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EFFECTS OF DIETARY SUPPLEMENTATION OF COPPER SULPHATE AND COPPER OXIDE ON SOME EGG QUALITY PARAMETERS OF LAYING HENS

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ABSTRACT

A total of two hundred and twenty-four (224) Bovan Nera pullets of 20 weeks old were used for the 12-week experiment to determine the implications of two sources of inorganic copper supplementation in layers' diets. They were divided into 8 groups of twenty-eight (28) birds per treatment. Eight (8) isocaloric and isonitrogenous experimental diets of 17.63 % Crude Protein and 2592.80 kcal.kg^{-1} Metabolizable Energy were provided, each was supplemented with Copper Sulphate and Copper Oxide at four levels of inclusion (0, 100, 200 and 300 mg.kg^{-1}) and the pullets were randomly assigned to the diets in a 2 x 4 factorial experiment. Each treatment was replicated seven (7) times with 4 birds per replicate. All data collected were, therefore, subjected to 2 x 4 factorial analyses of variance. It was observed that the interaction of the different levels of inclusion of the two sources of copper had a significant ($p < 0.05$) effect on all the external egg qualities except the shell ratio. The different levels of inclusion with the two sources of copper also had a significant ($p < 0.05$) influence on the albumen height, albumen weight, yolk length, yolk index and egg mass. It can therefore be concluded that the hen could tolerate the two copper sources up to 300 mg.kg^{-1} without any deleterious effects on the egg qualities.

Key words: layer; egg; copper; quality; diet

INTRODUCTION

The economy of egg production could be affected highly by egg quality. Egg quality is a factor which contributes for better economy price of fertile and table eggs. Egg quality was regarded by Stadelman (1977) as characteristics important for consumers. Egg quality is presented by its weight, percentage of eggshell, thickness and strength of eggshell (Hanusova *et al*., 2015). Eggshell weight correlates to size of egg and thickness (Harms *et al*., 1990). According to Hanusova *et al*. (2015), egg internal quality is influenced by factors such as egg storage, bird strain and age, induced moult, nutrition, ingestion of contaminants and disease.

Layer nutrition plays a significant role in affecting both the internal and external egg qualities such as egg size, length, shell strength, yolk and albumen (Saldanha *et al.*, 2009). Copper (Cu) has been found to play an important role in eggshell membrane formation, which in turn influences eggshell structure, texture, and shape (Baumgartner *et al.*, 1978). Studies have shown that dietary supplementation of various Cu sources, such Cu Sulphate, Cu citrate, Cu chloride etc. in laying hens improved egg production, egg weight, reduced egg deformities, total cholesterol, triglycerides, low density-lipoprotein cholesterol and increased high density-lipoprotein cholesterol (Pesti and Bakalli, 1996; Miles *et al.*, 1998; Lim and Paik, 2003; Jegede *et al.*, 2011). Cu supplementation (125-250 ppm) in broiler diet was reported to confer improved feed consumption, body weight gain, feed conversion ratio and protein anabolism (Paik, 2001; Karimi *et al.*, 2011). Furthermore, Cu has a positive influence on the activities of some digestive enzymes such as trypsin, chemotrypsin, amylse and lipase (Tang *et al.*, 2013). However the maximum dietary tolerable level of copper for poultry

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was set at 300 mg.kg^{-1} (NRC, 1994) as excessive accumulation of copper in the body causes toxicity (Lapointe *et al*., 2011). The objective of the study was to evaluate the effects of two sources of dietary Cu and levels on some egg qualities of laying hens.

MATERIAL AND METHODS

Experimental Site

The study was carried out at the Poultry Unit, Teaching and Research Farm, The Federal University of Technology Akure, Nigeria. The geographical coordinates of the location is between 7° 17' North and 5° 9' East (Mapzoom, 2015). The climatic condition of Akure follows the pattern of southwest Nigeria where the climate is influenced mainly by the rain-bearing southwest monsoon winds from the ocean and the dry northwest winds from the Sahara desert. The rainy season lasts for about seven months (April to October). The rainfall is about 1524 mm per year. The atmospheric temperature ranges between 28 °C and 31 °C and mean annual relative humidity is about 80 % (Ajibefun, 2011). The experiment was conducted in accordance to the research ethics and guidelines of the Animal Production and Health Department of the institution.

Experimental Design and Diet

Eight (8) experimental diets were formulated in a 2 × 4 factorial arrangement such that they have varying inclusion levels of the two sources of copper, namely Copper Sulphate ($CuSO_4$) and Copper Oxide (CuO), sourced from BDH Chemical Ltd, Poole, United Kingdom. Each was supplemented at four levels of inclusion (0, 100, 200 and 300 mg.kg^{-1}) (Table 1). The formulated diets met the nutrient requirements of laying hens according to NRC recommendations (NRC, 1994). The proximate analyses of the diet samples were carried out according to AOAC (1995). The metabolizable energy (ME) of feed samples was calculated using the prediction equation by Pauzenga (1985) as follows: ME = (37 x CP + 81.8 x EE + 35.5 x NFE).

