Asian-Aust. J. Anim. Sci.
Vol. 23, No. 6 : 786 - 791
June 2010

www.ajas.info

Effects of Fermented Garlic Powder on Production Performance, Egg Quality, Blood Profiles and Fatty Acids Composition of Egg Yolk in Laying Hens

X. Ao, J. S. Yoo, J. H. Lee, H. D. Jang, J. P. Wang, T. X. Zhou and I. H. Kim*
Department of Animal Resource and Science, Dankook University,
#29 Anseodong, Cheonan, Choognam, 330-714, Korea

ABSTRACT : The effects of fermented garlic powder on production performance, egg quality, blood profiles and fatty acid composition of egg yolk in laying hens were studied in a 35-d experiment. Two-hundred and forty (ISA brown) layers aged 41 weeks were randomly allocated into the following four treatments: i) CON (basal diet); ii) G1 (CON+fermented garlic powder 1.0%); iii) G2 (CON+fermented garlic powder 2.0%) and iv) G3 (CON+fermented garlic powder 3.0%). There were no differences ($p>0.05$) among treatments in egg production, egg weight, eggshell breaking strength and eggshell thickness throughout the whole experimental period. However, yolk height was increased significantly ($p<0.05$) by the addition of fermented garlic powder during the 5th week while yolk color was greater ($p<0.05$) in G2 and G3 than in CON and G1 in the 5th week. Compared with CON, Haugh unit was increased ($p<0.05$) in response to fermented garlic powder treatments during the 5th week. No significant effects on total protein, albumin and IgG were observed in response to any of the treatments over the experimental period ($p>0.05$). There was a significant ($p<0.05$) reduction in plasma cholesterol concentration when the dietary level of fermented garlic powder was increased from 0.0 to 3.0%. The levels of saturated fatty acids (SFA) were significantly decreased ($p<0.05$) in response to G2 and G3 while monounsaturated fatty acids (MUFA) were higher ($p<0.05$) in G2 and G3 treatment groups than in CON and G1. Compared with other treatments, polyunsaturated fatty acids (PUFA) and PUFA:SFA ratio were higher ($p<0.05$) in G3. In conclusion, this study demonstrated that addition of fermented garlic powder reduced plasma cholesterol concentration and did not cause adverse effects on production performance. Moreover, addition of 3.0% garlic powder decreased SFA but increased PUFA and PUFA:SFA ratio in egg yolk. (**Key Words :** Blood Profiles, Egg Production, Egg Quality, Fatty Acids, Garlic Powder, Laying Hens)

INTRODUCTION

It has long been considered that garlic (*Allium sativum*) has several beneficial effects for both humans and animals having antimicrobial, antioxidant, as well as antihypertensive properties (Konjufca et al., 1997; Sivam, 2001). Previous research suggested that these functions were mainly attributed to the bioactive components of garlic, including sulphur-containing compounds, such as alliin, diallylsulphides and allicin (Amagase et al., 2001). Therefore, the different garlic preparations used in various studies might be one of the reasons for the inconsistent results. Sharma et al. (1979) observed that egg yolk cholesterol was decreased by 1% or 3% garlic powder supplementation to diets of laying hens for 3 weeks. Sklan et al. (1992) reported that hepatic cholesterol concentrations in chickens were reduced when 2% garlic was fed. The activity of hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase was decreased by 50-69% in 12-week-old broilers and by 72-83% in 12-week-old Leghorn pullets with concomitant decreases in serum cholesterol of 7-25 and 20-25%, respectively, by the feeding of diets containing garlic paste at 3.8% or solvent fractions of garlic oil equivalent to 3.8% garlic paste (Qureshi et al., 1983). Chowdhury et al. (2002) observed there were no effects on egg production but significantly decreased serum cholesterol by the addition of garlic. However, Birrenkott et al. (2000) reported that there were no significant effects on yolk and serum cholesterol concentrations by 3% garlic powder supplementation when laying hens were fed diets for 8 months. Egg production, egg weight, feed efficiency, total plasma lipids, plasma cholesterol and yolk cholesterol were

* Corresponding Author: I. H. Kim. Tel: +82-41-550-3652, Fax: +82-41-553-1618, E-mail: inhokim@dankook.ac.kr
Received October 24, 2009; Accepted December 17, 2009

not affected during or at the end of 8 weeks of feeding garlic oil to layers (Reddy et al., 1991). In addition, there were few data about the effects of garlic on egg quality and fatty acid composition in egg yolk.

