

The Improvement of Laying Productivity and Egg Quality according to Providing Germinated and Fermented Soybean for a Feed Additive

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Abstract

This study was performed to investigate the effects of laying productivity and egg quality according to providing germinated and fermented soybean (GFS) as feed additive. Among the strain, we selected *Monascus purpureus* KCCM 12002 so that inoculated in soybean and fermented for 48 h at 20°C. A total of two-hundred forty 70-wk-old Hy-Line Brown layers were divided into four groups (4 treatment×6 replication×10 birds each) and fed diets containing 0 (as control) (T1), 0.5% (T2), 1.0% (T3) or 2.0% GFS (T4) for 6 wk. The laying productivity, egg quality and blood property in the egg yolk were experimented. There were no significant differences in the laying productivity, relative liver and spleen weights, egg yolk color and eggshell strength among another groups. The eggshell color, eggshell thickness and haugh unit significantly increased in the GFS-supplemented group ($p<0.05$) compared to control. However, no significant differences were observed in the blood property after supplementation. The amount of lactic acid bacteria present during storage increased by providing of GFS ($p<0.05$) compare to control group. Our study results suggested that GFS can be used as a favorable feed additive and feedstuff for the productivity of high quality eggs and promoted relative industry.

Key words: soybean, fermentation, germination, egg quality, *Monascus*

Introduction

Soybean foods have generated a lot of interest recently based on findings that consuming large amounts of soybeans results in a lower risk of osteoporosis, cancer, cardiovascular diseases and heart disease. In addition, isoflavones can suppress the onset of arteriosclerosis because it improves the metabolism of lipids, such as cholesterol (Crouse *et al.*, 1999).

The isoflavones found in soybeans, such as daidzein, genistein, daidzin and genistin, are believed to possess antioxidant and anticarcinogenic activities and inhibit melanoma cell growth (Barnes, 1995; Fritz *et al.*, 2003; Lee *et al.*, 2005; Liu *et al.*, 2005b; Russo *et al.*, 2006).

Since early times, East Asia area nations including China, Japan and Republic of Indonesia have used *Monascus purpureus* as a natural matter in liquor, bean curd, fish product stains, and embalmment of fish. *Monascus pur-*

pureus has been used as a traditional fermented food and its metabolic products have also been used as red pigments, γ -aminobutyric acid, and monacolin K (Bianchi, 2005; Dieter *et al.*, Juzlova *et al.*, 1996; 2002; Liu *et al.*, 2005). Many studies have shown that *Monascus purpureus* Went rice contained HMG-CoA reductase inhibitors, large quantities of unsaturated fatty acids, beta-sitosterol, campesterol and stigmaterol (Heber *et al.*, 1999). Monacolin K, commercially known as lovastatin, mevinnolin, cholestin, and mevacor, is one of the secondary metabolites from the *Monascus* species. In the former case, monacolin K is therapeutically and preventatively effective in the treatment of major kind of diseases like atherosclerosis, sepsis, peripheral arterial disease, peripheral vascular disease, cerebrovascular disease, ischemic disease, and bone fracture.

Germination processes have been developed to overcome the disadvantages of soybean seed used in food products (Zhu *et al.*, 2005). Soybean is a complex matrix of several bioactive compounds, including peptides and proteins, isoflavones, saponins, and other compounds with cancer-preventive properties. For example, lunasin is a novel and promising chemopreventive peptide from soy-

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bean (Jeong *et al.*, 2003).

However, only limited information is available on the effect of germinated and fermented soybean consumption in poultry. Therefore, the objectives of this study were to evaluate egg production and egg quality in laying hens fed diets containing germinated and fermented soybean meal.

Materials and Methods

Preparation of soybeans

Soybeans (*Glycine max* (L.) Merrill), which are commonly grown in Korea, were purchased in a local market in Korea. In this study, soybeans, they were washed three times and soaked in distilled water at ambient temperature for 10 h. The soaked soybeans were drained and germinated in an incubator at 20°C for about 24 h and then cooked in an autoclave at 121°C for 20 min. The enriched *Monascus purpureus* KCCM 12002 was inoculated in cooled soybean and fermented in an incubator at 20°C for 48 h. After drying at 80°C for 24 h, germinated and fermented soybean were stored at 4°C until used.

