Effect of garlic (*allium sativum*) supplementation on egg quality and yolk cholesterol in layer hens

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Forty eight Hi-sex White laying hens were used to evaluate the effect of oral administration of garlic juice (*Allium Sativum*) on egg quality and yolk cholesterol. Hens were equally divided into a control and three experimental groups then randomly caged in an environmentally controlled house. Garlic juice was prepared by blending pealed garlic cloves with distilled water (1:1, w/v). Hens in the experimental groups were gavaged individually by garlic juice at 0.25, 0.50 and 1% of body weight three times a week through the 47th week of age. Egg and its components' weights and interior egg quality were evaluated on three consecutive days to investigate the effect of garlic juice and storage duration (1, 5 and 10 days) at room temperature. Also, garlic juice and storage duration influence on the bacterial count of *E.Coli* challenged eggs was tested. Egg yolk cholesterol was assayed for five successive weeks starting at 43 weeks of age. Garlic juice increased (P<0.05) egg weight and egg mass but the increase in egg production did not attain statistical difference. Garlic juice reduced (P<0.05) the $\log_{10}$ of bacterial count in egg contents linearly. Garlic juice did not change (P>0.05) yolk cholesterol concentration throughout the sampling period. Garlic supplemented groups recorded similar egg components, however they surpassed (P<0.05) what registered by the control-fed group. With storage, egg and albumen weights decreased (P<0.05), whereas yolk weight increased (P<0.05) with no change on shell weight. Also, garlic-fed groups maintained lower (P<0.05) albumen and yolk pH when compared to control-fed group. The results of this study conclude that the given levels of garlic juice improved performance characteristics, egg quality and showed lower bacterial count of the *E.coli* challenged eggs but failed to show influential effect on egg yolk cholesterol.

Keywords: Garlic juice; laying hens; egg quality; cholesterol

Introduction

Egg is considered one of the major sources of human dietary cholesterol. Numerous efforts had been made to lower the cholesterol content of eggs. Since the sixties of the past century literature compiled genetic, nutritional, biological and pharmaceutical attempts to reduce yolk cholesterol; sadly, very limited success accompanied these efforts. Many authors observed that when cholesterol levels go down more than 20-30%, hen over-taxes egg production by reducing egg size and some times it ended by complete cessation of egg production. The inability to select for lower cholesterol levels more than 5 –7% was indicative of natural selection Marks and Washburn (1977).

Garlic is widely consumed and gained the trust in the scientific media as hypolipidemic and hypocholesterolemic among wide range of species (Shoetan et al., 1984). However, when tested on yolk cholesterol contents of laying hen, the hypocholesterolemia property of garlic was controversial. Some workers demonstrated a reduction in yolk cholesterol contents while others did not. The results reported in the herein study join those who failed to acknowledge hypocholesterolemic effect of garlic on egg yolk. The studies that registered a reduction in yolk cholesterol, garlic accounted for at least 4% of the diet. Increasing laying hen production, egg weight and egg shelf life drove many

researchers to study the significant effects of various nutritional, environmental and managerial aspects to scale up laying hen performance and egg quality. For many years albumen height and albumen height as a function egg weight, for example Haugh unit, were used as measures of egg quality. Garlic (*Allium sativum*) was identified by many researchers as a potent inhibitor of food pathogens. Therefore, we postulate that the use of garlic in laying hen might increase egg shelf life and decrease the possibilities of food poisoning. Thus, the experimental hens were gavaged with garlic juice individually to insure that each hen receives its designated level of garlic and to confirm the integrity of some of garlic active components, allicin to be specific. Under these preparation and administration circumstances, we evaluated the effect of oral administration of garlic juice on the layer hen performance and production parameters, egg quality and yolk cholesterol level.