The present study was conducted to determine the effects of garlic powder on egg production, egg quality, blood profiles and fatty acid composition of egg yolk in laying hens.

MATERIALS AND METHODS

Experimental birds and diets

All animal-based procedures were in accordance with the Guidelines for the Care and Use of Experimental Animals of Dankook University. A total of 240 ISA brown laying hens were randomly assigned to one of four treatments. The experiment was conducted from 41 to 46 wk of age. There were 10 replicates for each treatment with 3 adjacent cages (2 hens/cage, 38 cm×50 cm×40 cm) representing a replication. The house was provided with programmable lighting and ventilation. Feed and water were provided *ad libitum* and diets were presented in mash form. The composition of the corn-SBM basal diet is shown in Table 1. Dietary treatments were as follows: i) CON (basal diet); ii) G1 (CON+fermented garlic 1.0%); iii) G2 (CON+fermented garlic 2.0%) and iv) G3 (CON+fermented garlic 3.0%). Diets were formulated to meet or exceed the nutrient requirements of laying hens (NRC, 1994).

Table 1. Basal diet composition (as-fed basis)

Ingredients	%
Corn	50.4
Soybean meal, CP 46%	18.7
Wheat grain	10.0
Corn gluten meal	2.00
Wheat bran	5.00
Animal fat	4.40
Limestone	7.50
Tricalcium phosphate, P 18%	1.40
Salt	0.30
DL-methionine , 50%	0.10
Vitamin premix[1]	0.10
Mineral premix[2]	0.10
Chemical composition[3]	
ME (Kcal/kg)	2,904
Crude protein (%)	15.00
Lysine (%)	1.80
Methionine (%)	0.32
Calcium (%)	3.25
Phosphorus (%)	0.61

[1] Provided per kg of diet: 125,000 IU vitamin A; 2,500 IU vitamin D_3; 10 mg vitamin E; 2 mg vitamin K_3; 1 mg vitamin B_1; 5 mg vitamin B_2; 1 mg vitamin B_6; 15 mg vitamin B_{12}; 500 mg folic acid; 35,000 mg niacin; 10,000 mg Ca-Pantothenate and 50 mg biotin.

[2] Provided per kg of diet: 8 mg Mn; 60 mg Zn; 25 mg Cu; 40 mg Fe; 0.3 mg Co; 1.5 mg I and 0.15 mg Se.

[3] Calculated values.

Preparation of garlic powder

Garlic powder was provided by Liisna Company (Korea). The mixture of garlic and water (v/v 1:1) was filtered through a 45 μm sieve and the filtrate was then centrifuged (1,000 g, 40 min) to obtain the suspension. After that, the suspension was inoculated with 0.10% *Weissella koreensis* (1.0×10^7 CFU/ml) for 24 h. The inoculated product was cultivated at 25°C and garlic powder was collected by vacuum concentration.

Sampling and measurement

Daily egg production was recorded. In addition, a total of 30 eggs were randomly collected at 17:00 from each treatment (n = 30, three per replicate) on day 0, 21 and 35 and used to determine the egg quality. Eggshell breaking strength was evaluated using an egg shell force gauge model II (Robotmation Co., Ltd., Japan). Egg shell thickness was measured on the large end, equatorial region and small end using a dial pipe gauge (Ozaki MFG Co., Ltd., Japan). Finally, the egg weight, egg yolk color and Haugh units were evaluated using an egg multi-tester (Touhoku Rhythm Co. Ltd., Japan).

Blood samples were collected from the wing vein of the same laying hens that were selected at random from each treatment (one hen per replication) on day 0, 21 and 35 of the experiment using a sterilized syringe and K_3EDTA vacuum tubes (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ, USA). The blood samples were then centrifuged at 2,000×g at 4°C for 20 min within one hour of collection to separate the plasma. The concentrations of total protein, albumin, and total cholesterol in the plasma samples were analyzed with an automatic biochemical analyzer (RA-1000, Bayer Corp., Tarrytown, NY) using colorimetric methods. The immunoglobin G (IgG) was analyzed using nephelometry (Behring, Germany).

