

Effects of Dietary Supplementation of Fermented Rice Bran (FRB) or Fermented Broken Rice (FBR) on Laying Performance, Egg Quality, Blood Parameter, and Cholesterol in Egg Yolk of Hy-Line Brown Laying Hens

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ABSTRACT This experiment was aimed at investigating the effects of dietary supplementation with fermented rice bran (FRB) or fermented broken rice (FBR) on egg-laying performance, egg quality, blood parameters, and cholesterol level in egg yolk of Hy-Line Brown egg-laying hens. Altogether, 144 Hy-Line Brown egg-laying hens (32-week-old) were randomly allocated to one of 4 dietary treatment groups, with 4 replicates per treatment. Of them, 3 treatments diets were prepared by supplementing the basal diet with 0.1% probiotics (PRO), 1% fermented rice bran (FRB), or 1% fermented broken rice (FBR) at the expense of corn. Hen-day egg production was higher ($P<0.05$) in PRO and FRB treatment groups than in the basal treatment groups. However, feed intake, egg weight, egg mass, and feed conversion ratio did not differ among the treatment groups. Additionally, supplementation with FRB or FBR did not affect eggshell strength, eggshell thickness, egg yolk color, and Haugh unit during the feeding trial. There was no significant difference in leukocyte count. Total cholesterol level was lower ($P<0.05$) in the FRB treatment group than in the basal treatment groups. Aspartate aminotransferase, alanine transferase, glucose, and albumin levels were unaffected by dietary supplementation with FRB or FBR. Egg yolk cholesterol level was lower ($P<0.05$) in the FRB and FBR treatment groups than in the basal treatment groups. In conclusion, dietary supplementation with FRB or FBR improved egg-laying performance, and reduced the levels of total serum cholesterol and cholesterol in egg yolk of Hy-Line Brown egg-laying hens.

(Key words: Hy-Line Brown laying hens, laying performance, fermented rice bran, fermented broken rice, total cholesterol)

INTRODUCTION

Rice bran is a major cereal by-product, which is widely used in rice-producing countries as a feed ingredient in layer diets. It contains considerable amounts of fat, protein, metabolizable energy and a good source of B-group vitamins (Kratzer et al., 1974; Warren and Farrell, 1990; Rezaei, 2006). Broken rice kernels have normally only half of the value of whole or head rice. The head rice yield, i.e., the weight percentage of whole kernels remaining after milling is one of the most important physical characteristics that determines rice quality (Van Dalen, 2004). Among these, broken rice separated out after the polishing stage has the same chemical composition as polished rice; even though the quantities available are not very high, this by-product is a palatable, energy-rich and easily utilized feed. Another characteristic of this by-product is that it has low-protein content. Its protein is richer in lysine than that of other major cereals (De Marco et al., 2014). Therefore,

it is a common feed supplement in animal production, including poultry (Mu et al., 2011). Previous research on the supplementation of rice bran to broiler chicken fed has shown to improve growth performance (Maust et al., 1972; Zombade et al., 1982). In addition, studies on the usefulness of fermented rice bran (FRB) and fermented broken rice (FBR) for improving growth are scarce. However, the response of animals to feed containing FRB or FBR with respect to laying performance, blood parameters, and cholesterol in egg yolk has yet to be described. The fermentation of materials with microbial inoculums has been widely adopted to develop novel functional ingredients because this process may promote their functional quality such as antioxidant (Lee et al., 2008; Dini, 2010; Wang et al., 2011; Cao et al., 2012; Kim et al., 2012). This study aimed to determine whether the dietary supplementation of FRB or FBR to laying hens feed affects laying performance, egg quality, blood parameter, and cholesterol in egg yolk. Such information is expected to confirm the utility

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of FRB or FBR as a dietary supplement for Hy-Line Brown laying hens.

MATERIALS AND METHODS

The protocol for this experiment was reviewed and approved by the Institute Animal Care and Welfare Committee of the National Institute of Animal Science, Rural Development Administration, Republic of Korea.

