Research Article

Effects of Dietary Supplementation of Fermented Garlic Powder on Productive Performance, Hatchability, and Biochemical Blood Parameters of Layer Breeders

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Abstract | The objective of this study was to investigate effects of dietary fermented garlic powder (FGP) on productive performance and blood biochemical parameters of layer breeders. A total of 360 layer breeders (312 females and 48 males) were randomly divided into four dietary groups: 1) CON, basal diet; 2) FGP0.5, basal diet with fermented garlic powder at 0.5 g/kg; 3) FGP1.0, basal diet with fermented garlic powder at 1.0 g/kg, and 4) FGP2.0, basal diet with fermented garlic powder at 2.0 g/kg. FGP addition had no significant effect on egg production (EP) or egg weight (EW). However, eggs from groups fed a diet supplemented with FGP showed linearly ($P < 0.05$) higher hatchability (HAT) than those from the CON group fed a basal diet. Regarding blood parameters, serum concentration of albumin (ALB) was significantly ($P < 0.05$) higher in the group fed diets supplemented with FGP than in the CON group fed a basal diet. FGP supplementation quadratically ($P < 0.05$) increased serum total protein (TP) level. Aspartate amino transferase (AST), cholesterol (CHOL), and triglyceride (TG) concentrations were significantly ($P < 0.05$) decreased in the FGP2.0 group than in the CON group. Serum IL-2 level was significantly ($P < 0.05$) higher in the FGP2.0 group than in the CON group, although IL-6 level showed no significant difference. These findings suggest that dietary supplementation of FGP as a feed additive might improve productive performance and health of layer breeder hens.

Keywords | Blood composition, Fermented garlic powder, Hatchability, Layer breeder, Performance

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INTRODUCTION

The layer breeder production is one of the most important poultry industries, attracting substantial investment worldwide. Consequently, there is an urgent need to improve birds' productivity and enhance their health using natural feed additives (El-Sabrout et al., 2022; El-Saadany et al., 2022).

Garlic (*Allium sativum*) is used as a spice to improve the flavor of various foods around the world. It can be used to treat vascular diseases such as hyperlipidemia, arteriosclerosis, and hypertension (Ried, 2016). It also has pharmacological effects such as antibacterial, antiviral, anticancer, and anti-aging activities (Amagase et al., 2001). Garlic is known as an excellent nutritional food because it contains various vitamins, minerals, and amino acids (Pandey, 2012). In particular, allin, a constituent of garlic, is converted into a physiologically active substance called allicin by intracellular alliinase, which stimulate lymphocytes to improve immunity (Borlinghaus et al., 2014).

When garlic is used as a poultry feed additive, the susceptibility of broilers to Newcastle disease (ND), avian influenza (AI), and infectious bursal disease (IBD) is decreased (Jafari et al., 2011; Ao et al., 2011). In addition, garlic can decrease serum triglycerides and cholesterol levels and suppress the number of harmful bacteria such as *Escherichia coli* in intestine (Singh et al., 2017). A diet supplemented with garlic powder can improve yolk weight, yolk diameter, yolk color, and chick length and lower mortality of laying hens (Asrat et al., 2018). In addition, garlic can improve sperm production and protect sperm by regulating testosterone metabolic activity and testosterone from oxidative damage when it is fed to cocks (Musavi et al., 2018). Similarly, it has been found that garlic can relieve oxidative stress of ovaries and regulate the release of ovarian hormones such as follicle stimulating hormone (FSH) and luteinizing hormone (LH) (Modaresi and Heidari, 2015). Garlic can improve semen quality parameters (such as sperm vitality) and survival rate of male breeders when it is fed to poultry (Mohan et al., 2007; Okoro et al., 2016). These results indicate that garlic can be used as a feed additive to increase productive performance such as egg production and egg hatchability for breeder chickens. Furthermore, fermentation of foods is an effective form of extending the shelf-life of foods, which holds potential for extension of safety improvement of a variety of foods (Khubber et al., 2022). when garlic is fermented, flavor and safety of garlic are improved due to metabolic activities of microorganisms. In addition, contents of essential amino acids required for poultry are increased (Montano et al., 2004; Ao et al., 2010). It has been confirmed that fermented garlic-fed laying hens show increased albumen height and haugh unit in eggs and improved cellular and humoral immunity in blood (Hossain et al., 2016; Lim et al., 2018). The objective of this study was to investigate effects of various levels of garlic fermentation powder on productive performances, hatchability, and blood compositions of layer breeders.