To determine the fatty acid composition of the egg yolk, two 10-g samples were collected and extracted using chloroform: methanol (2:1, vol/vol) mixture according to the method described by Folch et al. (1957). Next, 20 to 25 mg of the extracted fat was saponified with 0.5 *M* methanolic sodium hydroxide and then methylated with boron trifluoride in methanol using the method described by Metcalfe et al. (1966). The fatty acid methyl esters obtained were then separated and analyzed by gas chromatography. The fatty acid content was determined using an HP6890 gas chromatograph (Agilent, Waldbronn, Germany) equipped with a flame ionization detector and an HP 19091 to 136 capillary column (60 m×0.25 mm internal diameter) with a 0.25 μm film thickness of stationary phase. Helium was

Table 2. Effects of garlic powder on egg production in laying hens[1]

Item	Diet				SE[2]	p-value[3]	
	CON	G1	G2	G3		Linear	Quadratic
Egg production (%)							
1 week	82.86	81.93	82.86	82.14	1.94	0.89	0.96
2 week	80.92	80.48	80.86	80.80	0.92	1.00	0.84
3 week	81.87	81.55	81.01	81.40	1.32	0.75	0.79
4 week	81.66	81.13	80.95	81.13	1.05	0.71	0.74
5 week	81.07	81.44	81.69	81.58	1.12	0.73	0.83
Egg weight (g)							
1week	63.15	62.47	61.57	62.78	1.17	0.23	0.35
3 week	63.90	63.25	63.60	64.82	0.83	0.80	0.50
5 week	64.71	62.20	62.38	62.56	0.90	0.06	0.22

[1] CON = basal diet; G1 = basal diet with garlic 1.0%; G2 = basal diet with garlic 2.0%; G3 = basal diet with garlic 3.0%.
[2] Pooled standard error. [3] Linear and quadratic effects of garlic.

used as carrier gas. Oven temperature was programmed as follows: from 140 to 160°C at 1.5°C/min, from 160 to 180°C at 0.50°C/min, and from 180 to 230°C at 2.50°C/min. The other chromatographic conditions were as follows: injector and detector temperatures, 280°C; sample volume injected, 1 μl. Fatty acids were identified by matching their retention times with those of their respective standards, as well as by mass spectrometry (HP5973, Agilent) of each peak.

Statistical analysis

Data were statistically analyzed by ANOVA using the GLM procedure of SAS (SAS Institute, 1996) for a randomized complete block design. Differences among all treatments were separated by Duncan's multiple range tests. The linear and quadratic effect of fermented garlic powder among treatments was analyzed using a contrast statement. Mean values and standard error of means (SEM) are reported. Probability values of less than 0.05 were considered as significant.

RESULTS AND DISCUSSION

Layer performance

There were no differences due to dietary treatment in egg production and egg weight over the 5-week period (Table 2). In agreement with our results, Reddy et al. (1991) and Chowdhury et al. (2002) found that garlic paste or garlic oil had no effect on egg production and egg weight. Recently, Safaa (2007) indicated that egg weight and production performance were not affected by 2.0% garlic addition. In contrast, Sakine and Onbasilar (2006) reported that egg weight increased with dietary garlic powder supplementation ($p<0.01$). These different results may be due to the use of different garlic sources, methods of preparation of garlic powder, and strains or age of layers used in the experiments.

Egg quality

Table 3 shows the effects of fermented garlic powder on egg quality in laying hens. The supplementation of fermented garlic powder had no significant effects ($p>0.05$) on eggshell breaking strength and eggshell thickness. However, yolk height in garlic powder treatments was higher ($p<0.05$) than in CON during the 5th week. In addition, yolk color was greater ($p<0.05$) in G2 and G3 compared with CON and G1 in the 5th week. Haugh unit was increased ($p<0.05$) by the addition of fermented garlic powder during the 5th week. In agreement with our results, a study conducted by Safaa (2007) reported that 2.0% addition of dietary garlic increased yolk weight, yolk color and Haugh unit. However, these results are different from the findings reported by Sakine and Onbasilar (2006) who observed that the supplementation of garlic powder had no significant effect ($p>0.05$) on egg breaking strength, shell thickness, albumen index, yolk index and Haugh unit. Due to the inconsistencies in egg traits, further research is needed to study the effect of garlic powder on egg quality.

