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Garlic (*Allium sativum*) Supplementation: Influence on Egg Production, Quality, and Yolk Cholesterol Level in Layer Hens

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ABSTRACT : Forty-eight 40-wk-old Hi-sex laying hens were individually caged in an environmentally controlled house to evaluate the effect of garlic (*Allium Sativum*) juice administration on egg production, egg quality, and yolk cholesterol. Garlic juice was prepared by blending pealed garlic cloves with distilled water (1:1, w/w). Hens were randomly divided into four equal groups; one served as a control and the other three groups were individually gavaged, 3.75 ml, 7.5 ml, or 15 ml garlic juice, three times a week, which respectively represented 0.25, 0.50 and 1% of body weight. Egg production was recorded on a daily basis; egg weight, albumen height, albumen and yolk pH, Haugh unit, and bacterial count of *E. coli*-challenged eggs were recorded at day of oviposition (day-1) and after 5 and 10 days of storage at room temperature. Yolk cholesterol content was analyzed for five successive weeks. Garlic juice increased ($p<0.05$) egg weight and mass with no change in egg production intensity. Garlic juice administration recorded higher ($p<0.05$) albumen height and improvement in Haugh unit. Also, eggs from garlic-treated hens recorded lower ($p<0.05$) albumen and yolk pH when compared to eggs collected from control hens. Garlic reduced ($p<0.05$) the $\log_{10}$ of bacterial count in egg contents linearly when challenged with *E. coli*. Egg-yolk cholesterol content was not influenced by garlic juice administration. It is concluded that garlic juice improved performance characteristics and may increase egg shelf life as indicated by egg quality improvement and lower bacterial count of *E. coli*-challenged eggs. The levels of garlic juice used in this study were insufficient to influence egg yolk cholesterol. (**Key Words :** Garlic, Hen, Egg, Bacterial Count, Cholesterol)

INTRODUCTION

Garlic (*Allium sativum*) gained the trust of many scientists and cultural remedies all over the world for the prevention and treatment of many diseases and is broadly dispersed and consumed as a spice and herbal medicine for thousands of years. Recent studies have validated many of the medicinal properties attributed to garlic and its potential to lower the risk of diseases. Garlic has been shown to have anti-thrombotic activity (Block, 1985), lower blood lipids, blood tension, and had a cardio-protective effect (Neil and Sigaly, 1994; Sigaly et al., 1994), antibacterial properties and a potent inhibitor of food pathogens (Sivam, 2001; Lee et al., 2003). The mechanisms of garlic have been accredited to its effective antioxidants action (Yang et al., 1993), and its ability to stimulate immunological responsiveness (Reeve et al., 1993).

Laying hen performance and egg quality are all heritable traits of major concern not only to breeders but also to industry and consumers in different parts of world. This demand inspired many researchers to study the effects of various nutritional, environmental and managerial aspects to scale up these characteristics and to sustain high hen-production intensity as well. Eggs are a highly delicate food product, which could lose quality rapidly during the period between collection and consumption. Thus, improving and extending egg shelf life were included to the list of selection criteria for breeders and other researchers in the fields of production, management and nutrition. Numerous efforts have been made to lower the cholesterol content of eggs. Should the egg yolk cholesterol go down to minimal levels, it would be a win battle not only for poultry industry but also it would be beneficial to public's health. Since the sixties of the past century literature compiled genetic, nutritional, biological and pharmaceutical attempts

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to reduce yolk cholesterol; sadly, very limited success accompanied these efforts. It was in 1977 when Marks and Washburn concluded that natural selection pressure is expected to work against artificial selection in favor of a stable nutrient amount for a given egg size in order to provide the necessary environment to support the developing embryo. The inability to lower cholesterol levels more than 5-7% was indicative of such natural selection.

The hypolipidemic and hypocholesterolemic effects of garlic among wide range of species are well documented in the scientific media. However, when tested on yolk cholesterol contents of laying hen, the hypocholesterolemia property of garlic was controversial. Some workers reported a reduction in yolk cholesterol contents due to the use of garlic (Sharma et al., 1979; Chowdhury et al., 2002) while others did not. In 1991, Reddy et al. 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 was not effective in lowering yolk cholesterol or other lipid components of the serum of laying hens, even when fed for up to 8 months. Hen's age, garlic preparation form and hen's strain are among other sources of variation, to mention a few, that might provide an explanation of discrepancy between literatures. Chowdhury et al. (2002) have suggested that the contradictory results in the literature were due to use of different commercial garlic products; others insinuated that different commercial garlic products may not be hypocholesterolemic (Berthold et al., 1998). In 2001, Kasuga et al. tagged garlic products as allicin-rich and nonallicin-rich products. The allicin-rich product is made from raw garlic and the other is made from processed garlic.

