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EFFECT OF DIETARY COPPER ON PERFORMANCE, SERUM AND EGG YOLK CHOLESTEROL AND COPPER RESIDUES IN YOLK OF LAYING CHICKENS

A.V. JEGEDE,* A.O. OSO, A.O. FAFIOLU, R.A. SOBAYO, O.M.O IDOWU, O.O. ODUGUWA

*Federal University of Agriculture, Department of Animal Nutrition, Abeokuta, Nigeria

ABSTRACT

A 294-day study was conducted to determine the effects of organic and inorganic copper (Cu) sources on laying performance, blood and yolk cholesterol and Cu accumulation in the yolk of laying hens. 480 Kabir® laying hens (20 weeks old) reared for 294 days in three phases (early, mid and late lay) were used. They were randomly allocated to six dietary groups of 80 birds split into four replicates of 20 birds each The diets consisted of a basal diet (containing 37.24 mg kg^{-1} Cu) supplemented with organic Cu (Cu proteinate; Cu-P) or inorganic Cu (Cu sulphate pentahydrate; $CuSO_4$) fed at 3 dietary concentrations (50, 100 and 150 mg kg^{-1}). Data on laying performance, blood and yolk cholesterol and Cu accumulation in the yolk were collected and subjected to Completely Randomized Design of the Analysis of Variance, laid out in 2 × 3 factorial arrangements. $CuSO_4$ supplementation resulted in poor (P <0.05) feed conversion ratio (feed/dozen eggs and feed kg $eggs^{-1}$) and reduced (P <0.05) hen day egg production when compared to Cu-P. High concentration of Cu resulted in reduced hen day egg production. No significant (P >0.05) effect of Cu sources and concentration was observed for body weight, weight gain and daily feed intake. The blood cholesterol and triglyceride level were significantly (P <0.05) reduced in birds fed diets with Cu-P. The blood cholesterol level decreased as the Cu concentration increased. More Cu was accumulated in the yolk of birds fed Cu-P. The blood and yolk cholesterol levels were significantly lower (P <0.05) in birds fed Cu-P than $CuSO_4$. It was evident that Cu-P was more bioavailable than $CuSO_4$ owing to higher accumulation of Cu in the yolk of the birds fed Cu-P. Cu-P at 50 mg kg^{-1} is recommended in the diets for laying hen. A high level of Cu-P (150 mg kg^{-1}) in laying hens' diets was effective for cholesterol reduction in yolk and blood of experimental birds.

Keywords: blood; copper; chicken; cholesterol; performance; yolk

INTRODUCTION

Copper is a very important trace mineral required for proper functioning of the central nervous, immune and cardiovascular systems and in pigmentation of the skin (Close, 1998). It is also an essential component of several enzyme systems, such as cytochrome oxidase, lysyl oxidase, ceruloplasmin and superoxide dismutase (Klasing, 1998) and metalloenzymes which are important for cellular respiration.

The Cu requirement of laying hen is unknown (NRC, 1994). Dietary mineral supplementation in animal diets has traditionally being achieved through the use of inorganic sources such as sulphates, carbonates, chlorides and oxides. These salts are broken down in the digestive tract to form free ions which are absorbed. However, free ions are very reactive and can form complexes with other dietary molecules making them difficult to absorb or in some cases unavailable for absorption and, therefore, of little benefit to the animals (Close, 1998).

The experiments have shown that Cu regulates cholesterol biosynthesis by reducing hepatic glutathione concentration (Kim *et al.*, 1992). Feeding pharmacological level of Cu has been reported to play important roles in

***Correspondence:** e-mail: vinbjegede@yahoo.com
A.V. Jegede, Federal University of Agriculture, Department of Animal Nutrition, Abeokuta, Nigeria
phone: +2348 035 397 280

