Asian-Aust. J. Anim. Sci.
Vol. 21, No. 12 : 1766 - 1772
December 2008

www.ajas.info

Effect of Fermented Soybean, "Natto" on the Production and Qualities of Chicken Meat*

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ABSTRACT : Natto is a Japanese traditional soybean product fermented by *Bacillus natto*. The effect of natto supplement on the production and qualities of chicken meat was studied since the effective use of various waste foods is required in Japan. Dried natto prepared by heating at 60°C was added to a basic diet at an amount of below 2%. The supplementation of dried natto did not influence the weights of the carcass, breast and thigh meat, fillet or abdominal fat. Growth of the thighbone such as the length, thickness of cortex bone, and Ca/P ratio in bone ash were not altered by the addition of natto. However, the pH of male meat decreased following the supplementation of dried natto from days 28 to 80. The water-soluble protein content in male thigh meat increased in the group fed 2% natto from days 28 to 80. Free peptides increased in male thigh meat by feeding 2% natto from days 0 to 80. The supplementation of natto increased free glutamic acid in thigh meat regardless of sex. Moreover, the supplementation of natto specifically decreased meat cholesterol in female chickens though the effect was not shown in male chickens. (**Key Words :** Chicken, Cholesterol, Glutamic Acid, Natto, Soybean)

INTRODUCTION

Since ancient times fermented soybean products were made traditionally in Far East Asia including "Tempe" in Indonesia, "Doubanjiang" in China, "Duenjang" and "Chung-Kook-Jang" in Korea, and "Miso" and "Tofu-yo" in Japan, (Asututi et al., 2000; Hong et al., 2004; Mine et al., 2005). Natto is a Japanese cheese-like product also made from soybeans by fermentation with *Bacillus subtilis var. natto* (*Bacillus natto*), which is a gram-positive spore-forming bacterium (Ashiuchi et al., 1998).

Currently more than 80% of feedstuffs for poultry are imported in Japan (Ishibashi and Yonemochi, 2002). In addition, large quantities soybeans are imported and the self-sufficiency ratio is only 8% in Japan. Therefore, the effective use of waste fermented soybean products with no commercial value would be highly beneficial as feed (Cho et al., 2007; Kim et al., 2007). Moreover, there are advantages of the use of natto as feedstuff because the biological value of soybeans is relatively higher than other vegetable proteins and abundant nutrients remain after fermentation such as isoflavones, saponins, lecithin, and various vitamins.

In addition to residual nutrients, there are various functional ingredients such as nattokinase, polyglutamic acid, and dipicolinic acid in natto after fermentation. For example, nattokinase is known as a fibrinolytic enzyme, which cleaves directly cross-linked fibrin *in vitro* (Fujita et al., 1993; Suzuki et al., 2003; Yamashita et al., 2003), and polyglutamic acid is capable of calcium solubilization. (Tanimoto et al., 2001) It is reported that the anti-*Helicobacter pylori* activity of natto is derived from dipicolinic acid (Sumi et al., 2006). Moreover, *Bacillus natto* as a probiotic could be regarded as a functional ingredient in natto. If natto could be used as poultry feedstuffs, converting surplus natto into feedstuffs could reduce feedstuff imports and productivity reuse food waste material.

In this study, natto supplement was fed to "Tsukuba-jidori", which is a high-quality meat-type chicken. "Jidori"

* This research was supported by a research-aid fund of research projects for utilizing advanced technologies in agriculture, forestry and fisheries, Japan.

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Received November 12, 2007; Accepted March 17, 2008