MATERIAL AND METHODS

Fermented garlic powder

Garlic powder was supplied by HtO Life Incorporated (Republic of Korea). Fermented garlic powder (FGP) was prepared with the method of Ao et al. (2010). Briefly, suspension was obtained after centrifuging a mixture of garlic powder and water (v/v 1:1), and then was inoculated with 0.10% *Weissella koreensis* (1.0×10^7 CFU/ml) and incubated at 25°C for 24 h. FGP was collected by vacuum concentration.

Birds and Management

This study was carried out at the Poultry Experimental Station of the Department of Animal Sciences at Jeonbuk National University in the Republic of Korea. Protocols for the experiment were approved by Jeonbuk National University Institutional Animal Care and Use Committee (JBNU 2021-0168).

Table 1: Basal diet compositions for different treatment groups

Ingredients	Contents (g/kg)
Corn	661
Soybean meal	211
Corn gluten meal	17.4
Limestone	94.4
MDCP	8.70
Salt	3.70
DL-methionine	1.70
Mineral premix[1]	1.00
Vitamin premix[2]	1.00
Total	1000
Calculated chemical composition	
ME (kcal/kg)	2,750
CP (%)	16.0
Total lysine (%)	0.740
Total methionine (%)	0.400
Ca (%)	4.00
AvP (%)	0.350

[1]Mineral supplemented provided per kilogram of diet: Fe, 60 mg, Cu,10 mg; Zn 80 mg; Mn, 110 mg; Iodine, 0.48 mg; Se, 0.40 mg.
[2]Vitamin supplement provided per kilogram of diet: vitamin A, 10,000 IU; vitamin D, 2,000 IU, vitamin E, 30 IU; vitamin B_1, 3 mg; vitamin B_2, 7 mg; vitamin B_6, 0.15 mg; vitamin B_{12}, 0.025 mg; vitamin K_3, 3 mg; Niacin, 50 mg; vitamin C, 200 mg; Pantothenic acid, 12 mg; choline, 500 mg; biotin, 0.15 mg; folic acid, 1.5 mg.
MDCP: mono-di calcium phosphate, ME: metabolic energy, CP: crude protein, AvP: available phosphorus.

Male and female layer breeders at 29 weeks old were obtained from a grandparent operation (Hy-Line Brown parent). These breeders were weighed individually to exclude heavy and light birds and then equally distributed into windowless floor pens (3 birds/m^2). After birds were given a two-week acclimatization period to reduce stress between males and females, similar levels of egg production were maintained for pens. A total of 360 31-week-old layer breeder (312 females and 48 males) were allotted into 24 pens with each pen having 15 birds (13 females and 2 males). Dietary groups were as follows: 1) CON, basal diet; 2) FGP0.5, basal diet with fermented garlic powder at 0.5 g/kg; 3) FGP1.0, basal diet with fermented garlic powder at 1.0 g/kg; and 4) FGP2.0, basal diet with fermented garlic powder at 2.0 g/kg. The experimental house had a temperature of 25 ± 5°C and a photoperiod of 16/8