Experimental Animals

A total of two hundred and twenty-four (224) Bovan Nera pullets of 20 weeks old were divided into 8 groups of twenty-eight (28) birds per treatment and randomly assigned to the eight (8) treatment diets in a 2 x 4 factorial experiment. Each treatment was replicated seven (7) times with 4 birds per replicate. Feed was given according to body weight and age twice daily in line with the Bovan Nera management manual and drinking water was also provided *ad libitum*. All required managerial practices such as strict bio-security measures were ensured and also as and when due, appropriate vaccines and prophylactic treatments were administered. The birds were housed in an open-sided building in a thoroughly cleaned, washed and disinfected three tier cage system of 32 x 38 x 42 cm dimension. Four (4) birds were conveniently housed in a unit.

Data Collection

Egg collection was carried out thrice per day on days 21, 28, 35, 42, 49, 56 and 63 after the onset of lay from each treatment and taken for egg quality determination. A total of 35 eggs per treatment, 5 eggs per replicate were randomly selected on weekly basis within 24 hours of lay. Egg weight, yolk weight, shell weight and shell membrane were measured with sensitive scale calibrated in grams. The albumen weight was calculated by subtracting the sum of the weights of the shell and the yolk from the total egg weight. Shell thickness was measured with micrometer screw gauge and the shells air-dried for two days before weighting. Yolk index was determined as the ratio of the yolk height to the yolk width. Yolk height and width were measured with a ruler calibrated in centimeter with the aid of optical pins and mathematical compass. Yolk index and Haugh Unit (HU) was determined as described by Oluyemi and Robert (2000):

Yolk Index = (Yolk height/Yolk width) x 100

$HU = 100 \log (H + 7.57 - 1.7W^{0.37})$

Where H = observed albumen height in mm

W = observed weight of eggs in grams

The width and the length of the eggs were measured with the aid of vernier caliper calibrated in centimeters. Albumen heights in millimeters were taken with the aid of optical pin which was used for the calculation of Haugh Units.

The cholesterol levels of the eggs were evaluated using laboratory procedures for determining egg cholesterol. All the eggs from all the treatments on the last day of feeding trial were collected and weighed, hard cooked by immersion in boiling water for eight (8) minutes. The yolks were individually removed and individually weighed and oven-dried at 70 °C, pooled and blended.

Cholesterol determination was done using a commercial test kit for cholesterol analysis (Sigma diagnostic cholesterol reagent procedure No 352'Sigma Chemical Co., St Louis, MO, USA). All sample extracts were analyzed in triplicates. Cholesterol concentrations (mg) were determined from the absorbance read at 500 nm using a spectrophotometer (Idowu *et al*., 2006).

Table 1: Percentage composition of experimental diets

INGREDIENTS	Diets with CuO inclusion (%)			Diets with $CuSO_4$ inclusion (%)				
	T1	T2	T3	T4	T5	T6	T7	T8
Maize	40.00	40.00	40.00	40.00	40.00	40.00	40.00	40.00
Soya Meal	24.00	24.00	24.00	24.00	24.00	24.00	24.00	24.00
Corn Bran	14.00	14.00	14.00	14.00	14.00	14.00	14.00	14.00
Wheat Offal	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Limestone	8.52	8.52	8.52	8.52	8.52	8.52	8.52	8.52
Bone Meal	2.30	2.30	2.30	2.30	2.30	2.30	2.30	2.30
Lysine	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Methionine	0.35	0.35	0.35	0.34	0.35	0.35	0.35	0.34
Layer Premix*	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Salt	0.28	0.27	0.26	0.26	0.28	0.27	0.26	0.26
CuO	0.00	0.01	0.02	0.03	0.00	0.00	0.00	0.00
$CuSO_4$	0.00	0.00	0.00	0.00	0.00	0.01	0.02	0.03
Total	100	100	100	100	100	100	100	100
CALCULATED VALUES								
Crude Protein (%)	17.63	17.63	17.63	17.63	17.63	17.63	17.63	17.63
ME ($kcal.kg^{-1}$)	2592.80	2592.80	2592.80	2592.80	2592.8	2592.80	2592.80	2592.80
Ca (%)	3.90	3.90	3.90	3.90	3.90	3.90	3.90	3.90
Total Phosphorus (%)	0.51	0.51	0.51	0.51	0.51	0.51	0.51	0.51
Crude Fibre (%)	4.19	4.19	4.19	4.19	4.19	4.19	4.19	4.19
Crude Fat (%)	4.47	4.47	4.47	4.47	4.47	4.47	4.47	4.47
Lysine	1.21	1.21	1.21	1.21	1.21	1.21	1.21	1.21
Methionine	0.62	0.62	0.62	0.61	0.62	0.62	0.62	0.61
ANALYSED VALUES								
Crude Protein (%)	17.52	17.51	17.54	17.57	17.53	17.51	17.50	17.55
ME ($kcal.kg^{-1}$)	2602.77	2602.35	2604.93	2602.52	2603.85	2602.43	2603.25	2603.96
Crude Fibre (%)	7.15	6.98	6.99	7.05	6.99	7.35	7.42	7.51
Crude Fat (%)	2.16	2.25	2.16	2.59	2.39	2.13	2.01	2.20
Crude Ash (%)	11.43	11.57	11.28	11.98	11.60	11.87	11.43	11.78
Moisture (%)	11.66	11.82	11.91	11.78	11.92	10.99	11.18	10.97
NFE (%)	50.08	49.87	50.92	49.03	49.57	50.15	50.46	49.99