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lipid metabolism (Bakalli *et al.*, 1995; Chowdhury *et al.*, 2004; Pesti and Bakalli, 1996, 1998). Metal-amino acid chelates have also been reported to be more efficiently absorbed from gut than those provided by inorganic salts (Wedekind *et al.*, 1994). Previous studies with broilers showed considerably higher utilization of copper from organic sources compared to copper sulphate (Jegede *et al.*, 2011). Copper proteinate was also reported to be more effective in reducing plasma cholesterol in growing pullets than copper sulphate (Jegede *et al.*, 2012), although the effect of supplemental Cu levels on egg-yolk cholesterol of laying birds was reported in previous experiments (Lien *et al.*, 2004). Literature sources reporting about effects of various forms of Cu (organic and inorganic) on egg-yolk cholesterol and Cu residues are scarce. This study, therefore, aims to investigate the bioavailability and utilization of organic and inorganic copper sources and their effects on laying performance, blood and yolk cholesterol and copper residue in egg yolk of laying hens.

MATERIALS AND METHODS

This study was carried out according to the research ethics and guidelines of the College of Animal Science and Livestock Production of the Federal University of Agriculture, Abeokuta, Nigeria.

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^{1}49.46″ N and Longitude 3°26^{1} 11.98″ E). This area lies in the tropical climate with an average rainfall of 1037 mm, mean ambient temperature of about 34 °C and yearly relative humidity of 82 % (Google Earth, 2010).

Table: 1 Gross composition of experimental diets (g kg^{-1})

Ingredients	Layers mash
Maize	440.00
Soybean meal	80.00
Groundnut cake	75.00
Fishmeal	20.00
Wheat offal	270.00
Bone meal	40.00
Oyster shell	71.00
Common Salt	2.50
[a]Premix (Cu free)	2.50
Methionine	2.00
Lysine	1.50
Total	1000.00
Determined Analysis	
Dry matter	898.2
Crude protein	171.0
Crude fibre	58.8
Ether extract	78.8
Ash	93.4
*Energy ME (Kcal kg^{-1})	2661.6
Basal Cu in the diet (mg kg^{-1})	37.24

Vitamins / mineral premix (Godoye) based on 2.5 kg per ton; vit. A: 6000000 IU, vit. D: 400000 IU, vit E: 40000 mg, vit k_3: 800 mg, vit B_1: 2000 mg, vit B_2: 6000 mg, vit. B_6: 5000 mg, vit B_{12}: 25 mg, Niacin: 80000 mg, Panthotenic Acid: 20000 mg, Folic Acid: 1000 mg, Biotin: 8 mg, Manganese: 300000 mg, Iron: 80000 mg, Zinc: 20000 mg, Copper: Nill, cobalt: 80 mg, Iodine: 400 mg, Selenium:40 mg, Choline: 2000 mg, BTH: 25,000 mg Anticaking agent: 6,000 mg; *ME (Kcal kg^{-1}) = 37 × % CP + 81 × % EE + 35 × % NFE (Pauzenga, 1985)

A basal diet was formulated to contain 37.24 mg kg^{-1} Cu. Six experimental diets were subsequently formulated in a 2 × 3 factorial arrangements of two sources of Cu salts ($CuSO_4$, Cu-P) supplemented at three levels of inclusion (50, 100 and 150 mg kg^{-1}) (Table 1). Supplemental Cu salts were included in the basal diet to formulate the experimental diets. Birds included into each group were assigned to the respective experimental diets. The diets were formulated to meet the nutrient requirements of layers according to NRC recommendations (NRC, 1994) and nutrient requirements of poultry in the tropics (Olomu, 1995).

A total of 480 twenty week-old Kabir® layers were randomly allotted to six treatment groups of 80 birds per group. Each treatment consisted of four replications with 10 cages (2 birds per 30.5 × 40.6 cm wire cage) and subjected to a photoperiod of 15 hours light and 9 hours darkness per day. Each group was randomly allotted to one of the six dietary treatments in a 2 × 3 factorial arrangement. The birds were fed layers mash for 294 days (Early, Mid and Late laying periods). Feed and water were supplied *ad libitum*.