Feeding trial

A total of two-hundred forty 70-wk-old Hy-Line Brown layers were divided into four groups and fed diets containing 0 (as control) (T1), 0.5 (T2), 1.0 (T3) or 2.0% (T4) GFS (germinated and fermented soybean) for 6 wk. The layers were randomly placed in six replicates with 10 birds each per group in a wire cage. The experimental diets were formulated to meet or exceed the nutrient requirements of NRC as shown in Table 1 (NRC, 1994). Graded levels of GFS were substituted at the expense of the control diet concentrations of 0.5, 1.0 or 2.0% on a weight basis. The experimental diets and water were provided for *ad libitum* intake. A room temperature of 18±3°C and a photoperiod of 16/8 h light/dark cycle were maintained throughout the experimental period.

All animal care procedures were approved by Institutional Animal Care and Use Committee in Konkuk University. Fresh experimental diets were provided everyday and the feed intake of each group was recorded weekly. At the end of the experimental period, ten birds from each group were selected and weighed individually.

Egg Production

Egg production was recorded daily in replicate (number of eggs/number of live birds × 100) and the mean egg weight was determined by the daily average weight of eggs,

Table 1. Formula and chemical composition of the experimental diet

Ingredients	Starter (%)
Yellow corn	59.55
Soybean meal	17.76
Wheat bran	3.00
Lupine	2.00
DDGS	3.00
Rapeseed meal	2.00
Tallow	1.68
Vit. mixture ¹⁾	0.10
Min. mixture ²⁾	0.10
MDCP	1.20
DL-methionine (98%)	0.13
Limestone	9.13
Choline chloride (50%)	0.05
Salt	0.10
NaHCO ₃	0.20
Total	100.00
Calculated values	
MEn, kcal/kg	2713.00
Crude protein, %	15.50
Crude fat, %	4.39
Ca, %	3.70
Available P, %	0.35
Lysine, %	0.75
Met + Cys, %	0.65

¹⁾Vitamin mixture provided the following nutrients per kg: vitamin A, 40,000,000 IU; vitamin D₃, 8,000,000 IU; vitamin E, 10,000 IU; vitamin K₃, 4,000 mg; vitamin B₁, 4,000 mg; vitamin B₂, 12,000 mg; vitamin B₆, 6,000 mg; vitamin B₁₂, 20,000 µg; pantothenic acid, 20,000 mg; folic acid, 2,000 mg; nicotinic acid, 60,000 mg

²⁾Mineral mixture provided the following nutrients per kg: Fe, 30,000 mg; Zn, 25,000 mg; Mn, 20,000 mg; Co, 150 mg; Cu, 5,000 mg; Ca, 250 mg; Se, 100 mg

excluding abnormal eggs (soft-shell plus broken eggs).

Egg and eggshell qualities

Eggshell strength, eggshell thickness, and eggshell color were measured for 30 eggs collected randomly from 6 replicates of each treatment biweekly. The eggs were weighed individually and then exposed to a breaking force using an eggshell strength tester (FHK, Fugihira, Ltd., Japan). The eggshell strength was measured as the maximum force (N) required to fracture each egg. On breaking, the egg contents were poured into a glass plate to measure the albumen height. Haugh unit values, along with albumen height and egg weight, were determined using a QCM⁺ Tester (QCM⁺, Technical Services and Supplies Ltd., England). Eggshell thickness was measured with a digimatic thickness micrometer gauge (Digimatic

micrometer, Series 293-330, Mitutoyo, Japan) on a piece of shell obtained from the equatorial region. Egg yolk color was measured by comparing with Roche egg yolk color fan (Yolk color fan, Roche, Switzerland). Eggshell color was also measured using a QCM⁺ Tester.

Analysis of blood property

At the end of experimental period, eight birds from each group were randomly selected weighed individually. The blood was drawn from wing vein using sterilized syringes for determination of the various blood profiles. At necropsy, the liver and spleen were immediately removed and weighed. The concentration of total cholesterol and high density lipoprotein-cholesterol (HDL-C), the activity of glutamic-oxaloacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) were estimated according to the colorimetric method using cholesterol diagnostic kit (Cholesterol E kit, HDL-cholesterol kit, Youngdong Medical Corporation, Korea) and GOT-GPT assay kit (BCS GOT-GPT assay kit, Bio Clinical System Corporation, Korea), following the manufacturer's direction.

