplementation except low-density lipoprotein. The reduction of blood cholesterol levels (LDL) on the addition of copper (CuSO<sub>4</sub> and Cu-NP) to the diet in this study is in consonance with the report of the Zahedi *et al.* (2013) in a study on Japanese quail. The result obtained in this study is also in consonance with the result obtained in the study of Kaya *et al.* (2006) on the effect of copper deficiency on blood lipid profile and haematological parameters in broilers. Supplementation of Cu-NP (225 ppm) resulted in a reduced LDL level in meat as compared to CuSO<sub>4</sub> (250 ppm), this may be due to increased bioavailability of Cu-NP (Scott *et al.*, 2018) as copper is added with the ability to regulate cholesterol biosynthesis indirectly by decreasing the reduced form of glutathione and increasing the oxidized form of glutathione (Bakalli *et al.*, 1995).

**Table 3** Comparative effect of dietary supplementation of copper sulphate (CuSO<sub>4</sub>) and copper nanoparticles (Cu-NP) on blood and meat lipid profile of broiler chickens

Parameters	Copper sources				SEM	P-value
	Control	CuSO <sub>4</sub> (250 ppm)	Cu-NP (225 ppm)	Cu-NP (275 ppm)		
<b>Blood</b>						
TRIG (mg/dL)	99.70	93.50	101.63	108.13	13.36	0.84
HDL (mg/dL)	65.95	51.10	55.10	60.55	9.65	0.54
VLDL (mg/dL)	19.95	18.70	20.35	21.60	2.67	0.84
LDL (mg/dL)	41.90 <sup>a</sup>	25.80 <sup>b</sup>	26.15 <sup>b</sup>	23.55 <sup>b</sup>	7.97	0.002
CHOL (mg/dL)	127.80	95.90	101.60	105.70	16.45	0.24
<b>Meat</b>						
TRIG (mg/dL)	161.80	129.75	124.80	137.30	20.11	0.291
HDL (mg/dL)	80.45	75.90	72.00	81.30	9.65	0.841
VLDL (mg/dL)	32.35	25.95	24.95	27.45	4.00	0.295
LDL (mg/dL)	37.35 <sup>a</sup>	31.50 <sup>a</sup>	21.70 <sup>b</sup>	36.15 <sup>a</sup>	6.92	0.016
CHOL (mg/dL)	150.15	133.30	118.60	144.90	16.02	0.196

**Note:** <sup>a,b</sup> Means within a row with different superscripts differ significantly at P < 0.05

TRIG = triglyceride, HDL = high-density lipoprotein, VLDL = very low-density lipoprotein, LDL = low-density lipoprotein, CHOL = cholesterol, SEM = standard error of mean

## CONCLUSIONS

The study revealed that albumin and uric acid levels declined in birds fed copper supplemented diet. However, supplementation of 225 ppm Cu-NP induced increased total bilirubin, 275 ppm Cu-NP increased superoxide dismutase and 250 ppm CuSO<sub>4</sub> increased glutathione peroxidase which

indicates their potentials as antioxidant (i.e., ability to reduce oxidative stress) in broiler chickens. Also, copper supplementation (both copper sulphate and nanocopper) in excess of the requirement reduced the low-density lipoprotein level in the blood of broiler chickens. Nanocopper at the rate of 225 ppm further reduced low-density lipoprotein concentration in the broiler meat.

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