1. Preparation of *Lactobacillus*-fermented Rice Bran or Broken Rice

Rice bran and broken rice harvested in 2015 were obtained from a rice processing complex (Seonghwan-eup, South Korea). Strains of microbes-*Lactobacillus plantarum* KCTC 1048 (Korean Collection for Type Culture, Daejeon, Korea) was obtained and used to ferment rice bran in this experiment. A 2-mL aliquot of *Lactobacillus* strain with 10^6 cfu/mL viable counts, was cultured in medium (1 L) containing 10 g de Man, Rogosa, and Sharpe broth (Difco Laboratories, Francisco Soria Melguizo S. A., Madrid, Spain) and 1 L of distilled water. The mixture was then incubated at 36°C for 24 h. For fermentation, 4 kg of dried rice bran was inoculated with 5 L of prepared *Lactobacillus* inoculums in a fermentation vessel, and incubated at 36°C with periodic mixing for 24 h. At the end of the fermentation, fermented rice bran was dried at 60°C to contain approximately 76~77% dry matter (DM), and was subsequently used for the feeding trial. The final concentrations of *Lactobacillus* in the probiotic products were approximately 10^8 cfu/g fermented product.

The nutrient compositions of FRB and FBR were analyzed in duplicate for DM, crude ash, crude protein, and crude fiber (AOAC, 1990). The results are presented in Table 1.

2. Birds and Experimental Design

A total 144, 32-week old Hy-Line Brown laying hens were randomly allotted to 1 of 4 dietary treatments. Each treatment had 4 replicated with 3 cages and 3 hens per cage (36 cm × 40 cm × 42 cm = width × length × height) in each replication. A commercial type basal diet was formulated to meet or exceed nutrient recommendations of the National Research Council (1994) for laying hens (Table 2). Three treatments

Table 1. Analyzed composition of fermented rice bran (FRB) and fermented broken rice (FBR) (DM-basis)

Composition (g/kg)	FRB ¹	FBR ²
DM	774.0	769.5
Crude ash	70.0	2.4
Crude protein	136.0	56.6
Crude fiber	71.0	3.0

¹ *Lactobacillus*-fermented rice bran.

² *Lactobacillus*-fermented broken rice.

diets were prepared by supplementing 0.1% probiotics (PRO) that contained a mixture of *Lactobacillus reuteri*, 1% fermented rice bran (FRB), or 1% fermented broken rice (FBR) to the basal diet at the expense of corn. The experimental period was 8 weeks. During the experiment, hens were provided with feed and water ad libitum and were exposed to 16 h:8 h light: dark lighting schedule. The temperature and humidity of the laying house was maintained at 18±3°C and 65~70%, respectively, during the experiment.

3. Laying Performance

Hen-day egg production rate, oviposition rate, broken egg production rate and egg weight were recorded daily, whereas feed intake and the feed conversion ratio were recorded weekly. Egg mass was calculated as per Hayat et al. (2009).

$$\text{Egg mass} = \text{Weekly number of eggs in a replicate} \\ \times \text{Average egg weight}$$

4. Determination of Egg Quality Parameter

Ten eggs per replicate were randomly collected at the end of every week. Eggshell strength, eggshell thickness, egg yolk colour, and Haugh units (HU) were measured. Eggshell strength was measured by the Texture Systems Compression Test Cell (model T2100C, Food Technology Co., Ltd., Rockville, MD, USA) and expressed as units of compression force exposed to units of eggshell surface area (kg/cm²). Egg shell thickness is defined as the mean value of measurements at 3 different locations on the egg (air cell, equator, and sharp end) and was measured with a dial pipe gauge (model 7360, Mitutoyo Co. Ltd., Kawasaki, Japan) and calculated using the

Table 2. Composition and nutrient content of experimental diet (as-fed basis)

