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Effect of Fermented Soybean "Natto" Supplement on Egg Production and Qualities*

K. Fujiwara, Y. Miyaguchi[1,] **, A. Toyoda[1], Y. Nakamura[1], M. Yamazaki[2]
K. Nakashima[2] and H. Abe[2]
Laboratory of Poultry, Ibaraki Prefectural Livestock Research Center, Ibaraki, 311-3157, Japan

ABSTRACT : Natto is a Japanese traditional soybean product fermented by *Bacillus natto*. The effects of dried fermented soybean (natto) supplement on egg production and egg qualities of layer chickens was studied with regard to the effective use of various waste foods in Japan. Dried natto, prepared by heating at 60°C, was added to a basic diet at a level of up to 3%. Forty 166-wk-old layer chickens (Rhode Island Red) were randomly divided into 4 groups and five layer chickens were used in each group with two replicates. Layer chickens in group 1 were fed a basic diet as the control. The remaining 3 groups were fed the basic diet supplemented with dried natto at levels of 1, 2, and 3% (w/w), respectively. The result did not show improvements in egg production or feed conversion ratio of layer chickens even when 3% dried natto was added to the control diet. The egg qualities including egg weight, eggshell strength and thickness, yolk color, yolk weight, albumen height, and Haugh unit were also not improved. However, the feeding of dried natto changed the cholesterol content in the egg yolk. The supplementation of dried natto showed the tendency to decrease the yolk cholesterol after 12-wk of feeding compared to the control diet though it did not change plasma cholesterol levels in the blood. On the other hand, yolk cholesterol decreased significantly after 12-wk of feeding 3% dried natto ($p<0.05$). (**Key Words :** Cholesterol, Fermented Soybean, Haugh Unit, Natto, Chicken)

INTRODUCTION

Fermentation processes have long been used to prepare traditional soybean foods in Far East Asia including "Doubanjiang" in China, "Duenjang" in Korea, and "Miso" in Japan, (Hong et al., 2004). Natto is also a traditional Japanese health food prepared using soybean fermented by *Bacillus subtilis var natto* (*Bacillus natto*), which is a Gram-positive spore-forming bacterium (Ashiuchi et al., 1998). It is well known that fermented foods have various functional properties. For example, yogurt made from milk by fermentation with *lactobacillus* shows various physiological activities such as antioxidative (Songisepp et al., 2005) and antiallergenic activities (Veckman et al., 2003). Natto also has various functional properties. Natto extracts are known to include nattokinase, which has an approximately four-times stronger fibrinolytic activity than plasmin in clot lysis assays (Fujita et al., 1993). Kiers et al. (2003) found fermented soybeans could be beneficial in the control of diarrhea in *Escherichia coli*-challenged weaned piglets (*Rhizopus microsporus* fermented) and significantly improved weight gain and feed intake (*Bacillus subtilis* fermented).

In the poultry industry, supplementation of beneficial microorganisms has attracted interest recently. Antibiotics are in wide-spread use to prevent poultry pathogens and disease in order to improve meat and egg production. However, the continued use of dietary antibiotics has resulted in common problems such as the development of drug resistant bacteria (Sorum and Sumde, 2001), imbalance of normal microflora (Andermont, 2000), and drug residues in the body (Burgat, 1991). As a result of these problems, it has become necessary to develop alternatives using beneficial microorganisms. A probiotic is a live microbial feed supplement that beneficially affects the host animal by improving its intestinal microbial

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** Corresponding Author: Y. Miyaguchi. Tel: +81-29-887-1261, Fax: +81-888-8525, E-mail: miayguti@mx.ibaraki.ac.jp
[1] College of Agriculture, Ibaraki University, Ibaraki, 300-0393, Japan.
[2] National Institute of Livestock and Grassland Science, Ibaraki, 305-0901, Japan.
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Table 1. Nutritional composition of dried natto and experimental diets