In the herein study, experimental hens were gavaged with fresh 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 influential effect of oral administration of garlic juice on the layer hen performance, egg quality, eggs microbial contamination and cholesterol contents of egg yolk.

MATERIALS AND METHODS

Animals, treatments and experimental design

A total of forty-eight 40-wk-old Hi-sex laying hens, were obtained from a local commercial layer farm with an average body weight of (1,489±34.4 gram). Hens were housed randomly onto individual cages in an environmentally controlled house where they were allowed to acclimatize for two weeks and the experimental period continued for five more weeks. A corn-soybean layer ration (ME, 2,800 kcal/kg; CP, 18.5%; Ca, 3.5%; available phosphorus, 0.35%) and water were provided *ad libitum* consumption. The daily photoperiod consisted of 16 h of light and 8 h of darkness (16 L:8 D). Temperature was maintained at 22±1°C throughout the experimental period. After being acclimatized, hens were assigned into four treatment groups in a completely randomized design, so that there were 12 laying hens in each group. Groups were gavaged with four levels of garlic juice as a percent of body weight; 0% as a control group and 0.25, 0.50, and 1.0% as treatment groups. Hens were gavaged individually three times a week and control group of chickens were gavaged distilled water at rate of 0.5% of body weight so they would have the same gavage exposure of the other groups.

Fresh garlic bulbs were obtained from a local produce market. Garlic juice was prepared as described by Kasuga et al. (2001). Briefly, peeled garlic cloves were homogenized with an equivalent weight of distilled water in a food blender for 1 minute. The mixture was then allowed to stand for 30 minutes at room temperature. Garlic juice was collected by filtering the mixture through cheesecloth. Hens were gavaged garlic juice within 30 minutes of preparation using a plastic 10 ml pipette.

Performance and egg quality measures

Eggs were collected and labeled on a daily basis at 0800 h and 1400 h throughout the experimental period and the percentage of egg production was measured. On each of two separate weeks, the collected eggs for three consecutive days were used for egg components weight and quality evaluation. After each collection, eggs were assigned randomly to one of three storage times (1, or 5, or 10 days). Eggs were stored at room temperature with 65% relative humidity (RH). The interior quality of eggs assigned to 1-day storage time was measured within 4 hours of being laid. Eggs were weighed and then broken onto a flat surface. Albumen height was measured in the middle of the thick albumen equidistant from the outer edge of the albumen and the yolk. The yolk was separated and weighed and the albumen was collected into beaker, yolk and albumen pH was immediately measured (pH Spear, Eutech Instruments, USA). The shells were dried at room temperature for 6 days and then weighed. The weight of the albumen was calculated as the difference between the weight of the egg and the weight of the yolk plus shell. Haugh units were calculated using the formula: HU = 100 log (H+7.57-1.7$W^{0.37}$), where H is the height of the albumen, and W is the weight of the egg (Haugh, 1937). Dry matters of both yolk and egg-white were analyzed using international procedures of AOAC.

Bacterial count procedure

Eggs were collected for three consecutive days to

measure bacterial count and bacterial growth of inoculated egg contents from fresh laid (1-), or 5- or 10-day stored eggs at room temperature. Bacterial counts were undertaken as described by Gürler and Fehlhaber (2004). Briefly, eggs were surface sterilized by wiping with 70% ethanol and allowed to air-dry, then burned off. The end of the egg was broken by sterilized glass rod. Egg contents were collected and homogenized in a sterile Petri-dish then transferred into a sterile tube. The *E. coli* was grown overnight at 37°C in trypticase soy broth. The egg contents mixture was challenged with 0.50 ml (2.5×10^3 CFU/ml) *E. coli* then kept for 24 h at 4°C. The egg mixtures were plated on tryptone soya agar (TSA) and incubated at 37°C for 24 h. After incubation, the colony forming units were counted and expressed as $\log_{10}$ CFU/ml of egg content.

Egg yolk cholesterol analysis

Yolk cholesterol was assayed for five successive weeks starting at 43 weeks of age. A sample of 2-3 eggs from each replicate was used for cholesterol quantification. Egg yolks were completely separated from the albumen, adhering white and chalazae; then weighted, pooled and mixed. The cholesterol content of egg yolk was determined following colorimetric method based on Liebermann-Burchard color reaction as described by Huang et al. (1961). Briefly, chloroform:methanol (2:1 v/v) solvent was used to extract total lipids from egg-yolk. The harvested extracts, which contain free cholesterol and cholesterol esters, were allowed to react with acetic anhydride and concentrated sulfuric acid, resulting in the formation of a blue-green complex. Egg yolk cholesterol content was quantified by comparing the color absorbance at 550 nm resulting from the Liebermann-Buchard reactions in egg yolk lipid extracts with cholesterol standards (Cholesterol reagent, Gainland Chemical Company, UK). All the readings were blanked against a chloroform:methanol.

