EFFECT OF COPPER SALTS ON PERFORMANCE, CHOLESTEROL, RESIDUES IN LIVER, EGGS AND EXCRETA OF LAYING HENS

EFECTO DE SALES DE COBRE SOBRE LA PUESTA, COLESTEROL, ACUMULACIÓN Y RESIDUOS EN HÍGADO, HUEVOS Y EXCRETAS DE GALLINAS PONEDORAS

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Egg-production. Cholesterol. Residue.

PALABRAS CLAVE ADICIONALES

Producción de huevos. Colesterol. Residuos.

SUMMARY

Increasing the supplementation from 0 to 250 mgCu/kg with Copper sulphate pentahydrate, $CuSO_4.5H_2O$ and Copper proteinate (CuP) increased hen day egg production and Haugh unit while the feed intake, feed per dozen egg laid decreased. Whole egg and egg yolk cholesterol concentrations were significantly lowered and more cholesterol was excreted in CuP supplemented group. Liver and plasma (free, ester and total) cholesterol concentrations of hens fed $CuSO_4.5H_2O$ supplemented diets were higher. Egg, yolk cholesterol, plasma cholesterol, lipoprotein and liver cholesterol concentrations reduced linearly as Cu dosage increased. Hens fed diets supplemented with CuP had a higher liver and excreta Cu residue. There was no difference in Cu residue values of the excreta, whole egg and egg yolk in both Cu sources.

RESUMEN

Incrementando de 0 a 250 mgCu/kg en la dieta con $CuSO_4.5H_2O$ o proteinato de cobre (CuP), aumentó la producción diaria de huevos y unidades Haugh y disminuyó la ingestión de pienso por docena de huevos. La concentración de colesterol en el huevo y en la yema disminuyó y las gallinas excretaron más colesterol en el grupo suplementado con CuP. Las concentraciones de colesterol (ésteres libres y total) en el hígado y plasma, suplementando con $CuSO_4.5H_2O$, fueron mayores. Los niveles de colesterol del huevo, de la yema, del plasma así como las lipoproteínas y colesterol del hígado se redujeron linealmente al aumentar Cu de 0 a 250 mg. Las gallinas con dietas CuP tuvieron mayor (p<0,05) concentración de Cu en hígado. No hubo diferencia en los valores de los residuos de Cu en las excretas, huevo y yema.

INTRODUCTION

Commercial organic Cu sources and reagent-grade inorganic copper salts have been used for supplementation of feeds (Guo *et al*., 2001). Conflicting reports of their bioavailability in animal feeding experiments have been summarized (Baker and Ammerman, 1995). Copper requirement of the hens is unknown, as indicated by a question mark in **table II-III** of the Nutrient

Requirement of Poultry (NRC, 1994). The use of organic copper in the laying hens' diets is a recent research focus (Aoyagi and Baker, 1993 and Guo *et al*., 2001), consequently there is dart of information in this area.

Efforts have been made to lower egg cholesterol through manipulation of dietary protein and energy contents and the use of dietary fibre which are known to be hypocholesterolemic (Lirette *et al*., 1993 and Idowu *et al*., 2002). Pesti and Bakalli (1998) obtained a reduction in egg yolk cholesterol concentration when 125mg Cu/kg diet was fed to White Leghorn hens and further decline in egg cholesterol was noticed when 250mg Cu/kg was fed. Pesti and Bakalli (1998) demonstrated that pharmacological levels of Cu (>250mg/kg diet) caused changes in 17 ß-estradiol and enzymes involved in carbohydrate, lipid and amino acid metabolism in matured hens.

Research evidences have shown that Cu 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. Jackson *et al.* (1979) noted that concentration of copper in the liver increased rapidly at 600 and 800 mg/kg dietary level of copper. Chiou *et al.* (1997) reported that Cu residues in the liver and excreta were significantly ($p<0.05$) increased as dietary Cu increased. Cu residue in egg was reported by the same worker to be 4.7 mg/kg at 400 mg/kg dietary Cu. The objective of this study was to evaluate the effects of three levels of $CuSO_4.5H_2O$, and copper proteinate on laying performance, plasma and egg cholesterol levels, liver accumulation and residues in eggs and excreta of laying hens.

