Modulation of Cholesterol Levels in Broiler Meat by Dietary Garlic and Copper[1]

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ABSTRACT Male Ross × Ross 208 chickens were fed from hatching to 21 d of age either a control diet (based on corn and soybean meal) or the control diet supplemented with 0, 1.5, 3.0, and 4.5% of a commercial garlic powder in Experiments 1 and 2. Once the dose-response relationship was established, 3% garlic powder or 63 or 180 mg/kg copper as cupric citrate or cupric sulfate pentahydrate were supplemented to the diet (Experiments 3, 4, 5, and 6). In the first two experiments, reductions of plasma cholesterol (P = 0.006) and triacylglycerols (P = 0.013) and liver (P = 0.012) and breast muscle (P = 0.165) cholesterol were observed in garlic-supplemented birds. Feeding either garlic powder or copper (63 and 180 mg/kg) resulted in reduced levels of plasma cholesterol, liver cholesterol, blood reduced glutathione, and breast and thigh muscle cholesterol. Differences were significant at $P < 0.05$ in at least one experiment. 3-Hydroxy-3-methylglutaryl reductase activity was decreased due to dietary garlic (P = 0.0369), but not by pharmacological levels of dietary copper (P = 0.982). The activity of fatty acid synthetase was decreased in birds fed copper (P = 0.035). Both garlic and copper supplements decreased cholesterol 7 α-hydroxylase activity (P = 0.024 and P = 0.022, respectively). The results of these trials confirm the findings that garlic and copper alter lipid and cholesterol metabolism. However, they do not work by the same mechanism. Feeding dietary garlic or copper for 21 d reduced cholesterol levels of broiler meat without altering growth of the chickens or feed efficiency.

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

1997 Poultry Science 76:1264–1271

INTRODUCTION

Garlic (*Allium sativum*) is widely distributed and used in all parts of the world as a spice and herbal remedy for the prevention and treatment of a variety of diseases, ranging from infections to heart diseases. In the past two decades, particular attention has been focused on the cholesterol-lowering activity of garlic.

Bordia *et al.* (1975) reported that the essential oils of onion and garlic can prevent fat-induced hyperlipemia. A marked reduction of serum cholesterol levels (53 and 34%) were observed in rats fed a diet supplemented with 2 or 3% garlic powder. Similar effects of garlic were found in rats fed diets containing either cholesterol or lard. Plasma and liver cholesterol as well as total liver lipids were reduced by about 30% by garlic supplementation, whereas plasma triacylglycerols were reduced only in the group fed lard (Myung *et al.*, 1982). Depressed hepatic cholesterol levels in chickens fed 2% garlic for 14 d were observed by Sklan *et al.* (1992). Various garlic extracts exhibited hypocholesterolemic effects on chickens, mainly through the inhibition of the key enzymes in cholesterol and lipid synthesis (Qureshi *et al.*, 1983).

Copper deficiency was shown to induce hypercholesterolemia in rats (Klevay, 1973). Feeding chickens supranormal levels of copper for 35 and 42 d resulted in decreases of plasma and breast muscle cholesterol and plasma triacylglycerols (Pesti *et al.*, 1994; Bakalli *et al.*, 1995; Pesti and Bakalli, 1996). The reduction of plasma and hepatic cholesterol by dietary garlic and copper has been shown in many species, including chickens. Because of the link between increased plasma cholesterol levels and coronary heart disease (CHD), researchers focused their attention in studying changes of plasma cholesterol concentrations. Consequently the literature is limited regarding changes of muscle tissue cholesterol concentration by dietary supplements. The objective of these studies was to further study the cholesterol-lowering effects of dietary garlic and copper in plasma, liver, and especially in muscle tissues of broiler chickens. The secondary objective was to investigate the cholesterol-lowering mechanism(s) by comparing and contrasting the effects of garlic and copper on key enzymes involved in cholesterol metabolism.

