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Effect of Inclusion of Garlic (*Allium sativum*) Powder at Different Levels and Copper into Diets of Hens on Performance, Egg Quality Traits and Yolk Cholesterol Content

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Abstract: This experiment was conducted to investigate the effects of inclusion of oven dried garlic powder (*Allium sativum*) at different levels and copper into diets of hens on performance, egg quality traits, yolk and serum cholesterol content. A total of 240 Lohmann white layers, 38 wks of age, were allocated randomly eight groups, each formed 6 replicate cages as subgroups, comprising of five hens. Treatment groups were fed diets containing a standard commercial layer diet, basal diet plus 200 ppm copper ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$), 2% garlic powder, 2% garlic powder + 200 ppm copper, 4% garlic powder, 4% garlic powder + 200 ppm copper, 6% garlic powder and 6% garlic powder + 200 ppm copper from week 38th to 50th. Feed conversion efficiency and cracked egg were not affected by feeding garlic powder and copper. Egg weight, egg production and feed consumption decreased with garlic powder and copper supplementation. There were no differences in the egg quality traits except for shell stiffness and yolk index. Egg yolk cholesterol concentration decreased linearly with increased levels of garlic powder but serum cholesterol concentration increased. The supplementation of 200 ppm copper and combinations of garlic powder and copper did not have a significant effect on cholesterol and triglyceride concentrations of egg and serum. Consequently, without having a significant effect on laying performance and egg quality characteristics, oven dried garlic powder can be used up to 6% as a hypocholesterolemic agent in practical layer diets without copper.

Key words: Laying hens, Garlic powder, copper, performance, egg quality, cholesterol

INTRODUCTION

Garlic (*Allium sativum*) is used widespread and is known as a herbal remedy for the prevention and treatment of different diseases. Studies have shown that garlic contained active components lowering 3-hydroxy-3-methylglutaryl reductase activity and cholesterol 7 alpha-hydroxylase activity (Konjufca *et al.*, 1997), blood glucose and lipid levels (Chi *et al.*, 1982), level of serum and egg cholesterol (Lim *et al.*, 2006; Khan *et al.*, 2007; Azeke and Ekpo, 2008; Canogullari *et al.*, 2009) in animals and in human (Bordia, 1981; Jain *et al.*, 1993). But Birrenkott *et al.* (2000) showed that %3 garlic powder did not have any significant effect on cholesterol concentration. Similarly Reddy *et al.* (1991) observed that plasma and yolk cholesterol were not effected by 2% garlic oil.

Although many references have been made in the literature to the hypocholesterolemic activity of garlic, results obtained from different studies was controversial. Lawson *et al.* (1992) recognized that allicin is an active component in garlic and they demonstrated that allicin is unstable and poorly absorbed from the digestive tract. Also, garlic preparations that are produced by heat or solvent processes known to void allinase and hence allicin may not be formed (Yu *et al.*, 1989). Some studies suggested that commercial garlic oil, garlic powder and commercially available garlic

extract may not be hypocholesterolemic. Although the reason for this is unknown, it likely relates to low level of garlic, preparation methods, the stability of chemical components and the duration of study (Chowdhury *et al.*, 2002; Khan *et al.*, 2007).

Research evidences have shown that 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-methyl glutaryl coenzyme A (HMG-Co A) reductase in rats (Idowu *et al.*, 2006). Pesti and Bakalli (1998) obtained a reduction in egg yolk cholesterol concentration when pharmacological levels of Cu were fed to White Leghorn hens. Supplementation of 250 mg copper/kg broiler's diet from cupric sulfate for 35 or 42 d, reduced plasma and breast muscle cholesterol by about 12 and 21%, respectively (Bakalli *et al.*, 1995). The maximum dietary tolerable level of copper for poultry was set at 300 mg kg^{-1} according to NRC (1994).

Limited information is available on the combined effect of oven dried garlic and copper on laying hens. The aim of this study was to investigate the dietary effect of the single or combination feeding of different levels of oven dried garlic powder and copper on laying performance, egg quality traits, serum and egg cholesterol concentrations.