Table 2: Effects of dietary FGP on productive performance of layer breeder hens

Dietary group[1]	EP (%)	EW (g)	AH (mm)	HAU	HAT (%)
CON	88.4	63.5	7.42 [b]	84.7 [b]	85.2
FGP0.5	88.7	62.2	8.03 [ab]	87.7 [ab]	85.8
FGP1.0	88.5	63.6	7.80 [ab]	87.1 [ab]	86.0
FGP2.0	87.1	63.1	8.39 [a]	89.6 [a]	88.8
SEM	0.567	0.233	0.143	0.738	0.489
L	0.368	0.923	0.023	0.075	0.036
Q	0.568	0.961	0.033	0.099	0.107

[a,b] means in each column with no common superscript differs significantly at $P < 0.05$.
[1]CON: basal diet, FGP0.5: basal diet with fermented garlic powder 0.5 g/kg, FGP1.0: basal diet with fermented garlic powder 1.0 g/kg, FGP2.0: basal diet with fermented garlic powder 2.0 g/kg.
EP: egg production, EW: egg weight, AH: albumen height, HAU: haugh unit, HAT: hatchability. SEM: standard error of mean, L: linear effects, Q: quadratic effects.

Table 3: Effects of dietary FGP on biochemical blood compositions in layer breeder hens

Dietary group[1]	ALB (g/dl)	TP (g/dl)	AST (IU/l)	HDLC (mg/dl)	CHOL (mg/dl)	TG (mg/dl)
CON	2.03 [b]	6.35 [b]	149[a]	12.8	191[a]	2,342[a]
FGP0.5	2.36 [a]	7.28 [ab]	143[a]	13.1	185[a]	2,034[ab]
FGP1.0	2.32 [a]	8.50 [a]	129[b]	16.4	179[ab]	2,015[ab]
FGP2.0	2.45 [a]	7.51 [ab]	133[b]	15.1	157[b]	1,468[b]
SEM	0.048	0.251	3.10	0.791	0.696	131
L	0.005	0.103	0.044	0.209	0.003	0.014
Q	0.006	0.007	0.049	0.303	0.012	0.052

[a,b] means in each column with no common superscript differs significantly at $P < 0.05$.
[1]CON: basal diet, FGP0.5: basal diet with fermented garlic powder 0.5 g/kg, FGP1.0: basal diet with fermented garlic powder 1.0 g/kg, FGP2.0: basal diet with fermented garlic powder 2.0 g/kg.
ALB: albumin, TP: total protein, AST: aspartate amino transferase, HDLC: high density lipoprotein cholesterol, CHOL cholesterol, TG: triglyceride. SEM: standard error of mean, L: linear effects, Q: quadratic effects.

h light/dark cycle maintained throughout the experimental period. The litter was set in each pen at a height of about 5 to 7 cm before the arrival of birds. Each pan was equipped with rubber feeders and nipple drinkers. Diets were based on corn and soybean meal formulated to meet nutritional requirements for parent stock of laying hens as recommended by Korean Feeding Standard for Poultry (2017). Proximate compositions of the formulated basal diet are shown in Table 1.

Productive performance

Egg production (EP) and egg weight (EW) were recorded daily. Average values were then calculated. At the end of the experiment, three eggs from each replicate (18 eggs per group) were collected. After the eggs was stored for 24 hours at room temperature for subsequent measurements, Albumen height (AH) and Haugh unit (HAU) were semi-automatically measured using an egg multi-tester device (QCM+ System, TSS, UK).

From one week before the end of this experiment, a total of 720 eggs (30 eggs per pen) were collected to determine the hatchability (HAT). Eggs used for incubation were stored at 13°C up to in one week before incubation. Eggs were incubated at 37.8°C with humidity of 55-60% until the 18th day of incubation. After that, incubator conditions were changed to 37.2°C and 70-80% for the actual hatching process. HAT (%) was calculated by counting the number of hatched eggs per total eggs.

Serum profiles

Blood samples were taken between 8:00 and 9:00 AM by puncturing the wing vein of six hens from each group at the end of this experiment. Blood samples were collected with sterile syringes and centrifuged at 3,000 rpm for 15 minutes at 4°C. The separated serum was stored at -20°C. Serum concentrations of albumin (ALB), total protein (TP), aspartate amino transferase (AST), high density lipoprotein cholesterol (HDLC), cholesterol (CHOL), and triglyceride (TG) were measured via colorimetry using a biochemical analysis equipment (Automatic Biochemical Analyser, Thermo Scientitic, Konelab 20, Finland).

