

Influence of combined usage of garlic powder and copper on egg yolk cholesterol concentration in laying hens

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Abstract: A research was conducted to evaluate the effects of combined usage of garlic powder (GP) and cupric sulfate pentahydrate (CS) as two different cholesterol-lowering supplements on blood serum and egg yolk cholesterol concentration in laying hens. A total of one-hundred ninety two 40-wks-old Single Comb White Leghorn (SCWL) laying hens were selected in a completely randomized designed to four diets that included unsupplemental basal diet as control diet (CO), the basal diet supplemented with either 15 g/kg GP, 200 mg/kg CS, or both 15 g/kg GP and 200 mg/kg Cs for a period of 6 weeks. During the experimental period hens performance traits included body weight gain, egg production, egg and yolk weights, egg mass, feed consumption, and feed conversion ratio were recorded. For statistical analysis, data were averaged by replicate for 6 wks period. At the end of the experimental period blood serum and egg yolk samples were collected for cholesterol analysis. Supplementation of GP or CS to the basal diet had no significant ($p>0.05$) effect on overall performance in laying hens. A decrease in blood serum and egg yolk cholesterol concentration was found ($p<0.01$) in birds fed GP, CS, and GP/CS diets in comparison with the unsupplemented diet birds (CO diet). Serum cholesterol levels were reduced by feeding GP, CS, or both by about 14, 20, and 28% respectively ($p<0.01$). Feeding GP or CS decreased egg yolk cholesterol about 6 and 12.5%, respectively ($p<0.01$), but feeding both GP and CS diet/ supplements had no further beneficial effect on egg yolk cholesterol

Key words: hen, egg, garlic, copper, cholesterol.

Introduction

The coronary heart disease (CHD) is the major cause of human death in the United States and other industrialized countries (American Heart Association, 1997). Clinical data strongly support the relationship between dietary cholesterol and CHD (American Heart Association, 1990). These suggestions tend to have adverse effects in the eggs industry. This is demonstrated by the rapidly declining per capita consumption of egg over the past

three decades (Sim and Nakai, 1994).

Garlic intake with inhibition of cholesterol biosynthesis, depressed serum cholesterol levels in humans and animals (Bordia *et al.*, 1975; Chi *et al.*, 1982; Harenberg *et al.*, 1988). Dietary garlic paste (38 g/kg) reduced serum cholesterol by 23% in 12-wks-old Leghorn pullets, when diets were fed for 4 wks (Qureshi *et al.*, 1983). Egg yolk cholesterol was reduced by feeding of 10 or 30 g/kg GP to laying hens for 3 wks (Sharma *et al.*, 1979).

Dietary copper, when fed at levels above the nutritional requirement has been demonstrated to

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alter the lipid metabolism of chicks. Supplementation of 250 mg copper/kg broiler's diet from CS for 35 or 42 d, reduced plasma and breast muscle cholesterol by about 12 and 21%, respectively (Bakalli *et al.*, 1995). Pesti and Bakalli (1998) observed a reduction in plasma and egg yolk cholesterol concentration of 30-wks-old White Leghorn hens due to adding pharmacological levels of Cu to the diet.

The purpose of this experiment was to study the effects of dietary GP and CS on egg yolk and serum cholesterol concentration in laying hens.

Materials and Methods

A total of one-hundred ninety two 40-wks old SCWL hens were used in this study. The birds were placed two in a cage (30×40×45 cm), given feed and water for ad libitum and subjected to a photoperiod of 15-h light and 9-h dark/d. A completely randomized design (CRD) was used for analysis of results with four replicated pens of 12 birds per treatment (total of 192 birds).