1 $mg.kg^{-1}$ (ppm) of $CuSO_4$/CuO = 0.0001 kg or %; NFE = Nitrogen Free Extract; ME = Metabolizable Energy

*Composition of premix: 2.5 kg of premix contains: Vit. A (10000000 iu), Vit. D3 (2500000 iu), Vit. E (12000 iu), Vit. B1 (2000 mg), Niacin (15000 mg), Vit. B6 (1500 mg), Vit. B12 (10 mg), Vit. K3 (2000 mg), Biotin (20 mg), Folic Acid (600 mg), Panthothenic Acid (7000 mg), Chlorine Chloride (150000 mg), Manganese (80000 mg), Iron (40000 mg), Copper (0 mg), Zinc (60000 mg), Selenium (150 mg), Iodine (1000 mg), Magnesium (100 mg), Ethoxyquine (500 g), BHT (700 g)

T1: Control diet without supplementary copper oxide; T2: Experimental diet supplemented with copper oxide at 100 ppm ; T3: Experimental diet supplemented with copper oxide at 200 ppm; T4: Experimental diet supplemented with copper oxide at 300 ppm; T5: Experimental diet without supplementary copper sulphate; T6: Experimental diet supplemented with copper sulphate at 100 ppm; T7: Experimental diet supplemented with copper sulphate at 200 ppm; T8: Experimental diet supplemented with copper sulphate at 300 ppm

Table 2: External egg qualities of laying hens fed diets supplemented with two sources of copper (mean ± SD)

