NATIONAL AGRICULTURAL AND FOOD CENTRE
RESEARCH INSTITUTE FOR ANIMAL PRODUCTION NITRA

Slovak J. Anim. Sci., 47, 2014 (3): 164-171

ISSN 1337-9984

EFFECTS OF DIETARY SUPPLEMENTATION WITH COPPER SULPHATE AND COPPER PROTEINATE ON PLASMA TRACE MINERALS, COPPER RESIDUES IN MEAT TISSUES, ORGANS, EXCRETA AND TIBIA BONE OF COCKERELS

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ABSTRACT

The present study was carried out to investigate the effect of dietary supplementation with copper sulphate ($CuSO_4$) and copper proteinate (CuP) on plasma trace minerals, Cu residues in meat tissues, organs, excreta and tibia bone of cockerels using two hundred and forty (240) day-old cockerel chicks arranged in a 2 × 3 factorial arrangements involving 2 copper (Cu) sources ($CuSO_4$, CuP) supplemented with 3 dosages (0, 50 and 100 $mg.kg^{-1}$). There were 6 treatment groups of 40 birds each replicated 5 times with 8 birds each. Experimental diets were fed for starter (0-8 weeks) and grower phases (8-16 weeks) of the bird. Increasing Cu dosage in the ration for cockerel chicks (0-8 weeks) resulted in increased ($P<0.0001$) Cu intake and improved ($P<0.05$) Cu bioavailability in the serum. Chicks fed diet supplemented with CuP showed increased ($P<0.05$) serum Zn concentration and reduced ($P<0.05$) Cu intake when compared to those fed diet supplemented with $CuSO_4$. At the grower phase (8-16 weeks), cockerels fed control diet had reduced serum Cu, while those fed diet supplemented with Cu, showed increased ($P<0.05$) serum Cu concentration. Dietary supplementation with CuP resulted in reduced ($P<0.05$) Cu intake and increased ($P<0.05$) serum Cu concentration when compared with cockerels fed diet supplemented with $CuSO_4$. Residual Cu concentration in breast meat tissue increased ($P<0.05$) with increasing dietary dosage of Cu. Dietary supplementation with CuP resulted in reduced ($P<0.05$) excreta Cu concentration, hence reduced environmental Cu pollution unlike birds fed diet containing $CuSO_4$ which showed increased excreta Cu concentration. Dietary inclusion of CuP showed increased ($P<0.05$) liver, heart and tibia bone Cu concentration when compared with birds fed diet supplemented with $CuSO_4$. Cockerels fed diet supplemented with 100 $mg.kg^{-1}$ Cu sourced from CuP recorded the highest ($P<0.05$) liver, heart and tibia bone Cu concentration. It was concluded that dietary inclusion of 100 $mg.kg^{-1}$ Cu sourced from CuP is recommended for improved Cu bioavailability in blood, tissue, organs and tibia bone of cockerels. To achieve reduced environmental Cu pollution resulting from excreta of poultry birds, organic salts of Cu, such as CuP, is recommended as feed additive.

Key words: day-old cockerels; organic copper; plasma trace minerals; serum Cu levels; tibia bone

INTRODUCTION

Copper (Cu) is an essential trace element necessary for various enzymatic systems in the body (Lim and Paik, 2006). It is required for skin pigmentation, proper functioning of the central nervous system, immune and cardiovascular system (Jegede *et al*., 2011). Minimum daily requirement of Cu for most avian species was reported as 5-8 $mg.kg^{-1}$ (Leeson, 2009). Cu is normally added as a feed additive into the ration for poultry. However, due to its growth-promoting and antimicrobial effects, Cu has

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Received: November 25, 2013
Accepted: January 16, 2014

been included at rather high concentrations far above pharmacological levels in feed for poultry (Skrivan *et al*., 2002).

