

Reuse of Expired Natto (Fermented Soybeans) as an Animal Feed

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Japan's low rate of food self-sufficiency—particularly in terms of its livestock industry—is a cause for concern. In addition, Japan annually generates food waste equivalent to 20% of the food consumed. Many producers of natto—a fermented soybean product with high nutritional benefits—face difficulties in disposing of expired products or surplus stock. To address these issues of food self-sufficiency and food waste disposal, we have been exploring the possibility of adapting unused natto as a feed for swine and poultry. Here, we prepared dried natto (DN) from commercial expired natto and investigated its suitability as a feed supplement for layer chickens.

In preliminary studies, we confirmed that feed supplemented with 3% DN had adequate palatability for feeding to chickens. This study revealed that the egg productivity and feed conversion efficiency of chickens that received fed the diet with 3% DN did not differ significantly from those of a control group. In addition, DN supplementation did not affect various measures of egg quality, including shell strength and freshness, or alter the concentrations of isoflavones and vitamin E in eggs. However, yolk concentrations of vitamin K and cholesterol decreased after supplementation with DN; the water-insoluble fraction of DN caused the decrease in cholesterol. These results suggest that expired natto can be used as a feed supplement with decent effects on egg quality. Further study is needed to evaluate the reusability of various food wastes to build a worldwide food-recycling society.

Key words: cholesterol, feed, fermented soybean, layer chicken, natto

Introduction

Since the beginning of the high-growth period, Japan has imported much of its food. Therefore, Japan's food self-sufficiency rate remains at approximately 40%, and its livestock feed self-sufficiency fell dramatically from 47% in 1965 to 17% in 2005 (Ministry of Agriculture, Forestry and Fisheries, 2013). The rate of self-sufficiency in concentrated animal feeds is currently approximately 12% overall, with rates of 11% for wheat, 0% for corn, and 6% for soybeans in 2009 (Nakata, 2011). Additionally, there is an indicator of a domestic food supply, which is termed as "food miles". This is computed by multiplying the quantity of food transported (tonnes) by the transporta-

tion distance (km). Japan's food miles are by far the highest in the world: the total food miles for soybeans was 20.5 billion ton·km in 1960 but had increased to 67.3 billion ton·km by 2010 (Nakata, 2011).

Recently, lifestyle changes based on large-scale production and consumption have led to the sharp increase in food waste. Food waste is created at various stages of production. For example, soybean curd and breadcrumbs are regarded as food waste in food manufacturing. Surplus stock is accumulated during wholesale storage. Expired and unsold food products, such as boxed lunches and deli items, are accumulated as food waste by retail stores. Cooked waste and leftover foods are accumulated by restaurants. Japanese government survey say as follow. Approximately 18 mil-

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lion tons of food waste—an amount equivalent to 20% of all foods consumed—is generated annually in Japan. Food waste in the form of surplus stock, expired foods, and leftovers (so-called “food loss”) accounts for 5 to 8 million tonnes of this figure each year—an amount nearly equal to the total annual rice yield in Japan (8.5 million tonnes in 2012). To reduce and recycle various food wastes, Japan established a food recycling law in 2001, and target levels of 10% food waste reduction by August 2015 were established for 31 industry sectors. Currently, the recycling rate of food waste from food processing is relatively high (38%), but that from restaurants is very low (4%) (Ministry of the Environment Japan, 2013).

Preparation of livestock feed from food waste is considered superior to composting of the waste, because selling price of livestock feed is higher than that of compost. To increase the reuse rate of food wastes, however, candidate feedstuffs must be selected carefully. Therefore, information regarding food waste—including its ingredients, forage quality, and safety—is needed. If the food waste contains useful quantities of functional ingredients, the feed prepared from it can be marketed as a high value-added feed to increase the additional value of livestock products.