Table 1: Ingredients and chemical composition of experimental diets

Ingredients (%)	C	C + Cu	2% GP	2% GP + Cu	4% GP	4% GP + Cu	6% GP	6% GP + Cu
Corn	52.81	52.81	52.81	52.81	52.81	52.81	52.81	52.81
Soybean meal	18.13	18.13	18.13	18.13	18.13	18.13	18.13	18.13
Full fat soybean	1.65	1.65	1.65	1.65	1.65	1.65	1.65	1.65
Sun flower meal	7.50	7.50	7.50	7.50	7.50	7.50	7.50	7.50
Corn gluten meal	2.04	2.04	2.04	2.04	2.04	2.04	2.04	2.04
Soybean oil	1.60	1.60	1.60	1.60	1.60	1.60	1.60	1.60
Marble powder	6.82	6.82	6.82	6.82	6.82	6.82	6.82	6.82
Salt	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
DCP 18	2.65	2.65	2.65	2.65	2.65	2.65	2.65	2.65
D-L Methionine 99	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
L-Lisin	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Vit-Min	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Wheat brain	6.00	6.00	4.00	4.00	2.00	2.00	-	-
Garlic	-	-	2.00	2.00	4.00	4.00	6.00	6.00
Copper	-	200.00 ppm	-	200.00 ppm	-	200.00 ppm	-	200.00 ppm
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated nutrient content								
DM (%)	87.90	88.10	88.60	88.30	88.30	88.20	88.50	89.10
CP (%)	18.90	18.10	19.00	19.20	18.60	19.70	20.20	19.60
Ether extract (%)	3.10	3.20	3.00	3.10	3.10	3.10	2.90	2.90
Crude fiber (%)	4.90	4.90	5.40	4.80	5.10	4.70	4.50	4.50
Ash (%)	6.40	6.50	6.50	6.60	6.60	6.70	6.90	7.00
NSP	54.60	55.40	54.70	54.60	54.90	54.00	54.00	55.10
ME* (kkal/kg)	2660.00	2668.00	2667.00	2667.00	2666.00	2661.00	2653.00	2675.00

C: Control, Cu: Copper, GP: Garlic Powder. Each two kilogram of vitamin-mineral premix contained 12,000 IU of retinal palmitate, 2,500 IU of cholecalciferol, 30 IU of DL- α -tocopheryl acetate, 34 mg of phyloquinone, 3 mg of thiamin, 6 mg of riboflavin, 30 mg of nicotinic acid, 10 mg of Ca-D-pantothenate, 5 mg of pyridoxine, 15 mg of cobalamin, 1 mg of folic acid, 50 mg of D-Biotin, 300 mg of choline chloride, 50 mg of ascorbic acid, 80 mg of manganese, 60 mg of iron, 60 mg of zinc, 5 mg of copper, 2 mg of iodine, 5 mg of cobalt, 150 mg of selenium

MATERIALS AND METHODS

Diet, animal and management: Locally produced garlic bulbs were purchased from market. The garlic bulbs with husk were sliced and thinly spread on a mat in outside for a one day. After this garlic was dried on a hot air oven at 50°C for the grinding to make a powder form. The dried garlic powder used in this experiment contained 88.8% dry matter, 13.74% crude protein, 0.79% crude fat, 5.52 crude fiber, 6.64% crude ash. Garlic powder was substituted at the expense of wheat bran at 2, 4 and 6% levels on weight basis. Cupric sulfate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) was purchased from Farmavet Ltd. as a copper source. Two hundred and forty Lohman layers, 38 weeks of age, were blocked according to the location of cages (50 x 46 x 46 cm) and then assigned randomly to receive one of eight isocaloric and isonitrogenous dietary treatments for 12 wks. Each dietary treatment was replicated in 6 cages, each containing 5 hens. The experimental diets were formulated to meet or exceed the NRC recommendations (NRC, 1994). Ingredient and chemical composition of the experimental diets were given in Table 1.

Treatment groups were fed diets containing a standard commercial layer diet, basal diet plus 200 ppm copper, 2% garlic powder, 2% garlic powder + 200 ppm copper, 4% garlic powder, 4% garlic powder + 200 ppm copper, 6% garlic powder and 6% garlic powder + 200 ppm

copper respectively. During the experimental period, hens were fed *ad libitum* once daily at 08:30 hrs and water was available all the times. Hens were subjected to a 17L:7D cycle.

Sample collection and analytical procedure: Feed samples were analyzed for dry matter, crude protein, crude fiber and ether extract concentrations (AOAC, 1990). Feed intake and egg production were recorded daily, egg weight was measured bi-weekly and body weight was measured monthly. Before determination of egg weight, a sample of 12 eggs from each experimental group was stored for 24 hrs in room temperature. Feed Conversion Ratio (FCR) was expressed as kilogram of feed consumed per kilogram of egg produced.

