

## Performance characteristics, plasma lipids and copper residue in organs and tissues of cockerel chickens fed dietary organic and inorganic copper

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### Abstract

A 112-day study was conducted to determine the effect of dietary organic (Copper proteinate) and inorganic (Copper sulphate) Cu source on growth performance, plasma lipids and copper residue in organs and tissues of cockerel chickens. 240 day-old commercial Black-Harco cockerel chicks were randomly distributed to 6 dietary treatments of 40 birds each. Each treatment group was randomly allocated to 4 replicates of 10 birds each. The diets were formulated to contain a basal diet (containing 30.62 and 29.71 mg/kg Cu for starter and finisher phases respectively) supplemented with organic Cu (Cu proteinate; Cu-P) or inorganic Cu (Cu sulphate; CuSO<sub>4</sub>) fed at 3 dietary levels (50, 100 and 150 mg/kg). The birds were fed chicks mash and grower mash at 1-56 days and 57-112 days respectively in a deep litter pen at floor space of 0.06 m<sup>2</sup>/bird. Cu-P supplementation resulted in significantly higher ( $P < 0.05$ ) final live weight (FLW) and total weight gain (TWG) compared to CuSO<sub>4</sub> at starter phase. Better feed conversion ratio was noticed in birds fed Cu-P supplemented diets compared to CuSO<sub>4</sub> at 150mg/kg Cu concentration at both starter and finisher phases. Birds fed 150 mg/kg Cu recorded the highest ( $P < 0.05$ ) feed intake value at starter phase. There was significantly higher ( $P < 0.05$ ) accumulation of Cu in the blood, heart, lung, liver and thigh of birds fed Cu-P than those fed CuSO<sub>4</sub>. The liver Cu concentration increased as dietary Cu concentration increased. Cu-P supplementation resulted in significant reduction ( $P < 0.05$ ) in plasma cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL) and triglyceride in comparison to CuSO<sub>4</sub>. The blood cholesterol, LDL and triglyceride decreased with increased copper concentration. Organic form of copper promotes growth, more bioavailable and more effective in reducing cholesterol than copper sulphate.

**Keywords:** Cockerels chickens, copper residue, and performance characteristics

### Introduction

Copper is one of the essential trace minerals in poultry nutrition. Cu is contained in a number of enzymes and proteins, and its role has been defined for many physiological functions related mostly to a catalytic agent in the active sites of cuproenzymes (McDowel, 1992). Feeding pharmacological levels of copper has been

reported to play important role in lipid metabolism (Bakalli *et al.*, 1995). Cu supplementation significantly reduced the cholesterol in broiler meat (Bakalli *et al.*, 1995; Konjufca *et al.*, 1997; Skrivan *et al.*, 2000; Mondal *et al.*, 2007; Jegede *et al.*, 2011). There has been increasing interest in organic or chelated copper. This is the form in which it occurs in nature. Recent studies

suggest that organic sources of Cu may be more reactive in promoting growth than CuSO<sub>4</sub>. Reports from various workers (Hemken *et al*, 1993; Du *et al*, 1996; Jegede *et al*, 2011) confirm that metal chelates of amino acids and peptides can enhanced the bioavailability of trace element. However some authors (Paik *et al.*, 1999; Lee *et al.*, 2001; Paik, 2001) reported absence of such effect for copper sources. This study was carried out to investigate the utilization of organic and inorganic Cu sources and their effects on the performance, copper residue in organs and tissues and lipid profile of cockerel chickens.

