Effects of Supplementary Copper-Chelate on the Performance and Cholesterol Level in Plasma and Breast Muscle of Broiler Chickens*

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ABSTRACT : An experiment was conducted to determine the effects of supernormal level of copper (Cu) from different supplementary sources on the performance, cholesterol level in plasma and breast muscle, and accumulation of fat and Cu in broilers. In a 5 wk feeding trial, two hundred forty hatched male broiler chickens were assigned to four dietary treatments: control diet containing 10 mg/kg supplementary Cu, control diet plus 250 mg/kg Cu from $CuSO_4$ ($CuSO_4$-250), control diet plus 125 mg/kg Cu from Cu-methionine chelate (Cu-Met-125), and control diet plus 250 mg/kg Cu from Cu-methionine chelate (Cu-Met-250). Weight gain in Cu-Met-125 treatment and Cu-Met-250 treatment were not different, but they were significantly ($p<0.05$) greater than that in $CuSO_4$-250 treatment. Plasma total cholesterol and reduced glutathione (GSH) in blood were significantly reduced by supplementation of $CuSO_4$-250, but were not significantly affected by Cu-Met supplementations. Plasma HDL cholesterol, plasma triglycerides and breast muscle cholesterol were not significantly affected by Cu supplementation. $CuSO_4$-250 improved metabolizability of crude fat, which resulted in low abdominal fat pad weight. Cu from Cu-Met was better absorbed and accumulated more in the breast muscle and lesser in the liver compared with Cu from $CuSO_4$. (*Asian-Aus. J. Anim. Sci. 1999. Vol. 12, No. 5 : 794-798*)

Key Words : Copper Sulfate, Copper-Methionine Chelate, Cholesterol, Glutathione, Broiler

INTRODUCTION

Copper (Cu) is an essential mineral which serves as a cofactor in many enzyme systems in the body. It has been known that supernormal level (125 to 250 ppm) of Cu in the form of sulfate improves growth rate and feed efficiency in broilers (Choi and Paik, 1989; Baker et al., 1991) and in pigs (Roof and Mahan, 1982; Edmonds et al., 1985; Cromwell et al., 1989).

Growth-stimulating action of dietary Cu has been attributed to its antimicrobial actions (Fuller et al., 1960; Vogt et al., 1981; Burnell et al., 1988). However, the antimicrobial hypothesis alone can not fully explain the effects of Cu. It has been demonstrated that intravenous injection of Cu stimulates the growth of weanling pigs (Zhou et al., 1994). The results of this experiment indicated that Cu acts systemically to influence the growth regulatory system in many ways.

Supernormal level of Cu is commonly supplied in the form of sulfate. It has been reported that sulfate form ($CuSO_4$) is more effective than oxide form (CuO) (Cromwell et al., 1989), and copper sulfate in the form of pentahydrate ($CuSO_4 \cdot 5H_2O$) is more effective than monohydrate ($CuSO_4 \cdot H_2O$) (Kim et al., 1993). It is well known that chelated minerals are more effectively absorbed and stay longer in the body (Fouad, 1976). The growth promoting effects of Cu-methionine chelate (Min et al., 1993) and sequestered-Cu (algal polysaccharide and Cu complex) in pigs (Kim et al., 1991) and broilers (Kim and Paik, 1993) have been demonstrated.

There are reports indicating that Cu is involved in the fat metabolism. Supplementary Cu reduced the incidence of oily bird syndrome (Maurice et al., 1981). Feeding chickens supernormal level of Cu resulted in decreases of plasma and breast muscle cholesterol and plasma triglycerides (Bakalli et al., 1995; Pesti and Bakalli, 1996; Konjufca et al., 1997). Copper deficiency was shown to induce hypercholesterolemia in rats (Klevay, 1973). Evidence indicates that the liver copper 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-methylglutaryl coenzyme A (HMG-CoA) reductase in rats (Valsala and Kurup, 1987). The HMG-CoA reductase activity is the rate-limiting step of mevalonate and, ultimately, cholesterol biosynthesis.