TREATMENT	Level of Copper (mg)	Egg Weight (g)	Egg Length (mm)	Egg Width (mm)	Shell Weight (g)	Shell and Membrane (g)	Shell Membrane (g)	Shell Thickness (mm)	Shell Ratio	Egg Shape Index
CuO	0	51.51 ± 0.95	40.40 ± 0.61	28.63 ± 0.51	4.92 ± 0.11^{b}	6.00 ± 0.11	1.09 ± 0.03	0.30 ± 0.01	9.57 ± 0.15	0.71 ± 0.07
CuO	100	52.13 ± 0.70^{a}	41.20 ± 0.50	28.23 ± 0.52	4.76 ± 0.08	5.83 ± 0.10	1.07 ± 0.04	0.29 ± 0.01	9.14 ± 0.11^{b}	0.68 ± 0.07
CuO	200	50.90 ± 0.75	40.80 ± 0.52	27.81 ± 0.44^{b}	4.67 ± 0.09^{b}	5.68 ± 0.11^{b}	1.01 ± 0.03	0.28 ± 0.01^{b}	9.19 ± 0.16	0.68 ± 0.06^{b}
CuO	300	51.51 ± 0.64	40.10 ± 0.41^{b}	28.25 ± 0.41^{b}	4.90 ± 0.07	5.90 ± 0.08	1.00 ± 0.03^{b}	0.26 ± 0.01^{b}	9.53 ± 0.09	0.70 ± 0.05
$CuSO_4$	0	53.33 ± 0.70	41.10 ± 0.50	28.61 ± 0.51	5.18 ± 0.08^{a}	6.23 ± 0.09	1.05 ± 0.03	0.31 ± 0.01	9.73 ± 0.09	0.69 ± 0.09
$CuSO_4$	100	49.68 ± 0.59^{b}	40.50 ± 0.41	28.40 ± 0.43	4.73 ± 0.08	5.74 ± 0.09	1.01 ± 0.04	0.29 ± 0.01	9.52 ± 0.13^{a}	0.70 ± 0.06
$CuSO_4$	200	53.05 ± 0.92	42.30 ± 0.62	29.83 ± 0.50^{a}	5.07 ± 0.13^{a}	6.12 ± 0.14^{a}	1.06 ± 0.03	0.33 ± 0.00^{a}	9.64 ± 0.22	0.71 ± 0.07^{a}
$CuSO_4$	300	53.00 ± 0.75	43.00 ± 0.50^{a}	29.95 ± 0.51^{a}	5.01 ± 0.11	6.10 ± 0.11	1.10 ± 0.02^{a}	0.30 ± 0.01^{a}	9.44 ± 0.14	0.69 ± 0.07
MEAN SEPARATION										
Level of Copper										
0		52.49 ± 0.59^{a}	40.80 ± 0.40	28.62 ± 0.32	5.06 ± 0.07^{a}	6.13 ± 0.07^{a}	1.07 ± 0.02	0.30 ± 0.00^{a}	9.65 ± 0.09^{a}	0.70 ± 0.06
100		50.87 ± 0.47^{b}	40.90 ± 0.30	28.33 ± 0.31	4.74 ± 0.06^{b}	5.79 ± 0.06^{b}	1.04 ± 0.03	0.29 ± 0.00^{b}	9.34 ± 0.09^{b}	0.69 ± 0.05
200		52.03 ± 0.61^{ab}	41.60 ± 0.42	28.91 ± 0.41	4.88 ± 0.08^{ab}	5.91 ± 0.09^{b}	1.04 ± 0.02	0.30 ± 0.00^{a}	9.43 ± 0.14^{ab}	0.69 ± 0.05
300		52.18 ± 0.49^{ab}	41.40 ± 0.31	29.03 ± 0.32	4.95 ± 0.06^{a}	5.99 ± 0.07^{ab}	1.28 ± 0.05	0.28 ± 0.00^{b}	9.49 ± 0.08^{ab}	0.70 ± 0.04
Treatment										
CuO		51.54 ± 0.37	40.60 ± 0.22^{b}	28.24 ± 0.23^{b}	4.82 ± 0.04^{b}	5.86 ± 0.05^{b}	1.04 ± 0.02	0.28 ± 0.00^{b}	9.37 ± 0.06^{b}	0.69 ± 0.03
$CuSO_4$		52.20 ± 0.38	41.70 ± 0.32^{a}	29.23 ± 0.25^{a}	4.99 ± 0.05^{a}	6.04 ± 0.05^{a}	1.05 ± 0.02	0.31 ± 0.00^{a}	9.58 ± 0.07^{a}	0.69 ± 0.04
Statistical significance										
Treatment		0.20	0.002	0.004	0.01	0.01	0.59	< 0.0001	0.02	0.39
Level		0.13	0.27	0.45	0.01	0.01	0.78	< 0.0001	0.14	0.49
Treatment* Level		0.01	0.002	0.04	0.05	0.05	0.04	0.002	0.20	0.02

a,b: Means with different superscripts within column differ significantly ($p \leq 0.05$)

Data Analysis

All the data obtained were subjected to a 2 x 4 factorial analysis of variance (ANOVA) using the SAS Statistical Package (2008). The significant treatment means were compared using the Duncan Multiple Range test option of the same software. The procedure was reviewed and approved by the Federal University of Technology Akure Animal Care and Use Committee.

RESULTS

External egg quality

The external egg quality of laying hens fed diets supplemented with two sources of copper is shown in Table 2. The results revealed that the different levels of inclusion of the copper had a significant ($p \leq 0.05$) effect on the shell weight, shell + membrane, and the shell thickness. The highest significant values (5.06 ± 0.07 g, 6.13 ± 0.07 g and 0.30 ± 0.00 mm) for these qualities, were observed in the control. The other external egg qualities that were not significantly different ($p \geq 0.05$) had various highest numerical values at different levels of inclusion. The highest numerical values for egg weight (52.49 ± 0.59 g), shell ratio (9.65 ± 0.09) and egg shell index (70.18 ± 0.57) were observed in the control. The egg width (2.90 ± 0.03 cm) and shell membrane (1.28 ± 0.05 g) had numerical values ($p \geq 0.05$) observed at 300 mg of inclusion level while that of egg length (4.16 ± 0.04 cm) was noticed in 200 mg of inclusion level. There was significant difference ($p \leq 0.05$) in the qualities except for the egg weight, shell membrane and egg shell index due to the treatments with CuO and $CuSO_4$. Results showed significant and numerically higher values were observed in the hens treated with $CuSO_4$ for all the egg qualities examined.

