Korean J. Food Sci. Ani. Resour.
Vol. 30, No. 4, pp. 609~616(2010)

ARTICLE

The Effects of Dietary Soybean Fermented with *Aspergillus oryzae* or *Bacillus natto* on Egg Production and Egg Lipid Composition in Layer

Heeok Hong[1], Oliver D. Abanto[2,3], Ki-Hyun Kim[3], Ki-Taeg Nam[3], Jong-Youn Son[4], Woo-Suk Jung[5], In-Sik Nam[3,6], and Seong-Gu Hwang[3]

[1]*Department of Food Service Management, Sangmyung University, Seoul 110-743, Korea*
[2]*Animal and Dairy Sciences Cluster, University of the Philippines Los Baños, College, Laguna 4031, Philippines*
[3]*Division of Animal Life and Environmental Science/GRRC, Hankyong National University, Anseong 456-749, Korea*
[4]*Department of Food and Biotechnology, Institute of Food Industry and Biotechnology, Hankyong National University, Anseong 456-749, Korea*
[5]*Department of Crop Science, Konkuk University, Seoul 143-701, Korea*
[6]*Korea Livestock Products HACCP Accreditation Service, Anyang 430-731, Korea*

Abstract

This study was conducted to determine the effects of dietary low grade soybean, fermented with *Aspergillus oryzae* (FSB 1) or *Bacillus subtilis* var. *natto* (FSB 2), on egg production and quality, fat and cholesterol content, and the fatty acid (FA) profile of eggs by lipid layer. A total of 18 Hi-Line strain layers, 22 wk of age, were randomly assigned to three dietary treatments: no fermented soybean (control), control with 15% FSB 1 (C + FSB 1), and control with 15% FSB 2 (C + FSB 2). The rate of egg production and egg weight were evaluated between two periods: one was from the 1st to 4th wk and the other was from the 5th to 8th wk. At the 8th wk, a total of 30 eggs were randomly selected from each treatment group and analyzed for physical quality, fat content, fatty acid composition and cholesterol content. The results showed that egg production was increased in hens fed with diets containing fermented soybeans from the 5th to 8th wk period ($p<0.01$). A similar tendency was observed through eight weeks' cumulative egg production ($p<0.05$). There were no significant differences in egg production between the C + FSB 1 and C + FSB 2 treatment groups ($p>0.05$). Egg weight and other physical properties did not vary between treatment groups ($p>0.05$). Egg yolks among different treatment groups were similar in fat content, but egg yolks in the C + FSB 1 and C + FSB 2 groups had lower oleic acid ($p<0.05$), higher linoleic, α-linolenic, and arachidonic acids ($p<0.01$), and lower cholesterol content ($p<0.05$) than those in the control group. In conclusion, supplementation of fermented low grade soybeans might be useful as a functional feedstuff to improve egg production and quality for a healthy human diet.

Key words: fermented soybean, egg production, fatty acid composition, egg cholesterol, laying hen

Introduction

In the process of cleaning and grading of soybeans for soy products, more than 10% is rejected and considered as non-edible for human. Rejected soybeans include undersize, wrinkled, cracked, and broken soybean as well as some hulls, which are considered waste or low economic value. Their utilization as animal feed for monogastric animals is limited by price, highly variable nutrient composition, and high fiber content.

*Corresponding author: Seong-Gu Hwang, Division of Animal Life and Environmental Science/GRRC, Hankyong National University, Anseong 456-749, Korea. Tel: 82-31-670-5121, Fax: 82-31-670-5127, E-mail: sghwang@hknu.ac.kr

The study by Zamora and Veum (1979) has revealed that fermented raw soybeans contained only slightly higher crude fat and crude protein, and significantly higher antitrypsin activity than heated unfermented soybeans. However, rats fed with fermented soybeans (FSB) had higher average daily gain (ADG) and feed efficiency (FE) than those fed with heated unfermented soybeans. It has been demonstrated that inclusion of FSB in diets of broiler chicken (Feng *et al*., 2007a; Mathivanan *et al*., 2006), Japanese quail (Chah *et al*., 1976), and neonatal pig (Zamora and Veum, 1988) improved ADG and FE. Recent studies (Jiang *et al*., 2007; Sahin *et al*., 2007) have shown that the antioxidant and estrogenic activity of isoflavones (ISF) improved animal performance after animals were given FSB. As antioxidants, ISF may also pro-

tect low density lipoprotein (LDL) from oxidation (Wei *et al*., 1995) and may exert its anti-atherogenic effects (Anthony *et al*., 1995). It was previously reported that soy protein intake decreased the serum concentration of total cholesterol, LDL cholesterol and triglycerides when compared with protein of animal origin (Potter, 1998). However, some recent findings did not corroborate to those earlier reports (Rios *et al*., 2008; Thorp *et al*., 2008).

The estrogenic effect of ISF genistein on reproductive tissues of ovariectomized gilts was clearly demonstrated by Ford *et al*. (2006). They found significant increase of wet weight, DNA, dry weight, and protein weight of uterus and cervix of ovariectomized gilts in the negative control (treated by 200 to 400 mg/d of genistein for 10 d). They also observed morphological changes in uterine and cervical tissues as well as cell proliferation as response to genistein treatment. These findings are consistent with observations in rats (Diel *et al*., 2001).