Materials and methods

A total of forty eight, 40-week old Hisex laying hens, were obtained from a local commercial layer farm with an average body weight of (1489 ± 34.4 gram). Hens were assigned numbers and housed in an environmentally controlled house, where they were allowed to acclimatize for two weeks. A corn-soy bean based layer ration (ME, 2800 kcal/kg; CP, 18.5%; Ca, 3.5%) and water were provided *ad libitum*. The daily photoperiod consisted of 16 h of light and 8 h of darkness (16L:8D). Temperature was maintained at 22±1^{o}C throughout the experimental period. Eggs were collected daily and labeled with chicken number at 0800 and 1400 h throughout the experimental period and egg production was measured on a weekly basis. Twelve hens were assigned randomly to each of four treatment groups. One group served as a control and the other three groups were individually gavaged with fresh garlic juice at 0.25, 0.5 and 1% of body weight three times a week from the beginning of week 42 through 47 week of age. Garlic juice was prepared by homogenizing the garlic cloves with an equivalent volume of distilled water in a food blender for 1 minute. The mixture was then allowed to stand for 30 minutes at room temperature.

At 44 and 46 weeks of age, egg weight, 29 – 36 eggs from each experimental group were logged for three consecutive days and albumen weight, yolk weight, shell weight and egg quality (albumen pH and height and Huagh unit) were measured at day 1 (fresh laid eggs), after 5 and 10 day storage at room temperature. Egg mass was calculated on weeks where egg weights were measured.

Cholesterol content of egg yolk was determined following colorimetric method based on Liebermann-Burchard color reaction as described by (Huang et al., 1961) from eggs collected at 43 through 47 weeks of age. At 45 weeks of age, eggs were collected for three consecutive days to measure bacterial count and bacterial growth of inoculated egg contents from fresh laid (1-), 5- and 10-day stored eggs at room temperature. Bacterial counts were undertaken as described by Gürler and Fehlhaber, (2004). The egg mixtures were plated on Tryptone soya agar (TSA) and incubated at 37^{o}C for 24 hours. After incubation the colony forming units (CFU) were counted, the bacterial count was expressed as $\log_{10}$ CFU/ml of egg content.

The collected data were subjected to analysis of variance using the GLM procedure of SAS (SAS, 1996). Egg components, egg quality criteria and bacterial counts were subjected to analysis of variance using GLM procedure of SAS. The statistical analysis failed to show two-way interaction between garlic level and storage duration. Cholesterol data were sorted by week and bacterial count data were sorted by storage time before performing the analysis of variance. Means were separated by the least significant difference.

Results and discussion

Garlic juice supplementation improved layers performance in terms of egg weight and mass ($P<0.05$) with numerical increase in egg production (Table 1). Hen-day egg production values registered in control hens and those supplemented with garlic juice at 0.25, 0.50 and 1.0% of body weight were 77.38, 82.74, 84.52 and 86.8%, respectively. Very limited reports from literature had evaluated the effect of garlic supplementation on layer performance. In 1991, Reddy et al. (1991) reported the effect of supplementing 0.02% garlic oil to Babcock B-300 strain; Chowdhury et al. (2002) researched the effect of mixing layer diets with 2 –10 % sun-dried garlic paste. Neither of

these studies detailed significant alterations in egg production, egg weight or mass. The diversity of garlic preparation and administration methods makes it harder to contrast our results with those in literature. Lawson et al., (1992) recognized 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., 1989b). In our study we took this fact in consideration when garlic juice was prepared. Blending garlic cloves with distilled water provided enough time for allinase to be liberated and form allicin from alliin (Block, 1985 and Yu et al., 1989a). As for the increased layer performance parameters reported in the herein study, we do not have a solid elucidation. However, the stimulation salivary flow rate and gastric juice secretion and hence aid in digestion when such spice additives were added to the diet as reported by (Srinivasan and Sambaiah, 1991) might provide an explanation.

Table 1. Effect of different levels of garlic juice on performance characteristics of laying hens.

Garlic juice (%)	Egg weight (g)	Egg production (%)	Egg Mass (g /hen / day)
0.00	60.51^{b}	77.38	47.45
0.25	65.89^{a}	82.74	54.39
0.50	65.13^{a}	84.52	55.23
1.00	65.02^{a}	86.80	56.26
SEM	1.35	4.30	3.14
Contrast	*Estimate*		
Garlic Vs Control	4.94^{**}	7.14^{NS}	7.818^{*}