The interaction of the different levels of inclusion of the two sources of copper had a significant ($p \leq 0.05$) effect in all the external egg qualities excluding the shell ratio. For the interaction between 100 mg CuO and 100 mg $CuSO_4$, significant difference ($p \leq 0.05$) was only observed in the egg weight with the significantly higher value (52.13 ± 0.70 g) observed in 100 mg CuO. In addition, the interaction of CuO and $CuSO_4$ at 200 mg also had a significant effect ($p \leq 0.05$) in the shell + membrane and egg shell index with the higher values (6.12 ± 0.14 g and 70.49 ± 0.71) in $CuSO_4$. Also, for the interaction of CuO and $CuSO_4$ at 300 mg, egg length and shell membrane had the higher values (4.30 ± 0.05 mm and 1.10 ± 0.02 g respectively) in $CuSO_4$ supplemented group. The egg width and shell thickness were significantly different in both 200 and 300 mg of CuO and $CuSO_4$ with the significantly higher values ($p \leq 0.05$) observed in $CuSO_4$.

Internal egg quality

The internal qualities of the eggs laid by the hens fed diets supplemented with two sources of copper are presented in Table 3. For the treatment with CuO and $CuSO_4$, the results revealed that the albumen height (7.5 ± 0.01 mm), yolk height (1.77 ± 0.01 mm) and yolk weight (12.87 ± 0.11 g) of eggs collected from hens fed $CuSO_4$ were significantly ($p \leq 0.05$) higher than those collected from hens fed CuO. The higher values for the albumen weight (14.17 ± 0.32 g), yolk length (2.95 ± 0.02 cm), egg mass (36.29 ± 0.34) and Haugh unit (99.98 ± 0.01) were observed in the eggs collected from the hens fed $CuSO_4$ and they were insignificantly different ($p \geq 0.05$) from CuO. The albumen length (6.75 ± 0.05 cm) and yolk index (0.60 ± 0.01) were observed to be higher ($p \geq 0.05$) for eggs obtained from hens treated with CuO.

All the yolk parameters (yolk height, weight, length and index) with albumen height were significantly ($p \leq 0.05$) influenced by the different levels of copper inclusion. Significantly highest values for albumen height (7.5 ± 0.01 mm) and yolk weight (13.00 ± 0.19 g) were observed in the control while yolk height (1.78 ± 0.02 mm) and yolk index (0.062 ± 0.01) were noticed to be highest ($p \leq 0.05$) at the 200 mg of inclusion. Significantly highest yolk length (2.99 ± 0.02 cm) was seen at the 300 mg. The other parameters were insignificantly ($p \geq 0.05$) different with their numerically higher values noticed at different levels: albumen length (6.84 ± 0.09 cm) at the control, Haugh unit (100.00 ± 0.01) at 100 mg, albumen weight (14.31 ± 0.49 g) at 200 mg, and egg mass (36.85 ± 0.45) at 300 mg.

The interaction of the different levels of inclusion with the two sources of copper had a significant ($p \leq 0.05$) influence on the albumen height, albumen weight, yolk length, yolk index and egg mass. This significant effect was observed in albumen height at the interaction of control, 100 and 200 mg of CuO and $CuSO_4$, with the highest significant mean (7.8 ± 0.01 mm) observed in 0 mg $CuSO_4$. For the albumen weight, yolk length, yolk index and egg mass, the significant ($p \leq 0.05$) effect of the interaction was noticed at the different levels of $CuSO_4$ but $CuSO_4$ control had the highest mean for albumen weight (14.85 ± 0.59 g) and egg mass (37.21 ± 0.75). Yolk index and yolk length have their significant highest means (0.062 ± 0.01 and 3.03 ± 0.03 cm) at 200 mg $CuSO_4$ and 300 mg $CuSO_4$, respectively.

Egg and excreta cholesterol level

Egg and excreta cholesterol level of the laying hens fed diets supplemented with the two sources of copper is shown in Table 4. For copper oxide, it was observed that the control had consistently the highest cholesterol values for the yolk (15.70 mg), whole egg (227.17 mg) and albumen (211.47 mg) except for the excreta where the lowest value for cholesterol was

Table 3: Internal egg qualities of laying hens fed diets supplemented with two sources of copper (mean ± SD)