MATERIALS AND METHODS

ANIMALS AND EXPERIMENTAL DESIGN

One hundred and eighty Black Harco layer strain, 30 weeks of age from the Poultry Section of the Teaching and Research Farm, University of Agriculture, Abeokuta, Nigeria, were used. The birds were kept in a standard battery cage with automatic nipple drinkers and standard feeding trough and randomly assigned to one of the six treatments in a 2 x 3 factorial arrangement. Each of the six treatments was fed to triplicate groups of chickens for ten weeks. The basal contained (g/kg): ground white corn, 400; soyabean meal,100; groundnut cake, 100; fishmeal, 10; wheat, 185; palm kernel cake, 100; bone meal, 20; oyster shell, 80; vitamin-mineral premix (Roche Nutripol 5 ®), 2.5 and salt, 2.5. The diet contained per kilogram: 2598.98 kcal ME, 175.0g crude protein, 588.4g nitrogen free extract, 60g ether extract, 75g fibre, 61g ash, 38.9g Ca, 9.0g available P and 5.9 mg Cu/kg. Treatments were basal diet supplemented with inorganic Cu ($CuSO_4.5H_2O$, Sigma Chemical, St Louis, M.O.) or Cu Proteinate (Bioplex Copper, Alltech, Nicholasville, KY) at 0, 125 and 250 mg Cu/kg.

Egg quality determination

For egg quality assessments, fifteen eggs (5 per replicate) from each treatment were sampled. Weight of each egg sample and the albumen, yolk and shell weights were measured, respectively with a sensitive weighing scale (Mettler-Toledo® PB3002) to the nearest 0.01g. Egg albumen quality (Haugh units) was evaluated by a P6085 Spherometer (tripod micrometer) having an accuracy of 0.01mm. Egg shells weight and thickness were individually measured; shell thickness was measured by a 25 M-micrometer gauge (Ames, Waltham, M.A., USA). Egg shape index (ESI) and egg shell index (I) were calculated according to Sauver (1988) using the formula: ESI= EB/EL and I= 100SW/S. EB is the egg breath (mm) and EL is the egg length (mm) measured with a vernier caliper with accuracy of 0.01mm. SW is the shell weight (g) and S the surface (cm^2); S was calculated from egg weight (EW) from the equation: S= K.EW2/3, where K has a value of 4.67 for egg weight less than 60g, 4.68 for egg weight between 60g and 70g and 4.69 for egg weight greater than 70g respectively. Formula for estimating the egg specific gravity (ESG) was based on weight of egg and shell as used by Poultry Adviser (1992):

ESG=EW/[0.9680(EW-SW)+(0.4921 SW)]

Egg cholesterol analyses

The eighteen eggs (6 per replicate) per treatment sampled at the expiration of ten weeks for egg yolk cholesterol determination were weighed and hard cooked by immersion in boiling water for 8min. Yolks (6 per treatment) were individual weighed and oven-dried at 70°C, pooled and blend. Egg total lipid was extracted with chloroform: methanol (2:1 v/v) using the procedure described by Folch *et al.* (1957). 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 triplicate. Cholesterol concentrations were determined from the absorbance read at 500 nm using a spectrophotometer (Idowu, 2004)

Plasma lipid analyses

At the end of the experiment, 5ml of blood was drawn from the brachial vein of 18 birds per treatment (6 per replicate) into heparinized tubes. Plasma was immediately separated by centrifugation for 10 min at 1400g. Plasma triglyceride, total, free, and lipoproteins and LDL, HDL and VLDL cholesterol were determined using enzymatic-colorimetric method (according to Randox diagnostic reagent kit-CHOD-PAP®). The lipoprotein fractions were precipitated before assay using polyvinyl sulphate according to the Boehringer-Mannheim (Meylan, France) procedure (Rouanet *et al.*, 1993). Cholesterol determination was done as indicated above.

Copper residue determination

Eighteen samples per treatment of egg (whole egg and yolk) and excreta were collected weekly, pre-ashed and pooled together at end of the tenth week collection prior the dry ashing in muffle furnace. Three laying hens per replicate group were slaughtered and liver excised at the end of the feeding trial. Weights of liver samples excised were recorded . Part of the liver sample was pre-ashed before dry ashing. The ashed samples of the liver, whole egg, egg yolk and excreta were analyzed for Cu content by atomic spectrophotometry (Perkin-Elmer, Atomic Absorption Spectrophotometer) at 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 new multiple range tests.