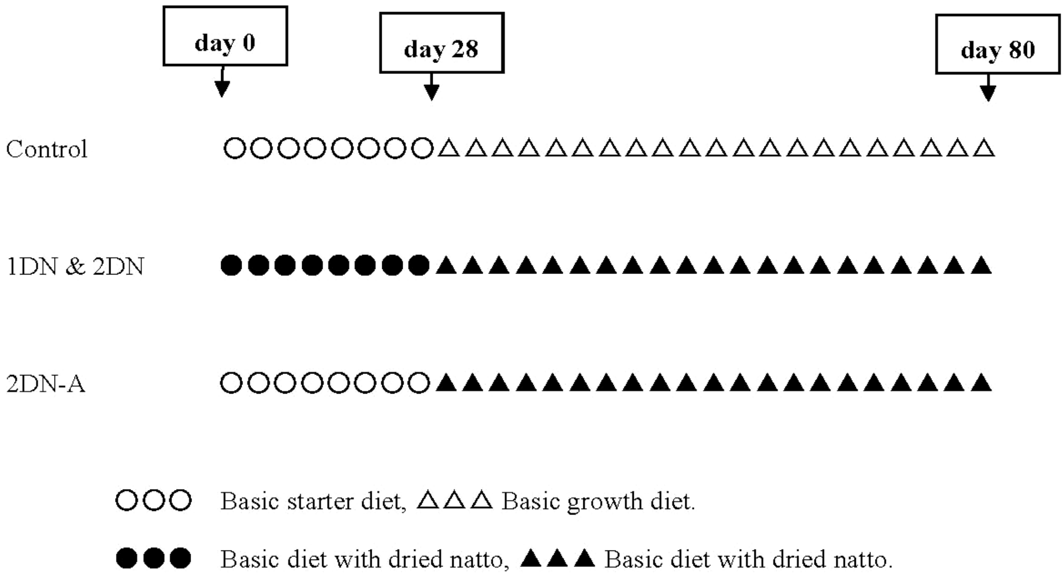

Figure 1. Preparation of dried natto according to the experimental design explained in MATERIALS AND METHODS. Control, a basic diet alone was supplied days 0 to 80. 1DN and 2DN, Basic diet with 1% and 2% dried natto was supplied for days 0 to 80, respectively. 2DN-A, Basic diet alone was supplied for days 0 to 27 and the diet with 2% dried natto was supplied for days 28 to 80.

chicken meat has recently become more popular in Japan because of its better taste and firmer texture than broiler meat. The chickens must be a Japanese species and have been bred free-range. "Tsukuba-jidori" was developed in Ibaraki Prefecture, Japan by crossbreeding between a male White Cornish and a female crossbred chicken between "Hinai-jidori" and Rhode Island Red.

To clarify the qualities of natto as a chicken feedstuff, the effect of natto supplement on the productivity and qualities of chicken meat were investigated in this study.

MATERIALS AND METHODS

Diets, birds, and conditions for feeding

Natto provided by Takano Foods Co. Ltd (Ibaraki, Japan) was dried in an oven at 60°C for 60 h followed by grinding into 1 mm fragments using a grinder. It was determined that the resultant dried natto contained more than 1×10^9 cfu/g. Forty newborn male or female Tsukuba-jidori in each group (2 replicates of 5 chickens making a subgroup) were randomly housed in 8 different cages. Each of the 4 groups of chickens was assigned randomly; a basic diet without natto was supplied to the control group. Experimental diets containing a basic diet plus dried natto at different dosages (1% and 2%) were supplied to the other 3 groups. For two groups, 1% and 2% natto supplemented-diets were supplied for days 0 to 80 (1DN and 2DN). In the remaining group, a basic diet without dried natto was supplied for days 0 to 27 and subsequently the 2% natto supplemented-diet was supplied for days 28 to 80 (2DN-A). During the experiment, chickens were fed on an *ad libitum* basis and had free access to water. Preparation of dried natto according to the experimental design described above is shown in Figure 1.

Preparation of meats and analytical procedure

After the feeding for 80 days, the chickens were slaughtered by decapitation. Breast and bone-in thigh meat, fillet, and abdominal fat were taken from the carcasses and weighed after exsanguination. The thighbone was also taken from the thigh and weighed, and the. Length of the bone and thickness of cortical bone were measured by slide gauge. About one-gram of cortical bone was pulverized and incinerated to ash at 500°C for 2 h. The bone ash was dissolved in HCl and the solution was used for the measurement of calcium (Ca) and phosphorus (P). Calcium was measured using an atomic absorption spectrophotometer (AA6500F, Shimazu Co. Ltd., Kyoto, Japan) and the phosphorus level was determined by a colorimetric method. The pH value of the meat was measured using a pH meter with a glass-electrode (Φ32PH meter, Beckman, CA, USA). Meat samples were minced and homogenized in 50 mM imidazole buffer. After centrifugation, the water-soluble protein in the obtained supernatant was measured by the Biuret method. Furthermore, the supernatant was added to trichloroacetic acid (TCA) and the amount of 10% TCA soluble peptides was determined by the Folin method. Free glutamic acid (Glu) in the meat was determined using an F-kit L-glutamic acid (Roche Molecular Biochemicals, Germany). Briefly, minced meat was dispersed in 1 mol/L perchloro acid and homogenized with an IKA ultra disperser. After