## **Materials and Methods**

### *Experimental Site*

The research work was carried out at the Poultry Unit of the Directorate of University Farms (DUFARM), Federal University of Agriculture, Alabata, Abeokuta, Nigeria (Latitude 7° 13' 49.46"N and Longitude 3° 26' 11.98"E). This area lies in the tropical climate with an average rainfall of 1037mm, mean ambient temperature of about 34°C and yearly relative humidity of 82% (Google Earth, 2010)

### *Copper Sources*

The copper sources used are copper proteinate and copper sulphate. The copper proteinate was obtained from Alltech® Inc. USA. It contained 10% copper. Feed grade copper sulphate used was obtained from Lucaris Limited Lagos Nigeria. It contain 25% copper. Other feed ingredients were purchased from a reputable commercial feed mill in Abeokuta.

### *Management of Experimental Birds*

240 day-old Black Harco cockerels were used. The birds were allocated into 6 treatment groups of 40 birds each. Each group had 4 replicates of 10 birds each. The birds were managed intensively in a deep

litter pen (0.06m<sup>2</sup> per bird) for 112 days. The diets were formulated such that the added copper sources (copper proteinate (Cu-P) and copper sulphate (CuSO<sub>4</sub>)) were included in the basal diet already containing 30.62mg/kg and 29.17mg/kg Cu for chicks (1-56 d) and finisher (57-112 d) phases respectively at three concentration (50, 100 and 150mg/kg) (Table 1). Feed and water were supplied *ad-libitum*.

### *Chemical Analysis*

The dietary ingredients were dried at 65°C for 36 hours in an oven and ground to pass through 1mm sieve and were analysed for dry matter (DM), crude fibre (CF), ether extract (EE), and total ash (AOAC, 1995, ID 7.101, 7.048, 7.016). The nitrogen fraction of feed samples was determined using kjeldahl destruction method and crude protein (CP) was determined by multiplying the N value by 6.25. The copper content of the basal diet was estimated by igniting the feed sample at 400°C for 4 hours in a muffle furnace. The ash was reconstituted using wet-ashing procedure (James, 1996). Analysis of Cu was estimated by using a Perkin Elmer Optima 4300DV ICP spectrophotometer (Perkin Elmer, Beaconsfield, UK)

### *Biochemical, Haematological and Cholesterol Analysis*

At the 16<sup>th</sup> week of the study, blood samples were collected through the wing veins of 2 birds per replicate (8 birds per treatment). 2.5ml of blood was collected into tubes containing Ethylene diamine tetra acetate (EDTA) and another 2.5ml was collected into a hypodermic syringe. The blood in EDTA bottles were used to determine haematological parameters, viz: packed cell volume (PCV), red blood cell (RBC), white blood cell (WBC) and haemoglobin (Hb) while those in the hypodermic syringe were used to determine serum parameters

**Table 1: Basal Composition of Experimental Diets (g/100g)**

Ingredients	Starter mash	Finisher mash
Maize	48.00	45.00
Fishmeal (72% CP)	2.00	2.00
Soyabean meal	15.00	12.00
Groundnut cake	11.00	8.00
Wheat offal	19.30	28.30
Bone meal	2.50	2.50
Oyster shell	1.50	1.50
Lysine	0.10	0.10
Methionine	0.10	0.10
Premix	0.25	0.25
Salt	0.25	0.25
Total	100.00	100.00
<b>Determined Analysis</b>		
Energy (ME/Kcal/kg)	2830.90	2655.00
Crude protein %	22.65	17.65
Ether extract%	4.36	4.62
Crude fibre%	9.48	8.90
Calcium%	1.32	1.34
Available Phosphorus%	0.48	0.48
Basal Cu (mgkg <sup>-1</sup> )	32.85	31.78

Vitamins / mineral premix (Godomix) based on 2.5kg per ton.