The feed samples were dried at 65 °C for 36 h in an oven and milled to pass through 1.0 mm sieve and were analyzed for dry matter (DM), crude fibre (CF), ether extract (EE) and total ash (AOAC, 1995). The nitrogen content of feed samples was determined using the Kjeldahl method and crude protein (CP) was determined by multiplying the N value by 6.25. The metabolizable energy (ME) of feed samples was calculated using the prediction equation M. E. = 37 × % CP + 81.8 × % EE + 35.5 × % NFE (Pauzenga, 1985). The Cu content of the basal diet was determined by igniting the feed sample at 400 °C for 4 h in a muffle furnace. The ash was reconstituted using wet-ashing procedure (James, 1996). Analysis of Cu was done by using a Perkin-Elmer Optima 4300DV ICP spectrophotometer (Perkin Elmer, Beaconsfield, UK). Data on body weight, weight gain and feed intake were collected weekly. Egg production was recorded daily.

Blood samples were obtained from the brachial veins of the birds at the end of early lay (0–14 weeks in lay), mid lay (15–28 weeks in lay) and late lay (29–42 weeks in lay). The blood at 2.5 ml was collected from 5 birds per replicate (20 birds per treatment) into a tube containing Ethylene-diamine-tetra-acetate (EDTA). Plasma was separated from the blood samples by centrifuging the whole blood samples at 3,000 rpm for 15 min into a test tube for plasma lipid analysis. The cholesterol and triglyceride assays of the blood plasma were done by enzymatic–colorimetric method using a RandoxR diagnostic cholesterol kit (BIOLAB with code 80106.2 × 100 ml cholesterol CHOD-PAPR) and a RandoxR diagnostic triglyceride reagent procedure (GPO-PAP Method Randox Laboratory Ltd., UK) respectively (Allain *et al.*, 1974).

Forty eggs per treatment (10 eggs per replicate), sampled at the end of each phase of the laying period, were weighed and hard cooked by immersion into boiling water for 10 minutes. The yolks were individually weighed, dried at 65 °C for 36 hrs, pooled, blended and digested using a modified wet-ashing procedure (James, 1996). Ash was reconstituted in 4 ml of 1N HCl solution and analyzed for copper via atomic absorption spectrophotometry (Perkin-Elmer, Optima 4300DV ICP spectrophotometer) at 324.7 nm (Chiou *et al.*, 1998). Egg total lipid was extracted with chloroform:methanol (2 : 1 v/v) from the dried yolk using the procedure described by Folch *et al.* (1957). Cholesterol determination was done using a commercial test kit for cholesterol analysis (Sigma Chemical Co., St Louise, MO USA). Cholesterol concentration was determined from absorbance read at 500 nm using a spectrophotometer. The data obtained were subjected to completely randomized design of the analysis of variance using the General Linear Models procedure of SAS (SAS Institute, 2002), The experiment was laid out in 2 × 3 factorial arrangement. Significant differences were determined using Duncan's multiple range test at the level of P <0.05 (Duncan, 1955). Each replicate was considered as an experimental unit. The Research Animal Ethic Committee approved this experimental protocol.

RESULTS AND DISCUSSION

The growth and production performance of laying birds fed experimental diet is shown in Table 2. The feed/kg egg and feed per dozen egg were significantly higher (P <0.05) in birds fed diets containing $CuSO_4$ compared to those fed Cu-P during the early lay period (0–14 weeks in lay). At the mid (15–28 weeks) and late (29–42 weeks) laying periods feed per dozen egg was significantly higher (P <0.05) in birds fed diets containing $CuSO_4$ compared to those fed Cu-P. The hen day egg production was significantly (P <0.05) improved by feeding Cu-P compared to $CuSO_4$ throughout the laying periods. There was no significant effect of Cu sources on the body weight, total weight gain and daily feed intake of the birds throughout the laying period. The levels of Cu concentration (Table 2) significantly (P <0.05) influenced hen-day egg production. Birds fed diets containing 50 mg kg^{-1} Cu concentration laid the highest (P <0.05) number of eggs during the early laying period. However, the birds fed diets containing 50 mg kg^{-1} and 100 mg kg^{-1} Cu concentration recorded higher number of eggs laid compared to those fed 150 mg kg^{-1} Cu concentration during the mid laying period. No significant effect (P >0.05) of Cu concentration on hen day egg production was noticed during the late lay period. The levels of Cu supplementation during 29–42