RESULTS AND DISCUSSION

LAYING PERFORMANCE AND EGG QUALITY

Table I displays the effects of source supplementary Cu on the laying performance and egg quality. Feeding inorganic Cu ($CuSO_4.5H_2O$) for 10 weeks resulted in a higher ($p<0.05$) feed intake and feed consumed per dozen egg lay. Funk and Baker (1991) reported a decrease in feed consumption in chicks fed 800mg/kg Cu added as copper sulphate in a corn-soybean meal diet. Feed consumed per dozen egg laid was improved ($p<0.05$) when laying hens were fed basal diet supplemented with organic copper-CuP this consequently translated into better hen day egg production. Sheideler and Ceyland (1999) and Tucker *et al.* (2003) noticed a similar improvement when hen diets were supplemented with organic minerals.

Increasing the dietary additive of Cu from 0 to 250mg/kg Cu **(table II)** significantly depressed feed intake and feed per dozen egg values ($p<0.05$) while the hen day egg production appreciated in dosage dependent manner ($p<0.05$). Chiou *et al.* (1997) also observed significant decrease in feed intake in younger laying hens when fed 600 mg/kg Cu in basal diets. Pesti and Bakalli (1988) observed a significant increase in egg production within 8 weeks of feeding laying hens with diet supplemented with pharmacological levels of Cu in form of sulphate. Increment in level of Cu supplementation has been observed to affect the reproductive physiology and lipid metabolism in matured laying hens.

Feed conversion efficiency was ($p<0.05$) improved when the Cu

Table I. *Main effect of copper source on egg laying performance and egg quality.* (Efecto de la fuente de cobre sobre la puesta y calidad del huevo).

Copper Source	Organic (CuP)	Inorganic ($CuSO_4$)
Performance		
Final weight (kg)	1.84±0.01	1.86±0.01
Feed/hen/day (g)	101.14±2.90[b]	109.09±3.15[a]
Egg production[1]	81.15±1.27[a]	73.08±1.58[b]
Feed/dozen egg (kg)	1.63±0.24[b]	1.88±0.30[a]
Egg weight (g)	62.16±1.06	62.07±1.09
Egg quality		
Egg shape index	0.69±0.00	0.69±0.01
Egg shell index	6.33±0.19	6.30±0.18
Egg specific gravity	25.50±0.01	25.59±0.01
Haugh unit	67.13±2.63	67.18±2.70
Shell thickness (mm)	0.34±0.01[a]	0.31±0.01[b]

[a,b]Means with different superscript are different ($p<0.05$); [1]percent.

Table II. *Main effect of copper dosage on laying performance and egg quality.* (Efecto de la dosis de cobre sobre la puesta y calidad del huevo).

Copper dosage (mg/kg of basal feed)	0	125	250
Performance			
Body weight (kg)	1.84±0.01	1.88±0.02	1.90±0.04
Feed/hen/day (g)	121.18±3.03[a]	107.53±3.01[b]	95.63±2.96[c]
Egg production (percent)	69.25±0.94[c]	76.99± 1.01[b]	85.12±1.08[a]
Feed/dozen egg (kg)	2.18±0.82[a]	1.68±0.80[b]	1.39±0.50[c]
Egg weight (g)	61.45±1.10	61.58±1.08	63.32±1.27
Egg quality			
Egg shape index	0.69±0.01	0.69±0.02	0.69±0.01
Egg shell index	6.67±0.16	6.70±018	6.79±019
Egg specific gravity	25.11±0.01	25.19±0.01	26.01±0.02
Haugh unit	76.20±2.53[b]	79.04±2.62[a b]	80.55±2.82[a]
Shell thickness (mm)	0.33±0.01	0.31±0.01	0.31±0.01

[a,b,c]Means with different superscript are different ($p<0.05$).

supplementation was increased from 125 to 250 mg/kg **(table II)**. The relatively lower feed conversion efficiency and egg hen day production values noticed in the control group when compared with either 125 or 250 mg Cu supplementation showed economic benefit from Cu supplementation (Agunbiade and Babatunde, 1988, and Tucker *et al.*, 2003). The linear improvement in the feed conversion efficiency and egg production **(table II)** also indicated that further increase in the supplementation beyond 250 mg/kg could result in further improvement in the feed conversion efficiency. Dietary concentrations as high as 480 mg/kg have been fed without adverse effect on egg production (Thomas and Goatcher, 1976). It was also evident that the inclusion of organic Cu at both 125 and 250 mg/kg enhanced egg production at a relatively lower fed intake which means better feed conversion efficiency compared with what was obtained at 250 mg inorganic Cu supplementation.