An experiment was conducted to compare the effects of supernormal level supplementation of copper sulfate and Cu-methionine chelate (Cu-Met) on the performance, cholesterol level in plasma and breast muscle and metabolism of fat and Cu in broiler chickens.

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* This research was supported by Chung-ang University Special Research Grants 1996.
Received September 1, 1998; Accepted November 14, 1998

MATERIALS AND METHODS

Experimental design and feeding

Two hundred forty hatched male Arbor Acres were raised in cages of 10 birds each. Six cages (replications) were randomly assigned to each of the four dietary treatments: control diet as the basal diet contained 10 mg of supplementary Cu from $CuSO_4$ $5H_2O$ per kg diet; $CuSO_4$-250 diet contained additional 250 mg Cu from $CuSO_4 \cdot 5H_2O$ per kg diet; Cu-Met-125 diet and Cu-Met-250 diet contained additional 125 mg and 250 mg of Cu from Cu-methionine chelate (Cu-Met) per kg diet, respectively. The composition of the basal diet used as the control is shown in table 1. Cu-Met was made in our laboratory by reacting $CuSO_4 \cdot 5H_2O$ and methionine at 1:2 molar ratio. About 50% of Cu-Met dissolved at pH 2. A quarter of dissolved Cu-Met was dissociated to ionic form (Cu^{++}) and remaining three quarters of dissolved Cu-Met was in a stable form which is considered to be chelated. Birds were given feed and water *ad libitum* and raised for 5 wk.

Collection and preparation of samples for analysis

The weekly weight gain and feed intake were measured by pen. On the termination of feeding, ten birds from each treatment were randomly chosen for blood and tissue analysis. Birds were killed by asphyxiation and blood samples of approximately 10 ml per bird were collected by heart puncture. Blood samples were placed in heparinized vacuum tube (Becton Dickinson/Vacutainer System, Rutherford, NJ, USA) and a portion of each blood sample was taken for glutathione analysis and the remainder was centrifuged at 3,000 rpm for 15 min. Plasma and tissue samples were stored at -20℃ until analysis. For the metabolic trial, three birds from each treatment were chosen and housed in the individual metabolic cage. After 3 days adjusting period, total excreta were collected for three days and dried immediately.

Analysis

Plasma total cholesterol, high density lipoprotein (HDL) cholesterol and triglyceride concentrations were determined using Sigma Diagnostic Kits (No. 352 for total cholesterol, No. 352-3 for HDL-cholesterol and No. 336 for triglycerides, Sigma Chemical Co., St. Louis, MO, USA). Muscle samples were homogenized and concentration of the total breast muscle cholesterol was determined by the method of Fenton and Sim (1991) using gas chromatography (HP6890, Hewlett Packard Co., USA). Blood reduced glutathione (GSH) was determined by the method of Beutler et al. (1963). For the analysis of Cu in the liver, muscle and excreta, samples were ashed with HNO_3 and HCl (AOAC, 1990) and assayed with ICP (Inductively Coupled Plasma) Emission Spectrometer (Model JY, Jobin Yvon, France). General compositions of feeds and excreta, and fat content of the liver were analyzed by AOAC (1990) procedures. The metabolizability of nutrients was calculated as the percent ratio of the amount of retained nutrient (ingested nutrient - excreted nutrient) to the amount of ingested nutrient. Abdominal fat pad was weighed and expressed as the percentage of the body weight (g/g×100). Ulceration of the gizzard lining was scored with gizzard erosion index. Gizzard erosion indices are arbitrary figures ranging from 1 for normal gizzard to 4 for severely eroded gizzard. The data were analyzed by ANOVA using General Linear Models (GLM) procedure of SAS (1985). Significant differences between treatment means were determined at $p<0.05$ using Duncan's new multiple range test (Duncan, 1955).