Blood profiles

The effect of garlic powder supplementation on blood parameters of laying hens is shown in Table 4. Garlic powder supplementation did not significantly affect the plasma concentrations of total protein, albumin and IgG. However, plasma total cholesterol decreased ($p<0.05$) with increasing levels of dietary garlic powder. These results are consistent with the beneficial effects of garlic on cholesterol metabolism in human health and agreed well with previous studies. Chowdhury et al. (2002) reported that plasma cholesterol concentration was decreased on average by 15, 28, 33, and 43% with increasing levels of dietary garlic paste of 2, 4, 6, or 8%, respectively.. Garlic powder

Table 3. Effects of garlic powder on egg quality in laying hens[1]

Items	Diet				SE[2]	p-value[3]	
	CON	G1	G2	G3		Linear	Quadratic
Eggshell breaking strength (kg/cm^2)							
1 week	3.81	3.80	3.83	3.86	0.18	0.60	0.71
3 week	3.93	3.90	3.81	3.71	0.12	0.46	0.80
5 week	3.74	3.63	3.61	3.85	0.11	0.33	0.72
Eggshell thickness (mm/10^2)							
1 week	38.12	36.60	36.21	37.00	0.01	0.46	0.52
3 week	38.00	38.00	39.00	37.00	0.01	0.10	0.25
5 week	36.10	36.17	36.33	36.20	0.01	0.48	0.32
Yolk height (mm)							
1 week	8.01	8.06	7.98	8.10	0.30	0.48	0.69
3 week	8.23	8.64	8.28	8.24	0.19	0.58	0.03
5 week	8.13^b	8.79^a	8.79^a	8.43^a	0.17	0.01	0.07
Haugh unit							
1 week	87.22	87.47	88.25	88.00	2.14	0.54	0.36
3 week	89.49	91.96	89.43	89.73	1.26	0.55	0.29
5 week	88.95^b	92.74^a	92.62^a	92.24^a	1.11	0.01	0.09
Yolk color							
1 week	10.68	10.33	10.55	10.65	0.20	0.36	0.56
3 week	10.80	10.62	10.38	10.77	0.18	0.42	0.53
5 week	10.36^b	10.23^b	11.17^a	11.27^a	0.23	0.01	0.11

[1] CON = basal diet; G1 = basal diet with garlic 1.0%; G2 = basal diet with garlic 2.0%; G3 = basal diet with garlic 3.0%.
[2] Pooled standard error. [3] Linear and quadratic effects of garlic.
[a, b] Means in the same row with different superscripts differ ($p<0.05$).

supplementation reduced significantly the plasma cholesterol concentration when laying hens were fed 0.5 and 1.0% garlic powder (Sakine and Onbasilar, 2006). Reduction of plasma cholesterol concentration by the addition of garlic powder was also observed in rats and broilers (Myung, 1982; Qureshi et al., 1983). The decrease of plasma cholesterol concentration by garlic powder supplementation might be due to the reduction of synthetic enzymes. Significant decreases in hepatic 3-hydroxy-3-methylglutaryl-CoA reductase, cholesterol 7α-hydroxylase, fatty acid synthetase, and in representative pentose-phosphate pathway activities accompanied the feeding of petroleum ether-, methanol- and water-soluble fractions of garlic (Qureshi et al., 1983). In contrast, Reddy et al. (1991) reported that feeding 0.02% garlic oil did not have any effect on plasma and yolk cholesterol in the Babcock B-300

Table 4. Effects of garlic on blood parameters in laying hens[1]