Total bacterial count in Cecal

The total bacterial count, *Lactobacillus* spp. and *coliforms* were determined on nutrient agar, MRS agar, and MacConkey agar, respectively, using the traditional method (Tuohy *et al.*, 2002). All plate was incubated under the aerobic or anaerobic condition at 37°C, during 24 h to 72 h. Results obtained were presented as base-10 logarithm colony-forming units per gram of cecal digesta.

Tibial mechanical test

The right tibia of each bird was removed as drumsticks with the flesh intact. The drumsticks were defleshed and the patella was removed by hand. They were then air-

dried for 24 h at room temperature and tibia length and weight were measured. The tibia breaking strength was determined using an All-digital electronic testing machine (Instron 3342, Instron, USA) fitted with a 3-point bend rig with a load cell capacity of 50 kg. To determine bone ash content, broken tibia were ashed in a muffle furnace at 600°C for 18 h.

Statistical analysis

All samples was performed at least three times and results were presented as the mean±SD. Statistical analyses were performed with SAS program (SAS Institute, 2002, USA) that using Duncan's multiple range test to compare treatment means was applied. Means with different letter within the table represent significant difference as $p < 0.05$ (Duncan, 1955).

Results and Discussion

The egg production and relative weights of various organs in laying hens fed experimental diets are shown in Table 2. The egg production in groups fed a diet containing 1% GFS was significantly higher than those of other groups, although there was no difference in feed intake. The layer fed diet containing 1% GFS showed the highest daily egg mass. The relative weights of various organs, such as liver, spleen and abdominal fat were not affected by dietary treatments.

The egg and eggshell qualities of the laying hens fed diets with varying levels of GFS are shown in Table 3. The Haugh unit and egg yolk color in hens fed diets containing GFS tended to be higher than the control. The eggshell strength, eggshell thickness, and shell color were not affected by dietary treatments. The improved shell-thickness together and shell strength indicate that isofla-

Table 2. Dietary Effects of germinated and fermented soybean on the laying performance and weights of organs from laying hens

Items	Control	GFSM ¹⁾		
		0.5%	1.0%	2.0%
Feed intake, g/d/bird	122.35±3.31 ²⁾	127.80±2.15	128.08±4.17	124.21±1.28
Egg production, %	54.01±0.25 ^b	53.91±0.62 ^b	67.31±0.84 ^a	57.32±0.52 ^b
Egg weight, g/egg	70.68±0.35 ^a	69.77±0.37 ^b	68.53±0.58 ^b	68.70±0.31 ^b
Daily egg mass, g/d/bird	38.16±0.42 ^b	37.53±0.24 ^b	46.28±0.22 ^a	39.37±0.36 ^b
Liver, g/100g BW	1.98±0.12	2.07±0.11	2.08±0.15	2.19±0.12
Spleen, g/100g BW	0.12±0.01	0.14±0.02	0.12±0.01	0.12±0.01
Abdominal fat, g/100g BW	4.10±0.21	3.46±0.13	4.59±0.14	4.86±0.20

¹⁾GFSM: *Monascus purpureus* (KCCM 12002)-germinated and fermented soybean

²⁾SEM: standard error of measurement

Values are presented as Mean±SD.

^{a-c}Mean values with different superscripts within the same row differ significantly ($p < 0.05$).

Table 3. Dietary Effects of germinated and fermented soybean on egg and eggshell qualities from laying hens

Items	Control	GFSM ¹⁾		
		0.5%	1.0%	2.0%
Eggshell color	29.67±0.21 ²⁾	31.10±0.23	31.10±0.18	29.41±0.21
Yolk color (R.C.F)	4.37±0.11 ^b	4.36±0.04 ^b	4.53±0.07 ^a	4.28±0.08 ^b
Eggshell strength (kg/cm ²)	2.18±0.01	1.95±0.05	2.17±0.07	2.09±0.03
Eggshell thickness (mm/100)	32.86±0.38	33.96±0.27	33.64±0.35	33.40±0.33
Haugh unit	74.36±0.93 ^b	75.77±0.72 ^b	76.53±0.59 ^{ab}	79.99±0.71 ^a

¹⁾GFSM: *Monascus purpureus* (KCCM 12002)-germinated and fermented soybean

²⁾SEM: standard error of measurement

Values are presented as Mean±SD.