	Dried natto	Natto added			
		+0% (Cont.)	+1%	+2%	+3%
Nitrogen-free extract (%)	13.4	56.3	55.8	55.4	55.0
Crude protein (%)	41.9	15.9	16.1	16.5	16.6
Moisture (%)	5.7	12.2	12.1	12.1	12.0
Ether extract (%)	21.3	4.8	5.0	5.2	5.3
Crude fiber (%)	10.5	2.5	2.6	2.7	2.8
Gross energy (Cal/g)	5,631	4,083	4,098	4,113	4,128

balance (Fuller, 1989), and is recommended as an effective alternative to antibiotics (Sissons, 1989). After feeding of probiotics, improvements in growth performance and feed efficiency have been reported in broiler chicks (Santoso et al., 1995; Cavazzoni et al., 1998). Furthermore, *Bacillus subtilis* has been studied as an antibiotic agent for poultry (Samanya and Yamauchi, 2002). Since there have been few investigations of the effects of the supplementation of natto prepared as a foodstuff in layer chicken diets, no information is available on the effects of natto supplement on egg production and egg qualities. The objectives of this study were to clarify the effects of the supplementation of dried natto containing living *Bacillus natto* on egg production and egg qualities of layer chickens during the feeding period and to evaluate the suitability of natto as layer chicken supplement.

MATERIALS AND METHODS

Birds, diets, and conditions for feeding

Forty 166-wk-old Road Island Red layer chickens (2 replicates of 5 layers per group) were housed randomly in 8 different cages. Each of the 4 groups of layers was assigned randomly to be fed a basic diet (Layer17S, Chubu Shiryo Co. Ltd, Aichi, Japan) without natto or one of 3 experimental diets with basic diet supplemented with dried natto at different dosages (1%, 2%, and 3%, respectively). The experiment lasted for 12 wk. Natto provided by Takano Foods Co. Ltd (Ibaraki, Japan) was dried in a drying oven at 60°C for 60 h followed by grinding into 1 mm granules using a grinder. It was confirmed that the resultant dried natto contained more than 1×10^9 cfu/g. During the experiment, layer chickens were fed on an *ad libitum* basis and had free access to water. The nutritional analysis of the control diet and dried natto are shown in Table 1.

Sample collection and analytical procedures

Egg production was calculated as the total number of eggs collected divided by the total number of live layer chickens on each day in each group. The feed consumption and feed conversion ratios were recorded for the last week of each month. The collected eggs were classified as either normal or damaged; the latter included broken eggs, cracked eggs, and shell-less eggs. Egg weights were determined every day.

An additional sample of 20 eggs was collected randomly from each experimental group (control and natto added) to examine the egg qualities after each 4-wk feeding period. The egg qualities included eggshell strength and thickness, yolk color and weight, albumen height, and Haugh unit. All eggs were weighed individually. Egg shell strength was measured by using of Eggshell force gauge (Model II Robotmation Co. Ltd. Tokyo, Japan). The eggs were broken onto a plastic plate to measure the Haugh unit value by the measurement of the albumen height and egg weight using an Egg multi-tester (EMC500C Robotmation Co. Ltd.). The yolk color was determined with a Roche yolk color fan. To measure the cholesterol content in the yolk, 5 eggs were selected randomly from each group, and the yolk was separated and weighed. Cholesterol was extracted from the yolks with 1 mol/L KOH containing 2-propanol and was determined using a cholesterol colorimetric assay kit (F-kit cholesterol, Roche Molecular Biochemicals, Germany). Blood was also collected from each bird at 12 weeks from the neck after slaughter. The plasma fraction was isolated using heparin and was kept at -20°C until cholesterol analysis. Plasma cholesterol was determined by the colorimetric method described above.

Statistical analysis

All data were subjected to a 1-way ANOVA (a 1-way ANOVA test was conducted for all data).

RESULTS AND DISCUSSION

Production performance

During the feeding period, the highest egg production (95.7%) was found in the 3%-natto group at week 5, and the lowest egg production (80.0%) was from the 1%-natto group at week 3. No significant effect of natto on egg production was observed in the periods from weeks 1 to 11. Egg production in the control and natto added groups were not significantly different (Figure 1A). The feed conversion ratios of the control and natto added groups were not significantly different during weeks 1 to 11 (Figure 1B).