Table 2: Main effects of Cu sources and Cu levels on the performance of lay (0–42 Weeks)

Parameters	Copper Sources		*P*-value	SEM	Copper concentration (mg kg^{-1})^{z}			*P*-value	SEM	Source × concentration
	Cu-P	$CuSO_4$			50	100	150			
					(0–14 weeks in lay)					
Body weight (g)	2064.9	2012.1	0.087	26.4	2026.8	2036.0	2052.5	0.770	7.52	0.857
Total weight gain (g)	374.0	358.7	0.085	7.65	329.3	370.8	398.8	0.767	20.19	0.855
Daily feed intake (g)	110.9	110.1	0.403	0.40	109.7	110.9	110.9	0.528	0.40	0.653
Feed / kg egg	2.58^{b}	2.63^{a}	0.018	0.05	2.59	2.62	2.58	0.257	0.02	0.097
Feed / dozen egg	1.78^{b}	1.99^{a}	0.017	0.11	1.83	1.92	1.91	0.572	0.03	0.999
HDEP (%)	62.5^{a}	58.7^{b}	0.004	1.93	63.04^{a}	59.9^{b}	58.9^{b}	0.021	1.25	0.185
					(15–28 weeks in lay)					
Body weight (g)	2334.6	2295.2	0.089	19.70	2322.8	2302.6	2319.2	0.727	6.22	0.872
Total weight gain (g)	269.7	283.1	0.410	6.70	296.0	266.5	266.7	0.247	9.80	0.773
Daily feed intake (g)	119.3	117.8	0.182	0.75	118.1	119.5	118.1	0.521	0.47	0.473
Feed / kg egg	2.86	2.98	0.459	0.06	2.90	2.95	2.90	0.949	0.02	0.915
Feed / dozen egg	1.89^{b}	2.03^{a}	0.007	0.07	1.91	1.97	2.00	0.220	0.27	0.958
HDEP (%)	72.3^{a}	67.8^{b}	0.0007	2.23	70.2^{a}	72.9^{a}	67.1^{b}	0.002	1.66	0.178
					(29–42 weeks in lay)					
Body weight (g))	2630.9	2588.9	0.131	21.00	2612.8	2624.4	2600.0	0.701	7.05	0.897
Total weight gain (g)	296.3	293.70	0.824	1.30	290.0^{b}	321.8^{a}	280.8^{b}	0.015	12.42	0.148
Daily feed intake (g)	120.5	118.6	0.120	0.95	118.1	120.5	119.9	0.252	0.72	0.478
Feed / kg egg	3.60	4.00	0.005	0.40	3.70	3.80	3.80	0.600	0.03	0.963
Feed / dozen egg	2.80^{b}	3.20^{a}	0.0001	0.20	3.00	3.00	3.10	0.118	0.03	0.063
HDEP (%)	58.5^{a}	49.5^{b}	0.0001	4.50	55.0	54.8	52.2	0.294	0.90	0.809
N	*12*	*12*			*8*	*8*	*8*			

^{ab} – mean on the same row having different superscripts are significantly different (P <0.05); HDEP – hen day egg production; z – added dietary copper to basal copper concentration; N – number of observation per mean; SEM – Standard Error of Mean

Table 3: Main effects of Cu sources and Cu concentration on the Cu in yolk and cholesterol in yolk and blood of layers (0–42 weeks)