All birds were fed isoenergetic and isonitrogenous basal diet for 2 wks prior to start of the experiment. The control diet (CO) contained 10.8 mg/kg Cu which determined by the atomic absorption spectrophotometry (Shimatzo Model 6050). The CO was supplemented with either 15 g/kg sun-dried GP, 200 mg/kg CS, or both 15g/kg GP and 200 mg/kg CS for 6 wks. Diets were formulated according to the National Research Council (NRC, 1994) requirements for energy and nutrient content for Leghorn hens. The composition of the basal diet is shown in (Table 1). The GP contained 132.8 g crude protein/kg, 4.8 g lipids/kg, 88.1 g water/kg and 3208 kcal/kg.

Body weights were measured at the beginning and end of the experiment period. Eggs were collected daily at the same time and hen-d egg production was calculated on a weekly basis. Egg weight and yolk weight were determined twice a week and egg mass was calculated by multiplying egg production by egg weight to determine actual egg yield. Feed consumption was recorded weekly and feed conversion ratio (kg feed/kg egg) was calculated. For statistical analysis, data were averaged by replicate

Table 1: Composition of the basal diet. ¹vitamine premix provides (per kilogram of diet): vitamin A, 5500 IU; cholecalciferol, 1200 IU; vitamin E, 11 IU; riboflavine, 40 mg; Ca pantothenate, 12 mg; nicotinic acid, 44 mg; choline chloride, 220 mg; vitamin B₁₂, 6.6µg; vitamin B₆, 2.2 mg; menadione, 1.1 mg (as menadione sodium bisulfate complex); folic acid, 0.55 mg; biotin, 0.11 mg; and ethoxyquin, 125 mg. ²Mineral premix supplied the following per kg of diet: Mn, 40 mg; Zn, 60 mg, Fe, 40 mg; Cu, 6 mg; and I, 1.5 mg. ³Estimated from NRC (1994) composition table. ⁴Mean ± SD of two assayed by atomic absorption spectrophotometry.

Ingredients and composition	Content (kg/100 kg)
Ground yellow corn	58.93
Soybean meal (44 g/kg protein)	13.18
Barley	10.00
Oyster shell	8.51
Corn gluten meal (60 g/kg protein)	6.98
Dicalcium phosphate	1.29
Vitamin premix ¹	0.25
Mineral premix ²	0.25
Common salt	0.30
L-Lysine	0.20
DL-Methionine	0.11
Composition by calculation ³	
Crude protein (g/kg)	157.0
ME (MJ/kg)	11.5
Calcium (g/kg)	36.0
Available phosphorus (g/kg)	3.6
Sodium (g/kg)	1.7
Lysine (g/kg)	7.2
Methionine + cyctine (g/kg)	6.6
Composition by analysis ⁴	
Cu (mg/kg)	10.8 ± 3.1

for 6 weeks period.

At the end of the experimental period, six hens per replicate were randomly selected for serum cholesterol level analysis. Blood was drawn from the wing vein by using sterilized syringe and needles. The blood samples were clotted for 30 min at room temperature and the serum was separated by centrifugation for 15 min at 2100 × g at room temperature. All samples were stored at -20°C until analyzed. Furthermore, 10 eggs from each replicate were chosen for egg yolk cholesterol concentration



Table 2: Effects of dietary garlic or copper, or both on performance of laying hens. Data are reported as least squares means \pm SD as averaged over 6 wks. Egg mass = (egg production \times egg weight)/100. CO = control; GP = 15 g/kg garlic powder; CS = 200 mg Cu/kg diet; GP/CS = 15 g/kg garlic powder plus 200 mg Cu/kg diet. NS: Not Significant at $p > 0.05$.