From a nutritional point of view, it is known that soybean meal has a variety of antinutritional factors, such as trypsin inhibitor, lectins, and soya globulins, which limit the

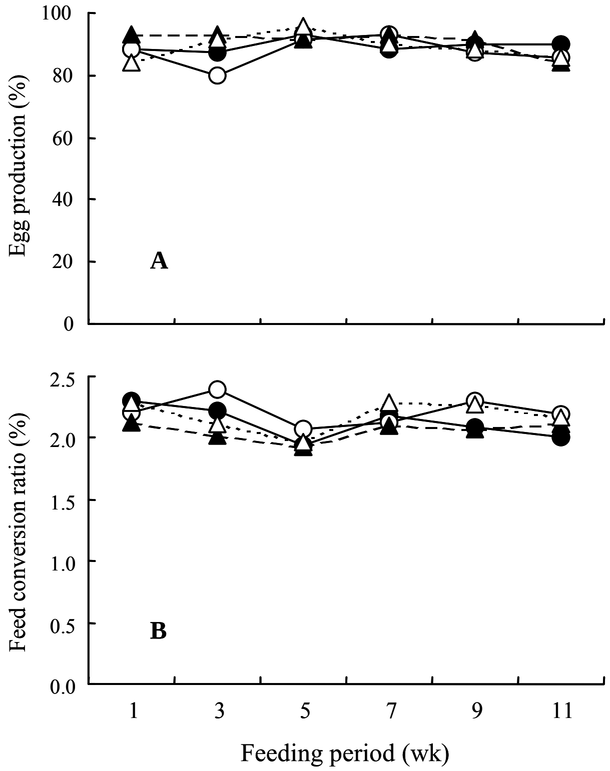

Figure 1. Effect of natto supplement on egg production and feed conversion ratio of layer chickens. A: Egg production; B: Feed conversion ratio. ●, Control (0% natto added); ○, 1% natto added; ▲, 2% natto added; Δ, 3% natto added.

application of soybean meal in animal feed especially for young animal (Dunsford et al., 1989; Li et al., 1990; Jiang et al., 2000). On the other hand, it was reported that protein hydrolysis is a suitable treatment for reducing the antigenic activity of soy products and improving nutritional performance in young calves (Lalles et al., 1995). Natto, which is prepared from soybeans by fermentation with *Bacillus natto*, has probiotic activities. However, few studies about the effect of natto as poultry feed have been reported. In this study, the effects of natto supplement on egg production and egg qualities were investigated showing that natto supplementation did not affect the egg production and feed conversion ratios (Figure 1). Miles et al. (1981) reported that *Lactobacillus* dose improved egg production and provided several explanations for the observations. These explanations included a possible increase in gut motility occurring in the presence of excessive numbers of organisms thereby altering nutrient availability for absorption at desired points, or that other beneficial bacterial populations may be altered so that cohabitation of the established microflora is unsettled. In our results, no effect of natto dose on egg production was shown, so further research should focus on enteric microorganisms to clarify the probiotic action of natto under other experimental conditions.

Egg qualities

Table 2 shows the effect of dried natto (3%) supplement on various egg qualities. The average egg weight in all