Table 1. Effect of natto supplementation on meat production

	Experimental groups			
	Cont.	1DN	2DN	2DN-A
Male (n = 10)				
Carcass (g)	3,405	3,457	3,463	3,435
Breast meat, bone- in (g)	799	819	823	857
Thigh meat, bone-in (g)	918	889	921	917
Filet (g)	129.1	129.2	127.4	128.6
Thigh bone (right) (g)	30.5	29.7	30.3	31.6
Abdominal fat (g)	88.4	89.0	88.4	64.9
Female (n = 10)				
Carcass (g)	2,538	2,495	2,510	2,504
Breast meat, bone-in (g)	637	616	603	620
Thigh meat, bone-in (g)	639	619	615	625
Fillet (g)	105.8	100.3	101.4	103.7
Thigh bone (right) (g)	19.6	18.6	19.0	18.1
Abdominal fat (g)	81.1	81.3	71.4	93.5

Cont., 1DN, and 2DN correspond to 0, 1, and 2% dried natto supplementation for days 0 to 80, respectively.
2DN-A, a basic diet was supplied for days 0 to 27 and subsequently 2% dried natto was supplied for days 28 to 80.

Table 2. Effect of natto supplementation on the bone growth of chicken

	Experimental groups			
	Cont.	1DN	2DN	2DN-A
Thigh bone length (cm)	10.68±0.33	10.76±0.12	10.95±0.23	10.91±0.34
Cortical bone thickness (mm)	0.33±0.03	0.35±0.07	0.39±0.05	0.37±0.06
Ca/P ratio of the thigh bone	2.51±0.14	2.36±0.10	2.94±0.60	2.45±0.03

n = 3, Values are mean±SE.
Cont., 1DN, and 2DN correspond to 0, 1, and 2% dried natto supplementation for days 0 to 80, respectively.
2DN-A, a basic diet was supplied for days 0 to 27, and subsequently 2% dried natto was supplied for days 28 to 80.

centrifugation, the resultant solution was added to 0.1 N NaOH to reach pH 8.0 and adjusted to a volume of 25 ml with water. The solution was used for the assay according to the F-kit instruction manual.

Statistical analysis

All data were subjected to a 1-way ANOVA test.

RESULTS AND DISUCUSSION

Production performance

The effect of natto supplement on meat productivity is shown in Table 1. The weight of the carcass was about 1.3 times higher in males than in females. The meat weights obtained (breast, thigh, and fillet) showed the same tendency as the whole carcass. The addition of 1% or 2% natto did not influence on the meat productivity. The effect of natto supplementation on thighbone growth is shown in Table 2. All data including the length of the thighbone, thickness of cortical bone, and the Ca/P ratio, were not altered by natto supplementation. It is known that natto is rich in isoflavones and vitamin K_2 (menaquinone-7); the former may help preventing bone loss by promoting calcium absorption (Yamaguchi, 2002), and the latter acts as a cofactor of gamma-carboxylase, which converts Glu residues in osteocalcin molecules to gamma-carboxyglutamic acid for promotion of normal bone mineralization (Tsukamoto et al., 2000). The expected results could not be obtained because of unsuitable experimental conditions in this study though we regarded the supplementation of natto should improve bone metabolism.

Meat qualities

The effect of natto supplementation on the pH of meat is shown in Figure 2. In male chickens, the pH of breast and thigh meat decreased significantly in the 2% natto group supplied for days 28 to 80 (2DN-A). This tendency was not found in female chickens on the same diet. Various qualities of meat depend on its pH. The nutritional composition of breast and thigh meats such as moisture, crude protein (CP), and fat content were investigated, showing that the protein concentration of female thigh meat tended to decrease with the supplementation of natto though the moisture and fat concentrations of meat in males did not change significantly (data not shown).