Statistical analysis

Collected data were subjected to analysis of variance using the GLM procedure of SAS (SAS, 1996); garlic level was used as a class statement. The results of egg components (yolk, albumen and shell), albumen and yolk pH, albumen height and Haugh unit were subjected to analysis of variance that included the main effects of garlic level and storage duration and their two-way interactions. Means were separated by the least-square method. Also, regression analysis using the regression procedure of SAS was used to model the relationship between garlic juice level or storage duration, as explanatory variables, and data from egg components, egg quality and bacterial count.

RESULTS

Hens' gavaged different levels of garlic held numerically higher performance when compared to control-fed group of hens and showed an improvement in egg quality with no sign of interaction with storage time (Table 1). All levels of garlic supplementation groups recorded similar egg weight (Ewt), yolk weight (Ywt), shell weight (Swt), and albumen weight (Awt); however, they surpassed what registered by the control-fed group. Interestingly, garlic juice administration did not affect the dry matter percentages of both yolk and albumin contents (data not shown). Contrasting the garlic-gavaged groups with the

Table 1. Effects of different levels of garlic juice and storage time on egg components, pH, and albumen quality

Garlic juice (%[1])	Performance (%)	Weight (g)				pH		Albumen quality	
		Egg[2]	Yolk	Shell	Albumen	Yolk	Albumen	Height (mm)	Haugh unit
0.00	77.38	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	82.74	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	84.52	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	86.80	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	4.30	0.77	0.16	0.07	0.63	0.01	0.03	0.15	0.90
linear	NS	***	**	NS	**	NS	NS	*	NS
Contrast	----------	----------	----------	----------	*Estimate*	----------	----------	----------	----------
Garlic vs. control	7.14^{NS}	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	41.76^{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	*	***	***	***	***

[1] Garlic juice as a percentage of hens' body weight. [2] Egg weight averaged over two-week collections.

[a, b, c] Means with different superscript within a column are significantly different ($p<0.05$).

* $p<0.05$; ** $p<0.01$; *** $p<0.001$. NS = Not significant.

control-fed group demonstrated an increase ($p<0.05$) by 3.53, 0.56, 0.27 and 2.71 gm in Ewt, Ywt, Swt and Awt, respectively. Similarly, yolk pH (YpH) and albumen pH (ApH) showed no statistical difference among garlic-gavaged groups; however, when compared with control-fed group YpH and ApH were suppressed ($p<0.05$) by 0.04 and 0.09, respectively. The main effects of garlic juice administration and storage period declared a significant effect on both albumen height (AH) and Haugh unit (HU) with no sign of interaction. Yolk weight increased linearly as storage time advances; on the other hand, Awt decreased linearly. Also, the decrease in ApH, YpH, AH, and HU followed a linear fashion as storage time progressed through day ten.

The effects of garlic juice administration and storage time on the bacterial count of egg contents challenged with *E. coli* are noted in Table 2. Both garlic supplementation and storage time ameliorated the bacterial count of egg contents with marginal warning of interaction between them. Garlic juice reduced ($p<0.05$) the $\log_{10}$ of bacterial count in egg contents linearly. Likewise, the storage time showed a similar effect. The decrease in bacterial count ranged from 3.465 in the control group to 3.399 $\log_{10}$ CFU/ml of egg contents in the highest garlic-juice gavaged group (1.0% of body weight), with no sign of differences in bacterial counts among the different levels of garlic administered groups. Bacterial counts were reduced linearly by increasing garlic dose ($p<0.05$) only when the colonies were counted from fresh eggs (1-day storage) as indicated in Figure 1.

Cholesterol concentrations per gram of yolk for all dietary groups are summarized in Table 3. Eggs were sampled for cholesterol quantification two weeks after the onset of garlic juice administration. The collected data from this experiment showed that yolk cholesterol concentration was not affected by garlic juice administration through out the sampling period.