Serum concentrations of interleukin-2 (IL-2) and interleukin-6 (IL-6) were analyzed using Chicken IL-2 ELISA Kit (My Bio Source, MBS2508525, USA) and Chicken IL-6 ELISA Kit (My Bio Source, MBS268769, USA), respectively, following the manufacturer's instructions. Absorbance of each well was read at a wavelength of 450 nm with a microplate spectrophotometer (BioTek ELX 800, Winooski, VT, USA).

Statistical analysis

All data obtained from this study were subjected to one-way analysis of variance (ANOVA) using the general linear model of Statistical Analysis System (SAS, Version 9.1) according to a completely randomized design. Polynomial contrasts were performed to test for linear (L) and quadratic (Q) relationships between FGP inclusion rate and the variable in question. Results are presented as mean values with pooled standard errors (SEM). Differences were considered significant at $P < 0.05$.

RESULTS

Productive Performance

Results concerning effects of supplementation with FGP on productive performance are shown in Table 2. Addition of FGP showed no significant effect on EP and EW. A linearly higher ($P < 0.05$) HAT was observed for eggs from groups fed a diet supplemented with FGP than HAT for eggs from the CON group fed a basal diet. AH and HAU were higher ($P < 0.05$) in the FGP2.0 group than in the CON group. FGP levels showed linear and quadratic effects ($P < 0.05$) on AH.

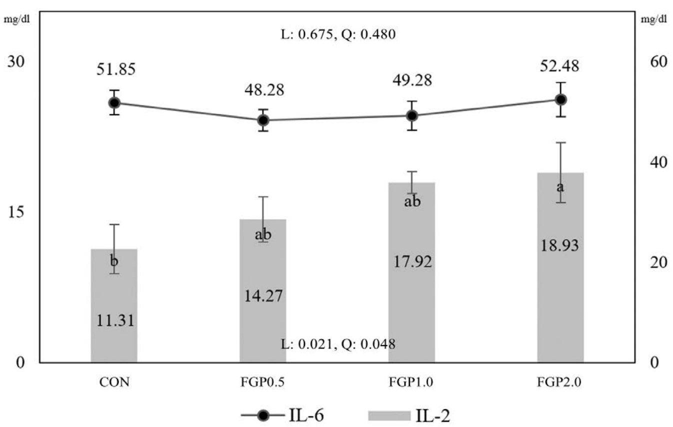

Figure 1: Effects of dietary FGP on serum IL-2 and IL-6 levels in layer breeder hens. [a,b] Values with different letters are significantly different at 5% level. CON: basal diet, FGP0.5: basal diet with fermented garlic powder 0.5 g/kg, FGP1.0: basal diet with fermented garlic powder 1.0 g/kg, FGP2.0: basal diet with fermented garlic powder 2.0 g/kg. IL-6: interleukin-6, IL-2: interleukin-2, L: linear effects, Q: quadratic effects.

Serum biochemical characteristics

Serum concentrations of ALB, TP, AST, HDLC, CHOL, and TG in hens fed experimental diets are shown in Table 3. Groups fed diets supplemented with FGP showed significantly ($P < 0.05$) increased ALB than the CON group. FGP inclusion in a diet quadratically increased ($P < 0.05$) serum TP levels. Serum AST levels were lower ($P < 0.05$) in FGP1.0 and FGP2.0 groups than in CON and FGP0.5 groups. In addition, AST levels were linearly and quadratically decreased ($P < 0.05$) with increasing FGP levels. However, the inclusion of FGP had no influence on HDLC level. Concentrations of CHOL and TG were significantly ($P < 0.05$) decreased in the FGP2.0 group than in the CON group. Concerning IL results (Figure 1), serum IL-2 levels were higher ($P < 0.05$) in the FGP2.0 group than in the CON group, although IL-6 levels showed no significant differences between groups.