*Vit. A: 3,200,000IU, vit.D: 640,000 IU, vit E: 2000mg, vit k<sub>3</sub>: 800mg, vitB<sub>1</sub>: 2000mg, vit B<sub>2</sub>: 6000mg, vit. B<sub>6</sub>: 5000mg, vit B<sub>12</sub>: 25mg, Niacin: 6000mg, Panthotenic Acid: 20000mg, Folic Acid: 1000mg, Biotin: 8mg, Manganese: 30000mg, Iron: 20000mg, Zinc: 20000mg, Copper: 80000mg BTH: 25,000mg Anticaking agent: 6,000mg*

(total serum protein, albumin, globulin, uric acid and creatinine). The PCV was determined by a microhaematocrit method (Baker and Silvertown, 1985) while Hb and RBC were determined using colorimetry cyanomethaemoglobin and improved Neubauer haemocytometer methods respectively (Jain, 1986). The Serum total protein, Albumin and Globulin were analysed colorimetrically using diagnostic reagent kit (Varley *et al.*, 1980).

Plasma was separated from the blood samples in EDTA bottles with a micro pipette into a test tube for plasma lipid analysis. The cholesterol and triglyceride assay of the blood plasma were done by enzymatic-colorimetric methods

(according to manufacturer's manual) using Randox<sup>R</sup> diagnostic cholesterol kit (BIOLAB with code 80106.2 x 100ml cholesterol CHOD-PAP<sup>R</sup> ) and Randox<sup>R</sup> diagnostic triglyceride reagent procedure (GPO-PAP Method Randox Laboratory Ltd, UK) respectively. Plasma HDL was determined by phosphotungstic acid method, LDL by colorimetric method and total lipids were analysed in the Automatic Blood Analyser using Commercial Kit (Labkit, Chemelex, S.A. Spain)

#### *Visceral Organ and Bone Copper Determination*

Livers, kidneys, hearts, lungs and tissues from thigh and breast were excised and stored at -20 °C. The samples were dried at

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100°C for 36hrs and digested using a modified wet-ashing procedure (James, 1996). Ash was reconstituted in 5ml of 1 N HCl solution and analyzed for copper Perkin Elmer Optima 4300DV ICP spectrophotometer (Perkin Elmer, Beaconsfield, UK)

*Data Collection*

Data on growth indices such as body weight, weight gain, feed intake and feed conversion ratio were collected weekly

*Experimental Design and Statistical Analysis*

The experimental layout was 2 (sources of copper) x 3 (concentration of copper) factorial arrangements. The data obtained were subjected to analysis of variance in completely randomized design using MINITAB statistical software (MINITAB, 2000). Significant means among variables were separated using Duncan Multiple Range Test of the same package at 5% level of significance

**Results**

The results of the performance characteristics of cockerel chickens fed

diets supplemented with dietary copper sources at 1-112 d is presented in Table 2. During 1-56 d, the final live weight (FLW) and the total weight gained (TWG) of the birds were significantly improved (P<0.05) by Cu-P supplementation compared to those fed CuSO<sub>4</sub>. Significantly better (P<0.05) feed conversion ratio (FCR) value was reported for birds that received Cu-P supplemented diets. The source of copper did not significantly (P>0.05) influence the feed intake. The total feed intake and FCR was significantly influenced by concentration of supplementary copper. There was a linear increase in total feed intake (TFI) as Cu concentration increased. A significantly better (P<0.05) FCR was observed when the birds were fed diets containing 50mg/kg dietary copper. The levels of Cu supplementation did not significantly influence the FLW and TWG, The interaction of Cu sources and Cu levels did not significantly influence the growth parameters measured (Table not shown). At 57-112 d, the TWG of the birds fed Cu-P was significantly higher than those fed CuSO<sub>4</sub>. Better (P<0.05) FCR was achieved