Table 2. Effects of different levels of garlic juice and storage time on *E. coli* count (log10) in egg contents

Garlic juice (%[1])	*E. coli* ($\log_{10}$)
0.00	3.465[a]
0.25	3.432[ab]
0.50	3.426[ab]
1.00	3.399[b]
SEM	0.015
Linear	0.0515
Contrast	---------- Estimate ----------
Garlic vs. control	-0.0463*
Storage (days)	
1	3.521[a]
5	3.420[b]
10	3.351[c]
SEM	0.013
Linear	***

[1] Garlic juice as a percentage of hens' body weight.
[a, b, c] Means with different superscript within a column are significantly different ($p<0.05$).
* $p<0.05$, *** $p<0.001$. NS = Not significant.

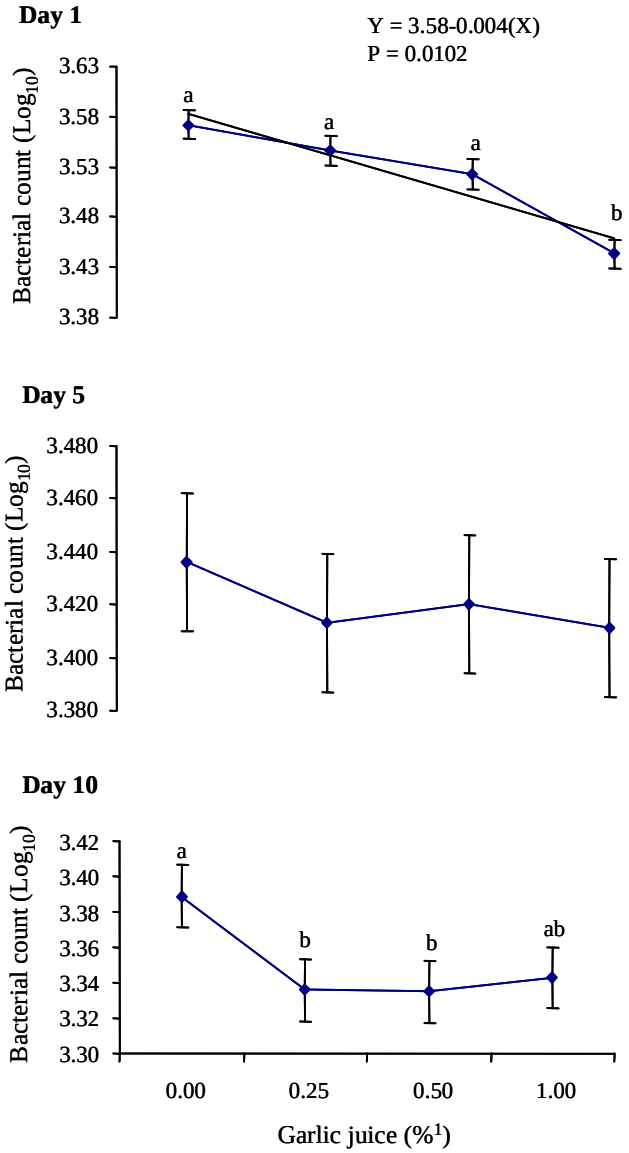

Figure 1. Effect of different levels of garlic juice on egg content *E. coli* count at1, 5, and 10 day storage time.

DISCUSSION

In the present study, the effects of oral administration of garlic juice on layer performance, egg components and quality, bacterial count of egg content, and yolk cholesterols levels in layer hens were investigated. Although the garlic administered groups had numerically higher laying performance values, the difference did not reach a significant level. In 1991, Reddy et al. reported the effect of

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

Garlic juice (%[1])	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. control	0.733^{NS}	-0.28^{NS}	-0.18^{NS}	-0.29^{NS}	-0.28^{NS}

[1] Garlic juice as a percentage of hens' body weight. NS = Not significant.

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. In more recent studies, Yalcin et al. (2006) showed that supplementing garlic powder at level of 5 or 10 g/kg showed numerical increase in hen-day egg production and a significant increase in egg weight, similar to what reported in the herein study. Also, Khan et al. (2007) reported that laying hens fed on dried garlic (2-8%) showed higher egg-production intensity with numerical increase in egg mass; however egg weight did not change significantly when compared to the control-fed group. The diversity of garlic preparation and administration methods makes it harder to contrast our results with those in literature. Also, the differences in experimental conditions between our trial and those described in the literature, such as the age of hens used, may provide another additive explanation to the results we report herein. Among the most potentially recognized active components in garlic is allicin. Lawson and Hughes (1992) demonstrated that allicin is unstable and poorly absorbed from the digestive tract. Also, garlic preparations that are produced by heat or solvent processes are known to void allinase, and hence allicin may not be formed. 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; Yu et al., 1989). Had we measured feed intake and efficiency or looked at the status of small intestine or their nutrient absorption abilities; we might have had an explanation to the increase in egg traits parameters. Adibmoradi et al. (2006) reported that garlic administration enhanced villus height and crypt depth and decreased epithelial thickness and goblet cell numbers in duodenum, jejunum and ileum of birds; similar results were reported by Nusairate (2007). They concluded that the morphological changes in the birds gut proclaim improvement in the digestive capacity. In 2003, Ramakrishna et al. reported that garlic supplementation probably enhanced the activities of the pancreatic enzymes and provided micro-environment for better nutrient utilization in rats. If this hold true on chicken, it partially explains the upward deviation in eggs' components weight due to garlic administration compared to the controlled-fed group of hens.