Egg quality traits such as weight, shape index, shell index, specific gravity and Haugh unit, were not affected by the main effects of Cu source **(table I)**. Shells from eggs laid by birds fed organic Cu were 6.5 percent thicker than those fed inorganic Cu ($p<0.05$). In a closely related study, Tucker*et al.* (2003) also noticed a relatively thicker shells in the hens fed organic minerals. Significant ($p<0.05$) effect was noticed in the Haugh units **(table II)** which indicated that the albumen quality was improved as a result of increase in the level of Cu supplementation. Egg shape index, shell index, specific gravity and shell thickness obtained for 125 and 250 mg/kg diets also did not differ from the control group (0 mg/kg diet).

The uniform egg shape index value (0.69) obtained in this study **(tables I and II)** indicated that supplementation of hen diets with either organic or inorganic Cu source at 0-250 mg Cu/kg diet sustained production of uniform shaped eggs. Egg composition values expressed as specific gravity was fairly uniform for both eggs from hens fed organic and inorganic Cu irrespective of level of supplementation. **Tables I, II** and **III** showed a numeric increase in the egg shell quality in terms of shell index and shell thickness. The interaction of Cu source and level of supplementation indicated significant influence on all the egg quality evaluated. The superior laying performance and better egg quality noticed in the hens fed organic Cu could be due to its ability to be absorbed and utilized in the same way as protein-amino acids chelated to it (Tucker *et al.*, 2003).

EGG AND PLASMA CHOLESTEROL REDUCTION

Table IV showed that the birds fed inorganic copper recorded significantly higher values in all the parameters measured; those values showed that proteinate form of Cu was more effective in reducing cholesterol level than sulphate when fed to laying birds. This observation agreed with Cromwell *et al.* (1989) who reported that sulphate form of Cu resulted in higher values than other forms. **Table VI** indicated some values that are dosage dependent. Higher values were obtained in the control group (0 mg Cu level). The values decreased as the level of inclusion of Cu increased (from 125 mg to 250 mg/kg of feed). Bakalli *et al*. (1995) noticed similar observation and reported that high level of Cu reduces GSH (Glutathione) which in turn stimulates the reduction of HMG-Co A (3-hydroxy-3methylglutaryl Coenzyme A) reductase activity which ultimately reduced cholesterol syn-

Table III. *Interaction effect of copper source and dosage on laying performance and egg quality.* (Interacción de la fuente y dosis de cobre sobre la puesta y calidad del huevo).

	Organic source (CuP) (mg/kg)			Inorganic source ($CuSO_4.5H_2O$) (mg/kg)		
	0	125	250	0	125	250
Performance						
Body weight (kg)	1.81±0.03	1.83±0.04	1.89±0.07	1.87±0.06	1.85±0.05	1.90±0.09
Feed/hen/day (g)	112.25±3.11[a]	105.3±2.28[c]	83.75±1.98[d]	110.12± 2.92[a]	109.64± 2.5[b]	107.50±2.38[b]
Egg production[1]	70.84± 1.05[b]	82.74±1.42[a]	84.98±1.63[a]	67.66±1.01[c]	71.24±1.09[b]	80.30± 1.12[a]
Feed/dozen egg (kg)	2.16±0.86[a]	1.52±0.60[bc]	1.12±0.41[c]	2.11±0.85[a]	1.84±0.72[b]	1.64±0.62[b]
Egg weight	63.09±1.22	61.16±1.06	62.23±1.13	63.55±1.27	61.74±1.09	60.92±1.02
Egg quality						
Egg shape index	0.70 ±0.03	0.69±0.01	0.69±0.01	0.70±0.02	0.70±0.03	0.70±0.03
Egg shell index[1]	6.81±0.13	6.91±0.18	7.01±0.20	6.82±0.15	6.88±0.16	6.98±0.19
Egg specific gravity	26.36±0.05	24.58±0.01	25.95±0.02	25.81±0.02	25.31±0.01	26.07±0.03
Haugh unit	74.70±2.20	78.99±2.56	79.70±2.61	78.70±2.49	79.63±2.46	80.91±2.70
Shell thickness (mm)	0.33±0.01	0.34±0.01	0.36±0.01	0.29±0.00	0.30±0.00	0.34±0.01

[abc]Means with different superscript are different ($p<0.05$); [1]percent.