Parameters	Cu Sources		*P*-value	SEM	Cu concentrations (mg kg^{-1})^{z}			*P*-value	SEM	Source × concentration
	Cu-P	$CuSO_4$			50	100	150			
0–14 weeks in lay										
Cu in yolk (mg kg^{-1})	1.01^{a}	0.85^{b}	0.0039	0.08	0.77^{b}	0.99^{a}	1.04^{a}	0.0001	0.08	0.7703
Yolk Cholesterol (mg g^{-1})	12.2^{b}	14.2^{a}	0.0003	1.00	14.4^{a}	13.3^{a}	12.1^{b}	0.0002	0.67	0.4509
Blood Cholesterol (mg g^{-1})	90.1^{b}	99.8^{a}	0.0024	4.82	100.1^{a}	94.5^{ab}	90.3^{b}	0.0260	2.82	0.0249
Triglyceride (mg dl^{-1})	54.4^{b}	90.7^{a}	0.0001	18.16	78.7	75.00	64.0	0.0023	4.41	0.0005
15–28 weeks in lay										
Cu in yolk (mg kg^{-1})	1.15^{a}	0.94^{b}	0.0311	0.11	0.89^{b}	1.06^{a}	1.19^{a}	0.0068	0.09	0.8713
Yolk Cholesterol (mg g^{-1})	13.2^{b}	15.2^{a}	0.0003	1.00	15.4^{a}	14.3^{a}	13.1^{b}	0.0020	0.67	0.4509
Blood Cholesterol (mg g^{-1})	103.8^{b}	114.9^{a}	0.0419	5.64	115.7	108.9	103.4	0.0557	3.56	0.8233
Triglyceride (mg dl^{-1})	55.8^{b}	91.7^{a}	0.0001	17.99	73.6^{a}	80.7^{a}	66.9^{b}	0.0192	3.99	0.2061
15–28 weeks in lay										
Cu in yolk (mg kg^{-1})	1.68^{a}	1.56^{b}	0.0311	0.06	1.51^{b}	1.61^{b}	1.75^{a}	0.0068	0.07	0.8713
Yolk Cholesterol (mg g^{-1})	15.5^{b}	17.5^{a}	0.0003	0.50	17.6^{a}	16.6^{a}	15.2^{b}	0.0016	0.68	0.4101
Blood Cholesterol (mg g^{-1})	120.8	127.8	0.0516	3.53	129.5^{a}	127.3^{a}	117.6^{b}	0.0412	3.66	0.8039
Triglyceride (mg dl^{-1})	71.2^{b}	100.9^{a}	0.0001	14.58	90.5^{a}	87.7^{a}	79.9^{b}	0.0164	3.19	0.2181
N	*12*	*12*			*8*	*8*	*8*			

ab – means on the same row having different superscripts are significantly different (P <0.05); *z* – added dietary copper to basal copper concentration; SEM – pooled standard of mean; *N* – number of observation per mean

weeks in lay significantly influenced the total weight gain. The birds fed diets containing 100 mg kg^{-1} Cu gained the highest (P <0.05) weight compared to those fed 50 mg kg^{-1} and 150 mg kg^{-1} Cu. Cu source × Cu concentration did not influence (P >0.05) the growth and production performance throughout the laying period.

Table 3 shows the main effects of Cu sources and Cu concentration on the Cu in yolk, and cholesterol in yolk and blood and yolk triglyceride of laying hens during 0–42 weeks in lay. During the early and mid periods in lay, birds fed Cu-P had higher (P <0.05) accumulation of Cu in the yolk compared to those fed $CuSO_4$. The yolk and blood cholesterol and triglycerides measured were significantly higher (P <0.05) in birds fed $CuSO_4$. However, the effect of Cu sources on blood cholesterol was not noticed during late lay. The Cu content in yolk increased with increased Cu concentrations in the birds fed 100 and 150 mg kg^{-1} Cu having significantly higher (P <0.05) Cu in the yolk compared to those fed 50 mg kg^{-1} Cu. The values of cholesterol in yolk and blood decreased as the levels of Cu supplementation increased. The triglycerides were not significantly (P >0.05) affected by Cu concentration during early lay, but were significantly reduced (P <0.05) as Cu concentration increased during mid and late lay. The interaction of Cu source and Cu concentration significantly influenced plasma triglycerides. Birds fed diets containing 50 mg kg^{-1} $CuSO_4$ had the highest (P <0.05) triglyceride value, while those fed 150 mg kg^{-1} Cu-P had the least (P <0.05) value. Other parameters measured were not significantly influenced (P >0.05) by the interaction "Cu source × Cu concentration" throughout the laying period.

