Studies on the Feeding of Cupric Sulfate Pentahydrate and Cupric Citrate to Broiler Chickens[1]

GENE M. PESTI and REMZI I. BAKALLI

Department of Poultry Science, The University of Georgia, Athens, Georgia 30602-2772

ABSTRACT Male commercial broiler strain chickens were fed either a control diet (based on corn and soybean meal) or the control diet supplemented with cupric sulfate pentahydrate or cupric citrate in seven experiments (six in floor pens, one in wire-floored batteries). In Experiment 1, feeding 125 or 250 mg/kg copper increased growth (4.9%) and decreased feed conversion ratios (3.4%), total plasma cholesterol (40.2%), and breast muscle cholesterol (37.0%). Feeding 375 mg/kg copper was without further beneficial effect. In Experiment 2, withdrawing growth promoting supplements of copper from the feed for the last 7 d caused a significant ($P < 0.05$) increase in breast muscle cholesterol at 42 d of age: 57.2, 48.0, and 43.2 mg/100 g meat for birds supplemented for 0, 35, or 42 d, respectively. Feeding 10 vs 260 mg/kg copper caused only small increases in tissue copper levels: 0.36 vs 0.41 mg/kg for breast meat, and 0.48 vs 0.60 mg/kg for thigh meat, respectively. Litter copper accumulations in these experiments were similar to those of earlier reports. Breast muscle cholesterol was reduced by feeding 125 mg/kg supplemental copper from cupric citrate (27.84 mg/100 g) or 125 mg supplemental copper from cupric sulfate pentahydrate (25.32 mg/100 g) compared to broilers fed the control diet (43.92 mg/100 g). Cupric citrate was efficacious for growth promotion at lower copper levels than cupric sulfate pentahydrate, resulting in reduced litter copper.

(*Key words*: copper, broiler, cholesterol)

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INTRODUCTION

Copper is often fed to broiler chickens at levels above the nutritional requirement. These "pharmacological" levels are fed because of copper's activity as a growth promotant (Fisher, 1973).

Dietary copper, when fed at physiological as well as pharmacological levels, has been demonstrated to alter the lipid metabolism of rats (Klevay, 1973; Murthy and Petering, 1976; Petering *et al.*, 1977), swine (Amer and Elliot, 1973a,b), nonhuman primates and humans (Lei, 1991), sheep (Todd and Thompson, 1963), and chickens (Bakalli *et al.*, 1995).

Metabolic changes resulting from dietary copper include changes in the rate of cholesterol biosynthesis and hepatic glutathione concentrations (Todd and Thompson, 1963; Kim *et al.*, 1992) and the relative distributions of various fatty acids in porcine depot fat (Amer and Elliot, 1973b). In studies with broiler chickens it was demonstrated that feeding 250 mg copper/kg diet (in addition to the copper needed to meet the classical nutritional requirement, 10 mg copper/kg) reduced plasma total cholesterol, (~ 26%), increased HDL cholesterol (~ 11%), reduced plasma triglycerides (~ 43%), reduced blood glutathionine (~ 40%), and reduced breast muscle cholesterol (~ 27%) (Bakalli *et al.*, 1995).

A series of experiments was conducted to confirm and extend some of the results of feeding pharmacological levels of copper to broiler chickens. The influence of higher levels of dietary copper on growth performance and tissue cholesterol was tested. To ensure that the active component of cupric sulfate pentahydrate was the copper, the responses to cupric sulfate pentahydrate and cupric citrate were compared. Cupric citrate was chosen because it is an organic form of copper that is inexpensive to manufacture. The deposition of copper in various edible tissues from birds receiving pharmacological levels of dietary copper was measured as was litter copper accumulation. The amount of copper remaining in the viscera of the birds was measured because it is a possible contaminant of processing plant waste water. When the growth responses to dietary copper from cupric citrate appeared to be better than from cupric sulfate pentahydrate, trials with increased replication were conducted to test the hypothesis that cupric citrate is a more efficient growth promotant than cupric sulfate pentahydrate.