**Table 2: Main effect of Cu sources and Cu levels on the performance characteristics of starter cockerels (1-112 d)**

	Cu Sources			Cu concentration			SEM
	Organic Cu	CuSO <sub>4</sub>	SEM	10ppm	50ppm	100ppm	
<b>1-56 d</b>							
Final weight	455.06 <sup>a</sup>	440.44 <sup>b</sup>	4.78	438.58	448.70	455.87	5.72
TWG	416.25 <sup>a</sup>	403.59 <sup>b</sup>	4.67	401.17	409.18	419.41	5.34
TFI	540.77	558.82	10.79	529.11 <sup>b</sup>	540.96 <sup>b</sup>	579.32 <sup>a</sup>	10.35
FCR	1.30 <sup>b</sup>	1.38 <sup>a</sup>	0.019	1.32 <sup>b</sup>	1.32 <sup>b</sup>	1.38 <sup>a</sup>	0.024
<b>57-112 d</b>							
FLW	1262.73	1205.55	20.05	1252.50	1239.84	1214.58	28.20
TWG	821.38 <sup>a</sup>	749.59 <sup>b</sup>	21.10	818.13	794.56	743.76	28.60
TFI	5349.37	5409.32	144.6	5407.08	5510.59	5670.37	156.66
FCR	6.52 <sup>b</sup>	7.22 <sup>a</sup>	0.23	6.62 <sup>b</sup>	7.00 <sup>ab</sup>	7.70 <sup>a</sup>	0.34
<i>n</i>	12	12		8	8	8	

<sup>ab</sup>Mean on the same row with different superscripts are significantly different (P<0.05)

ILW = Initial live weight, FLW = Final live weight, TWG = Total weight gain

TFI = Total feed intake, FCR = Feed conversion ratio, SEM= Standard error of mean

<sup>1</sup> Added dietary copper to basal copper concentration (31.78mg/kg for growing phase)

SEM – Standard Error of Mean (Pooled)

*n*- number of observation per mean

when the birds were given diets supplemented with dietary Cu-P. High concentration of Cu supplementation resulted in poor utilization of the diets at finisher phase. The interaction of Cu sources and Cu levels (Table not shown) did not significantly ( $P>0.05$ ) influence the growth parameters measured for the finisher cockerels.

The effect of copper source and copper concentration on the accumulation of copper in organs of the birds is shown in Table 3. Birds fed diets containing Cu-P had a higher accumulation of Cu in the blood, heart, liver and thigh. Cu concentration in breast muscle was not significantly ( $P>0.05$ ) influenced by the Cu sources. The liver Cu was significantly influenced by Cu concentration. High dosage of Cu supplementation resulted in significantly higher ( $p<0.05$ ) liver Cu. The accumulation of Cu in the liver of the cockerel chicken was significantly influenced ( $P<0.05$ ) by the interaction of Cu sources and Cu concentration (Table 4) with birds fed diets containing 150ppm Cu-P having the highest ( $P<0.05$ ) liver concentration.

The concentration of plasma lipid profile of cockerel chickens fed diets supplemented with dietary copper source is presented in Table 5. The cholesterol, HDL, LDL and triglyceride of birds fed  $\text{CuSO}_4$  were higher ( $P<0.05$ ) than those fed Cu-p supplemented diets. However, total lipid concentration was not effected ( $P>0.05$ ) by dietary Cu source and Cu concentration. There was a significant reduction ( $P<0.05$ ) of cholesterol, LDL, and triglyceride as the concentration of Cu supplementation increased.

### Discussion

The result of growth performance of cockerel chickens showed an increased final live weight and weight gain in birds fed Cu-P supplemented diets. This result

attests to the fact that organic form of Cu has a higher growth promoting potential than  $\text{CuSO}_4$ . The study of Zhou *et al.*, (1994) reported a significantly higher growth rate for pigs fed organic Cu than  $\text{CuSO}_4$ . Close, (1998) also reported 10.8% improvement in growth rate of pigs fed Cu-P compared to those fed  $\text{CuSO}_4$ . Lower FCR value of birds fed organic Cu based diets is an indication that the diet was better utilized by the birds. The organic Cu may have been absorbed through a route different from that of  $\text{CuSO}_4$ , avoiding its possible interference with dietary constituents such as phytates, zinc and Fe metabolism, thus, making the Cu more available for growth (Du *et al.*, 1996; Fairweather-Tait, 1996).