Received for publication September 9, 1996.
Accepted for publication March 12, 1997.
[1]Supported by state and Hatch funds allocated to the Georgia Agricultural Stations at The University of Georgia and a grant from the Southeastern Poultry and Egg Association.
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MATERIALS AND METHODS

Six experiments were conducted with day-old male Ross × Ross 208 broilers. The birds were randomly placed in electrically heated Petersime wire-floored battery brooders. The chicks were maintained on a 24-h light schedule and feed and water (0.5 μg Cu/L) were provided for *ad libitum* consumption throughout the 21-d experimental period (Table 1). At the end of each experiment, birds were weighed by pen and feed intake was determined. In Experiments 1 and 2, four levels of garlic powder (0, 1.5, 3.0, and 4.5%) were fed to three replicate pens of 6 birds each (total of 72 birds) in order to establish a dose-response relationship. A commercial garlic powder that was not deodorized[3] was used in all trials and supplemented at the expense of the total diet. The garlic powder contained 158.3 g crude protein/kg, 5.7 g total lipids/kg, 64.4 g water/kg, 3.998 kcal/g and 4.25 mg Cu/kg.

Experiment 3 had a completely randomized design with five replicate pens of 8 birds per treatment (total of 160 birds). Birds were fed two levels of garlic powder (0 and 3%) and two levels of copper (0 and 63 mg/kg) as cupric citrate, as it was observed in previous studies that 3% garlic powder and 63 mg/kg copper from cupric citrate would be sufficient to reduce tissue cholesterol levels.

Experiments 4 and 5 had completely randomized designs with six replicate pens of 6 birds each per treatment (total of 144 birds per trial). Experiment 6 also had a completely randomized design with three replicate pens of 10 birds each per treatment (total of 120 birds). Copper was supplemented as feed grade cupric sulfate pentahydrate in Experiments 4, 5, and 6.

At the end of each experiment (21 d) three birds per pen were randomly chosen for blood and tissue collection; all birds were sampled in Experiment 6. Blood samples were collected by heart puncture and placed in heparinized tubes. Birds were killed by cervical dislocation. Liver, thigh (*Biceps femoris*), and breast (*Pectoralis major*) muscle samples without skin were taken, chopped, ground, and frozen at –20 C until further analyses. After thawing, tissue samples were extracted with 2:1 chloroform:methanol (Bligh and Dyer, 1959). The total cholesterol content of the tissues was determined enzymatically by the method of Allain *et al.* (1974), as modified by Salé *et al.* (1984). Plasma high density lipoprotein (HDL) cholesterol concentrations were estimated using a Sigma Diagnostic Kit[4] (three birds per pen).

Liver samples were collected at the end of Experiment 6 for the determination of the activities of hepatic enzymes. Three samples from each pen (total of 36 samples) were assayed for the activities of 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase, cholesterol 7 α-hydroxylase and fatty acid synthetase (FAS). Because of the variability in HMG-CoA reductase values, an additional three samples from two pens per treatment were assayed on another day.

For the enzyme assays, tissues were thawed and liver homogenates were prepared in 0.1 potassium phosphate buffer, pH 7.4, containing 4 m*M* $MgCl_2$, 1 m*M* EDTA, and 2 m*M* dithiothreitol (Shapiro *et al.*, 1974).

The tissues were chopped, suspended in buffer (1:2, wt/vol), and homogenized in a Potter Elvehjem homogenizer. Prepared homogenate was centrifuged twice at 40,000 × *g* and the supernatant was centrifuged at 100,000 × *g*. The supernatant was separated to be assayed for FAS activity and the pellet was homogenized with 2 mL homogenizing buffer and sonicated. These procedures were performed at 4 C. Both microsomal fraction and 100,000 × *g* supernatant were stored at –20 C prior to assay for enzymatic activities.