In the past, Cu has been included in poultry feeds in form of inorganic salts like sulphates, carbonates, chlorides and oxides. Continuous use of these inorganic mineral salts as feed additives has been implicated in environmental mineral pollution arising from the accumulation of poultry wastes (Maheshwari, 2013). Intake of inorganic salts of minerals was reported to breaks down in the gastrointestinal tract to form very reactive free ions which binds dietary molecules and hinders absorption (Close, 1998). Hence, the use of organic sources of Cu has been advocated. Inclusion of organic salts of copper in feed for broilers has been reported to lead to high efficacy and improved bioavailability than their inorganic counterparts (Jegede *et al.*, 2011).

Dietary inclusion of mineral salts has been reported to show pronounced mineral residues in organs and animal tissues (Idowu *et al.*, 2006). Residues of heavy metals in poultry meat consumed by humans can be of public health importance to man's health. Dietary inclusion of zinc salts into the ration for poultry showed significant effect on Zn residues of meat tissues (Shyam Sunder *et al*., 2008), tibia bone, liver, excreta and egg shell of laying hens (Sahin *et al*., 2002). Idowu *et al.* (2011) reported that broilers fed diet supplemented with Cu showed some residual effect of Cu in the resultant meat tissues produced. However, no study has been conducted on the effect of inclusion of Cu salts on mineral residues in meat tissue, organs, excreta samples and tibia bone of cockerels. This study, therefore, aims to investigate the effect of dietary supplementation with copper sulphate (inorganic Cu salt) and copper proteinate (organic Cu salts) on plasma trace minerals, copper residues in meat tissue, organs, excreta samples and tibia bone of cockerels.

MATERIALS AND METHODS

Experimental site

The study was carried out at the Poultry Unit of the Teaching and Research Farm Directorate, Federal University of Agriculture, Abeokuta, Nigeria (Latitude 7° 13^{1} 49.46"N and Longitude 3° 26^{1} 11.98"E). Average temperature of 28.5 °C and mean annual rainfall of 1037 mm were recorded during the experimental period. The conduction of this study was agreed with the ethical policy and guideline of the Poultry Management Technical and Veterinary Committee of the university.

Cu sources

The Cu salts used in this study were Copper proteinate (CuP) and Copper sulphate ($CuSO_4 \bullet 5H_2O$). CuP was obtained from Alltech® Inc., USA (Alltech Inc. Kentucky, USA) and contained 10 % Cu (Bioplex Cu). Feed grade $CuSO_4$ containing 25 % Cu was purchased from Lucaris Limited, (Lucaris Nigeria Ltd, Lagos, Nigeria).

Experimental birds and dietary treatment

A total of 240 day-old cockerel chicks were assigned to 6 treatment groups of 40 birds each. Each treatment was replicated 5 times with 8 birds per replicate. The birds were managed intensively in a deep litter pen for 16 weeks. A basal diet containing no supplemental Cu (used as control) was formulated, each for the chick and growing phase of the birds. Six experimental diets were subsequently formulated in a 2 × 3 factorial arrangements involving two Cu sources ($CuSO_4$, CuP) supplemented with three dosages (0, 50 and 100 mg.kg^{-1}) for the chicks. The diets were formulated for the starter mash (0-8 weeks) and grower ration (8-16 weeks). Supplemental Cu was included into the basal diet containing basal Cu of 32.65 mg.kg^{-1} (chick diet) and 31.58 mg.kg^{-1} (grower diet) to formulate the respective experimental diets (Table 1). Diets were formulated to meet the NRC (1994) nutrient requirements of growing cockerels. Feed and water were supplied *ad libitum*. The experiment lasted for 16 weeks. The proximate compositions of the experimental diets were determined according to standard procedures (AOAC, 1995). Cu determination of feed was done using a Perkin Elmer Optima 4300DV ICP spectrophotometer (Perkin Elmer, Beaconsfield, UK). Feed samples were ignited at 400 °C for 4 h in a muffle furnace; resultant ash was reconstituted using wet-ashing procedure.

