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Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

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Modulation of Cholesterol and Copper Residue Levels in Muscles and Blood Serum of Finishing Broiler Chickens Fed Copper and Ascorbic Acid Supplements

O.M.O. Idowu[1], O.R. Ajuwon[3], A.O. Fafiolu[1], A.O. Oso[1] and O.A. Akinloye[2]
[1]Department of Animal Nutrition, College of Animal Science and Livestock Production, University of Agriculture, P.M.B. 2240, Abeokuta, Nigeria, West Africa
[2]Department of Biochemistry, University of Agriculture, P.M.B. 2240, Abeokuta, Nigeria, West Africa
[3]Department of Biomedical Sciences, Faculty of Health and Wellness Sciences, Cape Peninsula University of Technology, Bellville Campus, Bellville 7535, South Africa

Abstract: To study the effect of Copper (Cu) and ascorbic acid supplements on modulation of cholesterol and copper residue content of chicken muscles and blood serum, two hundred and forty finishing Anak broiler chickens of mixed sexes were randomly allotted to four dietary treatment groups with four replication of fifteen birds each. The feeding period was 5 weeks. The response to copper and ascorbic acid were such that dietary supplementation with 250 ppm Cu alone resulted in 30 and 19% reduction in pectoralis major and biceps femoris muscles cholesterol respectively. Supplementation with 250 ppm Cu + 100 ppm Ascorbic acid resulted in 29 and 12% reduction in muscles cholesterol while inclusion of 100 ppm ascorbic acid alone brought about 4 and 10% reduction in the two muscles respectively. Serum cholesterol reduced by 28% and the addition 100 ppm ascorbic acid reduced this to 26%. Ascorbic acid (100 ppm) exerted 12% reduction in serum cholesterol. Ascorbic lowered the hypocholesterolemic potency of copper by about 1-7%. Combination of 100 ppm ascorbic and 250 ppm Cu slowed down rate of weight gain by 11% when compared with 250 ppm Cu alone as supplement, Supplementation with 100 ppm ascorbic alone improved carcass yield by 4% compared to 250 ppm Cu. Metalloprotein enzyme (AST and ALP) activities were significantly increased with supplementation. Cu residue was more in biceps femoris than pectoralis major muscle. Above 30% reduction in Cu residue was noticed with addition of ascorbic acid.

Key words: Copper, ascorbic acid, cholesterol, reidues

INTRODUCTION

Copper (Cu) has often been added to poultry diets as growth promoting substance and the growth-promoting effect of Cu supplements has been reported by Skrivan *et al.* (2002) that supplementation with 200 mgCu/kg diet increased final body weight of chickens by 4.3%. Cu supplied as $CuSO_4$ $5H_2O$ is a cheap 'inorganic antibiotic'' that does not contribute to antibiotic resistance spreading among microorganisms. As a result of its antimicrobial activity and growth-promoting effects, copper has been added to feed mixtures for growing animals at rather high concentrations for several decades. Also Bakalli *et al.* (1995), Pesti and Bakalli (1996) and Idowu *et al.* (2006) revealed that high doses of Cu influenced lipid metabolism of poultry and decreased the cholesterol content in chicken meat and hen eggs up to 30%. Excessive intake of copper causes toxicity especially when fed for a long time (Ajuwon and Idowu, 2010). According to the European Union (2003), the Cu supplementation of animal diets is regulated by the EU Council Directive 70/524/EEC. It is therefore imperative to add other nutrients that could mop up the possible negative effects associated with higher inclusion of copper in livestock rations. Vitamin C, zinc and manganese are known to interferes with copper absorption and are possible attenuates. A part from the above nutritional role of ameliorating copper toxicity it also has beneficial effects on poultry performance in heat stress condition (Whitehead and Keller, 2003). Ascorbic acid is reported to improve carcass traits and affected blood constituents (Sahin and Kucuk, 2001; Lohakare *et al.*, 2005). Therefore the aim of this study was to investigate the effects of copper and vitamin C supplementation in the modulation of cholesterol content in muscles and serum, as well as its effect on growth performance, carcass traits and certain serum metabolites of the broiler chickens under the hot humid tropical conditions.