The ISF content of soybean is increased by fermentation. The increase in ISF content is affected by the type of microorganisms used in fermentation (Boue *et al*., 2000). A recent study has shown that fermentation of soybean and soybean meals (SBM) by *Aspergillus oryzae* GB-107 for 48 h eliminated trypsin inhibitor, reduced peptide size, and increased crude protein by as much as 10% without changing its essential amino acid profile (Hong *et al*., 2004). Dietary fermented SBM could also cause changes in digestive enzyme activities and intestinal morphology (Feng *et al*., 2007; Mathivanan *et al*., 2006) and reduction of phosphorus excretion in poultry (Hirabayashi *et al*., 1998).

Based on previous observations, the question emerged whether fermentation of low grade fermented soybean can utilize as functional feedstuff with both nutritional and physiological value, thus can be used as dietary source of layer chicken to improve egg production and quality, particularly the lipid composition of eggs. At present, there are several works demonstrating the effects of dietary fermented SBM on broiler chicken. However, there has been few investigation on the contribution of fermented soybean diets on egg production of layers. The objective of this study was to determine the effects of low grade soybean fermented by *Aspergillus oryzae* or *Bacillus subtilis* var. *natto* on egg production and quality, fat content, fatty acid composition and cholesterol content in laying hens. *A. oryzae* and *B. subtilis* var. *natto* (also known as *B. natto*) were used in this study because they are commonly used to produce FSB products for human consumption which are popular in China, Japan, and Korea such as fermented soy sauce, 'miso' (in case of *A. oryzae*), and 'natto' (in case *B. natto*).

Materials and Methods

Materials for fermented soybeans

Soybeans used in this study were the 'large original' strain of soybean grown in Korea, supplied by Rural Development Administration, consisting of low grade and rejected soybeans from food processing and considered inedible for human. *A. oryzae* (AO) and *B. natto* (BN) microbes used for fermentation of soybean were provided by the Institute of Fermented Food in Suwon, Korea.

Preparation of fermented soybeans

Soybeans were soaked in water for 8 h and then drained in a filtering screen or sieve. Soybeans were autoclaved for 1 h at 121°C and then cooled down to 50°C. AO and BN inoculums were prepared by culturing each microbe in nutrient broth at 35°C for 24 h. Cooled soybeans were inoculated with each microbe and cultured at 40°C for 24 h. The microbial count of each soybean fermented using either AO or BN were 7.5×10^6 and 1.8×10^8 CFU/g, respectively. To keep the antimicrobial activity of the microbes against enterobacteria, each sample was dried under blowing warm-air set at 60°C until their moisture content reached about 12%. After drying, fermented soybean samples were powdered prior to mixing with experimental diet.

Determination of chemical composition of soybeans

The moisture, crude fat, crude protein, crude fiber, and crude ash of unfermented and fermented soybeans were determined in triplicate according to AOAC procedures (AOAC, 1990). The crude protein was determined using the Kjeldahl method. Crude fat was analyzed through ether extraction using Soxhlet apparatus.

Experimental diet

The diets of each group were formulated including control (C), control with soybean fermented by AO (C + FSB 1), and control with soybean fermented by BN (C + FSB 2). Each experimental diet was prepared to satisfy the requirement of NRC feeding standard (NAS-NRC, 1984). The formula of each diet is shown in Table 1. Fermented soybeans were supplemented to each experimental diet at 15% inclusion rate substituting soybean meal and parts of

Table 1. Formula of each experimental diet (%) and the nutrient composition

Ingredients	Experimental diet[1]		
	Control (C)	C + FSB 1	C + FSB 2
Corn	60.0	60.5	60.0
Fermented soybean	0.0	15.0	15.0
Wheat bran	9.2	13.2	12.5
Soybean meal	15.0	0.0	1.3
Corn gluten meal	2.0	0.5	0.6
Tallow	3.5	0.5	0.3
Calcium bicarbonate	8.52	8.52	8.52
Dicalcium phosphate	1.0	1.0	1.0
Salt	0.3	0.3	0.3
L-Lysine 78	0.15	0.15	0.15
DL-Methionine 99	0.13	0.13	0.13
Choline 50	0.05	0.05	0.05
Premix[2]	0.15	0.15	0.15
Total	100.00	100.00	100.00
Nutrient composition			
Crude protein (%)	14.00	14.00	14.00
Crude fat (%)	6.29	6.23	6.03
Crude fiber (%)	3.35	3.54	3.64
Energy (Kcal/kg)	2,810	2,811	2,810

[1]FSB 1 = Soybean fermented with *Aspergillus oryzae*; FSB 2 = Soybean fermented with *Bacillus subtilis* var. *natto*.