3-Hydroxy-3-methylglutaryl-CoA reductase activity was determined by the method of Shapiro *et al.* (1974) as modified by Qureshi *et al.* (1982). Thirty to 50 μL of microsomal suspension (300 μg protein) were incubated at 37 C with 0.3 U (60 μL) glucose-6-phosphated dehydrogenase. After 10 min, 80 μL of cofactor-substrate solution containing 4.5 μmol glucose-6-phosphate, 5 μmol potassium phosphate buffer, pH 7.4, 0.3 μmol dithiothreitol, 25 nmol DL-3-hydroxymethyl-[3-^{14}C]glutaryl CoA (200,000 dpm)[5] and 0.45 μmol NADP+ were added. After 15 min of incubation at 37 C, 25 μL of 10*N* HCl were added to end the reaction. Samples were incubated at 37 C for at least 30 min to permit mevalonic acid to lactonize and denatured proteins were sedimented by centrifugation. One hundred microliters of the protein-free solution were applied to activated Silica Gel G plates. DL-Mevalonolactone standard was applied to the end channel plate. The plate was developed with 1:1 acetone:benzene. At the R_f value of 0.6 to 0.9, silica gel was scraped into counting vials, 4 mL of scintillation liquid were added, and radioactivity was measured. The activity of 3-hydroxy-3-methylglutaryl CoA reductase was expressed as picomoles of mevalonic acid synthesized per minute per microgram of microsomal protein.

Fatty acid synthetase activity was determined by the method of Nepokroeff *et al.* (1975) using 25 μL of protein fraction from the 100,000 × *g* supernatant. The reaction was initiated by the addition of the supernatant fraction to the mixture of substrate, previously equilibrated at 30 C for 5 min. Total volume of the reaction mixture (1 mL) contained: 500 μ*M* potassium phosphate buffer, pH 7.0; 33 n*M* acetyl-CoA, 100 n*M* malonyl-CoA, 100 n*M;* NADPH, 1 μM β-mercaptoethanol, and 25 μL supernatant fraction.

[3]Kroger Co., Cincinnati, OH 45202.

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

[5]3-Hydroxy-3-methyl[d-^{14}C] Glutaryl-Coenzyme A; Specific activity: 2.15 GBq/mmol, 58 mCi/mmol, Amersham North America, Arlington Heights, IL 60005.

TABLE 1. Composition of the basal diet, Experiments 1 to 6

Ingredients and composition	Content
	(g/100 g)
Ground yellow corn	57.34
Soybean meal	33.48
Poultry fat	3.15
Poultry by-product meal	3.00
Iodized sodium chloride	0.21
DL-methionine (98%)	0.19
Vitamin premix[1]	0.25
Mineral premix[2]	0.05
Defluorinated phosphate	1.54
Limestone	0.79
Composition by calculation[3]	
Protein	23.13
Energy, kcal/g	3.13
Ca, %	1.0
Total P, %	0.71
Available P, %	0.45
Na, %	0.20
Lysine, %	1.30
Methionine	0.57
Cystine	0.35
Composition by analysis[4]	
Cu	9.6 ± 2.3

[1]Vitamin premix provides (per kilogram): vitamin A, 5,500 IU from all trans retinyl acetate; cholecalciferol, 1,100 IU; vitamin E, 11 IU from all-rac-α-tocopheryl 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 (thiamine mononitrate); ethoxyquin, 125 mg.

[2]Mineral premix provided in milligrams per kilogram of diet: Mn, 60; Zn, 50; Fe, 30; Cu, 5; I, 1.5.

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

[4]Mean ± SE of three assays by atomic absorption spectrophotometry.

The oxidation of the NADPH was followed at 340 nm[6] at 25 C. The full-scale recorder tracing was set at 0.2 absorbance units. The initial slope of the recorder tracing was used to calculate the activity rate of fatty acid synthetase. One unit of FAS catalyzes the oxidation of 1 n*M* of NADPH/min at 30 C. The results are expressed as specific activity.