Plasma Cu and trace minerals

Blood samples (about 2.5 ml per bird) were collected from the wing veins of 2 birds per replicate (n = 10 per treatment) at 56 and 112 days of the study into heparinized tubes. Plasma was harvested (by centrifuging at 3200 rpm for 10 min) and used for the estimation of plasma Cu, Zn, Mn and Fe using an atomic absorption spectrophotometer (Perkin Elmer A Analyst 100).

Copper residues in meat tissues, organs and excreta sample

At the end of the study, two birds were selected from each replicate (n = 10 per treatment). Birds whose weight is a representative of the average weight of birds contained in each replicate were selected, slaughtered, de-feathered and dissected to separate

Table 1: Gross composition of basal diet

Ingredients	Chick starter mash (0-56 days)	Grower mash (57-112 days)
Maize	48.00	45.00
Soybean meal	15.00	11.00
Groundnut cake	11.00	8.00
Fish meal (72 % Crude protein)	2.00	1.00
Wheat offal	19.20	30.30
Bone meal	1.50	1.50
Oyster shell	2.50	2.50
Salt (NaCl)	0.25	0.25
*Premix (Cu free)	0.25	0.25
Methionine	0.20	0.10
Lysine	0.10	0.10
Total	100.00	100.00
Determined Analysis		
Crude protein (%)	22.04	17.20
Crude fibre (%)	4.26	4.75
Metabolizable energy ($MJ.kg^{-1}$)[a]	11.53	11.21
Ether extract (%)[a]	4.01	4.53
Calcium (%)[a]	1.45	1.30
Phosphorus (%)[a]	0.54	0.45
Lysine (%)[a]	0.91	0.75
Methionine (%)[a]	0.54	0.42
Cu level in basal diet ($mg.kg^{-1}$)[a]	32.65	31.58

Vitamins/mineral premix (Godomix©) included at the rate of 2.5 kg per ton of feed contains the following: Vitamin. A: 3.200.000 IU, vitamin D: 640.000 IU, vitamin E: 2000 mg, vitamin k3: 800 mg, vitamin B1: 2000 mg, vitamin B2: 6000 mg, vitamin. B6: 5000 mg, vitamin B12: 25 mg, niacin: 6000 mg, panthotenic acid: 20,000 mg, folic acid: 1000 mg, biotin: 8 mg, manganese: 30,000 mg, iron: 20,000 mg, zinc: 20,000 mg, copper: 0 mg, cobalt: 80 mg, iodine: 480 mg, selenium:40 mg, choline: 800,000 mg BTH: 25,000 mg. Supplementary dietary Cu was added to the basal diets at 0, 50 and 100 $mg.kg^{-1}$ Cu concentrations.
[a] Calculated value

the thigh, breast meat and organs (liver, kidney and heart). About 10 g cut sample from the thigh (*Biceps femoris*) and breast (*Pectoralis major*) muscle of each bird were collected (Jensen, 1984). Liver, heart and kidney samples were also collected and stored at -20 °C prior to analysis. For estimation of excreta Cu residues, two birds were randomly selected from each replicate at day 112 and housed individually in metabolic cages. Two days of acclimatization period were allowed for the birds in cages followed by 2 days of excreta collection. The samples of fresh excreta collected per replicate (n = 10 per treatment) were stored at - 20 °C until analysis.

Prior to laboratory analysis, samples of meat, organ and excreta were dried (100 °C for 36 h) and digested using a modified wet-ashing procedure (James, 1996). The ash was reconstituted in 5 ml of HCl solution and analysed for Cu using a Perkin-Elmer Optima 4300DV ICP spectrophotometer (Perkin Elmer, Beaconsfield, UK).

Cu residues in tibia bone

The right tibia of slaughtered birds was removed by cutting with a sharp knife. This was cleaned from adhering meat tissues and cartilage, placed into hexane for 48 h to remove fat and dried in an oven for 24 h until constant weight. The tibiae were weighed and ashed (at 600 ± 5 °C) for 4 h. Ash sample (0.2 g) of each tibia bone (n = 10 per treatment) was solubilized in 5 ml of 50 % HCl, mineral extract filtered into a volumetric flask and diluted using deionized water to a required volume. Cu concentration was determined

using the Perkin Elmer Optima 4300DV ICP spectrophotometer (Perkin Elmer, Beaconsfield, UK).