^{NS}Not significant; $^{*}P<0.05$; $^{**}P<0.01$

Table 2 summarizes the effect of garlic juice supplementation and storage duration on eggs' component weight and quality. The statistical analysis of our data clearly suggested that garlic supplementation and storage duration affected egg component weight and interior egg quality as well with no sign of interaction. Garlic supplementation increased ($P<0.05$) egg weight, albumen weight and yolk weight linearly (Table 2). Also, garlic increased albumen height ($P<0.05$) with no effect on albumen pH and Haugh unit. Chowdhury team in 2002 reported that sun-dried dietary garlic increased yolk weight. Our results confirm what Chowdhury team reported in 2002 although garlic was gavaged in the herein study. The lack of information about the nutrient use from garlic administration and/or the digestion and absorption abilities makes the increase in egg components weights less well understood and opens the door for further studies. Ramakrishna et al. (2003) reported that spices, such as garlic, supplementation enhances activities of the pancreatic enzymes, which provide microenvironment for better nutrient utilization in rats. Storage declined the scale of freshness as hinted by the decline in albumen's height and albumen pH. Also, stretching the storage period from 5 to 10 days, a drop in albumen weights and increased yolk weights were noted with no change in shell weights, in line with many reports (Scott and Silversides, 2000; Silversides and Scott, 2001; Monia et al., 2003; and Silversides and Budgell, 2004). Our results reported a shift in albumen pH from (8.35) at day one to (9.08) and (9.29) after 5 and 10 days of storage, respectively. In a recent report, Pappas et. al. (2005) characterize the decline in albumen deterioration rate as a function of the antioxidant status of egg contents. They proposed that organic selenium enhance the egg's antioxidant status by upgrading the glutathione peroxidase activity in yolk and albumen. This in turns slows the process of lipid and protein oxidation during storage period; hence more valuable egg quality by extended storage time. We also believe that this explanation might apply to our treatment since garlic is known of its antioxidant properties (Thomson and Ali, 2003 and Mirunalini et al., 2004).

Garlic administration failed to detect significant reduction in yolk cholesterol contents (Table 3). The lack of garlic juice effect on yolk cholesterol differ from the results of Sharma et al. (1979), who reported a reduction in egg yolk cholesterol by feeding 1 or 3% garlic powder. In 1991, Reddy et al. (1991) concluded that diet supplementation with garlic oil at 0.02% level did not affect yolk cholesterol. Similarly, Birrenkott et al. (2000) showed that diet supplementation with 3% of powdered

garlic revealed no difference in yolk cholesterol. However, comparing our results with the most recent report of garlic effect on yolk cholesterol (Chowdhury et al., 2002) an apparent contradiction was noted. When the levels of gavaged garlic juice were calculated on daily basis they measure about 0.80,

Table 2. Effects of different levels of garlic juice and storage time on egg components and quality.

Garlic juice (%)	Weight (g)				pH		Albumen	
	Egg	Yolk	Shell	Albumen	Yolk	Albumen	Height (mm)	Haugh unit
0.00	60.97^{b}	16.34^{b}	5.72^{b}	38.91^{b}	6.25^{a}	8.98^{a}	6.19^{c}	75.41^{c}
0.25	64.55^{a}	17.05^{a}	6.02^{a}	41.49^{a}	6.20^{b}	8.85^{b}	6.82^{b}	78.64^{b}
0.50	63.83^{a}	16.39^{b}	6.02^{a}	41.42^{a}	6.20^{b}	8.90^{ab}	6.57^{bc}	77.35^{bc}
1.00	65.11^{a}	17.26^{a}	5.92^{ab}	41.95^{a}	6.23^{ab}	8.90^{ab}	7.34^{a}	81.64^{a}
SEM	0.77	0.16	0.07	0.63	0.01	0.03	0.15	0.90
Linear	***	**	NS	**	NS	NS	*	NS
Contrast	*Estimate*							
Garlic Vs. Cont.	3.53***	0.56**	0.27**	2.71***	-0.04**	-0.09**	0.73***	3.8***
Storage (days)								
1 d	64.21	16.50^{b}	5.97	47.76^{a}	6.17^{c}	8.35^{c}	9.20^{a}	93.99^{a}
5 d	63.97	16.82^{ab}	5.97	41.19^{ab}	6.22^{b}	9.08^{b}	6.32^{b}	77.00^{b}
10 d	62.66	16.97^{a}	5.81	39.88^{b}	6.28^{a}	9.29^{a}	4.68^{c}	63.78^{c}
SEM	0.67	0.14	0.06	0.54	0.01	0.03	0.13	0.78
Linear	NS	*	NS	*	***	***	***	***