The performance of the laying birds during 0–42 weeks in lay show that the sources of Cu in the diet resulted in a significant variation in feed conversion ratio and hen´s day egg production. This trend was similar throughout the three phases (0–14, 15–28 and 29–40 weeks of lay) representing early, mid and late laying phases of the laying period, respectively. Less feed was consumed by birds fed Cu-P to lay a kilogram and a dozen egg compared to those fed $CuSO_4$. This suggests a better utilization of the diet containing the former and its effectiveness in enhancing better egg production. Idowu *et al.* (2006) reported a better feed conversion ratio (feed consumed per dozen eggs) when layers were fed diets supplemented with organic Cu. The hen´s day egg production was higher in birds fed Cu-P supplemented diets. The better feed consumption per kg egg and per dozen eggs converted to a better hen day egg production. Sheidder and Ceyland (1999) and Tucker *et al.* (2003) noticed significant improvement in egg production when laying hens diets were supplemented with organic minerals. Tanika (2004) reported that the addition of organic Cu increased egg production. Lim and Paik (2006) reported a variable effect of organic Cu, Zn and Mn on the egg production and egg quality. The body weight, weight gain and feed intake of laying birds were not influenced by Cu sources and Cu concentration throughout the laying period.

Increase in Cu concentration over 50 mg kg^{-1} during early lay and 100 mg kg^{-1} during mid lay resulted in a decline in the egg production. Hen´s day egg production decreased with increased levels of Cu. It is evident from this study, that high concentration of Cu inhibited egg production. The decrease in the egg production observed after feeding high Cu concentration for 28 weeks was unexpected though consistent with the results of Pearce *et al.* (1983) and Stevenson *et al.* (1983) who reported a decreased egg production following Cu supplementation in the diets of laying hens.

Moderately high levels of dietary copper seem to reduce cholesterol levels in eggs and meat products, although these effects are sometimes associated with loss of performance (Leeson, 2009). Ankari *et al.* (1998) reported that the reduced egg cholesterol was at the expense of 10 % reduction in the egg production when feeding high levels of Cu. The results of Jackson (1977), Thomas and Goatcher (1976) and Idowu *et al.* (2006) are contrary to the result of this study. The beneficial effect of additional Cu supplementation on egg production was not evident after the mid laying period.

The higher concentration of Cu in yolk of birds fed Cu-P suggests an increased bioavailability of Cu in Cu-P compared to $CuSO_4$. Organic Cu is reported to be more bioavailable in the tissues and organs of broiler chickens (Jegede *et al.*, 2011) The yolk cholesterol, blood cholesterol and triglyceride were significantly lower in laying birds fed Cu-P diets compared to those fed $CuSO_4$ diets throughout the laying period. Lien *et al.* (2004) reported a significant reduction in egg yolk and serum cholesterol of laying birds fed supplemental Cu. The significant reduction in yolk and blood cholesterol by feeding Cu-P shows that proteinate form of Cu was more effective in reducing cholesterol level than sulphate, when fed to laying birds. This observation agreed with the report of Idowu *et al.* (2006). Similar observation was reported by Chromwell *et al.* (1989), that sulphate form of Cu resulted in higher yolk and serum cholesterol level. 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 Shneider, 1991). Cu in yolk was increased as the level of Cu supplementation increased. Similar trend was observed throughout the laying phases. Yolk and blood cholesterol decreased as Cu levels increased from 50–150 mg kg^{-1} in each of the laying phases. This indicated that dietary Cu intake

reduced cholesterol concentration. It is possible that high Cu concentration reduced hepatic glutathione through the stimulation of the enzyme 3-hydroxyl-3-methylglutaryl coenzyme reductase (Kim *et al.*, 1992). The activity of the enzyme is the rate-limiting step of mevalonate and ultimately cholesterol biosynthesis (Valsala and Kurup, 1987). Eggs are rich source of dietary cholesterol and consumption of high level of dietary cholesterol increases the risk of coronary heart disease (CHD; Kritchevsky, 2004). Producing eggs low in cholesterol will be of great interest to egg consumers and this can be better achieved by supplementing the diets of laying birds with Cu-P.

CONCLUSION

More Cu was accumulated in the yolk of laying hens fed Cu-P. The cholesterol content in the yolk and blood of laying hens was reduced in birds fed Cu-P. The overwhelming evidence in this study was that Cu-P® is more bioavailable than $CuSO_4$ and it is more effective in reducing blood and egg yolk cholesterol.