To assess egg quality parameters, another sample of 12 eggs was randomly collected from each experimental group every month. Egg quality parameters were shape index, shell strength, shell thickness, albumen index, yolk index, yolk color (Yolk Colour Fan, the CIE standard colorimetric system, F. Hoffman-La Roche Ltd., Basel, Switzerland) and Haugh unit and they were calculated using following formulas as summarized by Ergun *et al.* (1987). Shape index (%) = (egg width, cm/egg length, cm)*100, Shell strength (kg/cm^2) was determined by using machine with the spiral pressure system, Shell thickness ($\text{mm} \cdot 10^{-3}$) was determined in 3 different parts

(upper and lower ends and middle) by using micrometer, Albumen index (%) = (albumen height, mm/average of albumen length, mm and albumen width, mm)*100, Yolk index (%) = (yolk height, mm/yolk diameter, mm)*100, Yolk color was determined by using commercially available yolk color fan according to the CIE standard colorimetric system, Haugh unit = $100 \cdot \log(H + 7.57 - 1.7 \cdot W^{0.37})$, where H = albumen height, mm and W = egg weight, g.

At the end of the experiment, blood and egg samples were taken from the each replicate in order to determine the ratio of triglyceride and cholesterol. Blood samples were taken from wing vena into blood tubes containing cloth activator. Tubes were centrifuged at +4°C, 3000xg for 5' and supernatant collected. The triglyceride and cholesterol ratio in serum and yolk was determined by HPTLC.

Isolation and homogenization of hen-egg yolk: Egg shells were broken manually, yolk were carefully separated from the white, washed gently with distilled water and rolled on Whatman 3 MM filter paper to remove any adherent egg white. The yolk membrane was then pictured with disposable pastor pipette and egg-yolk transferred into pre-weighted falcon tubes. 2 volumes of 20% SDS was added onto each g of isolated egg-yolk in falcon tubes and homogenized at 1000 rpm for 2 min using an ultra-turrax homogenizer. The homogenate was aliquoted and used for HPTLC analysis.

Total lipid extraction: The each volume of serum and egg-yolk lipids was shaken vigorously with 1 volumes of a mixture of n-hexane/2-propanol (3/2) (Merck KgaA, Darmstad/Germany). After, centrifugation of suspension at +4°C, 2000 x g for 10' upper phase was aspirated and used for HPTLC analysis (Hara and Radin, 1978; Koolman and Roehmn, 2005).

HPTLC: For separation and identification High Performance Thin-Layer Chromatography (HPTLC) plates (20 x 10 cm) (Merck KgaA, Darmstad/Germany) were used. To this end five-µl portions of extracted lipids of egg-yolk and serum were spotted with a micropipette 2 cm from the bottom of HPTLC plates. The lipids were

developed 6 cm from application point using a mobile phase of n-hexane: diethylether: formic acid (80:20:2 (v/v/v) (Merck KgaA, Darmstad/Germany). To visualize lipid classes, the entire plate was sprayed with a 10% CuSO₄ (w/v) in 8% H₃PO₄ (v/v) (Merck KgaA, Darmstad/Germany) and charred at 180°C. After cooling HPTLC plates evaluated by Phoretix 1D (TL120) software (Damyanova, 2002).

Statistical analysis: The data were statistically analyzed to ANOVA using the general linear models procedure of SPSS (SPSS for Windows, version 10.01). Polynomial contrasts to determine the feeding level of garlic powder in the diets were constructed.

RESULTS

Laying performance: The effects of single feeding of GP or Cu and combination of copper and garlic powder on the performance traits are shown in Table 2. Egg weight decreased in garlic powder and copper supplementation groups compared with the control group. Moreover, there were linear decreases in egg weight with increased supplemental garlic powder. Egg production rate was significantly affected by single feeding of GP or Cu and combination of copper and garlic powder. Increasing level of garlic powder quadratically decreased egg production. There was no effect in the feed intake of laying hens fed garlic powder. But supplementation of Cu and combination of copper and garlic powder significantly decreased feed intake. There were no significant differences in feed efficiency and damage egg rate in laying hens fed experiment diets.

Egg quality traits: The effects of experimental diets on the egg quality traits are shown in Table 3. The supplementation of garlic powder had no significant effect ($p>0.05$) on egg quality traits except for shell stiffness and yolk index. Garlic powder supplementation increased ($p<0.05$) shell stiffness quadratically and yolk index linearly (Table 3).