^{NS}Not significant; *P<0.05; **P<0.01; ***P<0.001

1.50 and 3.2 gm of garlic paste, respectively for 0.25%, 0.50% and 1.00% treatments. Although yolk cholesterol concentration (mg/g) reported in the herein study lags from 1 – 3 mg/g compared to what Chowdhury et al. (2002) described, they tabled significant drop in yolk cholesterol only to hens fed diets supplemented with garlic paste at 4% and above. The relative stability of chemical ingredients in garlic and the duration of the study may affect responses, since Lawson et al. (1992) reported that allicin, the potentially active component in garlic, is unstable and poorly absorbed from the digestive tract. Table 4 describes the effect of garlic juice on the bacterial count of egg contents. Garlic juice suppressed the bacterial counts linearly in a dose-dependent manner. Results of the present study are in harmony with several other studies (Guo et al., 2004 and Sarica et al., 2005) who reported the dietary herbal treatment resulted in lower *E. coli* counts compared to the control group in small intestines of animal. Also, garlic lowered the concentrations of total aerobic bacteria and *E. coli* in the small intestine in broiler chickens (Sarica et al., 2005).

Table 3. Effect of different levels of garlic juice on egg yolk cholesterol concentrations (mg/g).

Garlic juice (%)	Weeks of age				
	43	44	45	46	47
0.00	9.48	8.30	9.24	9.61	9.41
0.25	9.71	7.88	8.93	9.43	8.84
0.50	10.19	8.38	9.15	9.31	9.23
1.00	10.74	7.82	9.10	9.25	9.33
SEM	0.72	0.40	0.33	0.26	0.36
Contrast	*Estimate*				
Garlic Vs. Cont.	0.733^{NS}	-0.28^{NS}	-0.18^{NS}	-0.29^{NS}	-0.28^{NS}

^{NS}Not significant

Banerjee and Sarker (2003) showed that garlic inhibits the growth of bacterial pathogens (Gram-positive and Gram-negative), that were resistant to some commonly used antibiotics such as penicillin. Ankri and Mirelman (1999) reported that allicin is the main antimicrobial constituent of garlic, which is formed when the garlic clove is crushed. The bacterial count decreased linearly in all experimental groups as the storage time progressed, although one would expect that the bacterial growth would flourish after 5 and 10 days of storage. To explain these results we need to extend our focus to the physical and chemical changes that occur in egg during storage duration and conditions. Albumen pH has increased from 8.35 to 9.30 from freshly laid eggs and those stored for 10 days, respectively; while albumen height decreased from 9.20 mm to 4.68 mm respectively, from freshly laid eggs and those stored for 10 days.

Table 4. Effect of garlic juice and storage time on bacterial count (log_{10}) in egg contents of the layer hens with garlic juice

Garlic juice	Storage time (days)		
(%)	1	5	10
0.00	3.572^{a}	3.436	3.389^{a}
0.25	3.546^{a}	3.413	3.336^{b}
0.50	3.523^{ab}	3.420	3.335^{b}
1.00	3.443^{b}	3.411	3.343^{ab}
SEM	0.032	0.026	0.017
Linear	*	NS	NS
Contrast	*Estimate*		
Garlic Vs Cont.	-0.068^{NS}	-0.021^{NS}	-0.051^{*}

^{NS}Not significant; $^{*}P<0.05$

Many different types of proteins are found in egg albumen; most of them appear to possess antimicrobial properties or certain physiological functions to interfere with the growth and spread of invading microorganisms (Li-Chan et al., 1995). To mention few, lysozome is a relatively small enzyme that attacks the polysaccharide cell wall of Gram positive bacterial; avidin, other egg albumen protein, binds strongly to biotin thereby preventing bacteria from utilizing it as a nutrient. We believe that the role of albumen proteins in limiting bacterial contamination valid for a short period after oviposition. However, the less favorable condition created by the rise in albumen pH may provide an explanation for the lower bacterial growth after 5 and 10 days of storage.

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