To create a food-recycling system, each local resource should be used effectively in terms of food miles. We have been studying the effective use of crop and food-processing wastes in our local area of Ibaraki Prefecture, North Kanto, Japan. This region is known for hoshi-imo (dried sweet potato) and natto (fermented soybeans). Natto is produced by using the

Gram-positive spore-forming bacterium *Bacillus subtilis* var. *natto* (*B. natto*) to ferment soybeans (Ashiuchi *et al.*, 1998). The nutritional benefits of soybeans include their abundance of lysine (in which plant proteins typically are poor), and high functional ingredients such as isoflavones and nattokinase. In addition, natto is rich in fiber, carbohydrates, vitamins, and minerals.

To translate unused natto as a feedstuff, we have been studying its effect on the productivity and quality of chicken meat (Fujiwara *et al.*, 2008). In this study, we investigated on the reusability of natto, obtained as surplus stock, as a supplement for livestock feed and describe the effects of dried natto on egg production and quality.

Materials and Methods

Preparation of dried natto

Commercial natto 7 days past its expiration date was obtained from Takano Food Co. Ltd. (Ibaraki, Japan). The natto was dried in a drying oven at 60°C for 60 h, then ground into 1-mm particles by using a feed mill (Figure 1). The resulting dried natto (DN) was stored at 4°C until use. It was confirmed using nutrient agar media that viable bacteria in DN contained more than 1×10^9 cfu/g equivalent to that in natto before drying. To fractionate DN into its water-soluble and -insoluble fractions, DN was dispersed in 10 volumes of distilled water for 24 h. After filtration over filter paper (No. 2, ADVANTEC, Tokyo, Japan), the filtrate was freeze-dried and stored in a refrigerator as the water-soluble fraction of DN. The residue was treated with acetone,

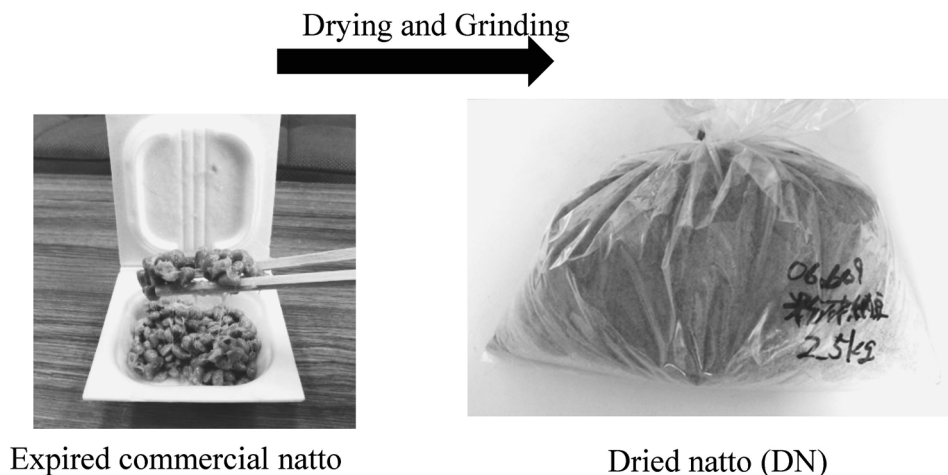


Fig. 1. Preparation of dried natto for layer chickens.

after which the acetone powder was recovered as the water-insoluble fraction of DN.

Nutritional composition analysis of natto

The chemical composition of untreated natto and DN, including proportions of moisture, crude protein, crude fat, crude fiber, and crude ash, was determined (AOAC, 2012). Briefly, moisture content was measured by using the oven-drying method at 135°C. Crude protein was determined by the Kjeldahl method of nitrogen analysis, and crude fat was determined by measuring the ethyl ether extract content using the Soxhlet extraction method. Crude ash content was measured as the weight of the minerals and other inorganic matter remaining after incineration at 550°C. Carbohydrate content was calculated as the nitrogen-free extract as follows: 100% – (crude protein + crude fat + crude ash + crude fiber). The gross energy of natto was determined the total amount of energy that the natto contained.

