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THE EFFECT OF DIETARY SUPPLEMENTATION WITH COPPER SULPHATE OR COPPER CHLORIDE ON LOCAL MAMOURAH STRAIN LAYING HENS:

2- EFFECT OF DIETARY COPPER SOURCE AND CONCENTRATION ON TOTAL LIPIDS, YOLK AND PLASMA CHOLESTEROL OF LAYING HENS.

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ABSTRACT

An experiment was conducted to test the hypothesis that pharmacological levels of dietary copper could reduce egg cholesterol content. Local Mamourah strain hens 36 weeks of age were fed corn and soybean meal diets with 0, 500, 750 and 1000 mg/kg diet as copper sulphate ($CuSO_4$. 5 H_2O) and copper chloride ($CuCl_2$. 2 H_2O). At the end of experimental periods (28 and 56 days, respectively) three eggs per group were randomly taken to determine yolk cholesterol and total lipids.

Results obtained were as follows:

- A significant ($P<0.05$) decrease in blood hemoglobin value (Hb) was observed at 500 mg/kg diet of copper source during the two experimental periods. Hb value was not affected by changing the dietary Cu source.
- A negative decrease in Cu plasma concentration was observed for different levels and sources of copper.
- Increasing dietary copper from zero to 1000 mg/kg diet the amount of cholesterol in plasma and yolk decreased linearly ($P<0.05$) and numerically. Levels at 750 and 1000 mg/kg diet decreased the plasma and yolk cholesterol by about 7.52, 19.40, 20.62 and 30.92%, respectively, during 8 weeks of experimental period.
- Copper supplementation positively affected plasma total lipids.
- Yolk lipids could be lowered substantially (15.78%) by the addition of 1000 mg/kg diet when compared with control group.
- Liver weight per unit body weight was depressed for 500 and 750 mg /kg diet of copper source.
- Cu concentration in the liver increased for all dietary levels.

These results suggested feed pharmacological levels and sources of copper to laying hens will produce some physiological responses depending on the strain type and duration of feeding.

Keywords: Copper, laying hens, lipids, cholesterol

INTRODUCTION

A series of experiments were conducted to confirm of results of feeding pharmacological levels of copper to broiler chicken. Bakalli *et al.*, (1995); Pesti and Bakalli (1996) and Konjufca *et al.*, (1997) observed changes in lipid metabolism and a reduction in plasma and meat cholesterol concentration of young broiler chickens due to adding pharmacological levels of cupric sulphate pentahydrate or cupric citrate to the diet. Pearce *et al.*, (1983) demonstrated that pharmacological levels of Cu (>250 mg/kg diet)

caused changes in 17β–estradiol and enzymes involved in carbohydrate, lipid and amino acid metabolism in mature laying hens. Their data suggested that copper supplements can affect reproductive physiology and lipid metabolism beyond changes simply due to reduced feed intake. Copper supplements decreased plasma lipid, 17β–estradiol and liver lipid concentrations and hepatic lipogenic enzyme activities. Mean liver glycolytic and amino acid metabolizing enzyme activities were affected by dietary copper additions.

The purpose of the study was to determine pharmacological levels of copper in laying hen diets and their effect on plasma and egg cholesterol.

MATERIALS AND METHODS

The experimental birds, design, management and statistical analysis were explained in the first part (El-Awady, *et al.*, 2002). At the end of experimental periods (28 and 56 days, respectively) three eggs per group were randomly taken to determine yolk cholesterol and total lipids. Eggs were placed in boiling water for 5 minutes. After cooling to room temperature, the yolk were separated from albumin and frozen at –20 °C. The yolk samples were thoroughly mixed and 0.5g was transferred to a conical-bottom centrifuge tube and extracted by the chloroform: methanol according to the method of Folch *et al.*, (1957) as modified by Washburn and Nix (1974). The filtrate was then analysed for cholesterol and total lipids by using a commercial kit of labkit (Plato, 6 E 08021 Barcelona, Spain).