The higher residue of copper in blood, heart, lung and thigh of the birds fed Cu-P than those fed  $\text{CuSO}_4$  suggest a more bioavailability and distribution of Cu-P into these organs than  $\text{CuSO}_4$ . Organic copper' that are most often chelates with amino acids or proteins are claimed to be preferentially absorbed and metabolized and provide less chance for antagonism of Cu with other minerals or substrates (Du *et al.*, 1996; Fairweather-Tait, 1996; Leeson and Summers, 2005).

The significantly higher accumulation of Cu in the blood, heart, lung, liver and thigh in birds fed Cu-P compared to those fed  $\text{CuSO}_4$  is an indication that Cu from Bioplex was more readily absorbed than the inorganic sources. The Cu in muscles (breast muscle) was not affected by Cu sources. The higher levels of Cu concentration resulted in higher level of liver Cu accumulation. Increased liver Cu of cockerels obtained with increasing dietary Cu levels implied high liver Cu accumulation. Previous studies also confirmed that liver Cu concentration is influenced by variations in dietary Cu

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**Table 3: Effect of Cu sources and Cu level on the Cu bioavailability in visceral organs and muscles of cockerels (mg/kg) at 112 d**

Parameters	Cu Sources			<sup>1</sup> Copper concentration (mg/kg)				Cu Source x Cu conc
	Cu-P	CuSO <sub>4</sub>	SEM	50	100	150	SEM	
Blood Cu	18.27 <sup>a</sup>	15.04 <sup>b</sup>	1.41	16.10	15.60	18.27	1.47	NS
Heart Cu	77.67 <sup>a</sup>	66.16 <sup>b</sup>	5.78	66.50	73.66	75.50	2.84	NS
Lung Cu	36.88 <sup>a</sup>	25.89 <sup>b</sup>	5.50	30.50	31.83	31.83	3.01	*
Liver Cu	103.56 <sup>a</sup>	87.44 <sup>b</sup>	9.06	83.35 <sup>b</sup>	90.45 <sup>b</sup>	109.03 <sup>a</sup>	2.84	**
Breast Cu	25.36	25.42	2.28	28.43	23.33	30.22	2.71	NS
Thigh Cu	43.45 <sup>a</sup>	38.67 <sup>b</sup>	2.64	39.67	36.50	47.00	3.23	NS
<i>n</i>	12	12		8	8	8		

<sup>ab</sup> Mean on the same row having different superscripts are significantly different (P<0.05)

<sup>1</sup> Added dietary copper to basal copper concentration (31.78mg/kg for growing phase)

SEM – Standard Error of Mean (Pooled)

*n*- number of observation per mean

**Table 4: Interactive effect of Cu sources and Cu level on the Cu bioavailability in visceral organs and tissues of cockerel chickens (mg/kg)**

Parameters	Cu-Proteinate (Cu-P)			<sup>1</sup> Cu Sulphate (CuSO <sub>4</sub> )			SEM
	50ppm	100ppm	150ppm	50ppm	100ppm	150ppm	
Blood Cu	20.00	21.00	24.00	19.00	17.00	19.33	0.873
Heart Cu	93.00	93.00	98.00	74.00	88.33	87.00	2.29
Lung Cu	40.00 <sup>b</sup>	52.33 <sup>a</sup>	42.33 <sup>b</sup>	37.00 <sup>b</sup>	27.33 <sup>c</sup>	37.33 <sup>b</sup>	2.32
Liver Cu	97.33 <sup>b</sup>	98.33 <sup>b</sup>	133.00 <sup>a</sup>	81.33 <sup>c</sup>	94.33 <sup>b</sup>	98.67 <sup>b</sup>	4.05
Thigh Cu	42.00	41.33	59.00	45.33	39.67	43.00	2.22
Breast Cu	32.33	26.67	26.67	31.33	26.00	39.33	1.74
Bone Cu	52.33	44.00	50.67	40.00	41.00	38.00	1.89
<i>n</i>	4	4	4	4	4	4	