Animals and test conditions for feeding

To investigate egg productivity and quality, 23-week-old layer chickens (Rhode Island Red) were housed in groups of 20 and were randomly assigned to receive a DN-unsupplemented diet (basic diet alone, Layer 17S; Chubu Shiryo Co. Ltd., Aichi, Japan) or a basic diet with DN supplementation (DN-supplemented diet) for 3 months. To confirm the yolk cholesterol-lowering effect on the other chicken breed, groups ($n = 16$ each) of 27-week-old layer chickens (White Leghorn) were fed a basic diet (Pureiya-tu; Toyohashi Shiryo Co. Ltd., Aichi) with or without natto supplementation for 1 month. During the experiments, the chickens had unrestricted access to feed and water. Daily egg production in each group was calculated as the total number of eggs collected divided by the total number of layer chickens. The feed conversion rate in each group was calculated as the weight of feed consumption divided by the weight of eggs produced. All procedures were approved by the guidelines of the Ibaraki University Animal Care and Use Committee.

Evaluation of egg quality

Egg weight was assessed as a basic property of eggs in the shell. Eggshell strength was tested by using an eggshell force gauge (Model 2; Robotmation, Tokyo). Egg freshness was reported in Haugh units and determined through the measurement of albumen height and

egg weight by using an egg multi-tester (EMC500C; Robotmation). Yolk color was evaluated according to the numbers on a Roche yolk color fan. Isoflavones in yolk were extracted by using 0.5 M hydrochloric acid and ethanol (Collison, 2008). α -Tocopherol in yolk was extracted as vitamin E by using a hexane-ethyl acetate mixture, followed by saponification with alkaline pyrogallol (Chase *et al.*, 1999). Menaquinone was extracted from yolk by using n-hexane (Kamao *et al.*, 2005). The concentrations of these compounds were determined through chromatographic separation by using an HPLC system (Chromaster; Hitachi High-technologies, Tokyo, Japan). Cholesterol in yolk was extracted with isopropanol after saponification with alkaline isopropanol and measured by using an enzymatic colorimetric assay kit (F-kit Cholesterol; Roche Diagnostics, Basel, Switzerland) according to the manufacturer's instructions.

Statistics

All data were subjected to 1-way ANOVA test using Microsoft Excel 2010.

Results

Nutritional profile of DN and its effect on egg production and quality

In terms of chemical composition, the moisture content of DN was only 5%, which was at most one-tenth that of untreated natto (Table 1). In addition, the crude protein, crude fat, crude fiber, and carbohydrate contents of DN were highly enriched by the drying of untreated natto. The gross energy of DN was approximately twice that of untreated natto. Dietary supplementation with 3% DN led to no significant differences in egg production or feed conversion rate compared with those of Rhode Island Red layers fed a DN-unsupplemented diet (Table 2). Egg weight, eggshell strength, and yolk color, and egg freshness of the DN-supplemented group were not significantly different from those of the control group.

Effect of feed supplementation with dried natto on various components in egg yolk

The isoflavone content in yolk, calculated as the sum of the amounts of daidzein, genistein, and glycitein, differed significantly between the control and DN-supplemented groups (Figure 2). DN supplementation for 3 months did not alter the vitamin E concentration in yolk but significantly decreased the levels

Table 1. Nutritional composition of natto before and after drying at 60°C for 60h

| | Before drying | After drying |
|------------------------|---------------|--------------|
| Moisture (%) | 59.5 | 5.3 |
| Crude protein (%) | 16.5 | 39.9 |
| Crude fat (%) | 10.1 | 22.0 |
| Crude fiber (%) | 6.7 | 12.8 |
| Carbohydrate (%) | 7.2 | 19.9 |
| Gross energy (Mcal/kg) | 2548 | 5864 |

The tested material was commercial natto that was 7 days past its expiration date.

Table 2. Egg production and egg-quality measures with and without dietary supplementation with dried natto (DN)

| | Without DN | | With DN | |
|---|------------|-------|---------|-------|
| | Before | After | Before | After |
| Egg production (%) | 88.6 | 90.0 | 84.3 | 85.7 |
| Feed conversion rate (kg/kg egg) | 2.30 | 2.00 | 2.29 | 2.17 |
| Egg weight (g) | 47.9 | 54.3 | 47.3 | 52.8 |
| Eggshell strength (kg/cm ²) | 3.29 | 3.38 | 3.18 | 3.22 |
| Yolk color | 12.0 | 12.4 | 12.1 | 12.9 |
| Egg freshness | 88.0 | 83.9 | 88.8 | 85.0 |

For 3 months, layer chickens received a control diet or a diet supplemented with 3% DN. Test parameters were assessed just before and immediately after the 3-month feeding period.

of vitamin K and cholesterol (Figure 3).