Item	Diet				SE[2]	p-value[3]	
	CON	G1	G2	G3		Linear	Quadratic
3 week							
Total protein (g/dl)	4.82	4.90	5.14	4.88	0.22	0.67	0.45
Albumin (g/dl)	2.18	2.22	2.26	2.20	0.07	0.76	0.51
IgG (mg/dl)	2.66	2.44	2.21	2.93	0.90	0.96	0.65
Total cholesterol (mg/dl)	205.40^a	202.00^{ab}	187.60^{bc}	181.40^c	5.12	<0.01	0.79
5 week							
Total protein (g/dl)	5.12	5.30	5.40	5.34	0.16	0.37	0.66
Albumin (g/dl)	2.28	2.40	2.50	2.38	0.07	0.29	0.24
IgG (mg/dl)	1.11	0.93	1.03	1.19	0.31	0.44	0.95
Total cholesterol (mg/dl)	209.40^a	203.20^a	185.80^b	183.20^b	4.58	<0.01	0.70

[1] CON = basal diet; G1 = basal diet with garlic 1.0%; G2 = basal diet with garlic 2.0%; G3 = basal diet with garlic 3.0%.
[2] Pooled standard error. [3] Linear and quadratic effects of garlic.
[a,b] Means in the same row with different superscripts differ ($p<0.05$).

Table 5. Effects of garlic on fatty acid composition of egg yolk in laying hens[1]

Fatty acids (%)	Diet				SE[2]	p-value[3]	
	CON	G1	G2	G3		Linear	Quadratic
Total SFA	34.24[a]	34.08[a]	32.04[b]	30.89[b]	0.44	<0.01	0.30
C14:0	0.39	0.39	0.37	0.39	0.02	0.80	0.57
C16:0	25.17[a]	25.40[a]	22.60[b]	22.58[b]	0.64	0.12	0.85
C18:0	8.60[ab]	8.25[ab]	9.05[a]	7.88[b]	0.32	0.07	0.23
C20:0	0.08[a]	0.03[b]	0.02[b]	0.04[b]	0.01	0.03	0.01
Total MUFA	49.41[bc]	48.42[c]	50.43[ab]	50.99[a]	0.41	0.08	<0.01
C14:1n-5	0.09[c]	0.14[a]	0.11[b]	0.10[bc]	0.01	<0.01	0.16
C16:1n-7	2.98	3.08	3.38	3.54	0.21	0.88	0.07
C18:1n-9	46.23[a]	44.99[b]	46.71[a]	47.10[a]	0.33	0.02	<0.01
C20:1	0.11[b]	0.21[a]	0.24[a]	0.25[a]	0.03	0.07	0.02
Total PUFA	12.56[b]	11.68[b]	12.20[b]	15.79[a]	0.45	<0.01	<0.01
C18:2n-6	11.53[b]	10.71[b]	11.12[b]	14.82[a]	0.44	<0.01	<0.01
C18:3n-3	0.03	0.03	0.03	0.03	<0.01	0.79	0.56
C20:2n-6	0.15	0.15	0.18	0.15	0.02	0.75	0.26
C20:4n-6	0.01	0.02	0.03	0.02	<0.01	1.00	0.08
C20:5n-3	0.20	0.18	0.20	0.20	0.02	0.21	0.79
C22:6n-3	0.62	0.58	0.63	0.62	0.02	0.25	0.87
PUFA:SFA	0.37[b]	0.34[b]	0.38[b]	0.51[a]	0.02	<0.01	<0.01

[1] CON = basal diet; G1 = basal diet with garlic 1.0%; G2 = basal diet with garlic 2.0%; G3 = basal diet with garlic 3.0%.
[2] Pooled standard error. [3] Linear and quadratic effects of garlic.
[a, b, c] Means in the same row with different superscripts differ (p<0.05).

strain. This result might be due to the strain used in the experiments because plasma cholesterol concentrations differ significantly among strains (Chowdhury, 2002). Besides, different analytical methods and garlic products might also result in this difference.