Item		Basal	PRO	FRB	FBR
Ingredients (g/kg)	Corn	595.3	594.3	585.3	585.3
	Soybean meal	224.9	224.9	224.9	224.9
	Wheat	40.0	40.0	40.0	40.0
	Limestone	98.5	98.5	98.5	98.5
	Tallow	14.0	14.0	14.0	14.0
	Mono dicalcium phosphate	19.0	19.0	19.0	19.0
	DL-methionine (99%)	2.4	2.4	2.4	2.4
	Lysin-HCl (78%)	1.4	1.4	1.4	1.4
	Salt	2.5	2.5	2.5	2.5
	Vitamin-mineral premix ¹	2.0	2.0	2.0	2.0
	Probitocis (PRO)	-	1.0	-	-
	<i>Lactobacillus</i> fermented rice bran (FRB)	-	-	10.0	-
	<i>Lactobacillus</i> fermented half crushed rice (FBR)	-	-	-	10.0
	Total		1,000.0	1,000.0	1,000.0
Energy and nutrient content ²	ME _n (MJ/kg)	11.66	11.66	11.66	11.66
	Crude protein (g/kg)	164.2	164.2	164.2	164.2
	Calcium (g/kg)	39.8	39.8	39.8	39.8
	Available P (g/kg)	7.2	7.2	7.2	7.2
	Lysine (g/kg)	9.5	9.5	9.5	9.5
	Methionine + cysteine (g/kg)	7.8	7.8	7.8	7.8

¹ Provided per kilogram of the complete diet: vitamin A (from vitamin A acetate), 12,500 IU; vitamin D₃, 2,500 IU; vitamin E (from DL- α -tocopheryl acetate), 20 IU; vitamin K₃, 2 mg; vitamin B₂, 5 mg; vitamin B₆, 3 mg; vitamin B₁₂, 18 μ g; calcium pantothenate, 8 mg; folic acid, 1 mg; biotin, 50 μ g; niacin, 24 mg; Zn (as ZnO), 60 mg; Mn (as MnSO₂ · H₂O), 50 mg; Fe (as FeSO₄ · 7H₂O), 50 mg; Cu (as CuSO₄ · 5H₂O), 6 mg; Co (as CoCO₃), 250 μ g; I [as Ca(IO₃)₂ · H₂O], 1 mg; Se (as Na₂SeO₃), 150 μ g.

² Nutrient contents in all diet were calculated were analyzed.

following equation:

$$\text{Eggshell thickness} = (\text{Sharp point thickness} + \text{Equator point thickness} + \text{Air cell thickness})/3$$

Egg yolk colour was evaluated by the Roche Yolk Color Fan (Hoffman-La Roche Ltd., Basel, Switzerland; 15 = dark orange; 1 = light pale). Hough unit values were calculated using a micrometer (model S-8400, Ames, Waltham, MA, USA) with the following equation described by Eisen et al. (1962):

$$\text{HU} = 100 \log (H - 1.7 W^{0.37} + 7.6)$$

where W is egg weight, and H is albumen height.

5. Sample Collection

At the end of the 8 week feeding trial, one bird with a BW close to the pen BW (i.e., 8 birds per treatment) was selected and killed by cervical dislocation. Immediately after death, a 5 mL blood sample was collected from the jugular vein of each bird using EDTA vacuum tubes (Becton Dickinson, Franklin Lakes, NJ), which were then stored on ice and subjected to immediate haematological analysis. Leukocytes: white blood cells, heterophils, lymphocytes, monocytes, eosinophils, and basophils were quantified using Hemavet Multispecies Hema-

tology systems (Drew Scientific Inc., Oxford, CT). The H:L ratios were determined by dividing the number of heterophils by that of lymphocytes. Serum samples obtained by centrifuging the samples for 20 min at 25,000 ×g and 4°C, were stored at -15°C. Total cholesterol, triglyceride, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and calcium in the serum were quantified using an ADVIA 1650 chemistry system (Bayer Diagnostic, Putraux, France).