Table IV. *Main effect of copper source on cholesterol contents.* (Efecto de la fuente de cobre sobre el contenido de colesterol).

Copper source	Organic (CuP)	Inorganic ($CuSO_4$)
Egg Cholesterol		
Yolk Cholesterol (mg/g)	11.81±1.08[b]	15.49± 1.80[a]
Cholesterol/Egg (mg/egg)	180.93±15.11[b]	207.57± 20.80[a]
Cholesterol excreted (mg/d/hen)	157.60±11.66[a]	150.44± 9.81[b]
Plasma Cholesterol		
Triacylglycerol	70.89± 4.01	71.00± 4.81
Free	34.67±2.11[b]	40.11±5.33[a]
Ester	76.00±6.60[b]	86.22±10.48[a]
Total	110.78±6.62[b]	125.89±8.50[a]
Lipoproteins		
HDL	22.44±1.21[b]	25.55±2.68[a]
LDL	74.11±1.86[b]	84.44±4.30[a]
VLDL	14.22±1.10[b]	16.33±3.20[a]
Liver		
Fresh liver wt.	29.84±2.01	33.57±2.50
Relative liver wt.	16.22±1.03[b]	18.05±3.08[a]
Liver Cholesterol (mg/g)	0.21±0.01[b]	0.37±0.05[a]

[a,b]Means with different superscript are different ($p<0.05$).

thesis. The esters of cholesterol contributed the greatest proportion of the total cholesterol and values followed a decreasing order (LDL,free cholesterol, HDL and VLDL). This observation corroborated the findings of Idowu *et al.* (2002). Results obtained in this study also agreed with Bakalli *et al*. (1995). Pesti and Bakalli (1988) and Konjufca *et al.* (1997) noticed a reduction in plasma concentrations of young broiler chickens due to dietary intake of pharmacological levels of copper pentahydrate or copper citrate. Paik *et al.* (1999) also reported that dietary intake of copper-chelate reduced plasma total cholesterol. The observed results may be due to the fact that Cu regulates cholesterol biosynthesis by reducing hepatic glutathione concentrations (Kim *et al.*, 1992). The interaction effects **(table VI)** of sources and Cu dosage showed no statistical significance on most of blood lipids parameters. Although VLDL was the last in decreasing order, however, egg yolk cholesterol has been reported to be synthesized in the liver of laying hens and transported to the developing follicles via plasma very low density lipoprotein (VLDL) where it is deposited by receptor-mediated endocytosis (Nimpt and Schneider, 1991).

Cu source, dosage and their interaction had no significant effect on egg weight. However, cholesterol concentration in the yolk, whole egg and excreta were significantly influenced by Cu source, dosage and their interaction **(tables IV, V** and

VI). Hens fed diets supplemented with organic Cu, laid eggs that had lower yolk cholesterol and cholesterol per egg values ($p<0.05$) than hen diets supplemented with $CuSO_4$ **(table IV)**. Daily cholesterol excreted was more in the treatment group fed diet supplemented with organic Cu source. Yolk cholesterol and cholesterol per egg also decreased ($p<0.05$) linearly as Cu dosage increased from 0 to 250mg/kg.The cholesterol excreted also tended to increase with Cu dosage **(table V)**. The interaction between Cu source and dosage showed that CuP reduced yolk cholesterol and cholesterol per egg than $CuSO_4$ at both 125 and 250 mg Cu levels respectively **(table VI)**. Cholesterol contents of the yolk and whole egg was highest in the control groups (0 mg Cu) while lowest cholesterol content was obtained in eggs laid by hens fed diet supplemented with 250 mg CuP this indicated that additive dietary copper intake actually reduced the cholesterol concentration. An inverse relationship was observed between the cholesterol contents of the yolk and the cholesterol excreted. Al-Ankari *et al.* (1998) also obtained a significant linear reduction in yolk and serum cholesterol as dietary Cu content increased from 0 to 250 mg/kg diet.

The relative liver weight values were significantly influenced by Cu dosage and the interaction between Cu source and dosage **(tables V and VI)**. Relatively heavier liver samples were excised from the hens fed diets supplemented with inorganic form of

Table V. *Main effect of copper dosage on cholesterol contents.* (Efecto la dosis de cobre sobre el contenido de colesterol).