Many studies have identified relationships between feed and meat qualities including color (Smith et al., 2002), texture (Kristensen et al., 2002), and drip loss (Young et al., 2004). However, effect of fermented soybean supplement on the production performance and pH of meat has not been investigated. Since the mechanism of the pH decrease by

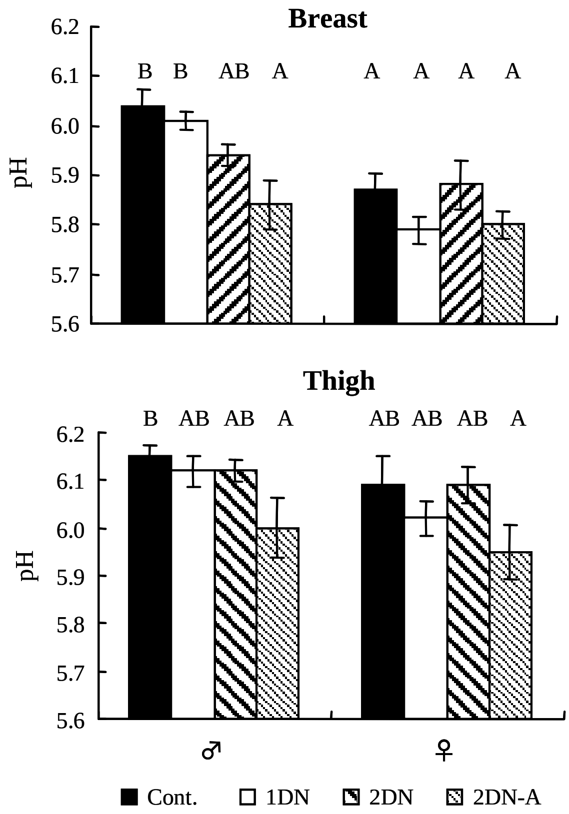

Figure 2. Effect of the supplementation of natto on the pH of the breast and thigh meat. Cont., 1DN, and 2DN correspond to 0, 1, and 2% dried natto supplementation for days 0 to 80, respectively. 2DN-A, a basic diet was supplied for days 0 to 27, and subsequently 2% dried natto was supplied for days 28 to 80. Vertical bar, standard error (n = 5). Data bearing the same superscripts are not significantly different (p>0.05).

natto supplementation was not clarified in this study, we intend to study the relationship between pH and physicochemical properties of the meat of natto fed chickens in our future research.

The water-soluble fractions such as the protein and peptides contents in meat increased with the supplementation of natto (Table 3). In male chickens, the water-soluble protein content of thigh meat was significantly higher in the 2DN-A treatment than in the control. In female chickens, the water-soluble protein content was not altered by natto supplementation. The TCA-soluble peptide content male thigh meat as higher in 2DN than in the control (p<0.05). The free amino acids and peptides that increase during post-mortem aging play an important role in the formation of meat taste. It is reported that the increase in free amino acids during the post-mortem storage of meat is caused by the action of aminopeptidases (Migita and Nishimura, 2006). Therefore, the free Glu contents in the breast and thigh meats after feeding of natto were determined, showing that the free Glu content of thigh meat was higher in 2DN than in the control regardless of sex (Figure 3). 2DN also increased the free Glu content in female thigh meat. This tendency was not shown in breast meat. Some di- or tri-peptides containing Ala, Asp, Val, Glu, Ser, and Pro were recognized to enhance the taste of 0.02% 5'-inosinic acid (IMP) (Maehashi et al., 1999). Fujimura et al. (2001) identified three compounds, free Glu, IMP, and potassium ions, as active taste components in chicken meat extracts. Glutamic acid and IMP, called “umami” taste, are preferred by consumers and constitute a characteristic taste of chicken meat. It is believed that free Glu is the most important ingredient among these three active taste compounds. Aging of meat also enhanced the increase in several free amino acids.

It was believed that active taste components were not influenced by diet (Farmer, 1999). However, it was recently reported that free Glu and sensory scores in meat were increased by a high CP diet, and the free Glu content was increased by elevated dietary CP levels for 3 to 10 days using Cobb strain female broilers (Fujimura and Kadowaki, 2006). Furthermore, the free Glu content was significantly increased by dietary leucine (Leu). In particular, compared

Table 3. Effect of natto supplementation on soluble protein and peptide levels in chicken

	Experimental groups			
	Cont.	1DN	2DN	2DN-A
Soluble protein (g/100 g)				
Breast (male)	9.48±0.15	9.93±0.29	9.58±0.22	9.80±0.01
Breast (female)	11.5±0.55	12.3±8.8	12.5±0.17	12.4±0.12
Thigh (male)	3.94±0.11	4.41±0.18	4.25±0.06*	4.88±0.29**
Thigh (female)	5.92±0.15	5.71±0.09	5.91±0.19	5.87±0.31
TCA-soluble peptide (μmol/100 g)				
Breast (male)	118.8±10.6	115.6±2.7	125.7±12.3	127.5±9.1
Breast (female)	1 04.5±2.8	107.2±4.9	115.6±3.1	109.0±8.3
Thigh (male)	84.6±5.5	103.9±8.3	129.2±19.1*	96.5±15.6
Thigh (female)	96.5±10.1	84.3±7.9	95.6±10.2	92.5±11.9

n = 3, Values are mean±SE.