6. Determination of Egg Yolk Cholesterol Level

Egg yolk cholesterol level was measured by using 40 eggs (10 eggs from each treatment) collected in the last week of the experiment. A sample of 2~3 eggs from each replicate was used for cholesterol quantification. Egg yolks were completely separated from the albumen, adhering white and chalazae; then weighted, pooled and mixed. The cholesterol content of egg yolk was determined following colorimetric method based on Liebermann-Burchard color reaction as described by Huang et al. (1961). Briefly, chloroform:methanol (2:1 v/v) solvent was used to extract total lipids from egg yolk. The harvested extracts, which contain free cholesterol and cholesterol esters, were allowed to react with acetic anhydride and concentrated sulfuric acid, resulting in the formation of a blue-green complex. Egg yolk cholesterol content was quantified by comparing the color absorbance at 550 nm resulting from the Liebermann-Buchard reactions in egg yolk lipid extracts with cholesterol standards (Cholesterol reagent, Gainland Chemical Company, UK). All the reading were blanked against a chloroform: methanol.

7. Statistical Analysis

All data were analyzed by one-way ANOVA as a completely randomized design using the PROC GLM procedure (SAS Institute Inc., Cary, NC). Each replicate was considered an experimental unit for the analysis of laying performance, blood parameter, and egg cholesterol. Outlier data were examined according to the method of Steel et al. (1997) using the UNIVARIATE procedure of SAS; however, no outliers were identified. The LSMEANS square means were separated using Fisher's LSD (Ott and Longnecker, 2009) and significance was set at $P \leq 0.05$ and $0.05 \leq P \leq 0.10$.

RESULTS AND DISCUSSION

Hen-day egg production was greater ($P < 0.05$) for PRO and FRB treatment groups than for the basal treatments. However, feed intake, egg weight, egg mass and feed conversion ratio did not differ among the treatments. Dietary supplementation of fermented rice bran has been reported improve BW gain and feed efficiency in broilers and pigs (Chu et al., 2011; Kang et al., 2015; Supriyati et al., 2015). The positive effects of fermented rice bran on growth performance of broiler chicken may be due to its high concentrations of protein, vitamin, minerals, complex carbohydrates, phytonutrients, phospholipids, essential fatty acids and more than 120 antioxidants (Saunders, 1985; Warren and Farrell, 1990; Ryan et al., 2011). To our knowledge, however, there have been no products of rice bran or half crushed rice on laying performance of birds, and therefore, it is difficult to compare previous data with those determined in this experiment. It is suggested, however, that fermentation processes of plant materials are able to elevate the efficacy of their antioxidant and anti-inflammatory properties to a level greater than that in the raw materials (Lee et al., 2008; Wang et al., 2011; Kim et al., 2012). In addition, fermented products were more palatable compared to the original materials as fermentation could produce preferred water soluble vitamin such as B₁, B₂ and B₁₂, and minerals (Kubad et al., 1997). This may be also the reason why inclusion of relatively small amounts (10 g/kg) of FRB or FBR in diets showed significant positive effects on laying performance of birds in this experiment. *Lactobacillus* spp. have been widely appreciated as potential probiotic bacteria and dietary supplementation of *Lactobacillus* spp. have been reported to improve growth performance of birds (Panda et al., 2006). There is also the implication that dead or inactive probiotic bacteria may improve performance and the health status of animals, possibly via similar mechanisms operating in animals fed viable probiotic bacteria (Wagner et al., 2000; Huang et al., 2004).

However, the supplementation of FRB or FBR did not have an effect on eggshell strength, eggshell thickness, egg yolk color, and HU during the feeding trial (Table 4). The lacking adequate data on the influence of supplementation with FRB

Table 3. Laying performance of laying hens fed the diet containing probiotics, fermented rice bran and fermented broken rice¹

Items	Dietary treatments ²				SEM ³	p-value
	Basal	PRO	FRB	FBR		
Hen-day egg production (%)	85.3 ^b	87.1 ^a	86.8 ^a	86.3 ^{ab}	0.68	0.04
Feed intake (g)	122.9	122.3	125.2	124.2	1.47	0.25
Egg weight (g)	63.2	62.7	62.4	62.9	0.60	0.32
Egg mass	53.9	54.6	54.2	54.3	0.92	0.12
Feed conversion ratio	2.28	2.24	2.31	2.29	0.05	0.18

^{a,b} Values in a row with no common superscripts letter are significantly different ($P < 0.05$).

¹ Data are least squares means of 4 observations per treatments.