Copper dosage (mg/kg of basal feed)	0	125	250
Egg Cholesterol			
Yolk Cholesterol (mg/g)	15.60±2.00[a]	12. 97±1.50[b]	12.40±1.27[b]
Cholesterol/Egg (mg/egg)	223.92±22.72[a]	193.40±17.20[b]	165.39±11.18[c]
Cholesterol excreted (mg/d/hen)	133.15±6.23[c]	147.36±8.32[b]	182.25±16.22[a]
Plasma Cholesterol			
Triacylglycerol	82.67±5.71[a]	76.17±4.82[b]	69.83±4.00[c]
Free	40.67±5.50[a]	37.17±4.91[b]	34.33±4.58[b]
Ester	87.83±10.44[a]	80.83±7.10[b]	74.67±5.93[c]
Total	128.67±9.41[a]	118.00±7.10[b]	108.17±6.00[c]
Lipoproteins			
HDL	26.00±2.83[a]	23.83±1.22[b]	22.17±1.15[b]
LDL	86.00±4.74[a]	79.00±3.68[b]	72.83±1.55[c]
VLDL	16.67±3.66[a]	15.17±2.02[b]	14.00±1.09[c]
Liver			
Fresh liver wt.	32.47±2.40	31.71±2.31	31.35±2.30
Relative liver wt.	7.65±1.33	16.87±1.09	16.50±1.06
Liver Cholesterol (mg/g)	0.34±0.03[a]	0.30±0.02[b]	0.23±0.01[c]

[a,b]Means with different superscript are different ($p<0.05$).

***Table VI.** Interaction effect of copper source and dosage on cholesterol contents.* (Interacción de la fuente y la dosis de cobre sobre el contenido de colesterol).

	Organic Cu (CuP) mg/kg			inorganic Cu ($CuSO_4$) mg/kg		
	0	125	250	0	125	250
Egg Cholesterol						
Yolk (mg/g)	12.17±1.34[c]	11.83±1.10[c]	11.43±1.04[c]	19.02±2.61[a]	14.11±1.88[b]	13.34±1.70[b]
Egg (mg/egg)	216.78±21.01[a]	182.56±16.22[d]	143.44±10.01[e]	231.06±23.11[a]	204.33±18.91[c]	187.33±16.70[d]
Excreted[1]	135.50±6.98[c]	153.60±9.77[b]	184.30±16.81[a]	130.80±5.44[c]	141.11±7.83[bc]	180.20±15.89[a]
Plasma Cholesterol						
Triacylglycerol	80.67±5.33	69.67±3.94	63.00±3.69	84.67±6.01	82.67±5.66	76.67±4.92
Free	39.33±5.00	34.00±4.50	30.67±3.88	42.00±5.68	40.33±5.40	38.00±5.19
Ester	85.67±10.62	74.67±5.99	67.67±5.21	90.00±11.30	87.00±10.91	81.67±9.74
Total	125.33±8.12	108.67±6.08	98.33±5.88	132.00±10.01	127.33±9.32	118.33±7.62
Plasma Lipoproteins						
HDL	25.33±2.44	21.67±1.10	20.33±1.00	26.67±2.89	26.00±2.80	24.00±2.20
LDL	83.67±4.80	73.33±1.80	65.33±1.28	88.33±5.01	84.67±4.40	80.33±3.92
\VLDL	16.33±3.52	13.67±1.00	12.67±0.82	17.00±4.00	16.67±3.67	15.33±3.00
Liver Cholesterol						
Fresh liver wt.	30.65±2.10	29.67±2.00	29.35±1.98	34.29±2.72	33.75±2.60	33.31±2.23
Relative liver wt.	16.93±1.10	16.21±1.01	15.53±0.95	18.34±3.10	18.24±3.10	17.53±1.30
Liver (mg/g)	0.25±0.01[c]	0.20±0.01[c]	0.16±0.00[d]	0.43±0.08[a]	0.39±0.06[b]	0.29±0.02[c]

[a,b]Means with the same superscript are not significantly different (p>0.05); [1](mg/d/hen).

Cu **(tables IV and V)**. The fresh and the relative liver weights decreased linearly (p>0.05) as the dosage level of Cu increased from 0 to 250 mg/kg **(tables V and VI)**. Lower (p<0.05) cholesterol contents were noticed in the groups fed CuP **(tables IV and VI)**. Liver cholesterol contents reduced linearly in a dosage dependent manner **(tables V and VI)**. Higher (p<0.05) liver cholesterol contents were noticed in the hens fed diets supplemented with inorganic Cu. Supplementation with CuP decreased (p<0.05) cholesterol content more than $CuSO_4$ **(tables IV and VI)** at 125 and 250mg dosages. The results obtained further corroborate the claims of Lubert (1981) that the rate of cholesterol formation by the liver was responsive to the amount of dietary Cu intake.