MATERIALS AND METHODS

Experimental birds management and design: Two hundred and forty (240) finishing Anak® broiler chickens of mixed sexes were randomly allotted to sixteen replicate groups and four of such groups were randomly

Corresponding Author: O.M.O. Idowu, Department of Animal Nutrition, College of Animal Science and Livestock Production, University of Agriculture, P.M.B. 2240, Abeokuta, Nigeria, West Africa

assigned to one of the four dietary treatment groups in a completely randomized experimental design. Treatments were basal broiler straight diet (control) supplemented with inorganic Cu ($CuSO_4.5H_2O$, Sigma Chemical, St! Louis, M.O.), Vitamin C (L-ascorbic acid, Rovimit®, Roche) and 250 mg Cu + 100 mg Vitamin C/kg basal diet respectively. The experimental birds were housed in adequately ventilated deep litter house that provided uniformly sized pens of 3.0 m x 3.0 m (1.21 m^2 per bird). The pens were equipped with standard feeding troughs and drinkers. Feed and water were provided *ad libitum*. There were four dietary groups:

Diet A : Cu nil, L-ascorbic acid nil (control)
Diet B : Cu 250 ppm, L-ascorbic acid nil
Diet C : Cu nil, L-ascorbic acid 100 ppm
Diet D : Cu 250 ppm, L-ascorbic acid 100 ppm

Feeding trial, carcass evaluation and data collection: The 4-week feeding trial began when all the birds were fed the control diet (un-supplemented basal diet) for one week to ensure equalization and adaptation. Water was supplied *ad libitum*. Data collected were body weights taken at the beginning and end of the feeding trial. Weekly records were kept on body weight and feed intake, while daily feed intake and feed conversion were calculated for each replicate group at the expiration of five weeks.

Serum metalloenzyme activities: The sera samples were analyzed for serum Alanine, Aminotransferase (ALT), Alkaline phosphatase (ALP) and Aspartate aminotransferase (AST) activities were determined spectrophotometrically using commercial test kit (Randox®). Total serum protein was determined using the Biuret method as described by Kohn and Allen (1995). Albumin was determined using Bromocresol Green (BCG) method.

Muscles and serum cholesterol determination: At the end of the feeding trial three birds per replicate pen were randomly chosen for carcass evaluation, blood serum and tissue collection; thigh (Biceps femoris) and breast (Pectoralis major) muscles were sampled without skin. Serum total cholesterol were determined using enzymatic - colorimetric method (according to Sigma diagnostic cholesterol reagent procedure No 352' Sigma Chemical Co., St Louis, MO, USA). Composite paste (100 g) of Biceps femoris and Pectoralis major muscle tissues were prepared by blending with a known amount of chloroform/methanol mixture (1:1 v/v). The resulting paste-solvent mixture was filtered and rinsed with an additional volume of the combined homogenate and was allowed to stand for 5 min with occasional stirring. The filtered homogenate was then equilibrated to remove non-lipid material. 2% (0.32M) w/v KCl solution was added to the aqueous layer to minimize the loss of liquid to the aqueous layer. The filtrate was centrifuged and lipid extract was decanted. The supernatant (extract) was made up to a final volume by adding chloroform so that the proportion of chloroform to methanol was 2:1 (v/v) and the final ratio of the solvent to tissue was 30:1. The supernatant fluid decanted was re-extracted before, cholesterol analysis (Folch *et al*., 1957; Gary, 1993). Concentration was expressed in mg/100 g wet weight of tissue and mg/dl of serum.

Proximate and Copper residue determination: The moisture (method 950.46), CP (method 992.15), crude fat (method 985.15) and ash (method 920.153) contents were determined in triplicate according to AOAC methods (AOAC, 1995). 100 g of composite paste of muscle tissue samples were oven dried, pre-ashed and pooled together according to replicates prior the dry ashing in muffle furnace. The ashed samples were analyzed chemically for copper content by Atomic Spectrophotometry (Perkin-Elmer, Atomic Absorption Spectrophotometer) at wavelength of 324.7 nm (Chiou *et al*., 1998).

Statistical analyses: Statistical analyses (ANOVA) were performed using General Linear Model (GLM) procedure of SAS (1985). Significant differences between treatment means were determined at $p<0.05$ using Duncan's multiple range tests.