To confirm the effect of DN on yolk cholesterol, we repeated the feeding test by using a different species of layer (White Leghorn) and several levels of DN supplementation (Figure 4). Yolk cholesterol decreased dose-dependently with DN supplementation, with a significant difference in yolk cholesterol concentration between 0% (basic diet alone) and 3% DN supplementation. Furthermore, supplementation with the water-insoluble DN fraction caused the same decrease in yolk cholesterol concentration as did supplementation with total DN.

Discussion

Natto is a Japanese food with health benefits. On the assumption that feeding surplus fermented soybean stock would incur no obvious health problems in layer chickens, we investigated the effect of feed supplementation with expired natto on egg productivity and

quality. We found that DN supplementation did not alter egg production by layers or the egg characteristics of shell strength, yolk color, and freshness. Thus, supplementation with 3% DN for 3 months did not increase these measures of egg quality in this study, suggesting that increased amounts of DN or prolonged feeding periods (or both) are required to achieve these effects.

It is known that some dietary components are transferred directly into meat and eggs. We found here that supplementation with DN did not alter isoflavone (daizein, genistein, and glycitein) or vitamin E concentrations in yolk but significantly reduced yolk vitamin K levels. In contrast to these findings, isoflavones (Suzuki and Okamoto, 1997), α -tocopherol (Grobas *et al.*, 2002), and menaquinone-7 (Saitoh *et al.*, 2001) reportedly are readily transferred into yolk. In addition, the observation that DN supplementation reduced vitamin K levels suggests that the vitamin K

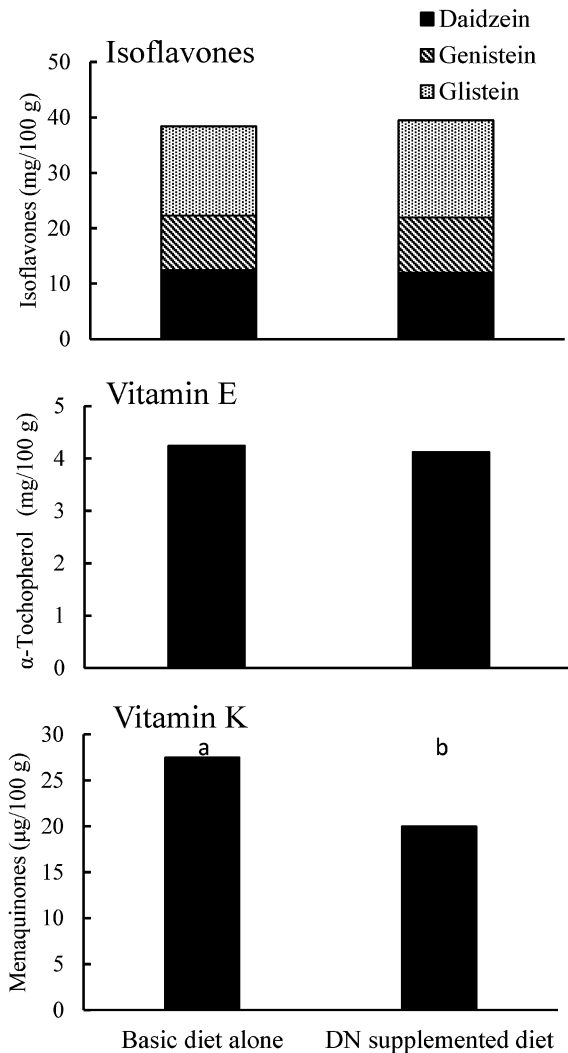


Fig. 2. Effects of dried natto (DN) supplementation on various nutrients in egg yolk. Basic diet alone, layer chickens were fed the basic diet without supplementation with dried natto. DN supplemented diet, layer chickens were fed the basic diet supplemented with 3% dried natto. Data are shown as means ($n=3$). Mean values with different letters are significantly different ($p<0.05$).