Diets	Egg production (eggs/hen-d)	Egg weight (g)	Yolk weight (g)	Egg Mass ² (g/d per hen)	Feed Consumption (g/hen-d)	Feed conversion ratio	Body weight gain (g/6 wk)
CO	79.05 \pm 2.05	58.84 \pm 1.71	17.36 \pm 0.38	45.59 \pm 2.77	86.52 \pm 1.31	1.91 \pm 0.10	81 \pm 0.2
GP	77.86 \pm 2.51	59.01 \pm 1.08	17.79 \pm 0.40	45.25 \pm 0.91	87.88 \pm 1.96	1.98 \pm 0.09	89 \pm 0.1
CS	80.11 \pm 1.03	59.00 \pm 0.54	17.77 \pm 0.36	46.13 \pm 0.63	88.57 \pm 1.73	1.89 \pm 0.06	104 \pm 0.6
GP/CS	77.88 \pm 2.26	58.80 \pm 0.89	17.91 \pm 0.46	45.79 \pm 1.41	87.50 \pm 1.60	1.92 \pm 0.10	94 \pm 0.3
	NS	NS	NS	NS	NS	NS	NS

analysis. The eggs were weighed and broken out and yolks were separated from albumin, weighed, pooled and blended prior to cholesterol analysis.

The cholesterol level of serum and cholesterol concentration of egg yolks were determined by enzymatic method as reported by Luhman *et al* (1990) by using the commercial kits (Biotrol Diagnostic Kit, France) and the Kone Specific Clinical Analyzer (Kone, Espoo, Finland). The yolk samples (1 ml) were mixed with 0.05M of NaOH (25ml) neutralized with 0.25N of HCl and then assayed (Luhman *et al.*, 1990).

All data were analyzed by one-way ANOVA using the General Multivariate Linear Regression Models (GLM) procedure of SAS[®] (SAS Institute, 2000). Significant differences among treatment's means were tested using Duncan's multiple range test (Steel and Torrie, 1960) and data were presented as means \pm SD, and differences were considered significant at $p < 0.01$.

Results

The results of this experiment showed that supplementation of GP or CS to the basal diet affected the cholesterol metabolism without having a significant ($p > 0.05$) effect on overall performance in laying hens.

Hen's body weight from 40 to 46 wks of age did not affect by the dietary treatments ($p > 0.05$). Hen-d egg production, egg weight, egg mass, yolk weight, feed consumption, and feed conversion ratio were also did not affect ($p > 0.05$) by adding GP or CS or

both to the basal diet during 6 wks of experiment. Hen's

performance data as averaged over the 6 wks period are presented in Table 2.

A significant decrease in blood serum cholesterol and egg yolk cholesterol concentration were found ($p < 0.01$) in birds fed GP, CS, and GP/CS diets in comparison with the CO diet (Table 3). It should be mentioned that the cholesterol-lowering effect of 200 mg/kg CS was more effective than 15g/kg dietary GP.

Discussion

In agreement with the current study, Chowdhury *et al* (2002) reported that egg production, egg weight, egg mass, feed consumption, feed efficiency, and body weight gain were not affect during 6 wks of study that 20, 40, 60, 80, or 100 g/kg sun-dried garlic paste were fed to six strains of laying hens. However Pesti and Bakalli (1998) reported that egg production increased by feeding 125 or 250 mg/kg CS to laying hens for 8 wks.

Reduction of serum cholesterol levels of the hens receiving CS diet was significantly higher than the hens fed GP diet (-20% vs. -14%, respectively; $p < 0.01$). However, the birds that fed both GP and CS in a diet, compared with the other treatments had the lowest concentration of cholesterol in their blood serum (-28%; $p < 0.01$). This is in contrast to findings of Konjofca *et al* (1997) which demonstrated that incorporation of both GP and CS in a diet did not have further effect in reduction of plasma total cholesterol level in a meat-type chicken's diet. This contraction



Table3: Effects of dietary garlic and copper, or both on the serum cholesterol levels and egg yolk cholesterol concentration of laying hens. ^{a-c} Values within a column with no common superscript differ significantly ($p < 0.01$). Mean \pm SD for four replicated of six cages, twelve hens each per treatment. Duncan's new multiple range test. CO = control; GP = 15 g/kg garlic powder; CS = 200 mg Cu/kg diet; GP/CS = 15 g/kg garlic powder plus 200 mg Cu.