TREATMENT	Level of Copper (mg)	Albumen Height (mm)	Albumen Weight (g)	Albumen Length (cm)	Yolk Height (mm)	Yolk Weight (g)	Yolk Length (cm)	Yolk Index	Egg Mass	Haugh Unit
CuO	0	7.30 ± 0.21^{b}	13.38 ± 0.59^{b}	6.90 ± 0.14	1.69 ± 0.03	12.96 ± 0.17^{b}	2.89 ± 0.06	0.059 ± 0.001^{b}	34.03 ± 0.98^{b}	99.97 ± 0.03
CuO	100	7.10 ± 0.20^{b}	14.46 ± 0.41	6.70 ± 0.08	1.75 ± 0.02	12.64 ± 0.17^{b}	2.94 ± 0.03	0.060 ± 0.001	36.57 ± 0.57^{b*}	99.95 ± 0.02
CuO	200	6.81 ± 0.22^{b}	13.81 ± 0.56	6.78 ± 0.09	1.77 ± 0.03	12.22 ± 0.19^{b}	2.90 ± 0.03	0.061 ± 0.001	35.40 ± 0.87	99.97 ± 0.02
CuO	300	7.10 ± 0.13	13.74 ± 0.43	6.66 ± 0.09	1.75 ± 0.02	12.77 ± 0.14^{b}	2.95 ± 0.03^{b}	0.060 ± 0.001	37.02 ± 0.58	99.98 ± 0.02
$CuSO_4$	0	7.82 ± 0.12^{a}	14.85 ± 0.59^{a}	6.78 ± 0.12	1.79 ± 0.02	13.03 ± 0.32^{a}	2.92 ± 0.03	0.061 ± 0.001^{a}	37.21 ± 0.75^{a}	99.97 ± 0.02
$CuSO_4$	100	7.40 ± 0.15^{a}	14.85 ± 0.59	6.78 ± 0.12	1.79 ± 0.02	13.03 ± 0.32^{b}	2.92 ± 0.03	0.061 ± 0.001	37.21 ± 0.75^{a*}	99.97 ± 0.02
$CuSO_4$	200	7.70 ± 0.25^{a}	14.76 ± 0.79	6.73 ± 0.18	1.79 ± 0.02	12.96 ± 0.21^{ab}	2.90 ± 0.03	0.062 ± 0.001	36.36 ± 0.73	99.97 ± 0.02
$CuSO_4$	300	7.41 ± 0.12	14.26 ± 0.58	6.80 ± 0.08	1.77 ± 0.01	13.03 ± 0.17^{ab}	3.03 ± 0.03^{a}	0.059 ± 0.001	36.65 ± 0.72	99.95 ± 0.02
MEAN SEPARATION										
Level of Copper										
0		7.52 ± 0.11^{a}	14.17 ± 0.42	6.84 ± 0.09	1.74 ± 0.02^{b}	13.00 ± 0.19^{a}	2.91 ± 0.03^{b}	0.060 ± 0.001^{ab}	35.74 ± 0.62	99.97 ± 0.01
100		7.21 ± 0.12^{b}	13.69 ± 0.36	6.66 ± 0.06	1.75 ± 0.02^{ab}	12.56 ± 0.11^{b}	2.95 ± 0.02^{ab}	0.060 ± 0.001^{b}	35.77 ± 0.36	100.00 ± 0.01
200		7.22 ± 0.20^{b}	14.31 ± 0.49	6.76 ± 0.10	1.78 ± 0.02^{a}	12.60 ± 0.14^{ab}	2.90 ± 0.02^{b}	0.062 ± 0.001^{a}	35.90 ± 0.56	99.96 ± 0.01
300		7.30 ± 0.12^{b}	13.97 ± 0.35	6.72 ± 0.06	1.76 ± 0.01^{a}	12.89 ± 0.11^{ab}	2.99 ± 0.02^{a}	0.059 ± 0.001^{b}	36.85 ± 0.45	99.96 ± 0.01
Treatment										
CuO		7.01 ± 0.13^{b}	13.86 ± 0.25	6.75 ± 0.05	1.74 ± 0.01^{b}	12.66 ± 0.08	2.92 ± 0.02	0.060 ± 0.001	35.89 ± 0.37	99.97 ± 0.01
$CuSO_4$		7.50 ± 0.10^{a}	14.17 ± 0.32	6.73 ± 0.06	1.77 ± 0.01^{a}	12.87 ± 0.11	2.95 ± 0.02	0.060 ± 0.001	36.29 ± 0.34	99.98 ± 0.01
Statistical significance										
Treatment		< .0001	0.43	0.85	0.03	0.03	0.20	0.58	0.41	0.25
Level		0.02	0.72	0.42	0.02	0.05	0.02	0.04	0.30	0.28
Treatment* Level		0.01	0.04	0.66	0.08	0.13	0.05	0.04	0.01	0.10

a,b: Means with different superscripts within column differ significantly ($p \leq 0.05$)

Table 4: Egg and excreta cholesterol levels of laying hens fed diets supplemented with two sources of copper (mean ± SD)