Cholesterol 7-α-hydroxylase was assayed by the method of Carlson *et al.* (1978) with few modifications (Qureshi *et al.*, 1982). Fifty microliters of previously prepared microsomal fraction were incubated in 145 μL of homogenizing buffer, with 5 μL buffer containing 0.1 U glucose-6-phosphate dehydrogenase for 10 min at 4 C. After incubation, 800 μL of cofactor mixture was added and incubated again at 37 C for 30 min. The cofactor mixture contained: 50 μM potassium phosphate buffer, pH 7.4, 5 μM cysteamine, 5 μM $MgCl_2$, 2 μM glucose-6-phosphate, 100 n*M* cholesterol[7] (0.1 μCi [4-^{14}C]), 1 mg Tween 80, and 50 n*M* NADP+.

One milliliter ethanol was added to terminate the reaction. The mixture was extracted twice with light petroleum ether. This extract was dried, dissolved in benzene-methanol 4:1, and applied with reference standards of 7 α-hydroxycholesterol to an activated Silica Gel G plate under nitrogen. The plate was developed with benzene-ethyl acetate 2:3. Identification of the cholesterol and 7α-hydroxycholesterol bands was done under ultraviolet light. Identified bands were scraped, placed into scintillation vials, and assayed for radioactivity. The enzyme activity was expressed as picomoles of [^{14}C] cholesterol converted into 7α-hydroxycholesterol per minute per milligram microsomal protein.

Proteins were quantified by the Bradford method (Bradford, 1976). The small effects due to EDTA and dithiothreitol in our samples were eliminated by running a buffer control with the assay.

Blood glutathione (GSH) (three birds per pen) were measured by the method of Beutler *et al.* (1963), which is based on the development of a relatively stable yellow color when DTNB [5,5′-dithiobis-(2-nitrobenzoic acid)] is added to sulfhydril compounds.

Statistical Analysis

Data were analyzed as one-way (Experiments 1 and 2) and two-way (Experiments 3, 4, and 5) designs by analysis of variance using the General Linear Models (GLM) procedure of SAS® (SAS Institute, 1985). Data from Experiments 1 and 2 were pooled for variables measured in both experiments (there were no treatment by experiment interactions). The experimental unit was the pen mean. For HMG-CoA reductase results, the effect of day of assay and the microsomal protein content of the tissue fraction assayed were also included in the statistical model.

RESULTS

Experiments 1 and 2

Garlic supplements had no effect on body gain or feed conversion ratio (Table 2). The concentration of blood reduced glutathione was not affected by dietary garlic ($P > 0.52$). Supplementation of 1.5% garlic was enough to reduce plasma total cholesterol. Addition of 3.0 or 4.5% garlic powder did not further affect plasma cholesterol levels. Linear reductions of plasma triacylglycerols and liver cholesterol levels were observed as garlic supplementation increased. In both trials, breast muscle cholesterol levels were reduced by approximately 15% but the reduction was not significant at $P > 0.05$.

Experiment 3

The incorporation of neither garlic nor copper (Table 3) in the diet affected body weight gain or feed efficiency. Both garlic and copper reduced blood reduced glutathione levels ($P = 0.05$ and $P = 0.01$). The effects on

[6]Beckman DU-6 Spectrophotometer, Beckman Instruments, Inc. Schiller Park, IL 60176.

[7][4-$^{-14}$C] Cholesterol. Specific activity: 1.96 GBq/mmol, 53 mCi/mmol, Amersham North America, Arlington Heights, IL 60005.

TABLE 2. Influence of dietary garlic on body weight gain, feed conversion ratio, and cholesterol and reduced glutathione contents of selected tissues (means ± SE), Experiments 1 and 2