Cholesterol content: The effects of garlic powder, copper and combination of garlic powder and copper supplementation on egg yolk and serum parameters of

Table 2: Effects of the garlic powder and copper on the performance of laying hens

Garlic powder (%)	0		2		4		6					
Copper ppm	0		200		0		200		SEM	GP	C	GP*C
Performance												
EW (g)	67.57	65.53	65.89	65.91	64.61	65.86	65.96	64.47	0.34	0.00L	0.02	0.00
EP (%)	89.62	84.51	90.53	88.51	90.51	88.70	86.80	88.74	0.96	0.02Q	0.01	0.00
FI (g/hen/day)	130.90	120.80	128.70	124.80	126.60	124.80	127.50	126.10	1.38	0.80	0.00	0.00
FE (kg feed kg ⁻¹ eggs)	2.17	2.21	2.17	2.15	2.17	2.14	2.24	2.21	0.03	0.07	0.70	0.56
DER (%)	0.13	0.05	0.22	0.27	0.23	0.32	0.26	0.57	0.12	0.07	0.32	0.48

EW = Egg Weight, EP = Egg Production rate, FI = Feed Intake, FE = Feed Efficiency, DER = Damage Egg Rate, GP = Garlic Powder, C = Copper, L = Linear effect of garlic powder supplementation; Q = Quadratic effect of garlic powder supplementation

Table 3: Effects of the garlic powder and copper on egg quality traits

Garlic powder (%)	0		2		4		6					
Copper ppm	0	200	0	200	0	200	0	200	SEM	GP	C	GP*C
EW (g)	67.14	67.11	66.84	66.53	63.41	67.94	66.18	64.53	0.96	0.22	0.35	0.01
SI (%)	74.28	73.47	74.78	73.86	74.17	74.44	74.42	74.42	0.47	0.67	0.28	0.51
SS (kg/cm ²)	0.73	0.84	1.14	0.81	1.06	1.05	0.76	0.91	0.06	0.00Q	0.65	0.00
ST(mm)	0.39	0.41	0.39	0.38	0.40	0.39	0.39	0.39	0.01	0.41	0.81	0.30
SW (g)	8.42	8.79	8.81	8.42	8.45	8.89	8.66	8.27	0.18	0.71	0.96	0.03
YC	8.67	8.22	7.83	8.22	8.56	7.94	8.39	8.06	0.23	0.33	0.12	0.13
YI (%)	42.54	44.09	45.30	44.61	44.80	45.16	44.07	45.30	0.61	0.02L	0.16	0.26
AI (%)	8.19	8.99	8.41	8.97	8.38	8.01	8.41	8.68	0.37	0.56	0.22	0.42
HU	81.24	84.23	82.07	85.38	82.62	80.84	82.93	83.82	1.58	0.62	0.23	0.36

EW = Egg Weight, SI = Shape Index, SS = Shell Stiffness, ST = Shell Thickness, SW = Shell Weight, YC = Yolk Color, YI = Yolk Index, AI = Albumen Index, HU = Haugh Unit, GP = Garlic Powder, C = Copper, L = Linear effect of garlic powder supplementation; Q = Quadratic effect of garlic powder supplementation

Table 4: Effects of the garlic powder and copper on cholesterol and triglyceride content of egg and serum (%)

GP (%)	0		2		4		6					
C (mg/kg)	0	200	0	200	0	200	0	200	SEM	GP	C	GP*C
Egg yolk												
Cho	18.36	18.07	18.10	18.69	17.57	17.97	15.68	17.38	0.53	0.01L	0.74	0.19
Trig	70.24	70.40	71.64	71.93	73.12	73.96	69.94	68.04	0.66	0.00Q	0.12	0.32
Serum												
Cho	18.59	18.15	18.62	18.99	19.81	20.41	20.32	20.53	0.37	0.00L	0.48	0.55
Trig	54.13	56.96	57.04	57.96	51.84	51.71	52.81	52.35	0.77	0.00L	0.15	0.16

Cho = Cholesterol, Trig = Triglyceride, GP = Garlic Powder, C = Copper, L = Linear effect of garlic powder supplementation; Q = Quadratic effect of garlic powder supplementation

laying hens are shown in Table 4. Egg yolk cholesterol concentrations decreased linearly ($p < 0.01$) with increasing levels of oven-dried garlic powder, but serum cholesterol increased linearly ($p < 0.01$). In this study, 2, 4 and 6% garlic powder reduced egg cholesterol concentration by 1.42, 4.30 and 14.60% respectively as compared with the control diet.