Table 1. Composition of the basal diets

Ingredients, %	Starter (0～3 wk)	Grower (4～5 wk)
Corn	60.69	68.53
Soybean meal	26.30	18.85
Corn gluten meal	7.44	7.59
Ca-phoshpate (18%)	1.79	1.57
Animal fat	1.50	1.50
Limestone	0.76	0.75
Vitamin premix[1]	0.53	0.38
Salt	0.40	0.28
Methionine (50%)	0.23	0.07
Trace mineral premix[2]	0.20	0.20
Lysine-HCl (78%)	0.17	0.29
Total	100.00	100.00
Chemical composition[3]		
ME (kcal/kg)	3,050	3,150
Crude protein (%)	21.5	19.0
Lysine (%)	1.10	1.00
Methionine-cystine (%)	0.86	0.71
Ca (%)	1.00	0.90
Total P (%)	0.71	0.64

[1] Vitamin premix provides per kg of starter diet: Vit. A, 16,000 IU; Vit. D_3, 3,200 IU; Vit. E, 50 IU; Vit. B_1, 2.6 mg; Vit. B_2, 10.4 mg; Vit. B_6, 6.5 mg; Vit. B_{12}, 0.4 mg; Vit. K_3, 6.5 mg; Niacin, 52 mg; Folic acid, 1.3 mg; Ethoxyquin, 125 mg.
Vitamin premix provides per kg of finisher diet: Vit. A, 12,000 IU; Vit. D_3, 2,400 IU; ; Vit. B_1, 2.0 mg; Vit. B_2, 8.0 mg; Vit. B_6, 5.0 mg; Vit. B_{12}, 0.3 mg; Vit. K_3, 5.0 mg; Niacin, 40 mg; Folic acid, 1.0 mg; Ethoxyquin, 125 mg.

[2] Trace mineral premix provides in mg per kg of diet: Mn, 70 mg; Zn, 50 mg; Fe, 60 mg; Cu, 10 mg; I, 0.5 mg; Co, 0.3 mg; Se, 0.2 mg.

[3] Calculated values.

RESULTS AND DISCUSSION

There were no significant differences among treatments in feed intake and feed/gain ratio. Weight gains at starter or grower period were not significantly different but overall weight gain for 5wk were different among treatments. Cu-Met-125 and Cu-Met-250 treatments showed significantly greater weight gain than $CuSO_4$-250 treatment. In the present study, Cu-Met-125 diet contained a half level of Cu, but the growth promoting effect was similar to that of Cu-Met-250. The $CuSO_4$-250 treatment did not show any growth promoting effect in this study (table 2).

Table 2. Weight gain, feed intake and feed conversion of male broiler chickens fed diets supplemented with different Cu sources

Item	Treatments[1]				SEM[2]
	Control	$CuSO_4$-250	Cu-Met-125	Cu-Met-250	
Weight gain, g/bird					
0~3 wk	574.9	581.4	618.5	609.8	14.9
4~5	730.5	704.0	746.6	747.5	18.2
0~5	1305.3[ab]	1285.4[b]	1365.1[a]	1357.4[a]	23.2
Feed intake, g/bird					
0~3 wk	834.4	819.9	863.7	858.0	17.8
4~5	1292.2	1259.5	1310.9	1319.6	30.7
0~5	2126.5	2079.4	2174.5	2177.6	41.6
Feed/Gain					
0~3 wk	1.46	1.41	1.40	1.41	0.02
4~5	1.77	1.79	1.76	1.77	0.03
0~5	1.63	1.62	1.59	1.60	0.01

[1] **Control:** basal diet contained 10 mg of supplementary Cu from $CuSO_4 \cdot 5H_2O$; **$CuSO_4$-250:** 250 ppm of supplementary copper in the form $CuSO_4 \cdot 5H_2O$; **Cu-Met-125:** 125 ppm of supplementary copper in form of Cu-methionine chelate; **Cu-Met-250:** 250 ppm of supplementary copper in form of Cu-methionine chelate.

[2] Standard error of the mean.

[a,b] Means with different superscript in the same row are significantly different at $p<0.05$.

It has been reported that the response to supernormal level of Cu in the form of $CuSO_4$ was different depending on the species (Paik et al., 1996; 1998). The responses have always been positive in pigs, inconsistent in broilers and negative in rats. Although variable results have been obtained with $CuSO_4$ in broiler experiments, Cu-Met treatment always outperformed $CuSO_4$ treatment. Although the control diet had enough methionine, extra methionine from Cu-Met might have influenced the result. Kassim and Suwanpradit (1996) indicated that dietary copper at the level of 375 mg/kg increased total sulfur amino acid requirement when assessed on the basis of feed intake. It is likely that systemic growth-promoting of Cu in pigs indicated by Zhou et al. (1994) may also act in broilers. However, other factors, such as severity of gizzard erosion (table 3), may have affected the performance.