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TABLE 1. Composition of the basal diet

Ingredients	Amounts
	(%)
Ground yellow corn	57.34
Soybean meal (dehulled)	33.48
Poultry fat (stabilized)	3.15
Poultry by-product meal	3.00
Iodized sodium chloride	0.21
DL-methionine (98%)	0.19
Vitamin premix[1]	0.25
Trace mineral premix[2]	0.05
Defluorinated phosphate	1.54
Limestone	0.79
Composition[3]	
Protein,%	23.13
Energy, kcal/g	3.13
Methionine	0.57
Cystine	0.35

[1]Vitamin premix provides the following per kilogram: vitamin A, 5,500 IU from all *trans*-retinyl acetate, cholecalciferol, 1,100 IU; vitamin E, 11 IU from all-*rac*-α-tocopherol acetate; riboflavin, 4.4 mg; Ca pantothenate, 12 mg; nicotinic acid, 44 mg; choline Cl, 220 mg; vitamin B_{12}, 6.6 μg; vitamin B_6, 2.2 mg; menadione, 1.1 mg (as MSBC); folic acid, .55 mg; d-biotin, .11 mg; thiamine, 1.1 mg (as thiamine mononitrate); ethoxyquin, 125 mg.

[2]Trace mineral premix provides the following in milligrams per kilogram of diet: Mn, 60; Zn, 50; Fe, 30; Cu, 5; I, 1.5.

[3]Estimated from NRC (1994) composition tables.

MATERIALS AND METHODS

Seven experiments were conducted with day-old broiler cockerels obtained from a local broiler producer. Peterson × Arbor Acres chicks were used in Experiments 1 to 6 and Ross × Ross chicks were used in Experiment 7. The chicks were randomly placed in floor pens with wood shavings (1.22 × 3.66 m) except for Experiment 2, when they were placed in Petersime wire floored battery brooders. The chicks were maintained on a 24-h light schedule, and feed and water (< 0.5 μg copper/L) were provided for *ad libitum* consumption throughout the experimental periods. Tissue, feed, and water copper were measured by flame atomic absorption spectrophotometry (Perkin Elmer 5000).[2]

The basal diet used is presented in Table 1. It was formulated to meet all National Research Council (1994) recommendations including copper. The basal diets averaged 10.4 ± 1.1 (mean ± standard deviation) mg copper/kg. The copper was supplemented as feed grade cupric sulfate pentahydrate or food grade cupric citrate.

Experiment 1 was a titration of four levels of copper from cupric sulfate pentahydrate (0, 125, 250, and 375 mg copper/kg). Four replicate pens of 15 birds each per treatment were started and 5 birds were randomly removed for blood and tissue analyses at 21 and 42 d.

Birds were bled by heart puncture with syringes containing sodium-heparin and killed by asphyxiation. Breast muscle samples were frozen at –20 C before analysis. Plasma total cholesterol was determined using Sigma Diagnostic Kits.[3] Muscle samples were thoroughly homogenized and extracted by the method of Folch *et al* (1957) modified by Bligh and Dyer (1959). The total breast muscle cholesterol content was determined by the enzymatic method of Allain *et al.* (1974) modified by Salè *et al.* (1984). Breast muscle tissue used for copper analysis was dried in a vacuum oven and dry ashed as described by Blanusa and Breski (1981).

In Experiment 2, four replicate pens of eight chicks each per treatment were placed in Petersime starting batteries shortly after hatching and moved to Petersime finishing batteries at 21 d. The birds were fed 250 mg copper/kg for 35 or 42 d. Tissue samples were taken after an overnight period without feed when the birds were 42 d old. The entire gastrointestinal tract was opened and gently scraped to determine the gastrointestinal tract content of copper. Experiment 3 had the same treatments and duration as Experiment 2. The only differences were that the birds were in floor pens and there were three replicate pens of 15 cockerels each. Only body weight, feed consumption, and litter copper measurements were made.

In Experiment 4 there were five levels of supplemental copper from cupric citrate (0, 63, 125, 185, and 250 mg copper/kg), and the experiment ended when the birds were 35 d old. Three replicate pens were fed each diet for 35 d. In Experiment 5 feeding 63 or 125 mg copper/kg from cupric citrate was compared to feeding 125 or 250 mg copper/kg from cupric sulfate pentahydrate. Four replicate pens were fed each diet for 42 d. After an overnight period without feed, the birds were killed by asphyxiation and breast muscle and gastrointestinal tract content samples were taken as before.

In Experiment 6, three levels of supplemental copper from cupric citrate (50, 75, or 100 mg/kg) and one level of copper from cupric sulfate pentahydrate (187 mg/kg) were compared to a control with no supplemental copper. Four pens of 15 birds each were fed each diet for 42 d. Birds were killed by asphyxiation on Day 42 and breast muscle samples were taken (there was no overnight period of feed deprivation). In Experiment 7, eight replicate pens were fed the basal diet or the basal diet supplemented with either 63 mg copper from cupric citrate or 125 mg copper from cupric sulfate pentahydrate.