The deterioration of albumen quality as assessed by albumen height and Haugh unit or the increase in albumen pH is a well known fact and had been cited by many researchers. Our results are in line with many reports (Li-Chan and Nakai, 1989; Ahn et al., 1999; Scott and Silversides, 2000; Silversides and Scott, 2001; Monia et al., 2003; Silversides and Budgell, 2004). Pappas et al. (2005) proposed that organic selenium enhances the egg's antioxidant status by upgrading the glutathione peroxidase activity in yolk and albumen; 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 (Yang et al., 1993; Thomson and Ali, 2003; Mirunalini et al., 2004). Thus, eggs collected from garlic-gavaged hens registered better albumen height, Haugh unit and pH probably because of less lipid and protein oxidation.

The results of our experiment did not detect significant reduction in yolk cholesterol contents as a result of garlic administration contrary to what one would expect. The lack of effect of garlic juice on yolk cholesterol differ from the results of Sharma et al. (1979), who reported a reduction in egg yolk cholesterol by feeding of 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. Chowdhury and his colleagues (2002) concluded that garlic paste in the diets of laying hens reduced serum and yolk cholesterol concentrations without affecting layer performance. In 2007, Khan et al. reported that dried garlic powder in the diets of commercial laying hens reduced serum and yolk cholesterol concentrations and skewed the layer performance upwards significantly. However, Birrenkott et al. (2000) showed that diet supplementation with 3% of powdered garlic was not

effective in lowering yolk cholesterol (mg/g) or other lipid components of the serum of laying hens, even when fed for up to 8 months. More recently, Yalcin et al. (2006) reported that total yolk cholesterol was not affected by garlic supplementation. It has been suggested that different commercial garlic products may explain the contradictory results in the literatures (Chowdhury et al., 2002). Understanding the metabolic process of cholesterol formation in egg yolk of laying hens may provide further explanation. Hargis (1988) cited many reports on the metabolism process of egg yolk cholesterol in domestic fowl and their modifying factors as well. The primary controlling factor in cholesterol synthesis is at the formation of mevalonic acid via HMG-CoA reductase. Vargas et al. (1986) concluded that the laying hen is capable of synthesizing cholesterol in excess of needs for yolk deposition and that the HMG Co-A reductase needs to be inhibited more than 43% to alter egg yolk cholesterol. Genetically, Marks and Washburn (1977) showed that selection for lower egg cholesterol has resulted in only modest (5-7%) reductions. Also, they failed to resume further reduction in egg cholesterol and concluded a minimum physiological level is reached, which might be controlled by natural selection pressure to maintain a certain level of cholesterol in the egg for use by the developing embryo.

The antibacterial property of garlic is a well-documented fact (Sivam, 2001; Lee et al., 2003). Garlic juice suppressed the bacterial counts linearly in a dose-dependent manner. Results of the present study showed harmony with several other studies (Tucker, 2002; Guo et al., 2004; Sarica et al., 2005). Saeed and Koons (1993) artificially inoculated eggs with bacteria also observed substantial growth in eggs stored at room temperature for 2-3 days. However, minimal or no growth occurred in refrigerated eggs at 4°C. The main antimicrobial constituent of garlic has been identified to be allicin, which is formed when the garlic clove is crushed (Ankri and Mirelman, 1999). Albumen pH has increased from 8.35 to 9.30 from freshly laid eggs and those stored for 10 days, respectively. This rise in pH probably increased the antimicrobial properties of albumen proteins, and made it less favorable for bacterial growth (Rosso et al., 1995).

In conclusions, the results of the present study show that administration of garlic juice to the laying hens resulted in improvement of egg quality and performance characteristics and decline in bacterial count. The increase in egg weight and mass add economical angle to the use of garlic in the egg poultry industry. The overall results of extended egg shelf life would be beneficial to the industry and the consumer as well. Further large scale studies are needed to confirm the outcome of this experiment, to measure feed efficiency, and to engineer a practical method of garlic administration without compromising its active component(s).

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