Variable	Experiment	Garlic supplementation 0%	1.5%	3.0%	4.5%	$P > F$[1] Experiment 1	Experiment 2
Body weight gain,[2] g/21 d	1	636 ± 33	600 ± 42	673 ± 33	635 29	0.842	0.733
	2	680 ± 27	651 ± 23	664 ± 14	664 ± 23		
Feed conversion ratio,[2] g feed:g gain	1	1.60 ± 0.01	1.52 ± 0.06	1.47 ± 0.07	1.57 ± 0.06	0.869	0.194
	2	1.55 ± 0.07	1.53 ± 0.13	1.53 ± 0.04	1.57 ± 0.02		
Plasma total cholesterol,[3] mg/100 mL	1	143 ± 5	118 ± 4	115 ± 5	120 ± 5	0.006	0.008
HDL-cholesterol,[3] mg/100 mL	1	27 ± 1	26 ± 1	25 ± 0	26 ± 1	0.037	0.075
Plasma triacylglycerols,[3] mg/100 mL	1	86 ± 9	76 ± 4	71 ± 9	58 ± 2	0.013	0.866
Liver cholesterol,[3] mg/100 g wet tissue	1	105 ± 10	91 ± 15	73 ± 15	54 ± 9	0.012	0.853
Breast muscle cholesterol,[3] mg/100 g wet tissue	1	46 ± 7	40 ± 3	39 ± 5	42 ± 5	0.165	0.552
	2	47 ± 4	46 ± 1	44 ± 4	40 ± 5		
Blood reduced glutathione,[3] mg/100 mL	2	52 ± 5	47 ± 4	55 ± 4	45 ± 1	0.588	0.623

[1]Indicates the probability that the linear and quadratic coefficients are not different from zero.

[2]Means of three replicate pens of six birds each.

[3]Means of three replicate pens of three samples each.

body gain or blood reduced glutathione were not additive when garlic powder or copper were added to the diet.

Breast muscle cholesterol was not significantly reduced in birds fed garlic, copper, or both garlic and copper.

Experiment 4

Supplementation of 3% garlic powder and/or 180 ppm copper as copper sulfate pentahydrate (Table 4) did not affect feed consumption or body weight gain. Plasma triacylglycerol levels were not altered by garlic or copper supplements. The HDL-cholesterol levels significantly increased in birds fed garlic. Only garlic significantly increased blood reduced glutathione levels. Plasma cholesterol level was decreased by about 20% in birds fed garlic, copper, and garlic and copper (P = 0.008 for the interaction). A decrease of about 28% in liver cholesterol was found in birds fed garlic, copper, and garlic and copper in comparison with the control (P = 0.020 for the interaction). Thigh muscle cholesterol levels were decreased by dietary garlic (P = 0.025) and copper (P = 0.024). Breast muscle cholesterol was reduced by dietary garlic (P = 0.081) and copper (P = 0.025).

Experiment 5

Copper-supplemented birds utilized feed more efficiently than unsupplemented birds (Table 5). Thigh muscle cholesterol level was significantly decreased by garlic and copper, but the garlic by copper interaction was not significant. Breast muscle cholesterol levels were significantly affected by garlic (P = 0.05) and by copper as evidenced by the significant garlic by copper interaction (P = 0.04).

Experiment 6

No differences among treatments in weight gain or feed conversion were found. The activity of the rate-limiting enzyme in cholesterol synthesis, HMG-CoA reductase

TABLE 3. Influence of dietary garlic and copper on the body weight gain, feed conversion ratio, blood reduced glutathione, and breast and thigh muscle cholesterol (mean ± SE), Experiment 3

Variable	0% Garlic 0 mg Cu/kg	3.0% Garlic 0 mg Cu/kg	0% Garlic 63 mg Cu/kg	3.0% Garlic 63 mg Cu/kg	$P > F$ Garlic	Cu	Garlic × Cu
Body weight gain,[1] g	749 ± 13	735 ± 18	731 ± 5	712 ± 21	0.31	0.21	0.84
Feed conversion ratio,[1] g feed:g gain	1.44 ± 0.02	1.44 ± 0.01	1.42 ± 0.02	1.49 ± 0.02	0.15	0.58	0.15
Blood reduced glutathione,[2] mg/100 mL	68.6 ± 2.4	59.9 ± 1.8	56.6 ± 3.5	58.5 ± 3.4	0.05	0.01	0.08
Thigh muscle cholesterol,[2] mg/100 g wet tissue	145 ± 17	121 ± 12	112 ± 8	99 ± 10	0.14	0.04	0.64
Breast cholesterol,[2] mg/100 g wet tissue	41 ± 3	38 ± 7	33 ± 2	35 ± 3	0.95	0.16	0.52

[1]Means of five replicate pens of eight birds each.