Data were analyzed one-way by analysis of variance using the General Linear Models (GLM) procedure of SAS® (SAS Institute, 1985). The experimental unit was the pen mean. When significant treatment effects were detected, means were separated using Duncan's new multiple range test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Feeding 250 mg/kg copper in excess of the basal level that satisfied the nutritional requirement (10 mg/kg),

[2]Perkin Elmer Corp., Norwalk, CT 06859-0012.

[3]Number 352, total cholesterol, Sigma Chemical Co., St. Louis, MO 63178-9916.

TABLE 2. Influence of copper (from cupric sulfate pentahydrate) supplementation on the performance and composition of broiler chickens, Experiment 1

Supplement Cu	Weight gain		Feed conversion		Cholesterol			
					Total plasma		Breast muscle	
	21 d	42 d	0 to 21 d	0 to 42 d	21 d	42 d	21 d	42 d
(mg/kg)	(kg)		(g:g)		(mg/DL)		(mg/100 g)	
0	0.587	1.879[a]	1.530	1.953[a]	135.2[a]	145.0[a]	48.6[a]	53.3[a]
125	0.621	1.895[a]	1.564	1.943[ab]	125.6[b]	105.6[b]	40.2[b]	41.8[c]
250	0.635	1.972[b]	1.496	1.889[c]	118.3[b]	103.4[b]	38.4[b]	38.9[d]
375	0.591	1.884[a]	1.505	1.916[bc]	121.0[b]	98.6[b]	45.3[a]	46.9[b]
Pooled SE	0.020	0.021	0.029	0.010	2.6	5.9	1.5	0.7

[a–d]Values with no common superscript differ significantly ($P < 0.05$) when tested by Duncan's new multiple range test following analysis of variance.

[1]Values represent the mean of four replicate pens of 15 cockerels each. Samples of 5 birds per pen were pooled for plasma and breast muscle determinations.

TABLE 3. Influence of dietary cupric sulfate pentahydrate supplementation (250 mg Cu/kg) on the performance and body composition of 42-d-old broiler chickens, Experiment 2[1]

Days of supplementation	Weight gain	Feed conversion	Blood		Cholesterol	
			Hemoglobin	PCV[2]	Total plasma	Breast muscle
	(kg/42 d)	(g:g)	(mg/DL)	(%)	(mg/DL)	(mg/100 g)
None	1.846[a]	1.993[a]	11.08	36.24	149.2[a]	57.2[a]
1 to 35	1.921[b]	1.920[b]	10.43	36.46	133.1[ab]	48.0[b]
1 to 42	1.963[b]	1.883[b]	11.18	36.10	129.9[b]	43.2[c]
Pooled SE	0.02	0.02	0.37	0.88	0.45	1.2

	Cu contents						
	Breast	Thigh	Heart	Liver	Gizzard	Carcass	Gastrointestinal tract
	(mg/kg as is basis)						
None	0.36	0.48[b]	2.78	3.12[b]	0.79[b]	1.51[c]	7.5 ± 1.0[b]
1 to 35	0.38	0.55[ab]	3.02	3.99[ab]	0.74[b]	1.89[b]	7.9 ± 1.3[b]
1 to 42	0.41	0.60[a]	3.04	5.02[a]	1.01[a]	2.44[a]	243.9 ± 62.1[a]
Pooled SE	0.02	0.02	0.02	0.10	0.34	0.06	21.2

[a,b]Values with no common superscript differ significantly ($P < 0.05$) when tested by Duncan's new multiple range test following analysis of variance.

[1]Values represent the mean of four replicate pens of eight cockerels each. Samples of three birds per pen were pooled for analysis.

[2]Hematocrit.

TABLE 4. Influence of dietary cupric sulfate pentahydrate (250 mg Cu/kg) on the 42-d performance and litter Cu of broiler chickens, Experiment 3[1]

Days of supplementation	Weight gain	FCR[2]	Litter Cu			
			0 d		42 d	
			As is	Dry	As is	Dry
	(kg)	(g:g)	(mg/kg)			
0	2.254[b]	1.783[a]	1.3	1.7	26.1[c]	31.9[c]
35	2.347[b]	1.696[b]	1.3	1.6	230.7[b]	298.7[b]
42	2.443[a]	1.708[b]	1.4	1.8	281.8[a]	372.2[a]
Pooled SE	0.019	0.022	0.1	0.1	5.3	7.87

[a–c]Values with no common superscript differ significantly ($P < 0.05$) when tested by Duncan's new multiple range test following analysis of variance.

[1]Values represent the mean of three replicate pens of 15 cockerels each. Samples of 3 birds per pen were pooled for analysis.