Cont., 1DN, and 2DN correspond to 0, 1, and 2% dried natto supplementation for days 0 to 80, respectively.

2DN-A, a basic diet was supplied for days 0 to 27, and subsequently 2% dried natto was supplied for days 28 to 80. * p<0.1, ** p<0.05.

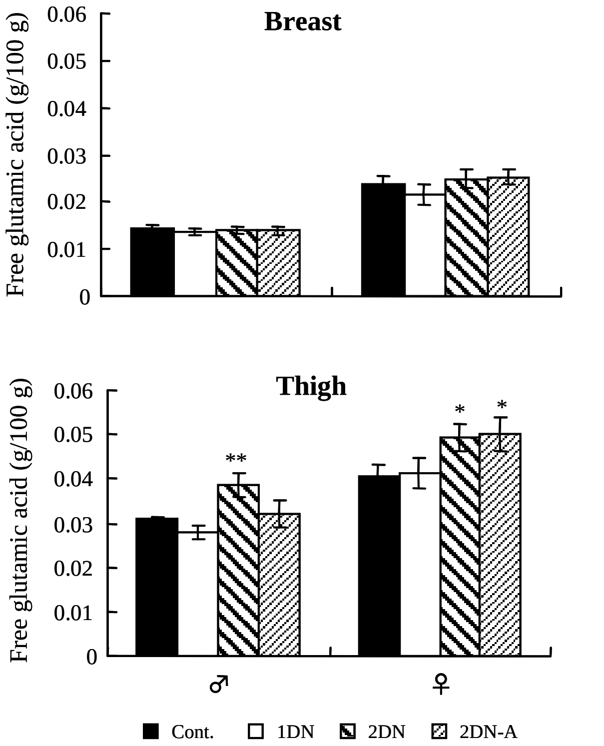

Figure 3. Effect of the supplementation of natto on the free glutamic acid concentration in the breast and thigh meat. Cont., 1DN, and 2DN correspond to 0, 1, and 2% dried natto supplementation for days 0 to 80, respectively. 2DN-A, a basic diet was supplied for days 0 to 27, and subsequently 2% dried natto was supplied for days 28 to 80. Vertical bar, standard error (n = 3). * and ** indicate that the values are significantly different at p<0.1 and p<0.05, respectively.

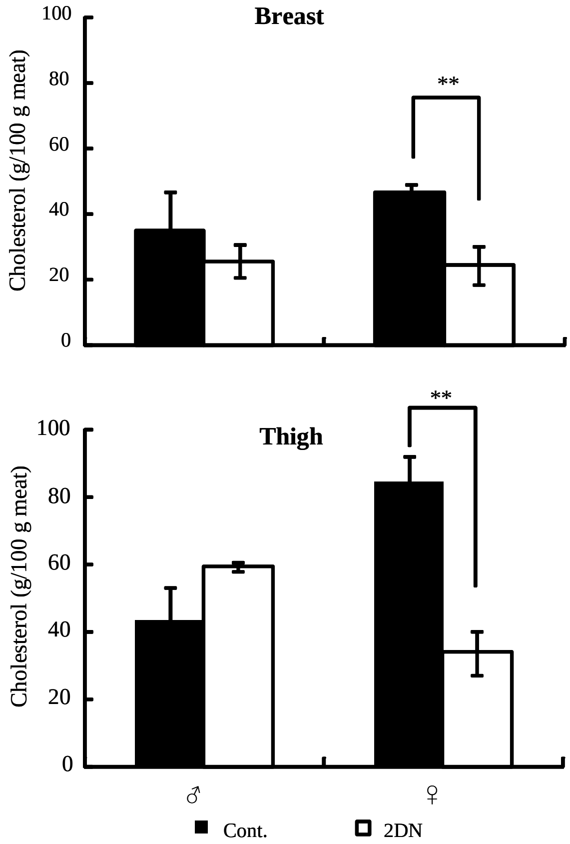

Figure 4. Effect of the supplementation of natto on the cholesterol content in the breast and thigh meat. Cont. and 2DN correspond to 0 and 2% dried natto supplementation for days 0 to 80, respectively. Vertical bar, standard error (n = 3). ** Indicate that the values between Cont. and 2DN are significantly different at p<0.05.

with the Leu 130% group, free Glu was increased by 17% in the Leu 100% group (Imanari et al., 2007).