^{a-b}Mean values with different superscripts within the same row differ significantly ($p<0.05$).

ones contained in GFS might play certain roles in the shell formation, as shown in other studies using synthetic isoflavones in laying hens. Sahin *et al.* (2003) reported that eggshell thickness increased in soy isoflavone supplemented quails and it is probably due to increased in calcium which was increased by supplemental isoflavones.

The blood profiles in laying hens fed diets with varying levels of GFS are shown in Table 4. There were no significant differences in the serum levels of GOT and GPT. Serum GOT and GPT levels are the most sensitive indicator of tissue damage in avian species (Lumeij, 1997). In addition, serum GOT and GPT is a valuable tool to determine a safe inclusion level for non-conventional feedstuff (Diaz *et al.*, 2003). The concentration of blood total cholesterol containing GFS tended to be higher than the con-

trol. This result suggests that the addition of GFS to chicken feed did not have negative effects.

The intestinal microbial populations in laying hens fed diets with varying levels of GFS are shown in Table 5. The number of lactic acid bacteria in layers fed diets containing 1% or 2% GFS was significantly higher than that of control. With the increase in the levels of GFS, the number of lactic acid bacteria linearly increased. There were no significant differences in the number of total microbes and coliform bacteria. This result was consistent with a previous report, in which broilers were fed diets containing *Lactobacillus* cultures (Jin *et al.*, 1998). However, further studies are needed to clarify the mechanism.

The mechanical and chemical properties of tibia in laying hens fed diets with varying levels of GFS are shown in Table 6. The tibial breaking strength in layers fed diets

Table 4. Dietary Effects of germinated and fermented soybean on the blood biochemical parameters of laying hens

Items	Control	GFSM ¹⁾		
		0.5%	1.0%	2.0%
GOT (U/dL)	119.53±3.21 ²⁾	117.69±4.43	121.73±5.18	124.39±4.91
GPT (U/dL)	8.76±0.50	7.50±0.28	8.24±0.23	7.96±0.42
Total-cholesterol (mg/dL)	169.20±5.33 ^a	149.12±5.18 ^b	161.50±6.22 ^a	165.00±5.94 ^a

¹⁾GFSM: *Monascus purpureus* (KCCM 12002)-germinated and fermented soybean

²⁾SEM: standard error of measurement

Values are presented as Mean±SD.

^{a-b}Mean values with different superscripts within the same row differ significantly ($p<0.05$).

Table 5. Dietary Effects of germinated and fermented soybean on the profiles of cecal microflora of laying hens

Items	Control	GFSM ¹⁾		
		0.5%	1.0%	2.0%
Total microbes (Log CFU/g)	7.21±0.32 ²⁾	7.18±0.90	7.63±0.73	7.45±0.98
Lactic acid bacteria (Log CFU/g)	7.25±0.24 ^b	7.71±0.43 ^{ab}	8.06±0.54 ^a	8.20±0.55 ^a
<i>Coliforms</i> (Log CFU/g)	5.07±0.51	5.60±0.38	4.30±0.49	4.70±0.29

¹⁾GFSM: *Monascus purpureus* (KCCM 12002)-germinated and fermented soybean

²⁾SEM: standard error of measurement

Values are presented as Mean±SD.

^{a-b}Mean values with different superscripts within the same row differ significantly ($p<0.05$).

Table 6. Dietary Effects of germinated and fermented soybean on tibial characteristics of laying hens

Items	Control	GFSM ¹⁾		
		0.5%	1.0%	2.0%
Tibia breaking strength (Kgf)	21.19±1.55 ²⁾	29.04±2.31	25.53±2.11	25.21±1.80
Ash, %	42.63±2.91	48.87±3.98	45.59±3.51	45.20±2.54

¹⁾GFSM: *Monascus purpureus* (KCCM 12002)-germinated and fermented soybean

²⁾SEM: standard error of measurement

Values are presented as Mean±SD.

with GFS tended to be higher than that of the control, but this difference was not significant. The layer fed diets with GFS had a slightly higher tibial ash content, but this increase was not statistical significant.

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