Blood samples were withdrawn from the wing vein of 3 hens per group before the start and at biweekly intervals (28 and 56 days) by using heparinized syringe. Hemoglobin value was immediately determined. The rest of blood samples was then immediately centrifuged at 3000 rpm for 10 minutes to separate plasma. Plasma samples were stored, frozen at –20 °C until assayed for cholesterol, total lipids and copper. Plasma cholesterol and total lipids concentrations were assayed by a colorimetric method using the commercial kits of labkit. Plasma copper content was assayed by using an atomic absorption sepectrophotometry apparatus. A slaughter test was operated on 3 hens per group to determine liver copper concentration.

RESULTS AND DISCUSSION

Blood hemoglobin value (Hb) of hens fed 500 mg/kg diet significantly ($P<0.05$) decreased (Table 1) during the two experimental periods (4 and 8 weeks) while Hb value was not significantly affected by the two higher levels during the first and second experimental periods. Cu sources did not affect Hb value. No significant differences in plasma Cu among supplemental Cu sources and levels were obtained in this study during the two experimental periods. Results recorded higher plasma Cu values at 8 weeks of experimental period than at 4 weeks of the experiment. There was no significant difference in plasma and yolk cholesterol content due to levels,

sources of Cu and their interaction between factors during first 4 weeks of experimental period.

Table (1): Blood hemoglobin (Hb), plasma Cu, plasma cholesterol, plasma lipid, yolk cholesterol and yolk total lipid of laying hens given control and Cu supplemented diet at 28 days.

Items	Hemo-globin	Plasma Cu ppm	Plasma cholesterol mg/dl	Plasma total lipid g/dl	Yolk cholesterol mg/g	Yolk total lipid g/g yolk
Before treatments at 28 days as range (basal diet)						
	-	0.05±0.0 to 0.15±0.1	105.7±4.7 to 143.0±4.5	2.2±0.19 to 4.8±0.51	7.3±1.4 to 32.0±10.8	0.29±0.04 to 0.41±0.01
After treatment at 28 days :						
Among levels mg/kg diet:						
0	8.5±0.3^{a}	0.05±0.00	112.5±13.7	2.6±0.57^{b}	14.9±2.2	0.38±0.01^{a}
500	7.3±0.3^{b}	0.16±0.04	115.2±11.5	3.0±0.47^{ab}	8.9±1.7	0.36±0.01^{ab}
750	7.9±0.2^{ab}	0.09±0.02	91.4±7.8	3.7±0.43^{ab}	13.9±3.1	0.38±0.01^{a}
1000	7.8±0.4^{ab}	0.11±0.03	102.3±5.6	4.2±0.37^{a}	15.7±3.2	0.32±0.02^{b}
Between source:						
Copper sulphate	8.0±0.3	0.12±0.02	101.9±7.2	3.4±0.37	13.6±1.9	0.37±0.01
Copper chloride	7.6±0.2	0.08±0.02	108.7±7.6	3.3±0.37	12.9±1.8	0.35±0.01
Interactions:						
Sulphate mg/kg:						
0	9.0±0.1^{a}	0.05±0.00	125.4±5.4	3.5±0.87^{ab}	14.9±1.9	0.41±0.01^{a}
500	7.3±0.7^{b}	0.17±0.02	110.6±17.2	2.0±0.24^{bc}	5.5±1.5	0.34±0.02^{ab}
750	8.2±0.3^{ab}	0.12±0.02	77.1±8.8	3.6±0.69^{ab}	13.2±3.5	0.38±0.02^{a}
1000	7.4±0.2^{b}	0.16±0.04	94.4±9.9	4.4±0.50^{a}	20.6±2.3	0.35±0.01^{ab}
Chloride mg/kg:						
0	7.6±0.1^{ab}	0.05±0.00	99.5±27.4	1.7±0.37^{c}	15.0±4.5	0.35±0.01^{ab}
500	7.1±0.4^{b}	0.15±0.01	119.8±18.7	4.0±0.25^{a}	12.3±1.0	0.37±0.01^{ab}
750	7.7±0.2^{ab}	0.06±0.03	105.7±4.7	3.8±0.67^{ab}	14.7±5.7	0.38±0.02^{a}
1000	8.3±1.1^{ab}	0.06±0.01	110.0±7.8	3.9±0.71^{a}	8.5±1.3	0.28±0.03^{b}

a, b and c Mean that are not followed by the same superscripts are significantly different (P<0.05).