REFERENCES

ALLAIN, C.C – POON, L.S – CHAN, C.S – RICHMOND, W. – FU, P.C. 1974. Enzymatic determination of total serum cholesterol. *Clinical Chemistry*, vol. 20, 1974, p. 470–475.

AOAC.1995. Association of Official Analytical Chemists, 15th ed. Official Method of Analyses, Washington DC.

ANKARI, A. – NAJIB, H. – AL HOZAB, A. 1998. Yolk and serum cholesterol and Production traits, as affected by incorporating a supra-optimal amount of copper in the diet of the Leghorn hen. *British Poultry Science*, vol. 39, 1998, p. 393–397.

BAKALLI, R.I – PESTI, G.M – RAGLAND, W.L. – KONJUFCA, V.H. 1995. Dietary copper in excess of nutritional requirement reduces plasma and breast muscle cholesterol of chicken. *Poultry Science*, vol. 74, 1995, p. 360–365.

CHIOU, P.S – CHEN, K.L. – YU, B. 1998. Effect of dietary organic arsenicals and Cu-Pric sulphate on copper toxicity, liver accumulation and residue in eggs and excreta of laying hens. *Animal Feed Science and Techology*, vol. 73. 1998. p 161–171.

CHOWDHURY, S.D. – PAIK, I.K. – NAMKUNG, H. – LIM, H.S. 2004. Response of broiler chickens to organic copper fed in the form of copper-methionine chelate. *Animal Feed Science and Technology*, vol. 115, 2004, p. 281–283.

CLOSE, W.H. 1998. The role of trace mineral proteinates in pig nutrition. In: Biotechnology In the Feed Industry, *Proceedings of the 14th Annual Symposium* (T.P. Lyons and K.A. Jacques, eds.) Nottingham University Press. Nottingham, U.K. 1998, p. 469–474.

CROMWELL, G.L. – STHLY, T.S. – MONGUE, H.J. 1989. Effect of source and levels of copper on performance and liver copper store in weanling pig. *Journal of Animal Science*, vol. 67, 1989, p. 296–302.

DUNCAN, D. B. 1955. Multiple range and multiple F test. *Biometrics*, vol. 1, 1955, p. 11–42.

FOLCH, G. – LEES, M. – STANLEY, G. H. S. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry*, vol. 226, 1957, p. 497–509.

IDOWU, O.M.O. – LANIYAN, T.F. – KUYE, O.A. – OLADELE-OJO, V.O. – ERUVBETINE, D. 2006. Effect of copper salts on performance, cholesterol, residues in liver, eggs and excreta of laying hens. *Separata de Archivos de Zootecnia*, vol. 55 (212), 2006.p. 327–338.

JACKSON, N. 1977. The effect of dietary copper sulphate on laying performance, nutrient intake and tissue copper and iron levels of the mature, laying, domestic fowl. *British Journal of Nutrition*, vol. 38, 1977, p. 93–100.

JAMES, C.S. 1996. Analytical Chemistry of Foods. Blackie Academic and professionals, Glascow. 1996.

JEGEDE, A.V. – ODUGUWA, O.O. – BAMGBOSE, A.M. – FANIMO, A.O. – NOLLET, L. 2011. Growth response, blood characteristics and Cu accumulation in organs of broilers fed on diets supplemented with organic and inorganic dietary Cu sources. *British Poultry Science*, vol. 52, 2011, p. 133–139.

JEGEDE, A.V. – ODUGUWA, O.O. – OSO, AO. – FAFIOLU, A.O. – IDOWU, O.M.O. – NOLLET, L. 2012. Growth performance, blood characteristics and plasma lipids of growing pullet fed dietary concentrations of organic and inorganic copper sources. *Livestock Science*, vol. 145, 2012, p. 298–302

KLASING, K.C. 1998. Nutritional modulation of resistance to infectious diseases. *Poultry Science*, (77), pp. 1119–1125.

KIM, J.W. – CHAO, P.Y. – ALLEN, A. 1992. Inhibitionof elevated hepatic glutathione abolishes copper deficiency cholesterolemia. *FASEB journal*, vol. 6, 1992, p. 2467–2471.