concentration in yolk is regulated by homeostatic control in spite of the excessive intake of natto, which is rich in vitamin K. Further study of the transfer of these components from natto to egg yolk is needed. In addition, DN supplementation reduced the cholesterol level in egg yolk; the same effect was achieved by using the water-insoluble fraction of DN, which included the fat, protein, and fiber derived from soybean or *B. natto* and their metabolic products. When dietary cholesterol intake increases, plasma cho-

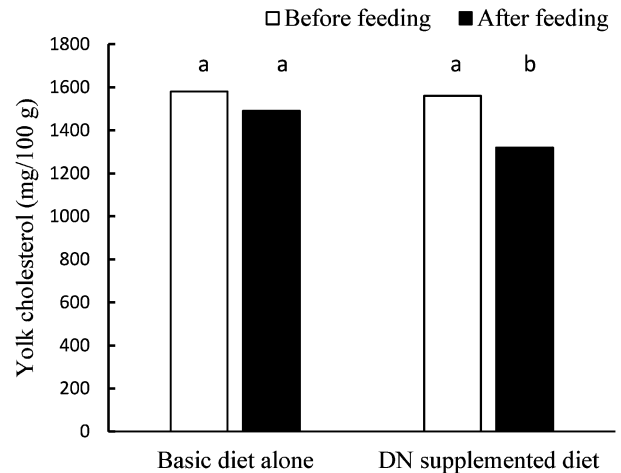


Fig. 3. Effects of dried natto (DN) supplementation on yolk cholesterol concentration. Basic diet alone, layer chickens were fed the basic diet without supplementation with dried natto. DN supplemented diet, layer chickens were fed the basic diet supplemented with 3% dried natto. Before feeding, Immediately before feeding the control or test diet. After feeding, After 3 months of feeding the control or test diet. Data are shown as means ($n=3$). Mean values with different letters are significantly different ($p<0.05$).

lesterol levels rise, and the risk of cardiovascular diseases and atherosclerosis increases (Connor and Connor, 2002). For this reason, low-cholesterol foods attract higher consumer demand than do conventional products.

Some beneficial bacteria (so-called “probiotics”), when administered in sufficient amounts, confer health benefits on the host (FAO/WHO, 2001). Part of the recent interest in probiotics may reflect their improvement of lipid metabolism (Endo *et al.*, 1999; Santoso *et al.*, 1995). In fact, the feeding of probiotics to chickens is known to lower yolk cholesterol levels (Mohan *et al.*, 1995; Abdulrahim *et al.*, 1996; Haddadim *et al.*, 1996), and feeding *B. subtilis* cultures to chickens decreases yolk cholesterol content (Xu *et al.*, 2006). According to theory, probiotics facilitate the digestion of cholesterol by enteric microorganisms. In this regard, Gilliland *et al.* (1985) reported that the ingestion of cholesterol by microorganisms decreased cholesterol levels in the feces. Fukushima and Nakano (1995) suggested that ingestion of probiotics leads to the inhibition of HMG-CoA reductase,