[2]The Premix supplies per kilogram diet: Vitamin A, 18,000 I.U; Vitamin D_3, 4,500 I.U; Vitamin E, 22.5 I.U; Vitamin K_3, 3.0 mg; Vitamin B_1, 2.25 mg; Vitamin B_2, 6.0 mg; Vitamin B_6, 4.5 mg; Vitamin B_{12}, 22.5 mcg, Oxyzero, 9.0 mg; Ca-Pantothenic acid, 12 mg; Niacin, 30 mg; Folic acid, 0.75 mg ; Biotin 0.15 mg; Manganese (Mn), 97.5 mg; Zinc (Zn), 97.5 mg; Iron (Fe), 75 mg; Copper (Cu), 13.5 mg; Cobbalt (Co), 0.15 mg; Iodine (I), 1.5 mg; Selenium (Se), 0.225 mg.

corn gluten meal and adjusting other components to equal contents of carbohydrates, protein and energy.

Each experimental diet was analysed for the fatty acid (FA) content following the procedures described below (see separate topic). The FA contents of the diets were presented in Table 3.

Feeding trial

A total of 150 Hy-Line strain layers, 20 wk of age, were purchased from breeding farm. Layers were allowed to adapt into the experimental facilities for 14 d before the experiment starts. From there, 18 layers showing constant egg production were selected and randomly divided into three groups (Control, C + FSB 1, or C + FSB 2). Each layer served as replicate of the experiment. Layers were housed in individual cages lightened from 6:00 AM to 10:00 PM. Layers were given 120 g of their assigned diet every day and had free access to water. The feeding trial lasted for 8 wk. Percent egg production of an individual layer was examined at the 1st to 4th, and the 5th to 8th wk of production. Cumulative egg production for the entire feeding period (8 wk) was also computed. The average weight of eggs of individual layer for each production period was also measured.

Table 2. Proximate chemical composition (%) of low grade soybean, unfermented or fermented with *Aspergillus oryzae* or *Bacillus subtilis* var. *natto*

Composition (%)	Soybean[1]		
	Unfermented	FSB 1	FSB 2
Moisture	11.0	11.2	9.7
Crude fat	14.2	20.2	20.3
Crude protein	41.5	43.8	40.7
Crude ash	6.5	5.4	5.2
Crude fiber	8.2	5.7	6.3

[1]FSB 1 = Soybean fermented with *Aspergillus oryzae*; FSB 2 = Soybean fermented with *Bacillus subtilis* var. *natto*.

Table 3. Fatty acid composition of dietary treatments

Composition	Experimental diet[1]		
	Control (C)	C + FSB 1	C + FSB 2
Myristic (C14:0)	3.23	2.93	3.08
Palmitic (C16:0)	31.13	29.24	30.77
Palmitoleic (C16:1)	1.81	1.86	1.77
Stearic (C18:0)	13.33	12.94	13.25
Oleic (C18:1)	30.27	33.09	29.59
Linoleic (C18:2)	10.83	11.40	9.69
α-Linolenic (C18:3)	1.24	1.20	1.18
SFA	47.7	45.1	47.1
USFA	42.9	46.4	41.0
SFA/USFA	1.11	0.97	1.14

[1]FSB 1 = Soybean fermented with *Aspergillus oryzae*; FSB 2 = Soybean fermented with *Bacillus subtilis* var. *natto*.

Evaluation of egg quality

Two wk prior to the last day of the feeding trial, a total of 30 eggs from each treatment group, composed of 5 representative egg samples from each layer, were randomly taken for eggshell strength, eggshell thickness, yolk color, albumin height and Haugh units analyses. Eggshell thickness and strength were measured using a FHK device (Fujihara Co. Ltd., Saitama, Japan). The egg yolk color, albumen height and Haugh units were measured using a egg quality control micro-process (QCM device) (TTS, Technical Services and Supplies, York, UK).

Total fat and fatty acid analysis

The total fat content of egg yolk was analyzed using AOAC (1990) procedure. Lipids of feed and egg yolk

were extracted with hexane:isopropanol (3:2, vol/vol) for fatty acid analysis. Fatty acids (FA) were converted into methyl esters following procedures described by Kim *et al*. (2007). Samples were analyzed for FA using a gas chromatograph (Model M600D, Younglin Co., Seoul, Korea). The FA methyl esters were separated using HP-INNO Wax column (crosslinked polyethylene glycol; 30 m×0.25 mm i.d.×0.25 μm film thickness; Hewlett-Packard, Wilmington, DE), with helium flow rate of 1 mL/min. The initial oven temperature was 50°C for 3 min and then programmed to increase by 10°C/min until final temperature of 250°C. The injector and detector temperatures were 220 and 275°C, respectively. One microliter sample was injected into the column in the split mode ratio of 1:100. The peak of each FA ($C_{14:0}$, $C_{16:0}$, $C_{18:0}$, $C_{18:1}$, $C_{18:2}$, $C_{18:3}$, and $C_{20:4}$) was identified and quantified by comparison with the retention time and peak area of each FA standard (Sigma Chemical Co., St. Louis, MO, USA).

Cholesterol analysis

The egg yolk methyl extract was prepared following the procedure described below. Briefly, 1 g of yolk sample was mixed with 20 mL 1 M KOH-MeOH and 10 mL isopropanol in a 250-mL condenser flask containing 1 g sea sand or boiling stone. The sample was heated in a reflux condenser and allowed to boil for 30 min. Then the sample was cooled inside a glass desiccator. The sample solution was pipette-out and transferred to a 50-mL screw-cap tube. The residue was washed with 6 mL isopropanol, boiled for another 5 min in the reflux condenser, allowed to cool inside the desiccator, and then added to the first methyl extract. The volume of methyl extract solution was adjusted to 25 mL with isopropanol, vortex-mixed, and then filtered using Whatman No. 1. (Cat. No. 1001 110, Whatman International Ltd., Maidstone, UK). Finally, the cholesterol content of egg yolk methyl extract was analyzed using procedures described in the cholesterol test kit (Cat. No. 10 139 050 035, r-biopharm, Darmstadt, Germany).