[2]Feed conversion ratio (grams of intake:grams of grain).

TABLE 5. Influence of dietary cupric citrate on the performance of broiler chickens, Experiment 4[1]

Supplemental Cu	Weight gain[2]	FCR[2,3]
(mg/kg)	(g/35 d)	(g:g)
0	1.801[c]	1.840[ab]
63	1.855[b]	1.763[bc]
125	1.928[a]	1.713[c]
185	1.914[a]	1.703[c]
250	1.789[c]	1.863[a]
Pooled SE	0.014	0.023

[a-c]Values with no common superscript differ significantly ($P < 0.05$) when tested by Duncan's new multiple range test following analysis of variance.

[1]Values represent the mean of three replicate pens of 15 cockerels each.

[2]Significant linear and quadratic effects ($P < 0.05$) of Cu on weight gain and feed conversion ratio were found.

[3]Feed conversion ratio (grams of intake:grams of gain).

improved the growth and feed conversion ratio of broilers. However, when copper was fed above 250 mg/kg (necessary for maximum growth and feed efficiency), the benefit was lost (Experiment 1, Table 2). One hundred and twenty-five or 250 mg/kg dietary copper reduced plasma and breast muscle cholesterol, but 375 mg/kg copper was without further beneficial effect.

Feeding 250 mg/kg copper for 35 or 42 d increased weight gains and decreased plasma total and breast muscle cholesterol with small increases in edible tissue copper (Experiment 2, Table 3). Withdrawing copper from the feed for 7 d allowed for an increase in breast muscle cholesterol, but tissue copper level decreases were small and nonsignificant except for the gizzard and ground whole carcass (which included the gastrointestinal tract contents). When copper was fed up to slaughter the concentration of copper in the gastrointestinal tract, even after an overnight period without feed, was very similar to what was in the feed, and also what was in the litter (Table 4). The 7-d feed withdrawal in Experiment 3 resulted in decreased litter copper, but also decreased performance (Tables 3 and 4).

Dietary copper from cupric citrate improved broiler performance (Experiment 4, Table 5), and lowered breast muscle cholesterol to the same degree as copper from cupric sulfate pentahydrate, but at lower levels of copper (Experiment 5, Table 6). This result establishes that the cholesterol lowering effect of cupric sulfate is indeed due to the copper and not the sulfate.

The level of copper from cupric citrate resulting in maximum growth was 125 mg/kg; and 250 mg/kg copper from cupric citrate was without effect (Table 5). This result led us to speculate that copper from cupric citrate is both efficacious and toxic at levels lower than copper from cupric sulfate.

The best growth responses in Experiments 5 to 7 were from 63 or 75 mg/kg copper (Tables 6 to 8). In each of Experiments 5 to 7, the performance was better from cupric citrate than from cupric sulfate, although we had limited resources and could not feed a range of levels of either source. In Experiment 7 the number of replicates was increased to increase confidence in the quantitative results. It could be that by feeding a higher level of copper from cupric sulfate in Experiment 7 a better response would have been observed. The response from 125 mg/kg copper was slightly better than 250 mg/kg copper in Experiment 5, so it was arbitrarily decided to use 125 mg/kg copper in Experiment 7. Nonetheless, it is apparent that copper from cupric citrate is efficacious at lower levels than are commonly fed for cupric sulfate. In Experiments 5 and 7, significantly better responses from copper from cupric citrate than from cupric sulfate were observed.

TABLE 6. Influence of dietary Cu source (sulfate vs citrate) on the performance, breast muscle cholesterol, litter, and visceral Cu contents of broiler chickens, Experiment 5[1]

Source	Supplemental Cu	Gain	FCR[2]	Breast muscle cholesterol	Litter Cu		Gastrointestinal tract content Cu	
					As is	Dry	As is	Dry
	(mg/kg)	(kg/42 d)	(g:g)	(mg/100 g)	(mg/kg)			
Basal	0	2.062[c]	2.000[a]	43.92[a]	24.2[d]	27.5[d]	7.9 ± 1.2	48.2 ± 6.7
Citrate	63	2.217[a]	1.874[b]	33.05[b]	81.4[c]	97.6[c]	52.9 ± 6.2	265.8 ± 30.5
Citrate	125	2.155[b]	1.905[ab]	27.84[b]	144.5[b]	169.8[b]	118.1 ± 32.9	618.1 ± 182.5
Sulfate	125	2.148[b]	1.967[ab]	25.32[b]	142.6[b]	171.3[b]	120.3 ± 10.5	614.5 ± 72.5
Sulfate	250	2.101[bc]	1.952[ab]	29.88[b]	240.6[a]	281.9[a]	106.3 ± 32.7	626.8 ± 109.7
Pooled SE		0.018	0.034	2.71	8.3	10.3		

[a-c]Values with no common superscript differ significantly ($P < 0.05$) when tested by Duncan's new multiple range test following analysis of variance.