Statistical analysis

Data generated were analyzed as a two-factor model (Cu levels × Cu source) consisting of two Cu sources (WMD and CGD) supplemented with 3 dosages (0, 50 and 100 mg.kg^{-1}). Data were analysed using the general linear models procedure of the SAS (SAS Institute, 2000) to determine the main effects (Cu levels, Cu source) and their interaction (Cu levels × Cu source). Significant differences were considered at $P < 0.05$.

RESULTS

Cu intake and plasma trace minerals

Main effect of Cu levels and source on Cu intake and plasma trace minerals of cockerels fed diet supplemented with $CuSO_4$ or CuP is shown in Table 2. Cu intake ($P<0.0001$) and serum Cu ($P<0.05$) of cockerel chicks (0-8 weeks) increased with increasing dietary dosage of Cu. However, feed intake, serum Zn, Mn and Fe concentration of the chicks were not affected by varying Cu dosage used in this study. Chicks fed diet supplemented with $CuSO_4$ had higher ($P<0.05$) Cu intake and lower ($P<0.05$) serum Zn than those fed with diet supplemented with CuP.

Cockerels fed control diet had the least ($P<0.05$) feed intake and serum Cu concentration. Cu intake increased ($P<0.001$) with increasing dietary dosage of Cu supplemented. All cockerels fed diet supplemented with Cu, irrespective of the dosage, showed high ($P<0.05$) serum Cu concentration. Grower cockerels (8-16 weeks) fed diet supplemented with CuP had lower feed intake ($P<0.05$), Cu intake ($P<0.0001$) and higher ($P<0.05$) serum Cu concentration than their counterparts fed with $CuSO_4$.

Interaction effect of Cu levels and source on Cu intake and plasma trace minerals of cockerels fed diet supplemented with $CuSO_4$ and CuP is shown in Table 3. Cockerel chicks fed diet supplemented with 100 mg.kg^{-1} Cu (irrespective of the source) recorded the highest ($P<0.0001$) Cu intake, whilst chicks fed diet, supplemented with 100 mg.kg^{-1} Cu sourced from CuP, had the highest ($P<0.05$) serum Cu concentration. Chicks fed control diet and groups fed diet supplemented with CuP (irrespective of Cu dosage) recorded the highest ($P<0.05$) serum Zn. Grower cockerels fed diet

Table 2: Main effect of Cu levels and source on Cu intake and plasma trace minerals of cockerels fed diet supplemented with copper sulphate or copper proteinate

Parameters	Main effect of Cu levels					Main effect of Cu source			
	0	50 mg.kg^{-1}	100 mg.kg^{-1}	Pooled SEM	P- value	$CuSO_4.5H_2O$	CuP	Pooled SEM	P- value
0-8 weeks									
Total feed intake (kg per bird)	2.20	2.30	2.53	0.10	0.750	2.43	2.25	0.09	0.900
Total Cu intake (mg per bird)	71.67^{c}	189.74^{b}	334.93^{a}	22.76	<0.0001	202.77^{a}	194.79^{b}	26.65	0.027
Serum Cu (µg.mL^{-1})	0.19^{c}	0.31^{b}	0.54^{a}	0.09	0.044	0.25	0.44	0.02	0.070
Serum Zn (µg.mL^{-1})	1.65	1.40	1.45	0.10	0.065	1.30^{b}	1.70^{a}	0.97	0.044
Serum Mn (µg.mL^{-1})	0.07	0.09	0.08	0.002	0.700	0.06	0.08	0.001	0.073
Serum Fe (µg.mL^{-1})	1.53	1.53	1.62	0.11	0.990	1.56	1.56	0.09	0.075
9-16 weeks									
Total feed intake (kg per bird)	2.92^{b}	3.36^{ab}	3.66^{a}	0.99	0.042	3.63^{a}	2.99^{b}	0.97	0.044
Total Cu intake (mg per bird)	92.24^{c}	274.24^{b}	481.58^{a}	47.66	<0.0001	303.16^{a}	262.21^{b}	65.60	<0.0001
Serum Cu (µg.mL^{-1})	0.18^{b}	0.40^{a}	0.49^{a}	0.09	0.040	0.20^{b}	0.51^{a}	0.08	0.041
Serum Zn (µg.mL^{-1})	1.30	1.43	1.31	0.04	0.140	1.20	1.49	0.15	0.095
Serum Mn (µg.mL^{-1})	0.11	0.10	0.09	0.010	0.085	0.10	0.10	0.008	0.105
Serum Fe (µg.mL^{-1})	1.73	1.78	1.73	0.14	0.099	1.73	1.75	0.11	0.990