Statistical analysis

The statistical differences between treatment groups were determined using one-way ANOVA in completely randomized design wherein the measurements were the dependent variables and the dietary treatments as the independent variables. Means were compared using Tukey test and $p<0.05$ were considered significant. All statistical analyses were performed using SPSS software version 11.5 (SPSS, Inc., Chicago, IL, USA).

Results

Chemical composition of fermented soybean

The chemical compositions of fermented and unfermented low grade soybeans are shown in Table 2. Fermented soybeans had generally higher crude fat (20.2% vs. 14.2%), lower ash (5.2 to 5.4% vs. 5.5%) and lower crude fiber (5.7 to 6.3% vs. 8.2%) compared with unfermented soybeans. About 42% increase in crude fat were observed as results of fermentation of soybeans. Soybean fermented with AO (FSB 1) tended to have slightly higher crude protein and lower crude fiber than unfermented soybeans (USB) and soybeans fermented with BN (FSB 2).

Fatty acid composition of the diet

In order to determine whether the substitution of soybean meal with fermented soybean affected the FA composition of the diet, a FA analysis of the three dietary rations was conducted. The result showed that the FA composition of C + FSB 1 diet was slightly different from the other dietary treatments. The C + FSB 1 had higher proportion of USFA and lower SFA than the other two treatment diets (Table 3). The control diet had similar FA composition with the C+FSB 2.

Egg production and egg quality

There were no significant differences observed in egg production between treatment groups from the 1st to 4th wk. The effect of fermented soybeans was only observed during the 5th to 8th wk (Table 4). During that period, layers fed FSB 2 diet, had significantly higher egg production ($p<0.01$) than those fed with control diets. Layers fed with C + FSB 2 diet had the highest egg production (95.2%), but not significantly different ($p>0.05$) from those fed with C + FSB 1 diet (91.7%). Overall (8 wk) cumulative egg production of both FSB diet groups was significantly higher egg production ($p<0.05$) than that of the control diet group. Egg production of layers fed with C + FSB 2 diet was similar to those fed with C + FSB 1 diet, with egg production of 94.6% and 93.4%, respectively. Egg production of layers fed with control diet was only 89.5%. The dietary treatments did not affect egg size and other egg physical quality parameters (eggshell strength, eggshell thickness, yolk color, albumen height and Haugh units). The physical quality attributes of eggs produced in this present study are presented in Table 5.

Table 4. Effects of dietary fermented soybean on egg production and egg weight in layer

Parameters	n	Dietary group[1]			SEM	*p* value
		Control (C)	C + FSB 1	C + FSB 2		
Egg production (%)						
1-4th wk	6	94.1	93.4	94.1	1.16	0.945
5-8th wk	6	85.1[b]	91.7[ab]	95.3[a]	1.44	0.006
Overall	6	89.6[b]	92.6[ab]	94.7[a]	0.81	0.032
Egg weight (g)						
1-4th wk	6	60.5	63.2	61.9	1.16	0.672
5-8th wk	6	60.8	59.3	59.8	0.89	0.805
Overall	6	60.6	60.7	60.9	0.86	0.994

[1]FSB 1 = Soybean fermented with *Aspergillus oryzae*; FSB 2 = Soybean fermented with *Bacillus subtilis* var. *natto*.
*Means in the same row with different superscript are significantly different (p<0.05).

Table 5. Physical quality attributes of eggs produced by layers fed diet with or without fermented soybean

Parameters	n	Dietary group[1]			SEM	*p* value
		Control (C)	C + FSB 1	C + FSB 2		
Shell strength (Mpa)	6	0.346	0.332	0.364	0.011	0.523
Shell thickness (mm)	6	0.344	0.331	0.350	0.038	0.088
Yolk color	6	6.8	7.0	7.0	0.07	0.519
Albumen height (mm)	6	6.91	6.50	7.05	0.30	0.760
Haugh units	6	81.3	79.4	82.3	1.99	0.855

[1]FSB 1 = Soybean fermented with *Aspergillus oryzae*; FSB 2 = Soybean fermented with *Bacillus subtilis* var. *natto*.

Fat content, fatty acid profile and cholesterol of egg yolks

The total fat content of egg yolk was not affected by the dietary treatments. The fat content of egg yolks obtained in this study ranged from 30.1 to 31.2%. However, significant differences between the control and FSB diet groups were observed in fatty acid composition particularly oleic ($C_{18:1}$), linoleic ($C_{18:2}$) and α-linolenic ($C_{18:3}$) acid. Egg yolks from the C + FSB 1 and C + FSB 2 groups had significantly lower (p<0.05) oleic acid (41.5 and 41.8%, respectively) than that from control group (45.9%).