[1]Values represent the mean of three replicate pens of 15 cockerels each. Samples of 5 birds per pen were pooled for gastrointestinal tract content and breast muscle analyses.

[2]Feed conversion ratio (grams of intake:grams of gain).

TABLE 7. Influence of dietary Cu citrate and Cu sulfate pentahydrate on the performance and breast muscle cholesterol of broiler chickens, Experiment 6[1]

Source	Supplemental Cu	Weight gain	FCR[2]	Breast muscle cholesterol
	(mg/kg)	(g/42 d)	(g:g)	(mg/100 g)
Control	0	2.15[b]	2.05[a]	68.8[a]
Citrate	50	2.29[a]	1.93[b]	46.5[b]
Citrate	75	2.31[a]	1.98[ab]	41.7[bc]
Citrate	100	2.19[ab]	1.99[ab]	34.6[c]
Sulfate	187	2.26[a]	1.99[ab]	39.6[bc]
Pooled SE		0.04	0.02	2.3

[a-c]Values with no common superscript differ significantly ($P < 0.05$) when tested by Duncan's new multiple range test following analysis of variance.

[1]Values represent the mean of four replicate pens of 15 cockerels each. Samples of 5 birds per pen were pooled for breast muscle analyses.

[2]Feed conversion ratio (grams of intake:gram of gain).

It remains to be proven why organic copper from cupric citrate would be more efficacious than inorganic cupric sulfate in improving the performance of broilers. Cupric citrate may prove to be better absorbed than cupric sulfate, although an organic complex of copper (copper lysine) is not (Baker *et al.*, 1991; Aoyagi and Baker, 1993). Zhou *et al.* (1994) demonstrated that pigs injected with copper histidinate grow faster than those injected with histidine solution. They injected levels similar to those absorbed copper from feeding growth promoting levels. Cupric citrate may be absorbed better than cupric sulfate or it may be a better co-factor for one of the mechanisms by which copper can promote growth. Zhou *et al.* (1994) proposed several such mechanisms: 1) release into the gut to affect microflora populations; 2) increased serum mitogenic activity; 3) increased pituitary growth hormone expression (La Bella *et al.*, 1973); 4) increased neuropeptide secretion (Tsou *et al.*, 1977; Barnea and Cho, 1987); 5) post-translational modification of regulatory peptides (Eipper and Mains, 1988); or 6) as a component of the growth factor Iamin (Parkart, 1987).

Although the mode of action of copper is not clear, pharmacological levels of dietary copper clearly affect both lipid metabolism and growth of poultry. However, the use of copper in poultry feeds may need to be minimized because of perceived environmental concerns. The levels of litter copper in Tables 4 and 6 are consistent with levels reported elsewhere (Kiker, 1974; Kunkle *et al.*, 1980; Vest and Dyer, 1993). Recent data suggest that litter copper may not be detrimental to soils or crops (Smith *et al.*, 1992). Soil copper was actually reduced by broiler litter application and resulting forage copper levels were increased slightly, but were still below the practical minimum copper requirement for cattle (NRC, 1988).

The results of Experiments 1 to 7 demonstrate that copper may be added to the diets of growing broiler chickens as either cupric sulfate pentahydrate or cupric citrate to improve growth and decrease meat cholesterol. When copper is fed at growth-promoting levels, meat copper increases but the levels are still very low. Cupric citrate may be used at lower levels than cupric sulfate pentahydrate, resulting in reduced litter copper.

TABLE 8. Influence of dietary cupric sulfate pentahydrate and cupric citrate on the performance of male broiler chickens, Experiment 7[1]

Supplemental Cu	Source	Weight gain		FCR[2]	
		21 d	42 d	21 d	42 d
(mg/kg)		(kg)		(g:g)	
0		0.710	2.189[c]	1.599	2.031[a]
63	Citrate	0.720	2.393[a]	1.606	1.891[b]
125	Sulfate	0.703	2.309[b]	1.653	1.938[b]
Pooled SE		0.007	0.025	0.032	0.028

[a-c]Values with no common superscript differ significantly ($P < 0.05$) when tested by Duncan's new multiple range test following analysis of variance.

[1]Values represent the mean of eight replicate pens of 30 cockerels each.

[2]Feed conversion ratio (grams of intake:gram of gain).

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