Diets	Serum Cholesterol (mg/100 ml)	Yolk Cholesterol (mg/g)	Egg Cholesterol (mg)
CO	137.30 \pm 6.50 ^a	12.92 \pm 0.50 ^b	224.3 \pm 11.72 ^b
GP	118.15 \pm 12.88 ^{ab}	12.30 \pm 0.61 ^{ab}	211.9 \pm 11.70 ^{ab}
CS	109.98 \pm 9.51 ^{bc}	11.41 \pm 0.49 ^a	196.4 \pm 10.69 ^a
GP/CS	99.22 \pm 8.74 ^c	11.43 \pm 0.55 ^a	198.2 \pm 13.27 ^a

may have been related to the differences in type of the birds.

In this experiment, changes in the egg yolk cholesterol concentrations somewhat were similar to those of serum cholesterol levels, so that the eggs from the group fed 200 mg/kg diet CS had significantly lower cholesterol levels ($p < 0.01$), compared with the eggs laid by the hens fed 15 g/kg dietary GP (196.38 \pm 10.69 mg vs 210.80 \pm 11.70 mg, respectively). However, by addition of these two supplements in a diet (GP/CS), in spite of further reduction in serum cholesterol levels, cholesterol content of eggs did not show significant difference with those of CS treatment (198.22 \pm 13.27 mg vs. 196.38 \pm 10.69 mg, respectively). These results indicated that combined usage of GP and CS as two hypocholesterolemia agents in a diet, could not tend to further beneficial effects in reduction of egg yolk cholesterol concentration in laying hens. Decrease in cholesterol content of egg yolk was also limited.

Decline in egg cholesterol content in the present study by feeding 15 g/kg dietary GP is consistent with findings of (Chowdhury *et al.*, 2002), which demonstrated that dietary garlic paste at 20 g/kg reduced egg yolk cholesterol, on average, by 5%. Sharma *et al.* (1979) reported that egg yolk cholesterol was reduced by 4.1 or 5.5% when laying hens were fed 10 or 30 g/kg GP for 3 wks. In contrast, decrease in egg yolk cholesterol concentration, by about 12.5% that found in the current study, when the birds were fed 200 mg Cu/kg diet were lower than those of Pesti and Bakalli (1998), who reported that after 8 wks of Cu supplementation in two experiments, egg

yolk cholesterol levels were reduced by 20 and 28% in experiment 1, and by 30 and 35% in experiment 2, from 125 and 250 mg/kg Cu, respectively. This contraction may have been related to the differences in age or strain of the hens, or differences in the percentage of copper in CS that was used in the present study with those of Pesti and Bakalli's experiments.

The differences between cholesterol-lowering effects of GP and CS pentahydrate were due to used dosage of GP in the present experiment. Amount of GP was based on the level that were observed in previous studies by Mottaghitalab and Taraz (2003), which demonstrated that 15 g/kg dietary GP would be sufficient to reduce egg yolk cholesterol concentration. Chowdhury *et al.* (2002) reported that cholesterol concentrations of egg yolk decreased linearly with increasing levels of dietary garlic paste, therefore, dietary garlic paste at 20, 40, 60, 80, or 100 g/kg reduced egg yolk cholesterol, on averaged, by 5, 9, 14, 20, and 24%, respectively.

Konjofca *et al.* (1997) investigated the cholesterol-lowering mechanism(s) of garlic and copper on key enzymes involved in cholesterol metabolism in broilers and demonstrated that both of these supplements alter lipid and cholesterol metabolism but, they do not work by the same mechanism. They reported that garlic supplements affected the major regulatory enzyme of cholesterol biosynthesis activity (3-hydroxy-3-methylglutaryl coenzyme A reductase or HMG-CoA reductase), but copper was not. However, both garlic and copper supplements were altered cholesterol 7α -



hydroxylase activity. These researchers observed HMG-CoA reductase and cholesterol 7 α -hydroxylase were depressed by about 40% in the microsomes of birds fed 30 g/kg dietary GP. Qureshi *et al* (1983) also, found more than a 40% reduction of these enzymes in chickens fed garlic.