Means in the same row having different letters are different significantly ($P<0.05$)

supplemented with CuP showed increased (P<0.05) serum Cu when compared with groups supplemented with $CuSO_4$.

Cu residues in meat tissues, organs, excreta and tibia bone

Main effect of Cu levels and source on residues of Cu in meat tissues, organs, excreta samples and tibia bone of cockerels fed diet supplemented with $CuSO_4$ and CuP is shown in Table 4. Thigh meat Cu level increased (P<0.05) with increasing dietary dosage of Cu. Control-fed birds had the least (P<0.05) thigh and breast meat Cu. However, Cu source showed no effect (P>0.05) on Cu residues of thigh and breast meat. Liver, heart, excreta and tibia bone Cu levels increased (P<0.05) with increasing dietary Cu dosage. Cockerels fed diet supplemented with CuP showed higher (P<0.05) liver, heart, tibia bone Cu and reduced (P<0.05) excreta Cu than birds fed with $CuSO_4$.

Table 5 shows the interaction effect of Cu levels and source on residual Cu concentration in meat tissues, organs, excreta samples and tibia bone of cockerels fed diet supplemented with $CuSO_4$ or CuP. CuP supplementation resulted in increased (P<0.05) thigh meat Cu. Control-fed birds showed the least (P<0.05) thigh meat Cu. Cockerels fed diet supplemented with 100 $mg.kg^{-1}$ Cu from CuP had the highest (P<0.05) liver and heart Cu concentration. Highest (P<0.05) excreta Cu was obtained from cockerels fed diet supplemented with 100 $mg.kg^{-1}$ Cu from CuSO4. Birds fed diet supplemented with 100 $mg.kg^{-1}$ Cu from CuP also showed the highest (P<0.05) tibia bone Cu.

DISCUSSION

Cu intake and plasma minerals

Improved feed intake of growing cockerels obtained in the present study following increasing Cu dosage agreed with previous work of Chowdhury *et al.* (2004) that dietary supplementation with Cu salts stimulated feed intake of poultry birds. Previous findings also confirmed improved feed efficiency following dietary supplementation of Cu at higher pharmacological levels (Baker *et al*., 1991).

Increased plasma Cu of cockerels obtained with increasing dietary Cu dosage agreed with previous findings that dietary Cu levels showed direct influence on plasma Cu (Luo and Dove, 1996). Supplementation with CuP in the present study, however, showed higher bioavailability (as reflected in increased plasma Cu), when compared with birds fed with $CuSO_4$. Previous studies confirmed that organic Cu was more absorbed than inorganic Cu (Baker *et al*., 1991).