Table 2. Effect of the supplementation of natto on egg qualities

	Feeding period (weeks)			
	0	4	8	12
Egg weight (g)				
Cont.	47.93±0.85	50.21±0.54	52.28±0.62	54.31±0.77
Natto added	47.30±0.50	49.64±0.59	52.36±0.38	52.83±0.51
Shell strength (kg/cm^2)				
Cont.	3.29±0.09	2.99±0.09	3.34±0.12	3.38±0.10
Natto added	3.18±0.11	3.13±0.13	3.12±0.09	3.22±0.08
Shell thickness (mm)				
Cont.	0.341±0.005	0.340±0.003	0.356±0.004	0.356±0.005
Natto added	0.328±0.005	0.329±0.005	0.335±0.005	0.336±0.004
Yolk color index				
Cont.	11.95±0.11	11.85±0.09	12.80±0.12	12.40±0.13
Natto added	11.75±0.16	11.95±0.13	12.80±0.14	12.89±0.23
Yolk weight (g)				
Cont.	10.58±0.17	11.82±0.12	12.85±0.20	13.65±0.21
Natto added	11.13±0.14	12.00±0.13	13.47±0.16	13.93±0.20
Albumen height (mm)				
Cont.	7.13±0.19	6.90±0.19	6.95±0.13	6.81±0.21
Natto added	7.24±0.17	7.07±0.24	7.15±0.18	6.92±0.23
Haugh unit				
Cont.	88.0±1.01	85.8±1.23	85.6±0.76	83.9±1.18
Natto added	88.8±0.97	86.8±1.40	86.7±1.00	85.0±1.44

n = 10, Values are mean±SE. Cont., basic diet alone; Natto added, three percent dried natto diets were given.

Table 3. Total-cholesterol concentrations in yolk and plasma after the feed supplementation with natto

	Natto added			
	+0% (Cont.)	+1%	+2%	+3%
Yolk (mg/100 g)	1,691±88	1,602±64	1,339±141	1,292±108*
Plasma (mg/100 ml)	105.5±9.3	119.1±11.8	116.0±9.3	136.4±18.9

Data are shown as mean±SE (n = 5).
* The value has a tendency to be lower than those of the other groups (p<0.1).

groups increased with increasing feeding period. However, during the 12-wk period of the experiment, the average egg weights among different treatments showed no statistical differences (p>0.1). After 12 wk of natto supplementation, the egg weight in the control and 3% natto groups were 54.3 and 52.8 g, respectively.

Egg shell thickness in the control increased slightly with increasing feeding period. At the end of 12 wk period, the egg shell thicknesses in the natto-added groups were lower than that of the control, but did not show statistical differences. Egg shell strength between the control and natto diet also showed no statistical differences (p>0.1).

The effects of dietary treatments on yolk color and weight were examined. Yolk color increased with increasing feeding period, but a significant difference in yolk color in the control and natto diets was not shown. In both groups, yolk weight also increased with feeding period, but the numerical differences in yolk weight in the control and natto diets were not statistically significant. The effect of the supplementation of natto on Haugh unit and albumen height was studied but showed no statistical differences in the values between control and natto diet (p>0.1).

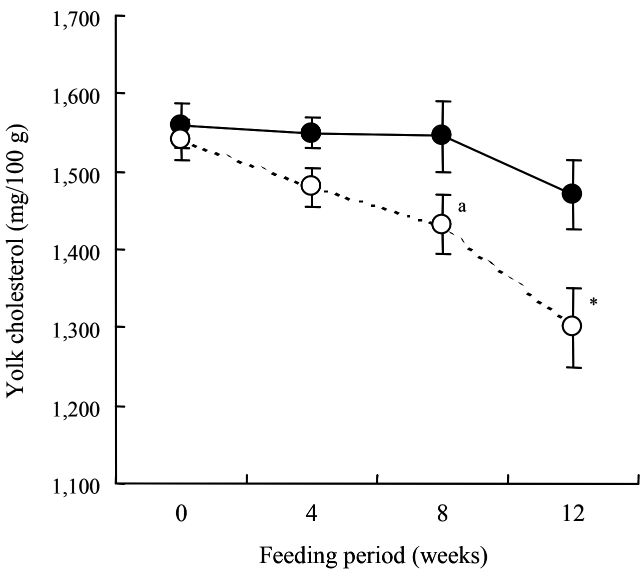

Figure 2. Change in yolk cholesterol content of layer chickens fed with natto supplement. Vertical bar, standard error (n = 3), ●, basic diet alone; ○, basic diet with 3% natto. [a] The value has a tendency to be lower than those of the other groups (p<0.1). * The value is significantly lower than those of the other groups (p<0.05).