The amount of polyunsaturated fatty acid (PUFA) in eggs, particularly the linoleic and α-linolenic, was greatly increased by diets containing fermented soybeans. Linoleic acid in the egg yolk from C + FSB 1 and C + FSB 2 groups (16.0 and 16.5%, respectively) was significantly higher (p<0.01) than from the control group (12.0%). There was 0.8 to 0.9% α-linolenic acid detected from eggs produced by layers fed with fermented soybean, while α-linolenic acid was not detected from eggs produced by the control group. Eggs from different treatment groups had similar contents of palmitic ($C_{16:0}$) and arachidonic ($C_{20:4}$) acids. However, egg yolk from the control group has a slightly lower arachidonic acid concentration than that from C + FSB 2 group (p<0.01). There were no significant differences in FA profiles of egg yolk between C + FSB 1 and C + FSB 2 groups.

Both FSB diet groups showed reduction in cholesterol content of egg yolk. Egg yolk of the C + FSB 1 and C + FSB 2 groups, had significantly lower (p<0.05) cholesterol content (1,411 and 1,387 mg/100g) than that of the control group (1,614 mg/100g). The cholesterol content

Table 6. Fat content and fatty-acid composition of egg yolk from eggs produced by layers fed diets with or without fermented soybean

Parameters	n	Dietary group[2)]		
		Control (C)	C + FSB 1	C + FSB 2
Total fat content (%)	6	31.2±1.1[1)]	31.2±1.2	30.1±1.3
Fatty acids (%)[3)]				
Palmitic (C16:0)	6	25.6±0.9	25.5±0.9	25.2±0.8
Stearic (C18:0)	6	9.3±0.5	10.1±0.5	9.7±0.6
Oleic (C18:1)*	6	45.9±1.7[a]	41.5±1.2[b]	41.8±1.2[b]
Linoleic (C18:2)**	6	12.0±0.3[b]	16.5±0.5[a]	16.0±0.6[a]
α-Linolenic (C18:3)	6	-	0.8±0.04	0.9±0.04
Arachidonic (C20:4)**	6	1.5±0.05[b]	1.6±0.10[ab]	1.8±0.08[a]

[1)]Data are presented as mean ± SE.
[2)]FSB 1 = Soybean fermented with *Aspergillus oryzae*; FSB 2 = Soybean fermented with *Bacillus subtilis* var. *natto*.
[3)]Values are expressed as percentage of the total fat.
*Means in the same row with different superscript are significantly different (p<0.05).
**Means in the same row with different superscript are significantly different (p<0.01).

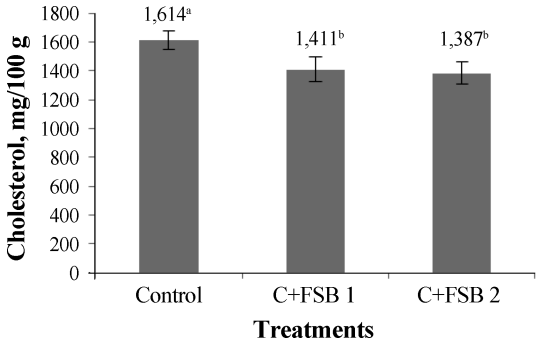

Fig. 1. Effect of dietary fermented soybean on cholesterol content of egg yolk (n = 6). Layers were fed with either control (0% fermented soybean), C + FSB 1 (control + 15% soybean fermented with *A. oryzae*), or C + FSB 2 (control + 15% soybean fermented with *B. subtilis* var. *natto*). Values are presented as mean; the vertical line represents standard error for individual observation. Means with different superscript are significantly different ($p<0.05$).

of egg yolk was not affected by the type of microorganisms used in fermentation of soybean in the present study.

Discussion

The effects of fermentation on the chemical composition obtained in the present study were consistent with the previous observations. The studies of Zamora and Veum (1979) and Feng *et al*. (2007b) have shown an increase in crude protein and crude fat of FSB more than of the heated unfermented soybean. Hong *et al*. (2004) have also reported 10% increase in crude protein of soybean meal as a result of fermentation. In the present study, crude protein of soybean fermented with AO was increased, while crude fiber of fermented soybeans particularly by AO was decreased. These results were consistent with the findings of Zamora and Veum (1979) when the same microorganism was used. The effect on fiber content was reversed when *Bacillus sphaericus* (Jeff-Agboola and Oguntuase, 2006) and *Rhizopus oligosporus* (Zamora and Veum, 1979) were used in the fermentation process. It is widely known that the effects of fermentation on the chemical composition vary with the type of microorganism and other fermentation conditions used. The *B. subtilis* var. *natto* used in *natto* fermentation usually causes substantial increase in amino acids and soluble carbohydrates, whereas the *Rhizopus* spp. used in the fermentation of various types of *tempe* are highly hydrolytic and increase soluble fats, protein and carbohydrates (PABTFF-NRC, 1992). Jeff-Agboola and Oguntuase (2006) suggested that the increase in protein in fermented soybean can be attributed to the protein synthesis and proliferation of the microorganism, but the increase in fat could be possibly due to transformation of carbohydrates to fats by the microorganisms. The degradation of oligosaccharides to more soluble carbohydrates by microorganisms (Wood, 1998) could be the cause of the substantial decrease in crude fiber.