DISCUSSION

Many scientists have investigated effects of long-term feeding of garlic and its' preparations on the performance of poultry. Our results are consistent with reports of Lim et al. (2018) and Hossain et al. (2016) showing that hens fed fermented garlic-supplemented diets (0.5 to 2.0 g/kg) show no difference in EP. Ao et al. (2010) have also confirmed similar levels of EP in laying hens fed a basal diet and high-dose FGP (10 to 30 g/kg)-supplemented diets.
Since breeders are used for the production of chicks, studies have been conducted to improve their hatchability by supplementing various additives (Aalaei et al., 2018; Darsi and Zhaghari, 2021). However, no studies concerning HAT of layer breeder fed a diet supplemented with FGP have been reported to the best of our knowledge. According to relevant reports (Mohan et al., 2007; Okoro et al., 2016), dietary supplementation of garlic to basal diet can increase sperm vitality and survival rate of cocks and egg fertilization of hens. Our findings were similar to those of a previous study (Hossain et al., 2016) that included 2.0 g/kg fermented garlic in the diet of laying hens, which significantly increased AH and HAU in subsequent tests of eggs. Hurnik et al. (1978) and Wolc et al. (2010) have reported a linear relationship of breeder's egg between HAU and HAT. Accordingly, the increase of HAT in the FGP-fed group might have been influenced by the HAU.

Garlic has been used to treat vascular diseases such as hyperlipidemia, arteriosclerosis, and hypertension of human (Yeh and Liu, 2001). Allicin in garlic can inhibit the synthesis of fat and cholesterol in the liver and decreased fatty acid liver level of laying hens (Mahmoud et al., 2010). Our results were consistent with those of Lim et al. (2018), who reported that serum concentrations of cholesterol and triglycerides of hens fed a diet supplemented with 0.2% fermented garlic were lower than those in hens of the control group fed a basal diet.

Several reports advocate strong immune potentiating effects of garlic. Major active ingredients in garlic are allicin, ajoene, diakyl polysulphides, s-allylcysteine, diallydisulphide, s-methyl-cysteine sulphoxide, and s-allylcysteine sulphoxide, which might be responsible for the therapeutic properties of garlic observed (Khan et al., 2012). Our results of IL-2 were consistent with earlier studies (Hossain et al., 2016; Lim et al., 2018) showing that dietary supplementation of 0.2% fermented garlic could increase serum IgG, leukocytes, IL-2 mRNA, and CD4+/CD8+ ratios of laying hens. Ried (2016) has reported that dietary garlic can regulate macrophage activity, lymphocyte proliferation, and antioxidant activity and increase immunity by releasing cytokines of T and B cells. ILs play an important role in intercellular communication among leukocytes (Akdis et al., 2016). IL-2 gives rise to an immune response that can lead to multiplication of activated C lymphocytes and B lymphocytes as well as antibody production (Yan et al., 2020). These results emphasize the fact that FGP has a global positive effect on serum biochemical parameters of layer breeders.

CONCLUSIONS

We confirmed that dietary supplementation of 2.0 g/kg FGP could be used as a feed additive for layer breeders to increase HAT and IL-2 and decrease AST, CHOL, and TG. Global results of this experiment highlight positive effects of FGP on productive performance and health of layer breeders.

CONFLICT OF INTEREST

The author has no competing interests relevant to this study to disclose.

NOVELTY STATEMENT

The study of dietary supplementtion of fermented garlic to improve productive performance and health of layer breeders is novel.

AUTHORS CONTRIBUTION

Lim, C.I. collected literature and drafted the manuscript. Kim, H.W. and You, A.S. conducted the experiment and provided the technical help. Heo, K.N., and Choo, H.J. reviewed the manuscript. All the authors read and approved the final manuscript.

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