Increasing dietary copper from zero to 1000 mg/kg diet decreased numerically and linearly (P<0.05) the amount of cholesterol in plasma and yolk. Levels of 750 and 1000 mg/kg diet decreased the plasma and yolk cholesterol by about 7.52, 19.40, 20.62 and 30.92%, respectively during 8 weeks of experimental period. The decrease in plasma and yolk cholesterol supported the hypothesis that the higher copper concentration decreased the formation of hepatic glutathione and ultimately cholesterol formation (Kim *et al.*, 1992). Glutathione acts in regulating cholesterol biosynthesis through the stimulation of enzyme 3-hydroxy-3-methylglutaryl co-enzymeA. reductase in rats (Valsala and Kurup, 1987). Liver copper regulates cholesterol

biosynthesis by reducing hepatic glutathione concentration (Kim *et al.*, 1992). If this hypothesis holds for rat, then chickens may respond similarly to the addition of copper. Kim *et al.*, (1992) indicated that reduced glutathione may play major role in cholesterol homeostasis. A recent study by Bakalli *et al.*, (1995) supported this hypothesis and agrees with this study.

Table (2): Blood hemoglobin (Hb), plasma Cu, plasma cholesterol, plasma lipid, yolk cholesterol and yolk total lipid of laying hens given control and Cu supplemented diet at 56 days.

Items	Hemo-globin	Plasma Cu ppm	Plasma cholesterol mg/dl	Plasma total lipid g/dl	Yolk cholesterol mg/g	Yolk total lipid g/g yolk
After treatment at 56 days:						
Among levels mg/kg diet:						
0	8.3±0.3^{ab}	1.7±0.01	87.8±4.2^{a}	2.2±0.28^{ab}	9.7±1.1	0.36±0.02
500	7.4±0.3^{b}	1.7±0.14	92.4±7.9^{a}	1.9±0.35^{b}	7.0±0.8	0.37±0.02
750	8.1±0.3^{ab}	1.6±0.2	81.2±6.8^{ab}	2.2±0.40^{ab}	7.7±1.7	0.37±0.02
1000	8.8±0.2^{a}	1.6±0.1	70.2±3.1^{b}	3.0±0.42^{a}	6.7±0.7	0.32±0.02
Between source:						
Copper sulphate	8.2±0.2	1.8±0.06	77.8±3.1	1.8±0.3^{b}	7.4±0.9	0.34±0.01
Copper chloride	8.1±0.3	1.6±0.08	88.5±5.3	2.8±0.13^{a}	8.1±0.7	0.36±0.01
Interactions:						
Sulphate mg/kg:						
0	8.7±0.1^{ab}	1.7±0.0	85.6±7.1^{ab}	1.7±0.16^{ab}	8.2±1.7	0.40±0.04
500	7.6±0.5^{bc}	1.9±0.3	78.8±7.6^{b}	1.3±0.17^{b}	5.7±1.2	0.37±0.03
750	8.2±0.5^{abc}	1.9±0.1	74.4±7.1^{b}	1.3±0.20^{b}	9.8±3.0	0.33±0.03
1000	8.3±0.2^{abc}	1.7±0.05	72.5±3.5^{b}	3.1±0.91^{a}	5.9±0.6	0.30±0.02
Chloride mg/kg:						
0	7.9±0.4b^{c}	1.7±0.0	90.1±2.7^{ab}	2.6±0.39^{a}	11.1±1.0	0.33±0.01
500	7.3±0.5^{c}	1.6±0.1	105.9±8.4^{a}	2.7±0.35^{a}	8.3±0.6	0.37±0.04
750	8.0±0.5^{abc}	1.3±0.1	89.9±11.0^{ab}	3.1±0.15^{a}	5.6±1.0	0.41±0.03
1000	9.3±0.2^{a}	1.6±0.2	67.9±5.4^{b}	2.9±0.24^{a}	7.5±1.1	0.35±0.03

a, b and c Mean that are not followed by the same superscripts are significantly different (P<0.05).