The dietary treatments given to the layers were formulated to be isocaloric. The perceived increase in egg production in layers fed diet containing FSB can therefore be attributed to the improvement in the functional components of soybean as a result of fermentation. The analysis of isoflavone content of the soybeans used in this study was conducted and reported by Chung *et al*. (2008). They found that fermentation of soybean slightly decreased the glycoside daidzein content by about 4 to 6%, but significantly increased genistin by 69 to 76%. Fermentation also tremendously increased the aglycone forms of isoflavonoids, the daidzein (20 to 25 fold) and genistein (3 to 4 fold). As a result, total isoflavone content of fermented soybeans was raised by 60 up to 70%. Furthermore, this finding was consistent with previous reports done by Mimura and Yasaki (1998). The type of microorganism did not affect the amount of daidzein in FSB. However, our study showed that FSB 2 had slightly lower genistin, higher daidzein, genistein and total isoflavone content than those of FSB. Isoflavonoids, such as genistein, are widely known as phytoestrogen eliciting various estrogenic effects in animals (Woclawek-Potocka *et al*., 2005; Ford *et al*., 2006). Even though the present study did not analyze the concentration of isoflavones in the blood, it might be possible that the positive increase in egg production of layers fed with fermented soybeans was due to the estrogenic effect of isoflavones. It is thought that an increase in the concentration of estrogen in circulating blood can trigger secretion of luteinizing hormone (LH) and, consequently, ovulation (Micevych and Sinchak, 2008).

Using broilers, Jiang *et al*. (2007) demonstrated that antioxidant activity of ISF may contribute to the improvement in layer performance. In addition, Sahin *et al*. (2007) reported that dietary ISF improved feed intake, egg production and egg quality in heat stressed quail, but the effects were not observed for those raised under thermo-neutral environment. Hence, those observations may be attributed to antioxidative effects rather than estrogenic effects of ISF.

There were no significant differences observed in the size of egg produced by layers under different dietary

treatments. This indicated that the nutritional status of the layers were sufficiently enough to support the requirements for high egg production. The eggs from different treatment groups were also similar in shell strength, shell thickness, yolk color, albumen height and Haugh units.

The amount of total fat in egg yolk was not affected by the diet in the present study. However, the PUFA of egg yolk in FSB diet groups was increased with a corresponding decrease in oleic acid content as compared to that of control. Although it is widely known that the FA profile of egg is highly affected by the lipid profile of the diet (Kim *et al*. 2007; Shang *et al*., 2005), changes in the FA composition of egg yolk in the present study cannot be attributed to such effect because only the FA profiles of the C + FSB 1 were different from the control and not that of C + FSB 2. The control and C + FSB 2 diets were similar in FA composition. Compared to the control group, both FSB diet groups showed higher PUFA contents of egg yolk. Changes in the FA composition of egg yolk, as a result of diet, can be caused either by an increase in the antioxidant activity among treated hens due to isoflavones or by intervention of the microorganisms in the gut as affected by FSB. Since the antioxidant activity of isoflavone, which is high in FSB, prevents oxidation of lipids in the blood (Wei *et al*., 1995), Meanwhile, recent reports have also shown that some probiotics affect serum and egg yolk lipid profiles (Salma *et al*., 2007; Xu *et al*., 2006). In the present study, the FSB contains live AO and BN organisms.

There was also significant decrease in egg cholesterol as a result of dietary fermented soybean. It is thought that fermented soybeans could increase antioxidative status in layers. Jackson *et al*. (2002) demonstrated that, as antioxidants, isoflavones protect blood lipids from oxidation as well as reduce serum cholesterol level. Even though the serum cholesterol was not analyzed in our study, it can be speculated that FSB supplementation in layer diet lowered serum cholesterol, thus leading to a decrease in the egg cholesterol level. The presence of live microorganisms in fermented soybeans may favorably changed lipid profiles of egg yolk. Xu *et al*. (2006) reported that supplementation of dried *B. subtilis* culture in layer decreased egg yolk cholesterol. Similar trends were observed with inclusion of *Lactobacillus acidophilus* (Abdulrahin *et al*., 1996) and *Rhodobacter capsulatus* (Salma *et al*., 2007) in layer diets. Our study showed similar cholesterol value to others (Chowdhury *et al*., 2002; Kim *et al*., 2007).

Therefore, our findings suggest that fermentation of low grade soybean can turn it into a functional feed source for layers, which improve not only egg production of layer but also egg quality to provide a healthy diet for human.