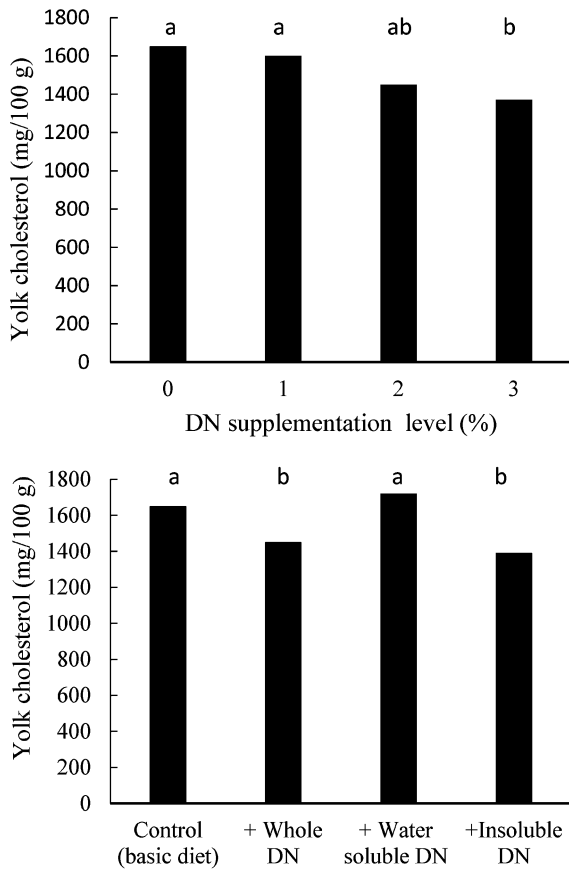


Fig. 4. Effects of supplementation with dried natto (DN) and its fractions on yolk cholesterol concentration.

Upper panel: Layer chickens were fed a basic diet supplemented with 0% to 3% dried natto for 1 month. Lower panel: Control, basic diet alone; + Whole DN, basic diet supplemented with 3% unfractionated dried natto; + Water-soluble DN, basic diet supplemented with 3% dried natto (water-soluble fraction only); + Insoluble DN, basic diet supplemented with 3% dried natto (water-insoluble fraction only). Data are shown as means. Mean values with different letters are significantly different ($p < 0.05$).

a rate-controlling enzyme of cholesterol synthesis, and subsequently decreases cholesterol concentrations. Supplementation with *Lactobacillus* is also considered a yolk-cholesterol-lowering tactic (Haddadin *et al.*, 1996).

In the animal industry, excessive use of antibiotics can lead to microbial resistance (Sorum and Sunde, 2001), imbalance in intestinal flora (Andremont, 2000), and retention of veterinary antibiotics in the food chain (Burgat, 1991). In this point, ingested probiotics

benefit host animals by improving the intestinal environment (Fuller, 1989). It was reported that adding dried *B. subtilis* to the diets of layers increases egg weight (Xu *et al.*, 2006) and dietary supplementation with *Lactobacillus* increases eggshell thickness (Nahashon *et al.*, 1994; Mohan *et al.* (1995). In addition, feeding of probiotics increases meat productivity and feed conversion ratios in broiler chickens (Cavazzoni *et al.*, 1998; Santoso *et al.*, 1995). By extension, fermentation products containing probiotics might constitute an excellent feed supplement to promote animal productivity and health. In this regard, Kiers *et al.* (2003) reported that feed supplementation with *B. subtilis*-fermented soybeans increased the body weight of piglets. In addition, we previously reported that DN supplementation modulated immune function in chickens, suggesting that natto might be useful as a feed additive (Seo *et al.*, 2015).

Fermented soybean products have been manufactured as traditional foods in Asia since ancient times. These foods include Japanese shoyu (soy sauce), miso (fermented soybean paste), and tofuyo (Okinawa-style fermented tofu); Chinese tobanjan; and Korean doenjang and cheonggukjang (Hong *et al.*, 2004; Mine *et al.*, 2005). Among other activities, the production of these and other foodstuffs and the dumping of expired products create massive amounts of food waste. To create a sustainable society, we must identify effective uses of various food wastes.

Conclusion

Produced through fermentation with *B. natto*, the soybean product natto has many nutritional benefits. Food waste equivalent to 20% of the total food consumed is produced annually in Japan. Considering that our local region of Ibaraki Prefecture is a natto-producing district, we investigated the suitability of DN as a feed supplement for layer chickens. Egg productivity, feed conversion ratio, and various markers of egg quality did not differ significantly between DN-supplemented and control layer chickens. Whereas DN supplementation did not alter the concentrations of isoflavones and vitamin E in eggs, it reduced the levels of vitamin K and cholesterol in yolk; the cholesterol-lowering effect was due to the water-insoluble fraction of DN. These results suggest that expired natto might be useful as a feed supplement that influences some aspects of egg quality.

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