[2]Means of five replicate pens of three samples each.

TABLE 4. Influence of dietary garlic and copper on the body weight gain, feed conversion, plasma triacylglycerols, and total cholesterol, high density lipoprotein (HDL)-cholesterol, blood reduced glutathione, and liver, thigh, and breast muscle cholesterol (mean ± SE), Experiment 4

Variable	0% Garlic 0 mg Cu/kg	3% Garlic 0 mg Cu/kg	0% Garlic 180 mg Cu/kg	3% Garlic 180 mg Cu/kg	*P* > *F* Garlic	Cu	Garlic × Cu
Body weight gain,[1] g	689 ± 26	671 ± 23	691 ± 16	672 ± 17	0.394	0.922	0.970
Feed conversion ratio,[1] g feed:g gain	1.51 ± 0.01	1.60 ± 0.06	1.48 ± 0.03	1.56 ± 0.06	0.098	0.491	0.942
Plasma tricylglycerols,[2] mg/100 mg	41 ± 2	41 ± 4	43 ± 4	43 ± 4	0.969	0.586	0.950
Plasma total cholesterol,[2] mg/100 mL	114 ± 5	88 ± 5	87 ± 5	95 ± 5	0.125	0.097	0.008
HDL-cholesterol,[2] mg/100 mL	28 ± 1	32 ± 1	30 ± 0	30 ± 1	0.035	0.791	0.160
Liver cholesterol,[2] mg/100 mL	208 ± 25	150 ± 10	152 ± 12	176 14	0.302	0.375	0.020
Blood reduced glutathione,[2] mg/100 mL	57 ± 1	62 ± 1	57 ± 1	64 ± 2	<0.001	0.604	0.539
Thigh muscle cholesterol,[2] mg/100 g wet tissue	137 ± 10	107 ± 5	107 ± 6	98 ± 9	0.025	0.024	0.180
Breast cholesterol,[2] mg/100 g wet tissue	56 ± 5	46 ± 5	43 ± 6	34 ± 5	0.081	0.025	0.961

[1]Means of five replicate pens of eight birds each.

[2]Means of five replicate pens of three samples each.

(Table 6) was suppressed by garlic ($P = 0.037$), but not by copper ($P = 0.98$) and there was no significant interaction ($P = 0.59$). Inspection of the data revealed that samples with higher microsomal protein contents had higher HMG-CoA reductase activities. Therefore, microsomal protein content was included as a covariable in the statistical method. Fatty acid synthetase activity was significantly suppressed by copper, but not garlic. The activity of the rate-limiting enzyme in bile acid synthesis (cholesterol 7α-hydroxylase) was significantly depressed by garlic ($P = 0.024$) and copper ($P = 0.022$).

DISCUSSION

The results of the first experiment (Table 1) showed that the garlic supplement was affecting lipid and cholesterol metabolism (reduced plasma and liver cholesterol and reduced plasma triacylglycerols) without having a significant effect on overall performance or breast muscle cholesterol. The second experiment had very similar results; and after pooling results from the two experiments, the data indicated about 17 chances of 20 that the difference in meat cholesterol was not due to random variability. Nonetheless, we decided to include dietary copper supplements in subsequent experiments to compare garlic supplementation to a substance known to decrease meat cholesterol (Bakalli *et al*., 1995; Pesti and Bakalli, 1996).

In Experiments 3, 4, and 5 (Tables 3, 4, and 5), copper supplements in excess of the nutritional requirement lowered breast and thigh muscle cholesterol as in our previous experiments (Bakalli *et al*., 1995; Pesti and Bakalli, 1996). Garlic supplements also lowered muscle cholesterol, but the mean depressions were not as large as from copper, nor were they large in comparison to observed variability: significant differences were found (at the $P < 0.05$ level) in only two of the five experiments. Across all five experiments, copper and garlic supplements lowered breast muscle cholesterol by 24 and 15%, respectively, and lowered thigh muscle cholesterol by 22 and 23%, respectively.