Fatty acid composition in egg yolk

In this study, the total SFA concentration of fatty acids in egg yolk decreased ($p<0.05$) in response to G2 and G3, the predominant fatty acid being palmitic, followed by stearic. This decrease was primarily due to the decrease in palmitic acid. Monounsaturated fatty acids (MUFA) were higher ($p<0.05$) in G2 and G3 treatment groups than in CON and G1. Furthermore, compared with other treatments, polyunsaturated fatty acid (PUFA) concentration was higher ($p<0.05$) in G3. This was mainly due to an increase in linoleic acid. Further, 3.0% fermented garlic powder supplementation resulted in a higher PUFA:SFA ratio compared with other treatments. This is beneficial for human health in that clinical data strongly support a relationship between SFA and the risk of coronary heart disease, and therefore there is a need to decrease the consumption of SFA and increase that of PUFA. However, no comparisons with other studies could be made because investigations of the use of garlic powder in relation to egg quality have not yet been reported. Therefore, further research is needed to study the effect of garlic powder on fatty acid composition in egg yolk.

Considering the data obtained herein and the above discussion, it can be concluded that the supplementation of garlic powder could reduce plasma cholesterol significantly without adverse effects on egg production. Moreover, dietary 3.0% garlic addition decreased SFA and increased PUFA and PUFA:SFA ratio in egg yolk. However, more studies are needed to determine the effects of garlic on egg yolk composition, especially the fatty acids and the potential cholesterol-depressing mechanism.

REFERENCES

Amagase, H., B. L. Petesch, H. Matsuura, S. Kasuga and Y. Itakura. 2001. Intake of garlic and its bioactive components. J. Nutr. 131:955-962.

Birrenkott, G., G. E. Brockenfelt, M. Owens and E. Halpin. 2000. Yolk and blood cholesterol levels and organoleptic assessment of eggs from hens fed a garlic-supplemented diet. Poult. Sci. 79 (Suppl. 1):75 (Abstr.).

Chowdhury, S. R., S. D. Chowdhury and T. K. Smith. 2002. Effects of dietary garlic on cholesterolmetabolism in laying hens. Poult. Sci. 81:1856-1862.

Folch, J., M. Lees and G. H. S. Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226:497-509.

Konjufca, V. H., G. M. Pesti and R. I. Bakalli. 1997. Modulation of cholesterol levels in broiler meat by dietary garlic and copper.

Poult. Sci. 76:1264-1271.

Metcalfe, L. D., A. A. Schmitz and J. R. Pelka. 1966. Rapid preparation of fatty acid esters from lipids for gaschromatographic analysis. Anal. Chem. 193:514-515.

Myung, S. C., T. K. Eunsook and T. J. Stewart. 1982. Effects of garlic on lipid metabolism in rats fed cholesterol or lard. J. Nutr. 112:241-248.

National Research Council. 1994. Nutrient Requirements of Poultry. 9th rev.ed. National Academy Press, Washington, DC.

Qureshi, A. A., Z. Z. Din, N. Abuirmeileh, W. C. Burger, Y. Ahmad and C. E. Elson. 1983. Suppression of avian hepatic lipid metabolism by solvent extracts of garlic: Impact on serum lipids. J. Nutr. 113:1746-1755.

Reddy, R. V., S. F. Lightsey and D. V. Maurice. 1991. Effect of feeding garlic oil on performance and egg yolk cholesterol concentration. Poult. Sci. 70:2006-2009.

Safaa, M. Hosam. 2007. Effect of dietary garlic or fenugreek on cholestrol metabolism in laying hens. Egypt. Poult. Sci. 27: 1207-1221.

Sivam, G. P. 2001. Protection against Helicobacter pylori and other bacterial infections by garlic. J. Nutr. 131 (3S), 1106S-1108S.

Sharma, R. K., R. A. Singh, R. N. Pal and C. K. Aggarwal. 1979. Cholesterol content of chicken eggs as affected by feeding garlic, sarpagandha, and nicotinic acid. Haryana Agric. Univ. J. Res. 9:263-265.

Sklan, D., Y. N. Berner and H. D. Rabinowitch. 1992. The effect of dietary onion and garlic on hepatic lipid concentrations and activity of antioxidative enzymes in chicks. J. Nutr. Biochem. 3:322-325.

SAS institute. 1996. SAS User's Guide: Statistics. Version 7.0. SAS Institute, Cary, NC.

Sakine Yalc and E. Ebru Onbasilar. 2006. Effect of garlic powder on the performance, egg traits and blood parameters of laying hens. J. Sci. Food Agric. 86:1336-1339.