RESULTS AND DISCUSSION

The effects of dietary supplementation of Cu alone, Vitamin C alone and Cu vitamin C combined on moisture content, total musclefat, cholesterol concentrations in the muscles and serum of finishing broiler chickens are shown in Table 2. No significant difference was noticed in the moisture content among the chicken muscle types ($p>0.05$). However, dietary supplementations resulted in a significantly greater total muscle fat, total cholesterol and Cu residue in the biceps femoris muscle of the experimental chickens when compared to the pectoralis major muscle from chickens fed same experimental diets ($p<0.05$). This observation corroborated the previous findings of Araujo (1990) and Zanini *et al*. (2003) who reported that the contents of lipids and cholesterol were greater for the biceps femoris muscle than for Pectoralis major muscle. The explanation for this difference was probably in the muscular composition. The amount of lipids in the biceps femoris and pectoralis major muscle muscles could be related to the different muscular functions and the need for giving skeletal support and movement. The pectoralis major muscles of domesticated broiler chickens were never used for flying and when excised after killing were found to be white meat while the biceps femoris muscles were mainly red meat. According to

Murray *et al.* (1993) classification, white muscles (classified as type 2) are known to lack myoglobin and contain few mitochondria but have a bigger amount of glycogen and they derive their energy from anaerobic glycolysis and exhibits relatively short duration of contraction. The red meat (biceps femoris muscles) contain myoglobin and mitochondria, their metabolism is aerobic in which the major energy sources are blood glucose, free fatty acids derived from the breakdown of triacylglycerol in the tissue which requires more blood flow to the leg muscle tissue, hence the leg muscles have more lipids and cholesterol content than the pectoralis major muscle.

Copper and vitamin C inclusion both reduced significantly muscle and serum cholesterol (Table 2). Dietary supplementation with 250 ppm Cu alone resulted in 30 and 19% reduction in pectoralis major and biceps femoris muscles cholesterol respectively. Addition of Cu vitamin C combined resulted in 29 and 12% reduction in muscles cholesterol while vitamin C alone brought about 4 and 10% reduction respectively. Serum cholesterol reduced by 28% and with the addition 100 ppm ascorbic acid reduced this to 26%. Ascorbic acid (100 ppm) exerted 12% reduction in serum cholesterol (Table 2). Stronger hypocholesterolemic effect was therefore noticed in copper than ascorbic acid. The addition of vitamin C to Copper in this experiment reduced the hypocholesterolemic potency of Cu. This observation could be adduced to the claims of Tsuchiya and Bates (1997) that vitamin C has the ability as a powerful oxidizing agent to reduce Cu II (Cupric) ion at absorption site which may result in marginal reduction in available Cu and thus modify its biological properties. The hypocholesterolemic effect exerted by Cu is premised on the fact that copper regulates cholesterol biosynthesis by reducing hepatic glutathione concentrations (Kim *et al*., 1992). Glutathione is known to regulate cholesterol biosynthesis through the stimulation of the enzyme 3-hydroxy-3-methyl glutaryl coenzyme A (HMG-Co A) reductase in rats (Valsala and Kurup, 1987). The HMG-Co A reductase activity is the rate limiting step of mevalonate and ultimately, cholesterol synthesis. Copper supplementation (250 mg/kg) to broiler diet has been reported to decrease Pectoralis major muscle and plasma cholesterol concentrations (Konjufca *et al*., 1997; Pesti and Bakalli, 1996; Bakalli *et al*., 1995). No synergy in terms of hypocholestromic effect of Cu-vitamin C mixture was noticed. HDL cholesterol was generally higher than LDL cholesterol.

Copper residue level values are expressed on a dry matter or wet weight basis in tissues. Lower levels have been found in muscle because chickens are characterized by a high level of copper clearance (Aoyagi and Baker, 1993). Values (2.05-5.81 mg/100 g wet wt.) obtained in this experiment was within the range (0.2-1.6 mg/kgDM) reported by Jackson (1977); Stevenson and Jackson (1981); Ledoux *et al*. (1991). The corresponding values for fresh weight represented about one third of the values observed in dry matter (25% for muscle). Low cholesterol concentrations were noticed at higher Cu residue concentration in the muscles and serum (Table 2) which was a reflection of amount of Cu intake and feed intake. Cu residue was more in biceps femoris than pectoralis major. Above 30% reduction in Cu residue was noticed with addition of ascorbic acid.