Table 3: Interaction effect of Cu levels and source on Cu intake and plasma trace minerals of cockerels fed diet supplemented with copper sulphate or copper proteinate

Parameters	$CuSO_4.5H_2O$			Cu Proteinate			Pooled	
	0	50 $mg.kg^{-1}$	100 $mg.kg^{-1}$	0	50 $mg.kg^{-1}$	100 $mg.kg^{-1}$	SEM	P - value
0-8 weeks								
Total feed intake (kg per bird)	2.37	2.41	2.50	2.02	2.18	2.55	0.09	0.060
Total Cu intake (mg per bird)	77.38^{d}	199.29^{b}	331.63^{a}	65.95^{f}	180.18^{c}	338.23^{a}	46.50	<0.0001
Serum Cu ($\mu g.mL^{-1}$)	0.18^{c}	0.22^{c}	0.35^{b}	0.20^{c}	0.40^{b}	0.72^{a}	0.09	0.043
Serum Zn ($\mu g.mL^{-1}$)	1.60^{a}	1.20^{b}	1.10^{b}	1.70^{a}	1.60^{a}	1.80^{a}	0.75	0.042
Serum Mn ($\mu g.mL^{-1}$)	0.06	0.08	0.07	0.07	0.09	0.08	0.002	0.095
Serum Fe ($\mu g.mL^{-1}$)	1.55	1.52	1.60	1.50	1.54	1.64	0.10	0.059
9-16 weeks								
Total feed intake (kg per bird)	3.41^{ab}	3.65^{a}	3.83a	2.43^{c}	3.07^{b}	3.49^{ab}	0.95	0.041
Total Cu intake (mg.bird)	107.75^{e}	297.77^{c}	503.95^{a}	76.73^{f}	250.70^{d}	459.21^{b}	52.44	<0.0001
Serum Cu ($\mu g.mL^{-1}$)	0.18^{b}	0.20^{b}	0.22^{b}	0.18^{b}	0.60^{a}	0.75^{a}	0.09	0.035
Serum Zn ($\mu g.mL^{-1}$)	1.20	1.40	1.00	1.40	1.45	1.62	0.095	0.064
Serum Mn ($\mu g.mL^{-1}$)	0.11	0.09	0.09	0.10	0.10	0.09	0.01	0.070
Serum Fe ($\mu g.mL^{-1}$)	1.75	1.80	1.65	1.70	1.75	1.80	0.10	0.099

Means in the same row having different letters are different significantly (P <0.05)

The interaction of plasma trace minerals with Cu supplementation noted in the current study was also reported in previous studies on rat (Van Campen and Scaife, 1967). Increased Cu concentration was reported to trigger improved Fe retention (Prohaska, 1991). Reduced plasma Zn concentration obtained in this study with increasing $CuSO_4$ supplementation corroborated previous findings that Cu and Zn mutually hamper the absorption of each other (Gipp *et al*., 1974).

Copper residues in meat tissues, organs and excreta sample

The Cu concentration values obtained in the thigh and breast meat in this study was within the range reported in the literature (Ledoux *et al.*, 1991). Increased tissue Cu concentration recorded with increasing Cu dosage confirmed the assertion that

Table 4: Main effect of Cu levels and source on Cu concentration in meat tissues, organs, excreta samples and tibia bone of cockerels fed diet supplemented with copper sulphate or copper proteinate

Parameters	Main effect of Cu levels					Main effect of Cu source			
	0	50 mg.kg^{-1}	100 mg.kg^{-1}	Pooled SEM	P- value	$CuSO_4.5H_2O$	CuP	Pooled SEM	P- value
Meat tissue Cu									
Thigh (mg.100g^{-1})	2.10^{c}	3.40^{b}	3.73^{a}	0.97	0.025	2.97	3.18	0.21	0.060
Breast (mg.100g^{-1})	1.85^{b}	2.15^{ab}	2.55^{a}	0.92	0.042	2.20	2.17	0.18	0.055
Cu in Organs									
Liver (mg.100g^{-1})	3.53^{c}	4.98^{b}	6.45^{a}	1.01	0.044	4.51^{b}	5.46^{a}	1.20	0.044
Kidney (mg.100g^{-1})	2.10	2.25	2.10	0.11	0.090	2.10	2.20	0.10	0.106
Heart (mg.100g^{-1})	2.30^{c}	2.80^{b}	3.83^{a}	0.95	0.019	2.18^{b}	3.77^{a}	0.98	0.029
Excreta Cu (mg.100g^{-1})	3.11^{c}	4.73^{b}	5.90^{a}	0.99	0.040	5.20^{a}	3.96^{b}	0.95	0.035
Tibia bone Cu (mg.100g^{-1})	4.66^{c}	5.85^{b}	8.10^{a}	1.42	0.030	5.52^{b}	6.89^{a}	1.36	0.022