Copper supplementation positively affected plasma level of total lipids, the highest value recorded by feeding 1000 mg/kg diet. Plasma total lipids concentration of hens fed 500, 750 and 1000 mg/kg diet significantly increased by about 15.38, 42.30 and 61.53% when compared with the control group at the first 4 weeks experimental period, while those hens fed the same supplemental Cu levels significantly (P<0.05) reduced yolk total lipids. This experiment clearly demonstrates that yolk lipids can be lowered substantially by addition of 1000 mg/kg diet by about 15.78% when compared with control group (Table 1). The same trend was noticed at the end of 8 weeks experimental period. Copper chloride significantly (P<0.05) increased plasma total lipids during this period (Table 2).

The liver weight expressed as g/kg body weight was given in Table (3). Liver weight/kg body weight numerically reduced by the *ad libitum* feeding of diet containing 500 and 750 mg/kg compared with those of control birds fed on diet without copper. These results agree with those reported by Jackson, 1977 and Jackson *et al.*, 1979. The Cu analysis of liver is shown in Table (3).

Table (3): Mean body weight, dressing weight, fresh weights (g/kg body weight) of liver and gizzard and copper concentration (ppm) in liver of laying hens given control and copper supplemented diets.

Treat-ments	Body weight	Dressing weight	Liver	Gizzard	Liver Cu DM (ppm)	Dry weight of liver (g)	Total liver Cu (ppm)
Among levels mg/kg diet:							
0	1863.3±25.9^{a}	1213.2±32.6	16.7±0.61	12.8±0.94	1.07±0.03^{b}	7.9±0.4	8.6±0.6^{c}
500	1740.0±47.3^{ab}	1148.4±29.9	14.8±0.94	12.5±0.40	1.40±0.05^{b}	7.4±0.4	10.7±0.7^{b}
750	1803.0±54.9^{ab}	1156.2±21.8	15.4±0.72	11.2±0.77	1.80±0.02^{b}	8.6±0.5	15.8±0.3^{b}
1000	1701.7±33.7^{b}	1108.6±35.3	16.0±0.93	12.2±0.93	3.10±0.07^{a}	8.3±0.7	24.5±0.3^{a}
Between source:							
Copper sulphate	1796.7±31.7	1151.5±26.8	15.1±0.55	11.9±0.51	2.3±0.50^{a}	8.1±0.3	18.9±3.5^{a}
Copper chloride	1757.5±34.9	1161.7±19.2	16.3±0.57	12.4±0.60	1.3±0.06^{b}	8.0±0.4	10.9±0.9^{b}
Interactions:							
Sulphate mg/kg:							
0	1876.7±39.3^{a}	1211.2±42.8	16.7±1.10	11.2±1.10	1.06±0.06^{b}	8.1±0.3	8.70±0.7^{c}
500	1793.3±37.1^{ab}	1171.6±59.8	13.7±0.29	12.4±0.56	1.5±0.30^{b}	7.4±0.5	10.8±1.46^{c}
750	1860.0±30.5^{a}	1146.3±27.8	14.9±0.61	10.8±0.24	2.1±0.40^{b}	8.8±0.7	19.5±5.2^{b}
1000	1656.7±57.8^{b}	1076.9±69.7	15.1±1.60	13.4±1.50	4.8±0.90^{a}	7.9±1.0	36.6±3.2^{a}
Chloride mg/kg:							
0	1850.0±40.4^{ab}	1215.2±59.1	16.7±0.87	14.4±0.82	1.08±0.04^{b}	7.8±0.7	8.5±1.0^{c}
500	1680.7±83.5^{ab}	1125.3±19.3	15.8±1.70	12.6±0.68	1.40±0.05^{b}	7.5±0.6	10.5±0.9^{c}
750	1746.7±104.8^{ab}	1166.1±39.0	15.8±1.40	11.5±1.60	1.50±0.10^{b}	8.3±0.9	12.1±2.1^{bc}
1000	1746.7±17.6^{ab}	1140.2±19.4	16.8±0.90	11.2±1.00	1.40±0.10^{b}	8.6±1.1	12.3±2.8^{bc}

a, b and c Mean that are not followed by the same superscripts are significantly different (P<0.05).