In this study, the increase in free Glu in meat was shown by 2% natto supplementation though the 2% dried natto diet did not have a high level of CP or Leu. It is reported that *Bacillus* as a probiotic enhanced not only intestinal flora but also meat production and qualities and lipid metabolism (Santoso et al., 1995; Cavazzoni et al., 1998). Several researchers have shown previously that the peptide content of soy-fermented products is greater than that of unfermented soybeans (Okamoto et al., 1995). Various reports suggested that fermentation increased protein content, eliminated trypsin inhibitors, and reduced the peptide size in soybeans, soybean meal, and fermented soybean might be of more benefit to livestock as a novel feed ingredient (Hong et al., 2004). Therefore, natto supplementation could enhance the meat qualities not by providing additional protein but by other factors such as biofunctional peptides in natto and *Bacillus natto* as a probiotic.

Meat cholesterol levels of chickens fed with natto supplement are shown in Figure 4. The cholesterol content of thigh meat was higher in females than in males. The supplementation of natto decreased meat cholesterol in female thigh meat significantly (p<0.05). The same tendency was shown in breast meat. On the other hand, significant differences were not found in males. It is reported that the supplementation of red mold rice decreased the serum and meat cholesterol levels of broiler chickens (Wan et al., 2005). It was also reported that feeding of high levels of copper reduced the cholesterol content by approximately 25% in the edible muscle tissue of broiler chickens for 42 d without altering the growth of the chickens or substantially increasing the copper content of the edible meat (Bakalli et al., 1995). The high molecular weight fraction of soybean protein hydrolyzates showed hypocholesterolemic effect in rats (Sugano et al., 1989).

In the studies of bacterial cultures, egg with lower cholesterol levels in the yolk were produced by feeding of a dried culture of *Bacillus subtilis*, which is the same strain as *Bacillus natto* (Xu et al., 2006). It is known that probiotics in intestinal flora digest cholesterol for their own cell metabolism. Gilliland (1985) reported that microorganisms absorb cholesterol and cause a decrease in cholesterol in host animals. Fukushima and Nakano (1995) suggested that the serum cholesterol decrease in host animals fed probiotics was caused by inhibition of HMG-CoA reductase activities. Haddin et al. (1996) showed that yolk cholesterol levels decreased by feeding of 4×10^8 million living *Lactobacillus*/g diet for 48 week.

However, our results showed that the decrease in meat cholesterol level was found in females specifically, suggesting that natto components act as female hormones such as isoflavones with estrogen-like functions. Kishida et al. (2006) reported that dietary isoflavone-rich fermented soybean extract decreased the serum cholesterol concentrations in female rats but did not affect the concentrations in male rats. Furthermore, in accordance with the deglycosylation of isoflavone glycosides, the estrogenic activity of black soymilk by *Bacillus natto* on the ERβ estrogen receptor increased threefold (Kuo et al., 2006). The meat cholesterol lowering effect of natto requires further study.

Recently, it has been well-shown that bioactive peptides, which possess diverse and unique health benefits including the prevention of age-related chronic disorders, such as cardiovascular disease, cancer, obesity, and decreased immune function, are produced by fermented soybeans in human diets (Mejia and De Lumen, 2006). In the poultry industry, the supplementation of probiotics has recently attracted interest from the point of poultry health. Antibiotics are in widespread use to prevent poultry pathogens and disease so as 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 balance (Fuller, 1989), and is recommended as an effective alternative to antibiotics (Sissons, 1989). Since it is possible that natto, which contains bioactive peptides and living *Bacillus natto* as a probiotic for poultry, we are also investigating the immunomoduratory effects of natto from the point of view of animal health.

ACKNOWLEDGMENTS

We are grateful to the manager of Takano Foods Co. Ltd., Ken-Ichiro Kaneko for providing fermented soybean products. We wish to thank Mr. Kazuo Namai of the Laboratory of Poultry, Ibaraki Prefectural Livestock Research Center.

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