The literature is limited regarding changes in muscle cholesterol due to the effect of cholesterol-reducing agents, especially in the chicken. Changes of cholesterol concentrations in tissues such as the aorta, heart (Hassel *et al*., 1987, 1988) and kidney (Hassel *et al*., 1988) have been studied in copper-deficient rats; unlike plasma and liver cholesterol concentrations, no changes in cholesterol concentrations were observed in these tissues.

Changes in plasma and liver cholesterol contents are more frequently observed, perhaps because plasma and liver cholesterol belong to the "fast turnover cholesterol pool" (Field *et al*., 1960; Chobanian and Hollander, 1962). The muscle cholesterol pool comprises the slow turnover pool and equilibrates slowly with the plasma cholesterol pool. The muscle cholesterol pool is larger and perhaps less active and it may take a longer feeding period to show a significant reduction of cholesterol levels.

Age and sex have a bearing on the cholesterol metabolism and cholesterol concentrations in pigeons (Wagner and Clarkson, 1974). Our findings of lower plasma cholesterol levels in 3-wk-old male birds in comparison with 12-wk-old layers (Qureshi *et al*., 1983) are in agreement with findings (Lorenz *et al*., 1938) that laying hens have higher blood cholesterol levels than immature hens or mature roosters. Similarly, average plasma triacylglycerol levels were lower in 3-wk-old broilers (Tables 2 and 4) than in the laying hens (Qureshi *et al*., 1983). In one of the two experiments, we found decreased plasma triacylglycerol levels. Other research has also shown variability in triacylglycerol level response to garlic. In a study conducted with rats, garlic decreased plasma triacylglycerols only when animals were fed 1% lard (Chi, *et al*., 1982).

TABLE 5. Influence of dietary garlic and copper on the body weight gain, feed conversion ratio, and breast and thigh muscle cholesterol (mean ± SE), Experiment 5

Variable	0% Garlic 0 mg Cu/kg	3% Garlic 0 mg Cu/kg	0% Garlic 180 mg Cu/kg	3% Garlic 180 mg Cu/kg	*P* > *F* Garlic	Cu	Garlic × Cu
Body weight gain,[1] g	721 ± 25	736 ± 10	711 ± 24	771 ± 17	0.075	0.534	0.261
Feed conversion ratio,[1] g feed:g gain	1.68 ± 0.04	1.65 ± 0.03	1.57 ± 0.06	1.58 ± 0.03	0.852	0.037	0.635
Thigh muscle cholesterol,[2] mg/100 g wet tissue	149 ± 7	105 ± 9	118 ± 11	100 ± 7	0.002	0.049	0.140
Breast cholesterol,[2] mg/100 g wet tissue	55 ± 4	32 ± 4	40 ± 5	40 ± 8	0.048	0.486	0.042

[1]Means of five replicate pens of eight birds each.

[2]Means of five replicate pens of three samples each.

Cholesterol concentrations were found to be much higher in the thigh than in breast muscle (Tables 3, 4, and 5). A possible explanation is that cholesterol is usually associated with adipose tissue, which is more abundant in thigh than in breast muscle. Also, thigh muscles have a much greater content of slow-twitch fibers than breast muscles. Slow-twitch fibers have many more mitochondria, their mitochondria are bigger, and the metabolic rate much faster in comparison to fast-twitch fibers. Slow twitch sarcoplasmic reticulum are found to contain two to three times as much cholesterol as fast-twitch *Caudofemoralis* sarcoplasmic reticulum in rabbits (Bloch, 1991). The higher cholesterol concentration reduces membrane fluidity (Yeagle, 1989), lowers Ca+-ATPase activity (Madden *et al*., 1979), and regulates contraction and relaxation rates.