Results show significant (P<0.05) increase of liver copper by dietary copper level. The two sources of copper showed significant increase by copper sulphate than copper chloride.

The effect of high levels of dietary copper in increasing liver Cu concentration in the present experiment agree with that obtained by Mehring *et al.*, (1960) they found that liver Cu concentration increased from 14 μg/g in control (26 mg Cu/kg diet) to 820 μg/g in group receiving 1176 mg Cu/kg diet. Results by Jackson *et al.*, (1979); Stevenson and Jackson (1981) reported the same trend.

This experiment clearly demonstrates that yolk cholesterol can be lowered substantially by the addition of 1000 mg/kg diet copper as sulphate

chloride. The main effects directly attributable to the copper sulphate are the high liver Cu. The variation in the tested prameters values could be attributed to differences in analytical method and strain.

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التأثير الغذائي لإضافة كبريتات النحاس أو كلوريد النحاس على سلالات المعمورة المحلية للدجاج البياض ٠

٢- التأثيرات الغذائية لمصادر وتركيزات النحاس على الدهون الكليـــة و كوليســترول الصفار والبلازما للدجاج البياض.

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صممت التجربة لمعرفة إمكانية تقليل كوليسترول صفار البيض وذلك باستخدام ســلالة المعمورة للدجاج البياض عند عمر ٣٦ أسبوع وتمت التغذية على عليقة أساســية مــن الــذرة وكسب فول الصويا مضاف إليها أربع مستويات مــن النحــاس صــفر، ٥٠٠ ، ٧٥٠ و ١٠٠٠ ملليجرام/ كيلو جرام عليقة على هيئة كبرتيات نحاس مائية وكلوريد نحاس مائي ومع نهايــة كــل فترة من فترات التجربة تم اخذ ٣ بيضات بطريقة عشوائية من كل مجموعة لتقدير محتوى الصفار من الكوليسترول والدهون الكلية.

- أظهر مستوى إضافة (٥٠٠ ملليجرام/كيلوجرام عليقة) انخفاض معنوي (مستوى ٥%) فــى هيمجلوبين الدم خلال فترتي التجربة و لم تتأثر قيم هيمجلوبين الدم بتغيير مصادر النحاس.
- أظهرت كل من مستويات النحاس ومصادره فى العليقة تأثير سلبي على محتوى البلازما من النحاس.
- أظهرت النتائج أن زيادة النحاس من (صفر إلى ١٠٠٠ ملليجرام/كيلوجرام عليقة) انخفاضــا منتظما على محتوى البلازما من الكوليســترول. وأن كــل مــن مســتوى (٧٥٠ و ١٠٠٠ ملليجرام/كيلوجرام عليقة) أدى إلى انخفاض كــل مــن محتــوى البلازمــا والصــفار مــن الكوليسترول بحوالي (٧,٥٢، ١٩,٤٠، ٢٠,٦٢، ٣٠,٩٢% على التــوالي) خــلال فتــرة ٨ أسابيع من مدة التجربة.
- كان لإضافة النحاس تأثيرا ايجابيا على محتوى البلازما من الدهون الكلية.
- أوضحت النتائج انخفاضا فى محتوى صفار البيض من الدهون الكلية وأن مســتوى إضــافة ١٠٠٠ ملليجرام/كيلوجرام عليقة اظهر انخفاضا يقدر بحوالي ١٥,٨٧% مقارنة بالكنترول.
- أنخفض وزن الكبد (جم/كيلوجرام وزن حى) (عند مستوى ٥٠٠ ، ٧٥٠ ملليجرام/كيلــوجرام عليقة).
- زاد تركيز النحاس بالكبد بزيادة مستويات النحاس فى العليقة.
- هذه النتائج تؤكد على أن التغذية للدجاج البياض على مستويات ومصادر مختلفة من النحاس يؤدى إلى الاستجابة الفسيولوجية تبعا لنوع السلالة وطول فترة التجربة.