References

1. Abdulrahim, S. M., Haddadin, S. Y., Hashlamoun, E. A., and Robinson, R. K. (1996) The influence of *Lactobacillus acidophilus* and bacitracin on layer performance of chicken and cholesterol content of plasma and egg yolk. *Br. Poult. Sci*. **37**, 341-346.
2. Anthony, M., Clarkson, T., Weddle, D., and Wolfe. M. (1995) Effect of soy protein and phytoestrogens on cardiovascular risk factors in rhesus monkey. *J. Nutr*. **125**, 803S-804S.
3. AOAC (1990) Official Methods of Analysis. 15th ed., Association of Official Analytical Chemists, Washington, DC.
4. Boue, S. M., Carter, C. H., Ehrlich, K. C., and Cleveland, T. E. (2000) Induction of the soybean phytoalexins coumestrol and glyceollin by *Aspergillus*. *J. Agric. Food Chem*. **48**, 2167-2172.
5. Carroll, K. (1991) Review of clinical studies on cholesterol-lowering response to soy protein. *J. Am. Diet Assoc*. **91**, 820-827.
6. Chah, C. C., Nelson., R. A. Carlson, C. W., Semeniuk, G., Palmer, I. S., and Hesseltine, C. W. (1976) Fungus-fermented soybeans benefit the life cycle of Japanese quail (*Coturnix coturnix japonica*). *Poult. Sci*. **55**, 975-981.
7. Chowdhury, S. R., Chowdhurry, S. D., and Smith, T. K. (2002) Effects of dietary garlic on cholesterol metabolism in laying hens. *Poult. Sci*. **81**, 1856-1862.
8. Chung, W.-Y., Kim, S.-K., and Son, J.-Y. (2008) Isoflavones contents and physiological activities of soybeans fermented with *Aspergillus oryzae* or *Bacillus natto*. *J. Korean Soc. Food Sci. Nutr*. **37**, 141-147.
9. Diel, P., Smolnikar, K., Schultz, T., Laudenbach-Leschowsky, U., Michna, H., Bolt, H. M., and Vollmer, G. (2001) Phytoestrogens and carcinogenesis – Differential effects of genistein in experimental models of normal and malignant rat endometrium. *Hum. Reprod*. **16**, 997-1006.
10. Fasina, Y. O., Garlich, J. D., Classen, H. L., Ferket, P. R., Havenstein, G. B., Grimes, J. L., Qureshi, M. A., and Christensen, V. L. (2004) Response of turkey poults to soybean lectin levels typically encountered in commercial diets. 1. Effect on growth and nutrient digestibility. *Poult. Sci*. **83**, 1559-1571.
11. Feng, J., Liu, X., Xu, Z. R., Liu, Y. Y., and Lu, Y. P. (2007a) Effects of *Aspergillus oryzae* 3.042 fermented soybean meal on growth performance and plasma biochemical parameters in broilers. *Anim. Feed Sci. Technol*. **134**, 235-242.
12. Feng, J., Liu, X., Xu, Z. R., Lu, Y. P. and Liu, Y. Y. (2007b) Effects of *Aspergillus oryzae* fermented soybean meal on growth performance, digestibility of dietary components and activities of intestinal enzymes in weaned piglets. *Anim. Feed Sci. Technol*. **134**, 295-303.
13. Feng, J., Liu, X., Xu, Z. R., Wang, Y. Z., and Liu, J. X. (2007c) Effects of fermented soybean meal on digestive enzyme activities and intestinal morphology in broilers.