As described above, egg qualities such as egg shell, yolk, and albumen were not affected by the supplementation of dried natto (Table 2). It has been suggested that medullary bone in the marrow cavity of long bones is the major contributor of skeletal calcium involved in egg shell formation (Taylor and Moore, 1954). Estrogen induces the early formation of the medullary bone with the maturation of female birds (Bloom et al., 1958). Soybean isoflavones contained in soy products acts as an estrogen-like substrate (Miksicek, 1994). Xu et al. (2006) reported that the egg mass from the antibiotics differed significantly in a group fed with dried *Bacillus subtilis culture* (p<0.05). Nahashon et al. (1994) and Mohan et al. (1995) reported a slight improvement in egg shell thickness in hens fed with supplement containing *Lactobacillus*. Therefore, we hypothesized that dried natto, which contains isoflavones and living *B. natto*, would also enhance egg shell thickness and weight. However, natto supplement did not influence the egg shell thickness. Therefore, it was clarified that 3% and below of dried natto did not alter the properties of egg shell thickness.

Yolk cholesterol concentration in the 3% natto group decreased with increasing feeding period. In the 3% natto group, the cholesterol concentration after the 12-wk feeding period was significantly lower than that at week 0 (p<0.05) (Figure 2). Moreover, after the 12-wk feeding period the average cholesterol concentration in the yolk decreased in a natto dose-dependent manner (Table 3). The tendency of the reduction of yolk cholesterol (p<0.1) required the addition of 3% dried natto. On the other hand, natto supplement did not influence plasma cholesterol levels.

Recently, considerable attention has been paid to the potential of feed supplement in altering lipid metabolism. Suppression of cholesterol in cocks (Endo et al., 1999; Wenjun et al., 2004; Lim et al., 2006) and in broilers (Santoso et al., 1995) has been reported. It was reported that probiotics reduce cholesterol concentrations in egg yolk (Mohan et al., 1995; Abdulrahim et al., 1996; Haddadin et al., 1996) and in the serum in chicks (Mohan et al., 1995; Jin et al., 1998). Our finding of the reduction in yolk cholesterol agrees with these studies. It was also reported that the yolk cholesterol levels were reduced by dried *Bacillus subtilis* culture supplementation (Xu et al., 2006). It is possible that some of the organisms present in the probiotic preparation could assimilate cholesterol present in the gastrointestinal tract for their own cellular metabolism, thus reducing the amount absorbed, as suggested by Gilliland et al. (1985). Kalavathy et al. (2003) indicated that lactic acid bacterial strains are able to alter the enterohepatic cycle and reduce cholesterol through the assimilation of dietary cholesterol into the bacterial cells and the bile salt hydrolate activity in the intestine. Another reason for the decrease of cholesterol in probiotic-fed hosts, as suggested by Fukushima and Nakano (1995), is that probiotics are able to inhibit hydroxymethyl-glutaryl-coenzyme A, an enzyme involved in the gastrointestinal tract. Haddadin et al. (1996) reported that cholesterol levels in yolks were decreased by 18.8% when layer hens were fed for a 48-wk period with Lactobacillus at up to 4 million viable cells per gram of feed.

In conclusion, the results demonstrate that supplementation of dried natto to the diet of layer chickens has a beneficial effect. Natto supplement reduced the content of yolk cholesterol during the 12-wk feeding period, although plasma cholesterol levels were not changed by the supplementation of natto. When female chickens become sexually mature, estrogen stimulates the hepatic production and secretion of huge amounts of smaller VLDL, which have been called “yolk targeted VLDL”, containing large amounts of apolipoprotein VLDL II (apoVLDL II) (Schneider et al., 1990). So the lowering of yolk cholesterol by feeding of natto could be due to the decrease in yolk targeted VLDL or apoVLDL II. Therefore, further work should focus on investigating cholesterol metabolism and its related gene expression in the liver and in gastrointestinal flora to clarify the suitability of natto supplement for the development of low-cholesterol eggs.

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