TREATMENT	Level of Copper (mg)	Yolk (mg)	Whole Egg (mg)	Albumen (mg)	Excreta (mg)
CuO	0	15.70 ± 0.16	227.17 ± 2.44	211.47 ± 2.30	134.16 ± 1.97
CuO	100	13.64 ± 0.18	192.20 ± 0.83	178.55 ± 0.72	148.86 ± 3.68
CuO	200	13.64 ± 0.08	187.55 ± 3.32	173.91 ± 3.25	183.90 ± 1.77
CuO	300	12.88 ± 0.11	177.58 ± 3.44	164.70 ± 3.52	202.28 ± 3.83
$CuSO_4$	0	15.27 ± 0.42	228.25 ± 1.94	212.98 ± 2.15	134.91 ± 1.21
$CuSO_4$	100	13.37 ± 0.29	189.11 ± 3.78	175.74 ± 3.99	156.53 ± 4.29
$CuSO_4$	200	12.35 ± 0.04	172.01 ± 4.15	159.66 ±4.12	185.33 ± 0.50
$CuSO_4$	300	12.26 ± 0.06	169.99 ± 4.67	157.74 ± 4.68	207.38 ± 0.59
MEAN SEPARATION					
Level of Copper					
0		15.49 ± 0.22[a]	227.71 ± 1.41[a]	212.23 ± 1.45[a]	134.28 ± 1.03[a]
100		13.51 ± 0.16[b]	190.65 ± 1.86[b]	177.15 ± 1.92[b]	152.70 ± 3.06[b]
200		12.99 ± 0.29[c]	179.78 ± 4.21[c]	166.79 ± 3.96[c]	184.62 ± 0.88[c]
300		12.57 ± 0.15[c]	173.79 ± 3.10[c]	161.22 ± 3.05[c]	204.83 ± 2.07[d]
Treatment					
CuO		13.96 ± 0.32[a]	196.12 ± 5.75[a]	182.16 ± 5.44[a]	167.30 ± 8.27
$CuSO_4$		13.31 ± 0.38[b]	189.84 ± 7.23[b]	176.53 ± 6.89[b]	170.91 ± 8.42
Statistical significance					
Treatment		0.0001	0.016	0.029	0.071
Level		< 0.0001	< 0.0001	< 0.029	< 0.0001
Treatment* Level		0.111	0.112	0.149	0.496

a,b: Means with different superscripts within column differ significantly ($p \leq 0.05$)

observed (134.16). Also it was observed that 300 mg copper oxide inclusion level consistently had the lowest cholesterol values for yolk (12.88 mg), whole egg (177.58 mg) and albumen (164.70 mg) except for excreta (202.28 mg) that had the highest cholesterol value. The same trend was also observed for copper sulphate where the control had the highest cholesterol values for yolk (15.27 mg), whole egg (228.25 mg) and albumen (212.98 mg) except for excreta (134.91 mg). The 300 mg inclusion level had consistently the lowest cholesterol values for yolk (12.26), whole egg (169.99) and albumen (157.74) but the highest cholesterol value was observed for the excreta (204.83). From the results, it was observed that an inverse relationship existed between values of egg cholesterol parameters and waste cholesterol values.

It was also observed that the cholesterol values for the parameters of the birds fed different levels of copper sulphate supplementary diet compared with the cholesterol levels in the parameters of the birds on copper oxide supplemented diet were lower. However, the cholesterol levels for the excreta for birds on copper sulphate diets had higher values than their counter parts on copper oxide supplementary diet (Table 4). This shows that copper sulphate was more effective in reducing yolk, whole egg and albumen cholesterol than copper oxide. In addition, it was observed from the results that dietary copper sulphate at the different levels of inclusion contributed to more loss of cholesterol in the excreta than copper oxide. Significant differences ($p \leq 0.05$) were observed for yolk, whole egg and albumen except for the excreta for the treatment. For the level of inclusion, the four parameters were significant ($p \leq 0.05$). However, for the interaction, significant difference was not observed for any of the parameters.

DISCUSSION

External egg quality

Copper (VI) sulphate ($CuSO_4$) had an increasing influence on the majority of the external qualities examined in this study as it was seen in the egg length,

egg width, shell weight, shell and membrane weight, shell thickness and shell ratio. Although these results were in contrast to Kaya and Macit (2012) who observed that the supplementation of copper in laying chicken diets did not have significant effect on egg quality traits, supplementation of $CuSO_4$ in the hens produced eggs with better external qualities. Egg quality traits such as weight, shell membrane and shell index were not affected by the main effects of Cu source. Shells from eggs laid by birds fed $CuSO_4$ were thicker than those of CuO. This was not in accordance with Holoubek *et al*. (2002) who reported an insignificant effect of copper on the values of eggshell weight and shell thickness.