Plasma total cholesterol and GSH in blood were significantly reduced by supplementation of $CuSO_4$-250, but were not significantly affected by Cu-Met supplementation at either level (table 3). Plasma HDL cholesterol tended to be high in Cu supplemented groups than the control. However, plasma triglycerides, and breast muscle cholesterol were not significantly affected by Cu supplementation. Current data for plasma total cholesterol and blood GSH is in agreement with that of Bakali et al. (1995). It may be interpreted that high level of Cu from $CuSO_4$ reduced GSH, which reduced stimulation of HMG-CoA reductase activity and, ultimately, reduced cholesterol synthesis. However, breast muscle cholesterol level was not significantly affected like the results reported by other researchers (Bakalli et al., 1995; Pesti and Bakalli, 1996; Konjufca et al., 1997). They used basal diets containing 5 mg/kg supplementary Cu, while the current experiment used a basal diet containing 10 mg/kg supplementary Cu. NRC (1994) requirement for Cu is 8 mg/kg for broilers. The level of Cu supplemented in the current experiment excludes any possibility of hypercholesterolemia caused by Cu deficiency. Thus, the difference in cholesterol parameters between the control and Cu supplemented groups may not be as great as those of other researchers.

Liver fat content tended to be low in Cu supplemented groups than the control. Weight of abdominal fat pad was lowest in $CuSO_4$-250 treatment but that of Cu-Met treatment at either level was not different from that of the control. Supernormal level of Cu supplementation reduced plasma triglycerides in some experiment (Bakalli et al., 1994), but not in others (Konjufca et al., 1997) like the current experiment. It is not well understood how Cu affects fat metabolism. It was observed that Cu stimulated the growth of longissimus muscle and speculated that Cu might be involved in the growth regulatory system such as pituitary growth hormone gene expression, secretion of several neuropeptides in the hypothalamus, and stimulation of growth hormone secretion from bovine pituitary explants (Zhou et al., 1994). Such roles of Cu in the system will influence fat metabolism as well as the growth of the animals.

Cu concentration in the liver, breast muscle and excreta were significantly affected by treatments. Liver Cu in $CuSO_4$-250 treatment was higher than the control, but that of Cu-Met-125 or Cu-Met-250 treatment was not significantly different from the control. Breast muscle Cu was higher in Cu-Met-250

Table 3. Influence of dietary Cu sources on the plasma total cholesterol, high density lipoprotein (HDL)-cholesterol, breast muscle cholesterol, plasma triglycerides, blood reduced glutathione (GSH), liver fat, abdominal fat pad, gizzard erosion index and Cu in the liver, breast muscle and excreta of broiler chickens

Variable	Treatments[1]				SEM[2]
	Control	$CuSO_4$-250	Cu-Met-125	Cu-Met-250	
Plasma total cholesterol (mg/100 mℓ)	151.5[a]	126.8[b]	138.7[ab]	146.3[a]	5.50
Plasma HDL cholesterol (mg/100 mℓ)	90.5	105.5	104.1	105.0	5.15
Breast muscle cholesterol (mg/100 g wet tissue)	44.8	48.8	39.6	39.5	3.86
Plasma triglycerides (mg/100mℓ)	78.8	62.1	58.0	81.1	9.02
GSH (mg/100mℓ blood)	92.6[a]	75.9[b]	92.1[a]	90.2[a]	3.10
Liver fat (%, DM)	16.2	13.3	11.2	14.7	1.79
Abdominal fat pad (% body wt.)	2.03[ab]	1.73[b]	2.32P	2.08[ab]	0.14
Gizzard erosion index	1.00[c]	3.10[a]	1.50[bc]	2.20[d]	0.26
	Cu (mg/kg, DM)				
Liver	19.3[b]	36.3[a]	15.2[b]	22.3[b]	3.95
Breast muscle	2.25[b]	2.29[b]	2.27[b]	2.97[a]	0.09
Excreta	155.0[d]	808.7[a]	358.7[c]	543.3[b]	16.24