KRITCHEVSKY, S.B. 2004. A review of scientific research and recommendations regarding eggs. *Journal of American College of Nutrition*, vol. 23 (6), 2004, p. 596S–600S

LEESON, S. 2009. Copper metabolism and dietary needs. *World's Poultry Science Journal*, vol. 65, 2009, p. 353–366. 2009.

LIEN, T.F. – CHEN, K.L. – WU, C.P. – LU, J.J. 2004. Effect of supplemental copper and chromium on the serum and egg traits of laying hens. *British Poultry Science*, vol. 45 (4), 2004, p. 535–539.

LIM. H.S. – PAIK, I.K. 2006. Effect of dietary supplementation of copper chelates in the form of methionine, chitosan and yeast in laying hens. *Asian-Australian Journal of Animal Science*, vol.19, 2006, p. 1174–1178. 2006.

NATIONAL RESEARCH COUNCIL. 1994. Nutrient Requirements of Poultry, 9th Revised edition. National Academy Press, Washington, D.C. 1994.

NIMPT, J. – SCHNEIDER, W.J. 1991. Receptor – mediated lipoprotein transport in laying hens. *Journal of Nutrition*, vol. 121, 1991, p. 1471–1474.

OLOMU, J. M.1995. Monogastric Nutrition: Principle and practice. 1st edition, Benin, Nigeria. 1995, p. 75–82.

PAUZENGA, U. 1985. Feeding parent stock. *Zootechnica International*, pp. 22–24.

PEARCE, J. – JACKSON, N. – STEVENSON, M.H. 1983. The effects of dietary intake and of dietaryconcentration of copper sulphate on the laying domestic fowl: Effects of some aspects of lipid, carbohydrate and amino acid metabolism. *British Poultry Science*, vol. 24, 1983, p. 337–348.

PESTI, G.M. – BAKALLI, R.I. 1996. Studies on the feeding of Cu-Pric sulfate pentahydrate and Cu-Pric citrate to broiler chickens. *Poultry Science*, vol. 75, 1996, p. 1086–1091.

PESTI, G.M. – BAKALLI, R.I. 1998.Studies on the effect of feeding Cu-Pric sulfate pentahydrate to laying hens on egg cholesterol content. *Poultry Science*, vol.77, 1998, p. 1540–1545.

SHEIDDER, S.E. – CEYLAND, N. 1999. Effects on Mn and Zn Proteinates (Eggshell 49) on egg shell quality of laying hens. *Proceedings of the VIII Euopean Symposium* on the quality of eggs and egg products, Bologna, Italy. vol. 2, 1999, p.187–192.

STEVENSON, M.H. – PEARCE, J. – JACKSON, N. 1983.The effects of dietary intake and of dietary concentration of copper sulphate on the laying domestic fowl: Effects on laying performance and tissue mineral contents. *British Poultry Science*, vol. 24, 1983, p. 327–335.

TANIKA, O.D. 2004. Mineral utilization in poultry as affected by virginiamycin or mineral source. An MSc thesis submitted to graduate faculty of the Louisiana State University. 2004.

THOMAS, M.C – GOATCHER, W.D. 1976. High levels of copper in layers diets. *Poultry Science*, vol. 55, 1976, p. 1608. (Abstract).

TURKER, L.A. – KENYON, S. – WILSON, S. 2003. Benefits of organic minerals in Commercial laying hen performance and egg quality. *Proceedings of the 16th European Symposium* on the quality of eggs and egg products. Sept. 23–26 Ploutragan France, 2003. p. 198–202.

VALSALA, P. – KURUP, P.A. 1987. Investigation on the mechanism of hypercholesterolemia observed in Cu deficiency in rats. *J. Bioscience* (Bangalore), vol. 12, 1987, p. 137–142.

WEDEKIND, K.J. – LEWIS, A.J. – GIESMANN, M.A. – MILLAR, P.S. 1994. Bioavailability of zinc from inorganic and organic sources for pigs fed corn-soybean meal diets. *Journal of Animal Science*, vol. 72, 1994. p. 2681–2689.