LIVER COPPER ACCUMULATION AND RESIDUE

Table VII shows the main effects and the interaction effects of Cu source and dosage of copper on the contents of the liver, egg yolk, whole egg and excreta of laying hens. The main effects of Cu source was not significant on yolk, whole egg and excreta Cu contents, however more Cu accumulated in the liver of hens fed diets supplemented with inorganic Cu form (p<0.05). This may be due to higher feed intake noticed among hen receiving diets supplemented with inorganic Cu form **(table I)**. Copper concentration of the liver was higher than those of the yolk and whole egg. This is expected because the liver is the major site of Cu metabolism and storage (Chiou *et al.*, 1998). Increasing the level of Cu supplementation resulted in a linear increase in the Cu concentration of the liver, yolk, whole egg and excreta ($p<0.05$). The interaction of the source and level of supplementation of Cu was significant on all the parameters measured **(table VII)**. The Cu concentrations of the liver, yolk and whole egg were significantly higher in the hen fed inorganic Cu at all levels of supplementation than those on organic Cu. This implied that the interaction between Cu source and levels of supplementation significantly influenced Cu accumulation in the liver and egg.

The Cu content of the excreta from the laying hens significantly increased as the level of supplemental Cu increased ($p<0.05$) according with observations of Chiou *et al.* (1998). There was no significant difference between mean values of the Cu sources used ($p>0.05$). The interactive effects between Cu source and supplemental level showed significant differences ($p<0.05$) in the amount of Cu excreted, as more organic copper was excreted than the inorganic form

TableVII. *Effects of source of copper and dosage on liver accumulation and residue.* (Efecto de la fuente y dosifis de cobre sobre la acumulación y residuo en el hígado).

Source	Cu Level (mg/kg)	Liver Cu (mg/kg)	Yolk Cu (mg/kg)	Whole egg (mg/kg)	Excreta (mg/kg)
Main effects of source of supplementary Cu					
Inorganic Cu		70.94±0.11[a]	1.01±0.08	7.49±0.22	782.45±16.66
Organic Cu		60.24±2.05[b]	0.94±0.03	7.13±0.13	820.62±17.30
Main effects of supplementary levels of Cu					
	0	14.00±1.01[c]	0.77±0.01[c]	6.46±0.10[b]	173.74±8.72[c]
	125	38.77±3.24[b]	0.99±0.05[b]	7.53±0.40[a]	1021.11±24.33[b]
	250	142.44±9.22[a]	1.17±0.09[a]	7.93±0.41[a]	1236.78±31.11[a]
Interaction effects of source and levels of Cu supplementation					
Inorganic Cu	0	14.00±1.00[e]	0.81±0.01[b]	6.61±0.10[c]	169.44±7.04[d]
	125	48.44±1.56[c]	1.00±0.06[a]	7.86±0.49[b]	971.10±18.66[c]
	250	150.37±10.07[a]	1.23±0.10[a]	8.00±0.63[a]	1198.23±28.14[a]
Organic Cu					
	0	14.00±1.00[e]	0.73±0.01[c]	6.32±0.09[d]	178.03±19.01[d]
	125	29.10±1.56[d]	0.98±0.04[b]	7.19±0.14[ab]	1017.11±24.01 [b]
	250	134.50±7.43[b]	1.11±0.07[a]	7.87±0.40[b]	1275.32±36.10[a]

[a,b]Means with the same superscript are not significantly different ($p>0.05$).

of Cu. The difference between the excreta Cu concentration of the control group and that of 125-250mg Cu groups were about 7-9 fold increase.

CONCLUSION

The egg laying performance improved due to Cu supplementation, egg quality parameters were grossly unaffected by the supplementation while plasma cholesterol and egg cholesterol reduced in a dosage dependent manner. Supplementation of hen diets with 125 or 250 mg/kg in form of copper proteinate also resulted in laying performance that was superior to supplementation with 250 mg/kg in form of copper sulphate pentahydrate. Copper proteinate was more effective and efficient in the reduction of plasma cholesterol and egg cholesterol. Less of the organic Cu was accumulated in the egg and live than the organic Cu form.

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