[1] **Control:** basal diet contained 10 mg of supplementary Cu from $CuSO_4 \cdot 5H_2O$; **$CuSO_4$-250:** 250 ppm of supplementary copper in the form $CuSO_4 \cdot 5H_2O$; **Cu-Met-125:** 125 ppm of supplementary copper in form of Cu-methionine chelate; **Cu-Met-250:** 250 ppm of supplementary copper in form of Cu-methionine chelate.

[2] Standard error of the mean.

[a,b] Means with different superscript in the same row are significantly different at $p<0.05$.

treatment than other treatments. Cu concentration of excreta was highest in $CuSO_4$-250 followed by Cu-Met-250, Cu-Met-125 treatment and the control. Current data shows that Cu in the liver is more readily accumulated by $CuSO_4$ supplementation than Cu-Met, and Cu in the breast muscle is more readily accumulated by Cu-Met than $CuSO_4$. Fecal data indicates that Cu in Cu-Met is more absorbed than that in $CuSO_4$. Gizzard erosion index was highest in $CuSO_4$-250 treatment followed by Cu-Met-250, Cu-Met-125 treatment and the control. It is interesting to note that gizzard erosion due to supernormal Cu supplementation is less severe with Cu-Met than with $CuSO_4$. Gizzard erosion is caused by strong acidic nature of $CuSO_4$ (Miyazaki and Umemura, 1987). Cu-Met is less acidic than CuSO4 and methionine supplementation alleviated gizzard lining erosion (Miller et al.1975). Although such methionine effect on gizzard erosion was not evident in other studies (Jensen and Maurice, 1978; Robbins and Baker, 1980), present study clearly shows that Cu-Met afflicts less damage to gizzard lining than $CuSO_4$.

Metabolizability of crude fat was significantly different among treatments with $CuSO_4$-250 group being the highest and Cu-Met-250 group the lowest (table 4). Metabolizability of other nutrients were not significantly different among treatments. High metabolizability of crude fat in $CuSO_4$ treatment may have resulted in low abdominal fat pad weight.

It can be concluded that Cu-Met-125 is more effective than $CuSO_4$-250 in promoting growth of broilers. Supplementation of $CuSO_4$-250 reduces plasma cholesterol and blood GSH, but the reduction of breast muscle cholesterol is not conclusive. $CuSO_4$-250 also improves metabolizability of crude fat, which resulted in low abdominal fat pad weight. Cu in the form of Cu-Met is better absorbed than that of $CuSO_4$. Different accumulation rate of Cu in the liver and breast muscle is indicative that Cu from CuSO4 and Cu-Met may have different pathway in the body of broilers.

Table 4. Nutrients metabolizability of the experimental diets

Treatment[1]	Metabolizability[2] (%)				
	DM	Crude protein	Crude fat	Crude fiber	Crude ash
Control	81.5	63.3	87.6ab	32.9	24.7
$CuSO_4$-250	80.4	62.5	92.2a	27.2	30.9
Cu-Met-125	81.8	63.2	82.5bc	30.7	22.0
Cu-Met-250	82.5	65.0	81.7c	35.8	26.5
SEM[3]	1.13	2.16	1.58	5.09	5.79

[1] **Control:** basal diet contained 10 mg of supplementary Cu from $CuSO_4 \cdot 5H_2O$; **$CuSO_4$-250:** 250 ppm of supplementary copper in the form $CuSO_4 \cdot 5H_2O$; **Cu-Met-125:** 125 ppm of supplementary copper in form of Cu-methionine chelate; **Cu-Met-250:** 250 ppm of supplementary copper in form of Cu-methionine chelate.

[2] The ratio of the amount of retained nutrient (ingested nutrient - excreted nutrient) to the amount of ingested nutrient.

[3] Standard error of the mean.

[a,b,c] Means with different superscript in the same row are significantly different at $p<0.05$.

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