<sup>abc</sup> Mean on the same row having different superscripts are significantly different (P<0.05)

<sup>1</sup> Added dietary copper to basal copper concentration (31.78mg/kg for growing phase)

SEM – Standard Error of Mean (Pooled)

*n*- number of observation per mean

**Table 5: Effects of Cu source and Cu concentration on plasma lipids of finisher cockerels at 112 d)**

Parameters	Cu Sources			<sup>1</sup> Copper concentration (mg/kg)				Cu Source x Cu conc
	Cu-P	CuSO <sub>4</sub>	SEM	50	100	150	SEM	
Cholesterol (mg/g)	145.43 <sup>b</sup>	211.23 <sup>a</sup>	3.06	207.04 <sup>a</sup>	176.69 <sup>b</sup>	151.28 <sup>c</sup>	3.74	NS
HDL(mg/g)	114.65 <sup>b</sup>	125.36 <sup>a</sup>	2.10	119.67 <sup>ab</sup>	111.14 <sup>b</sup>	129.17 <sup>a</sup>	2.58	NS
LDL (mg/g)	31.02 <sup>b</sup>	52.13 <sup>a</sup>	1.12	47.47 <sup>a</sup>	41.94 <sup>b</sup>	35.07 <sup>c</sup>	1.37	NS
Triglyceride( mg/g)	52.37 <sup>b</sup>	63.95 <sup>a</sup>	2.41	71.39 <sup>b</sup>	59.72 <sup>b</sup>	43.36 <sup>a</sup>	2.95	*
Total lipid (mg/dL)	308.82	311.67	3.43	313.72	313.45	303.57	4.20	NS
<i>n</i>	8	8		12	12	12		

<sup>abc</sup> Mean on the same row having different superscripts are significantly different (P<0.05)

<sup>1</sup> Added dietary copper to basal copper concentration (31.78mg/kg for growing phase)

SEM – Standard Error of Mean (Pooled)

*n*- number of observation per mean

\*- significant

NS- Not significant

concentrations (Guo *et al.*, (2001; Luo *et al.*, 2005). Liver Cu is a more reliable criterion of Cu status and relative bioavailability. Increased liver Cu concentration obtained with cockerels fed diet supplemented with Cu-P implied higher bioavailability. Chowdhury *et al.* (2004) reported high liver Cu concentration in broilers fed with organic Cu compared to those receiving inorganic Cu.

The lower values of total cholesterol, LDL and triglyceride observed following supplementation of Cu-P showed that Cu the form of proteinate was more effective in reducing cholesterol than sulphate when fed to cockerel chickens. Jegede *et al.* (2011) reported a similar finding that proteinate form of Cu was more effective in reducing cholesterol level than in sulphate form when fed to broilers. The previous studies of Skrivan *et al.*, (2000); Mondal *et al.*, (2007) and Jegede *et al.*, (2011) showed supplemental Cu at pharmacological levels in the diets decreased plasma and breast muscle cholesterol. This observation may be due to the fact that Cu regulates cholesterol biosynthesis by reducing hepatic glutathione concentration and changing oxidized glutathione ratio, which decrease the activity of 3-hydroxyl-3-methylglutaryl Co- (HMG-CoA) reductase (Kim *et al.*, 1992). Cu-P supplementation significantly reduced triglycerides concentration compared to CuSO<sub>4</sub> supplementation in cockerel chickens. This observation agreed with the findings of Mondal *et al.* (2007) who reported a better reduction of plasma triglycerides by organic Cu than inorganic Cu.

### Conclusion

It is evident from the study that, organic Cu in the form of copper proteinate played a superior role in terms of growth

performance, availability and distribution into organs and reduction of cholesterol in cockerel chicken

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