Table 1: Gross composition of experimental diet

Maize (%)	44.00
Soya bean full fat (%)	36.00
Rice husk (%)	12.33
Palm oil (%)	4.00
Bone meal (%)	2.50
Oyster shell (%)	0.50
Vitamin/mineral premix	0.25
Salt (NaCl) (%)	0.25
Methionine (%)	0.17
Total	100.00
Determined analysis	
Crude protein (%)	19.90
Fat (%)	11.00
Ash (%)	14.31
Fibre (%)	4.78
Copper (mg/kg)	5.90
Calculated analysis	
Energy (ME) (kcal/kg)	3203.76
Energy/protein ratio	160.20
Methionine (%)	0.45
Calcium (%)	1.33
Phosphorus (%)	0.69

Table 1 shows the gross and proximate compositions of the basal diet. The determined proximate values were within the recommended feeding standard for finishing broiler chickens (NRC, 1994). The basal copper content (5.90 ppm) was in the lower border of the range (6-8 ppm) recommended by NRC (1994). As shown in Table 3 the body weight, daily weight gain and feed conversion were significantly ($p<0.05$) improved with supplementation of the basal diet. Performance response in terms of the final body weight and daily weight gain was statistically similar for treatment groups supplemented with 100 ppm ascorbic acid alone and 100 ppm ascorbic plus 250 ppm Cu. The group supplemented with 250 ppm of Cu alone grew faster (56.30 g/bird/day) than the other three groups by about 24%. This was an improvement over 4.3% reported by Skrivan *et al.* (2002) who supplemented with 200 ppm Cu. Copper between 125 and 250 mg/kg feeding stuff was also effective in promoting growth of broiler chicken (Pesti and Bakalli, 1996; Ewing *et al*., 1998; Skrivan *et al*., 2002). Supplementation of finishing broiler diet with either Cu alone or Cu and vitamin C combined also resulted in a significantly optimum feed conversion ratios (2.79-2.93). Combining 250 ppm Cu with 100 ppm

Table 2: Cholesterol content and copper residue in chickens muscles and serum (Mean±SE)

Measurements	Control	$CuSO_4$	Vitamin C	$CuSO_4$ + Vitamin C
Pectoralis major muscle				
Moisture content (%)	70.01±1.30	70.77±1.12	70.13±1.10	70.82±1.20
Total fat (%)	3.10±0.22^{a}	2.08±0.14^{c}	2.62±0.20^{b}	2.02±0.18^{c}
Cholesterol (mg/100 g wet wt.)	57.80±0.34^{a}	40.40±0.29^{b}	55.60±0.36^{a}	41.00±0.30^{b}
Cu (mg/100 g wet wt.)	2.98±0.10^{b}	3.88±0.20^{a}	2.05±0.10^{b}	3.11±0.20^{a}
Biceps femoris muscle				
Moisture content (%)	74.01±1.66	74.11±1.70	73.89±1.48	74.12±1.80
Total fat (%)	4.12±0.28^{a}	2.11±0.18^{c}	3.07±0.24^{b}	2.42±0.20^{c}
Cholesterol (mg/100 g wet wt.)	134.20±0.51^{a}	108.10±0.40^{d}	120.20±0.55^{b}	118.40±0.47^{c}
Cu (mg/100 g wet wt.)	3.48±0.15^{b}	5.81±0.20^{a}	2.90±0.18^{c}	4.12±0.20^{a}
Serum				
Total cholesterol (mg/dl)	156.27±0.78^{a}	112.50±0.44^{c}	137.50±0.66^{b}	116.27±0.46^{c}
HDL cholesterol (mg/dl)	74.10±0.38^{a}	54.40±0.30^{c}	69.10±0.32^{b}	58.20±0.30^{c}
LDL cholesterol (mg/dl)	46.32±0.04^{a}	40.88±0.02^{c}	42.72±0.02^{b}	41.20±0.03^{c}
Cu (µg/ml)	2.35±0.25^{b}	4.48±0.35^{a}	2.31±0.21^{b}	3.05±0.19^{c}