Kim *et al* (1992) demonstrated that copper deficiency causes hypercholesterolemia by elevating hepatic GSH (reduced glutathione) levels and changing the G- S-S-G (oxidized glutathione) ratio, which decreases the activity of the HMG-CoA reductase. Konjofca *et al* (1997) expected that supplemental copper would decrease GSH levels, and subsequently the HMG-CoA reductase activity. Fields and Lewis (1997) reported that 100-200% increase in hepatic lipid synthesis associated with Cu deficiency may not be due to Cu depletion but rather iron accumulation. It is well recognized that a Cu deficiency increases the iron content of the liver approximately 100% (Fields and Lewis 1997, Wilson *et al.*, 1997). Moreover, overloading the liver with iron increases the expression of a number of genes, and leads to hypercholesterolemia that is comparable to that observed in Cu-deficient animals (Dabbagh *et al.*, 1994, Fields *et al.*, 1994, Fields *et al.*, 1996, Fields and Lewis 1997).

In conclusion, the results of this experiment showed that the cholesterol-lowering effect of 200 mg/kg CS diet was more than 15 g/kg dietary GP. However, combined usage of GP and CS as two hypocholesterolemia agents in a diet was more effective in reducing the serum cholesterol level, but it did not tend to further beneficial effects in reduction of egg yolk cholesterol concentration in laying hens.

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تأثیر مصرف توأم پودر سیر و مس در کاهش غلظت کلسترول زرده تخم مرغ در مرغان تخمگذار

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در این مطالعه به منظور کاهش کلسترول سرم و زرده تخم مرغ از پودر سیر و سولفات مس به عنوان دو عامل کاهش دهنده کلسترول، استفاده گردید. تعداد ۱۹۲ قطعه مرغ تخمگذار لگه‌ورن به طور کاملاً تصادفی در گروه‌های جداگانه مساوی به مدت ۶ هفته تحت تأثیر چهار جیره غذایی زیر قرار گرفتند: گروه شاهد (CO) دریافت کننده جیره پایه بدون پودر سیر و سولفات مس، گروه دریافت کننده پودر سیر (GP) به میزان ۱۵g/kg، گروه دریافت کننده سولفات مس (CS) به میزان ۲۰۰g/kg و گروه دریافت کننده مخلوط پودر سیر و سولفات مس (GP+CS) به ترتیب ۱۵g/kg و ۲۰۰g/kg جیره غذایی. در مدت آزمایش عملکرد گله از نظر افزایش وزن بدن، تولید تخم مرغ، وزن تخم مرغ‌ها و زرده آنها مصرف خوراک و ضریب تبدیل جیره غذایی ثبت شد. اطلاعات جمع آوری شده در مدت ۶ هفته مورد تجزیه و تحلیل آماری قرار گرفت. در پایان دوره برای تعیین میزان کلسترول سرم و زرده تخم مرغ نمونه‌گیری انجام شد. نتایج نشان دادند که افزودن پودر سیر و یا سولفات مس تأثیری بر عملکرد مرغان تخم‌گذار نداشته است ($p > 0.01$) میزان کلسترول سرم در تیمارهایی که پودر سیر، سولفات مس و یا مخلوط آنها را مصرف کرده بودند. به ترتیب ۱۶، ۲۰ و ۲۸ درصد کاهش یافته بود ($p < 0.01$) میزان کلسترول زرده تخم مرغ با مصرف پودر سیر و سولفات مس به ترتیب ۶ و ۱۲/۸ درصد کاهش پیدا کرده بود ($p < 0.01$)، ولی مصرف توأم آنها تأثیر چندانی در کاهش کلسترول زرده نداشته است.

واژه‌های کلیدی: مرغ، تخم مرغ، کلسترول، مس، سیر.