Means in the same row having different letters are different significantly (P <0.05)

Table 5: Interaction effect of Cu levels and source on Cu concentration in meat tissues, organs, excreta samples and tibia bone of cockerels fed diet supplemented with copper sulphate or copper proteinate

Parameters	$CuSO_4.5H_2O$			Cu Proteinate			Pooled	
	0	50 mg.kg^{-1}	100 mg.kg^{-1}	0	50 mg.kg^{-1}	100 mg.kg^{-1}	SEM	P - value
Meat tissue Cu								
Thigh (mg.100g^{-1})	2.01^{c}	3.20^{b}	3.70^{a}	2.18^{c}	3.60^{a}	3.76^{a}	0.99	0.044
Breast (mg.100g^{-1})	1.90^{b}	2.20^{ab}	2.50^{a}	1.80^{b}	2.10^{ab}	2.60^{a}	0.89	0.027
Cu in Organs								
Liver (mg.100g^{-1})	3.52^{c}	4.96^{b}	5.06^{b}	3.53^{c}	5.00^{b}	7.84^{a}	0.94	0.019
Kidney (mg.100g^{-1})	2.10	2.20	2.00	2.10	2.30	2.20	0.09	0.107
Heart (mg.100g^{-1})	2.20^{c}	2.10^{c}	2.25^{c}	2.40^{c}	3.50^{b}	5.40^{a}	0.97	0.032
Excreta Cu (mg.100g^{-1})	3.17^{d}	5.18^{b}	7.25^{a}	3.05^{d}	4.28^{c}	4.55^{bc}	1.02	0.033
Tibia bone Cu (mg.100g^{-1})	4.60^{c}	5.50^{bc}	6.45^{b}	4.72^{c}	6.20^{b}	9.75^{a}	1.64	0.022

Means in the same row having different letters are different significantly (P <0.05)

dietary Cu levels directly influence tissue Cu residues (Komprada *et al*., 1999). Increased thigh and breast meat Cu obtained with increased Cu dosage could be attributed to low level of copper clearance in the muscle tissue of birds (Aoyagi and Baker, 1993).

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

Excreta Cu concentration followed a different pattern unlike the trend obtained for liver Cu. Increasing dietary Cu from $CuSO_4$ resulted in increased excreta Cu, whilst inclusion of CuP showed reduced excreta Cu. Reduced excreta Cu with birds fed with CuP agreed with previous findings that inclusion of inorganic Cu resulted in reduced excreta Cu and environmental Cu pollution (Chowdhury *et al.*, 2004). Reduced excreta Cu was also obtained with metal-amino acid chelates in comparison to inorganic Cu salts (Lee *et al*., 2001). This low excreta Cu concentration in CuP-fed group could be linked to improved retention of Cu.

Dietary supplementation with CuP increased tibia bone Cu with the highest value obtained with cockerels fed with 100 $mg.kg^{-1}$ Cu from CuP. Banks *et al*. (2004) also reported that birds fed with Cu-lysine had highest tibia Cu compared with those fed with copper citrate or sulphate. Cao *et al.* (2000) showed that tibia bone Zn was higher in birds supplemented with organic Zn compared with those supplemented with inorganic Zn.

CONCLUSION

The findings of this study showed that dietary inclusion of up to 100 $mg.kg^{-1}$ CuP improved Cu bioavailability in the blood, meat tissues, organs and tibia bone of cockerels. To achieve reduced environmental Cu pollution resulting from excreta of poultry birds, organic salts of Cu, such as CuP, is recommended as feed additive.

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