The different levels of inclusion of the two copper sources in the diet of the hens showed alterations in few of the external egg qualities. These are the shell weight, shell and membrane weight as well as the shell thickness. Egg shell weight and shell thickness obtained for 200 $mg.kg^{-1}$ diets did not differ from the control group (0 $mg.kg^{-1}$ diet) but numerically lower shell weight was recorded in comparison with the control. Meanwhile, the linear improvements in majority of the external egg qualities, as a result of the three levels of copper sources, indicated that the hens can tolerate both CuO and $CuSO_4$ up to 300 $mg.kg^{-1}$ without adversely affecting the external qualities of the eggs. This was supported by the report of Thomas and Goatcher (1976) that dietary Cu concentrations as high as 480 $mg.kg^{-1}$ had no adverse effect on egg production and external qualities. The interaction of Cu source and level of supplementation indicated significant influence on all the external egg qualities evaluated excluding shell ratio. The better external egg qualities noticed in the hens fed $CuSO_4$ could be due to its ability to be absorbed and utilized more than CuO. Though the egg weight was not influenced statistically, it was observed to reduce with the different levels of the copper salts when compared with the control. This was in support of Pekel and Alp (2011) that postulated that supplementation with 250 $mg.kg^{-1}$ of $CuSO_4$ decreased egg weight. It means that the different levels of copper inclusion did not have any significant negative effect on the egg weight.

Internal egg quality

In this study, eggs laid by the hens fed $CuSO_4$ had longer albumen with no significant effect on the Haugh unit but a slight numerical improvement. This was against the findings of Idowu *et al.* (2006) who noted a significant effect in the Haugh unit of eggs collected from laying hens fed copper salts. Similarly, Jensen *et al.* (1978) found that adding Cu to the diet of laying domestic fowl affected egg quality significantly; this was reflected in an increase in the Haugh units of the eggs. This report was against the results of this study where the Haugh unit was not affected by the inclusion of copper.

Moreover, layers fed the diets supplemented with $CuSO_4$ presented improved yolk yield relative to the CuO treatment. This was in consonance with the observation reported by Jegede *et al.* (2011), that sulphate form of Cu resulted in higher yolk level. Meanwhile, in the experiment carried out by Kaya *et al.* (2013), no significant effect on egg quality traits, except for yolk color was observed following the dietary inclusion of copper into layers' diet. The different levels of copper supplementation had a great influence on the albumen height and all the yolk parameters examined. This inclusion of copper salts caused the albumen height and yolk weight to reduce relative to the control while the yolk height and length improved but Kaya *et al*. (2013) reported that it was only the yolk color, among the internal egg qualities, that was influenced when layers were fed diets supplemented with copper.

Egg and Excreta Cholesterol Level

Copper, according to Kim *et al.* (1992), Pekel and Alp (2011) and Jegede *et al.* (2015) regulates cholesterol biosynthesis by reducing hepatic glutathione concentration and thereby reducing cholesterol content of egg. The regulatory effect of copper was observed in the cholesterol level of egg; yolk, whole egg and albumen from birds fed copper diets as they were lower compared to those of eggs in birds on the control diet. These results support the findings of other authors (Al Ankari *et al*., 1998; Pesti and Bakalli, 1998; Balevi and Coskun, 2004; Idowu *et al.*, 2006; Lim *et al*., 2006; Jegede *et al.*, 2015). However, our results are not in support of Pekel and Alp (2011) who worked on laying hens and reported that dietary copper intake through the use of different copper compounds did not affect the cholesterol of egg yolk. It was observed from the results that egg yolk cholesterol reduced linearly as copper concentration increased which supports the report of Idowu *et al.* (2006).

The cholesterol content of egg has an inverse relationship with the cholesterol content in excreta in animals fed copper diet even due to the fact that excess cholesterol removed in the system of the animal is present in the excreta. The higher the concentration of copper in the diet of hens, the lower the cholesterol contents of their eggs and the higher the content in excreta. This explains the trend in excreta cholesterol in the results of the experiment. The results of excreta cholesterol was found to consistently increase as the copper dosage in diets increased linearly. These results support the findings of Leeson (2009). From the results, an inverse relationship was observed between the cholesterol values for the egg yolk and the cholesterol values for excreta for the different treatments and at different inclusion levels. This supports the findings of Idowu *et al.* (2006) in their work on laying hens who reported increase in the values of excreta cholesterol as the cholesterol values

in the egg parameters decreased. It was also observed that inclusion levels of copper in the diet of laying hens had significant effect on the cholesterol levels in their excreta in this study which supports the report of Idowu *et al.* (2006).

CONCLUSIONS

The two sources of copper did not influence the external egg qualities negatively but hens fed $CuSO_4$ had eggs with better external qualities especially shell thickness. Though the albumen height was influenced, the Haugh unit, being the unit for measuring the quality of an egg, was not affected. Yolks of eggs obtained from hens fed $CuSO_4$ were longer and heavier than those from hens fed CuO. Copper oxide was more effective in reducing the cholesterol level in eggs compared to copper sulphate, hence it promotes production of healthier eggs for consumption. Therefore, it can be concluded that the hen could tolerate the Cu sources up to 300 $mg.kg^{-1}$ which also supports the claim of NRC (1994).

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