^{a,b,c}Means on a row with different superscript are significantly different ($p<0.05$)

Table 3: Performance of birds fed vitamins and copper supplements (Mean±SE)

Measurement	Control	$CuSO_4$	Vitamin C	$CuSO_4$ + Vitamin C
Performance traits				
Initial BWT (g/bird)	600.00±0.87	600.00±0.62	600.00±0.54	600.33±0.03
Final BWT (g/bird)	1870.00±10.90^{c}	2176.23±16.79^{a}	2033.30±19.64^{b}	2008.75±15.47^{b}
BWT gain (g/bird/day)	45.18±0.39^{c}	56.30±0.11^{a}	50.36±0.12^{b}	50.34±0.66^{b}
Feed intake (g/bird/day)	140.50±0.23	157.10±0.58	154.10±0.52	147.50±0.14
Feed conversion ratio	3.11±0.32^{a}	2.79±0.09^{b}	3.06±0.21^{a}	2.93±0.26^{b}
Carcass evaluation				
Dressed BWT (%)	74.88±1.73	75.52±1.70	78.82±1.45	75.58±1.83
Abdominal fat (%)	1.08±0.08^{a}	0.37±0.02^{c}	0.84±0.03^{b}	0.79±0.02^{b}

^{a,b,c}Means on a row with different superscript are significantly different ($p<0.05$). BWT-Body Weight

Table 4: Serum metabolites and enzymes (Mean±SEM)

Measurements	Control	$CuSO_4$	Vitamin C	$CuSO_4$ + Vitamin C
Total protein	41.00±1.67	42.87±1.65	44.87±1.32	45.30±1.40
Albumin	25.00±1.29	26.90±1.29	26.97±1.32	26.00±1.23
Globulin	16.00±1.39^{b}	15.97±1.36^{b}	17.90±1.84^{b}	19.30±1.17^{a}
Uric acid (mg/dL)	3.50±0.09	3.57±0.03	3.10±0.06	4.40±0.06
SPGT (ALT) (IU/L)	10.30±0.10	14.21±0.14	11.09±0.15	12.34±0.16
SGOT (AST)(IU/L)	105.70±0.44^{a}	107.57±0.15^{a}	100.67±0.90^{b}	85.67±0.58^{c}
ALP (IU/L)	92.57±0.52^{a}	94.50±0.98^{a}	82.77±1.18^{b}	71.10±2.14^{c}

^{a,b,c}Means on a row with different superscript are significantly different ($p<0.05$)

of vitamin C in the diet appeared to cause a reduction in the feed conversion efficiency when compared to what was obtained when 250 ppm Cu alone was used as the only supplement (Table 2). Feed intake increased ($p>0.05$) with supplementation across treatments with highest feed consumption noticed in the group on 250 ppm Cu supplementation. Percentage dressed weight did not differ across treatment group. Significantly lowest abdominal fat was obtained in the chickens fed diet supplemented with 250 pp Cu while the controlled group had the highest abdominal fat deposit. The abdominal fat for the treatment groups supplemented with Cu and vitamin C and vitamin C alone were statistically similar. This implied that both Cu and vitamin C were hypolipolemic and were capable of influencing lipid metabolism.

Supplementation with either Cu or Vitamin C also significantly influenced globulin, SGOT and ALP concentration in the serum (Table 4). Inclusion of either 100 ppm L-ascorbic alone or mixed with 250 ppm of Cu significantly increased values of globulin and reduced SGOT and ALP serum concentrations respectively. Addition of 250 ppm of Cu alone increased significantly those enzymes. Total serum protein, albumin, uric acid and SGPT values were not different ($p>0.05$) across treatment groups. In conclusion addition of copper and vitamin C modulated cholesterol content in muscles of broiler chickens with vitamin C lowering the hypocholesterolemic potency of copper by about 1-7%. Combination of 100 ppm ascorbic and 250 ppm Cu slowed down rate of weight gain by 11% when compared with 250 ppm Cu alone as supplement, while supplementation with 100 ppm ascorbic alone improved carcass yield by 4% compared to 250ppm Cu. Metalloprotein enzyme (AST and ALP) activities were significantly increased with supplementation.

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