Poult. Sci. **86**, 1149-1154.
14. Ford, J. A. Jr., Clark, S. G., Walters, E. M., Wheeler, M. B., and Hurley, W. L. (2006) Estrogenic effects of genistein on reproductive tissues of ovareictomized gilts. *J. Anim. Sci*. **84**, 834-842.
15. Hirabayashi, M., Matsui, T., Yano, H., and Nakajima, T. (1998) Fermentation of soybean meal with *Aspergillus usamii* reduces phosphorus excretion in chicks. *Poult Sci*. **77**, 552-556.
16. Hong, K. J., Lee, C. H., and Kim, S. W. (2004) *Aspergillus oryzae* GB-107 fermentation improves nutritional quality of food soybeans and feed soybean meals. *J. Med. Food*. **7**, 430-435.
17. Jackson, C.-J. C., Dini, J. P., Lavandier, C., Rupasinghe, H. P. V., Faulkner, H., Poysa, V., Buzzell, D. and DeGrandis, S. (2002) Effects of processing on the content and composition of isoflavones during manufacturing of soy beverage and tofu. *Process Biochem*. **37**, 1117-1123.
18. Jeff-Agboola, Y. A. and Oguntuase, O. S. (2006) Effects of *Bacillus sphaericus* on proximate composition of soybean (*Glycine max*) for the production of Soy Iru. *Pakistan J. Nutri*. **5**, 606-607.
19. Jiang, R., Chang, X., Stoll, B., Ellis, K. J., Shypallo, R. J., Weaver, E., Campbell, J., and Burrin, D. G. (2000) Dietary plasma proteins used more efficiently than extruded soy protein for lean tissue growth in early-weaned pigs. *J. Nutr*. **130**, 2016-2019.
20. Jiang, Y. Z., Jiang, S. Q., Lin, Y. C., Xi, P. B., Yu, D. Q. and Wu, T. X. (2007) Effects of soybean isoflavone on growth performance, meat quality, and antioxidation in male broilers. *Poult. Sci*. **86**, 1356-1362.
21. Kim, J. H., Hwangbo, J., Choi, N.-J., Park, H. G., Yoon, D.-H., Park, E.-W., Lee, S.-H., Park, B.-K., and Kim, Y. J. (2007) Effect of dietary supplementation with conjugated linoleic acid, with oleic, linoleic, or linolenic acid, on egg quality characteristics and fat accumulation in the egg yolk. *Poult. Sci*. **86**, 1180-1186.
22. Li, D. F., Nelssen, J. L., Reddy, P. G., Blecha, F., J. Hancok, D., Allee, G., Goodb, R. D., and Klemm, R. D. (1990) Transient hypersensitivity to soybean meal in the early weaned pig. *J. Anim. Sci*. **68**, 1790-1799.
23. Liu, J., Chang, S. K. C., and Wiesenborn, D. (2005) Antioxidant properties of soybean isoflavone extract and tofu *in vitro* and *in vivo*. *J. Agric. Food Chem*. **53**, 2333-2340.
24. Mathivanan, R., Selvaraj, P., and Nanjappan, K. (2006) Feeding of fermented soybean meal on broiler performance. *Int. J. Poult. Sci*. **5**, 868-872.
25. Micevych, P. and Sinchak, K. (2008) Estradiol regulation of progesterone synthesis in the brain. *Mol. Cell Endocrinol*. **290**, 44-50.
26. Mimura, A. and Yasaki, S. (1998) Microbial transformation of soybean germ components to antioxidative isoflavonoids. *Soy Protein Res*. **1**, 46-51.
27. NAS-NRC (National Academy of Science, National Research Council). (1984) Nutrient Requirements of Poultry. Washington, DC.
28. PABTFF-NRC (Panel on the Application of Biotechnology to Traditional Fermented Foods, National Research Council). (1992) Application of Biotechnology in Traditional Fermented Foods. The National Academies Press, Washington, DC.
29. Potter, S. (1998) Soy protein and cardiovascular disease: the impact of bioactive components in soy. *Nutr. Rev*. **56**, 231-235.
30. Rios, D. R. A., Rodrigues, E. T., Cardoso, A. P. Z., Montes, M. B. A., Franceschini, S. A., and Toloi, M R. T. (2008) Lack of effects of isoflavones on the lipid profile of Brazilian postmenopausal women. *Nutr*. **24**, 1153-1158.
31. Ruiz-Larrea, M. B., Mohan, A. R., Paganga, G., Miller, N. J., Bolwell, G. P., and Riceevans, C. A. (1997) Antioxidant activity of phytoestrogenic isoflavones. *Free Radic. Res*. **26**, 63-70.
32. Sahin, N., Onderci, M., Balci, T. A., Cikim, G., Sahin, K., and Kucuk, O. (2007) The effect of soy isoflavones on egg quality and bone mineralization during the late laying period of quail. *Br. Poult. Sci*. **48**, 363-369.
33. Salma, U., Miah, A. G., Tareq, K. M. A., Maki, T., and Tsujii, H. (2007) Effect of dietary *Rhodobacter capsulatus* on egg-yolk cholesterol and laying hen performance. *Poult. Sci*. **86**, 714-719.
34. Thorp, A. A., Howe, P. R., Mori, T. A., Coates, A. M., Buckley, J. D., Hodgson, J., Mansour, J., and Mever, B. J. (2008) Soy food composition does not lower LDL cholesterol in either equol or nonequol producers. *Am. J. Clin. Nutr*. **88**, 298-304.
35. Wei, Q.-K., Chen, T.-R., and Chen, J.-T. (2008) Use of *Bacillus subtilis* to enrich isoflavone aglycones in fermented natto. *J. Sci. Food Agric*. **88**, 1007-1011.
36. Wei, H., Rowen, R., Cai, Q., Barnes, S., and Wang, Y. (1995) Antioxidant and antiproliferative effect of soybean isoflavon genistein. *Proc. Soc. Exp. Biol. Med*. **208**, 124-130.
37. Woclawek-Potocka, I., Bah, M. M., Korzekwa, A., Pistula, M. K., Wiczkowski, W., Depta, A., and Skarzynski, D. J. (2005) Soybean-derived phytoestrogens regulate prostaglandin secretion in endometrium during cattle estrous cycle and early pregnancy. *Exp. Biol. Med*. **230**, 189-199.
38. Wood, B. J. B. (1998) Microbiology of Fermented Foods. Vol. 2. 2nd ed. Springer, USA.
39. Xu, C.-L., Ji, C., Ma, Q., Hao, K., Jin, Z.-Y., and Li, K. (2006) Effects of a dried *Bacillus subtilis* culture on egg quality. *Poult. Sci*. **85**, 364-368.
40. Yousef, M. I., Kamel, K. I., Esmail, A. M., and Baghdadi, H. H. (2004) Antioxidant activities and lipid lowering effects of isoflavon in male rabbits. *Food Chem. Toxicol*. **42,** 1497-1503.
41. Zamora, R. G. and Veum, T. L. (1988) Nutritive value of whole soybean fermented with *Asperdillus oryzae* and *Rhizopus oligosporus* as evaluated by neonatal pigs. *J. Nutr*. **118**, 438-444.
42. Zamora, R. G. and Veum, T. L. (1979) The nutritive value of dehulled soybeans fermented with *Aspergillus oryzae* or *Rhizopus oligosporus* as evaluated by rats. *J. Nutr*. **109**, 1333-1339.

(Received 2010.2.5/Revised 2010.5.26/Accepted 2010.7.14)