DISCUSSION

In agreement with the present study, Lim *et al.* (2006) reported that there were no differences in the feed consumption and feed efficiency of laying hens fed 0%, 1%, 3% and 5% garlic powder, 200 ppm of Cu, or combination of 3% garlic powder and 200 ppm of Cu. Similarly Chowdhury *et al.* (2002) observed that body weight, body weight gain, feed consumption, egg production and feed efficiency were not significantly affected by 0-100 g kg⁻¹ sun-dried garlic paste over 6 weeks. Reddy *et al.* (1991) showed that egg production, egg mass, BW, feed intake and feed efficiency were not affected during the 8 wks that 0.02% garlic oil was fed to the Babcock layer.

Canogullari *et al.* (2009) fed layer diets containing 0, 0.5, 1 and 2% garlic powder for 12 weeks period and also reported that feed consumption and feed efficiency were not affected by the diets. However, Khan *et al.* (2007) showed that the inclusion of 0%, 2%, 6% and 8% garlic powder significantly ($p < 0.01$) increased the feed consumption with increasing levels of dietary garlic in laying hens. Pesti and Bakalli (1998) reported that dietary copper at the level of 250 per kg feed had favourable effect on feed intake and egg production. Balevi and Coskun (2004) also noticed that no

significant effect on feed intake, egg production and feed conversion of chicks fed diets supplemented with copper sulphate.

Yalcin *et al.* (2006) reported that the supplementation of garlic powder had no significant effect ($p > 0.05$) on egg shell index, egg breaking strength, egg albumen index, egg yolk index and egg Haugh unit. In contrast of finding of Yalcin *et al.* (2006), Canogullari *et al.* (2009) observed that there were significant differences ($p < 0.05$) in the egg albumen index, egg shell index and egg Haugh unit in layer fed 0, 0.5, 1 and 2% garlic powder. The supplementation of copper did not have significant effect on egg quality traits. Combination of garlic and copper had significant effect on egg weight, shell stiffness and shell weight in present study.

The finding of Khan *et al.* (2007) who found that oven dried garlic at 2%, 6% and 8% decreased the egg yolk cholesterol by 5.7%, 14.28% and 23.57%, respectively compared with the control diet is consistent with our study. Similarly Canogullari *et al.* (2010) reported that mean egg yolk cholesterol levels reduced by 19.37, 29.58 and 46.40% in the 1, 2 or 4% levels garlic powder purchased commercially supplemented diets, respectively. Reddy *et al.* (1991) concluded that yolk cholesterol was not influenced when the laying hens were fed garlic oil at 0.02% level. Lim *et al.* (2006) observed that there were further reduction in egg and serum cholesterol concentration when laying hens were fed 3% garlic powder with supplementation of 200 ppm of Cu.

In the present study, egg yolk triglyceride responded quadratically ($p < 0.01$). Also serum triglyceride reduced linearly ($p < 0.01$) with increasing levels of garlic powder.

Similarly, Azeke and Ekpo (2008) showed that the inclusion of garlic powder at 1% and 2% levels into diets of hens significantly decreased the egg yolk triglyceride and cholesterol in laying hens. The supplementation of 200 ppm copper and interaction of garlic powder and copper did not have significant effect on cholesterol and triglyceride concentrations of egg and serum. Balevi and Coskun (2004) reported that the use of supplementary copper to provide 150 mg/kg in poultry diets decreased yolk cholesterol concentrations without any adverse effect on production performance. Similarly, laying hens given diets supplemented with 0, 125 and 250 mg/kg Cu for 28 d revealed yolk cholesterol concentrations of 11.6, 9.0 and 8.0 mg/g respectively (Pesti and Bakalli, 1998). Previous studies have shown that garlic decreased activities of key enzymes in supplying substrates, hepatic 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA reductase) and cholesterol 7 α -hydroxylase activities which controls the rate of cholesterol biosynthesis in the liver and consequently reduced serum and egg cholesterol content (Qureshi *et al.*, 1983; Konjufca *et al.*, 1997). Konjufca *et al.* (1997) reported that garlic supplements affected the major regulatory enzyme of cholesterol biosynthesis activity but copper was not. These researchers observed that HMG- CoA reductase and cholesterol 7 α -hydroxylase were depressed by about 40% in the microsomes of birds fed 30 g/kg dietary GP.

Consequently, supplemental oven dried garlic decreased linearly yolk cholesterol concentration without having a significant effect on laying performance and quality characteristics of egg but not copper. At the end of the study it was observed that oven dried garlic powder can be used up to %6 as a hypocholesterolemic agent in practical layer diets without copper.

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