The decreased HMG-CoA reductase and cholesterol 7 α-hydroxylase activities, by about 40% in the microsomes of birds fed garlic (Table 6), confirms the findings of Qureshi *et al.* (1983), who found more than a 40% reduction of the activities of HMG-CoA reductase and cholesterol 7 α-hydroxylase in chickens fed garlic.

The large variability of HMG-CoA reductase that we observed compared to Qureshi *et al.* (1983) may be related to our experimental conditions. Because birds in the present study received continuous light, their HMG-CoA reductase diurnal activity was probably not synchronized. Our observation that the amount of microsomal protein was a significant covariant, affecting HMG-CoA reductase results, is interesting: some uncontrolled factor that alters enzyme activity must be present in the microsomes. In spite of the amount of variability between birds, we ran enough assays to be confident that garlic supplements were affecting HMG-CoA reductase activity ($P = 0.037$), but copper was not ($P = 0.982$).

TABLE 6. Effects of garlic powder and copper sulfate on hepatic enzyme activities in 12-d-old broilers, Experiment 6

Garlic	Copper	HMG CoA reductase[1]	Fatty acid synthetase[2]	Cholesterol 7-α-hydroxylase[3]
(g/100 g)	(mg/kg)			
0	0	458 ± 198	50 ± 4	1.08 ± 0.19
3	0	272 ± 81	50 ± 4	0.65 ± 0.07
0	180	511 ± 220	45 ± 2	0.64 ± 0.05
3	180	209 ± 51	38 ± 3	0.53 ± 0.07

Analysis of variance Source	df[4]	*P* > *F*	df[4]	*P* > *F*	df[4]	*P* > *F*
Garlic	1	0.0369	1	0.359	1	0.0237
Copper	1	0.9824	1	0.035	1	0.0222
Garlic × copper	1	0.5984	1	0.353	1	0.1877
Microsomal protein	1	<0.001				
Time	1	0.019				
Error	15		8		8	

[1]Data expressed as means ± SE; picomoles of mevalonic acid synthesized per minute per milligram microsomal protein; means of three pens of three samples each. HMG CoA reductase = 3-hydroxy-3-methylglutaryl coenzyme A reductase.

[2]Nanomoles of NADPH oxidized per minute per milligram of cytosolic fraction. Means of three replicate pens of three samples each.

[3]Picomoles of [^{14}C] cholesterol into 7 α-[^{14}C]hydrocholesterol per minute per milligram microsomal protein. Means of three replicate pens of three samples each.

[4]Degrees of freedom.

Kim *et al.* (1992) demonstrated that copper deficiency causes hypercholesterolemia by elevating hepatic GSH (reduced glutathione) levels and changing the GSH: GSSG (oxidized glutathione) ratio, which decreases the activity of the HMG-CoA reductase. If this mechanism is functional in copper deficiency, we expected that supplemental copper would decrease GSH levels and subsequently the HMG-CoA reductase activity. As hypothesized, both garlic and copper in Experiment 3 (Table 3) reduced blood GSH levels (Table 3). However, these results were not repeated in Experiment 4 (Table 4) although tissue cholesterol concentrations were still depressed.

Because garlic was confirmed to alter HMG-CoA reductase activity (Table 6), but copper was not, the supplements must not lower tissue cholesterol by the same mechanism. It is very interesting to note that both garlic and copper supplements depress cholesterol 7 α-hydroxylase activity. This result is consistent with the hypothesis of substrate availability regulating cholesterol 7 α-hydroxylase activity (Bjorkhem and Akerlund, 1988). Copper depressed FAS in our studies, as in those of Qureshi *et al.* (1983), which would suggest that more substrate would be available for HMG CoA reductase.

It is not known which ingredients were fed to broilers in current meat composition tables (USDA, 1979). It is likely that they were fed growth promoting levels of copper sulfate, but the tables may need to be reevaluated to reflect the types of diets that were fed. Results reported here support the hypothesis that it is possible to produce meat with different cholesterol contents by either feeding copper or garlic powder. Although feeding the levels of garlic powder in the present study most likely imparted off-flavors in the meat, feeding only the active compounds might not.

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