² Basal = basal diet; PRO = basal diet + 1 g/kg probiotics; FRB = basal diet + 10 g/kg *Lactobacillus*-fermented rice bran; FBR = basal diet + 10 g/kg *Lactobacillus*-fermented broken rice.

³ Pooled standard error of the means.

Table 4. Egg quality of laying hens fed the diet containing probiotics, fermented rice bran and fermented broken rice¹

Items	Dietary treatments ²				SEM ³	p-value
	Basal	PRO	FRB	FBR		
Eggshell strength (kg/cm ²)	3.94	3.42	3.80	3.92	0.28	0.28
Eggshell thickness (µm)	408.60	401.00	401.30	400.00	8.48	0.35
Egg yolk color	7.56	7.97	7.80	7.78	0.28	0.13
Haugh unit	86.90	91.8	89.90	85.50	3.03	0.29

¹ Data are least squares means of 4 observations per treatments.

² Basal = basal diet; PRO = basal diet + 1 g/kg probiotics; FRB = basal diet + 10 g/kg *Lactobacillus*-fermented rice bran; FBR = basal diet + 10 g/kg *Lactobacillus*-fermented broken rice.

³ Pooled standard error of the means.

or FBR to laying hens feed on egg quality of poultry requires further research.

Table 5 presents the concentrations of leukocytes in the whole blood. There were no significant differences in the level of leukocyte. Blood parameters are good indicators of physiological, pathological and nutritional status of an animal and changes in hematological parameters have the potential of being used to elucidate the impact of nutritional factors and additives supplied in diet on any living creature. For example, leukocytes are known increase sharply when infection occurs, as they are one of the first lines of defence of the body (Ganong, 1999; Alzawqari et al., 2011; Masoudi et al., 2011). Leukocyte counts also have been used as a measure of immune function in birds (Johnson and Zuk, 1998). Many factors such as exposure to various microbes and che-

micals can cause changes in both granulocytic white blood cells (Lucas and Jamroz, 1961). The lack of adequate data on the influence of dietary supplementation of FRB or FBR of laying hens in altering blood parameters in poultry requires further research.

Total cholesterol was less ($P < 0.05$) for FRB treatment group than for the basal treatments. AST, ALT, glucose, and albumin were not affected by supplementation of FRB or FCR in diets. The supplemental fermented rice bran diets decreased the concentrations of total cholesterol in this experiment. This is in contrast with previous studies. Chu et al. (2011) and Kim et al. (2006) reported that an addition of fermented materials to the feed decreased total cholesterol in pigs. Kitawaki et al. (2009) reported that *Lacobacillus* spp. fermented soymilk and soy yogurt decreased the concentration of plasma

Table 5. Blood parameter of laying hens fed the diet containing probiotics, fermented rice bran and fermented broken rice¹

Items	Dietary treatments ²				SEM ³	p-value	
	Basal	PRO	FRB	FBR			
White blood cells (WBC)	6.83	11.48	12.85	6.05	1.85	0.65	
Heterophils (HE)	0.62	1.40	1.20	0.83	0.59	0.79	
Leukocytes (K/ μ L)	Lymphocytes (LY)	5.62	8.97	7.06	5.16	0.85	0.85
	Monocytes (MO)	0.55	1.01	1.29	0.75	0.23	0.69
	Eosinophils (EO)	0.05	0.08	0.04	0.03	0.05	0.36
	Basophils (BA)	0.01	0.01	0.03	0.01	0.85	0.35

¹ Data are least squares means of 4 observations per treatments.

² Basal = basal diet; PRO = basal diet + 1 g/kg probiotics; FRB = basal diet + 10 g/kg *Lactobacillus*-fermented rice bran; FBR = basal diet + 10 g/kg *Lactobacillus*-fermented broken rice.

³ Pooled standard error of the means.

Table 6. Blood biochemistry of laying hens fed the diet containing probiotics, fermented rice bran and fermented broken rice¹

Items	Dietary treatments ²				SEM ³	p-value
	Basal	PRO	FRB	FBR		
Total cholesterol (mg/dL)	128.2 ^x	113.4 ^{xy}	95.8 ^y	112.8 ^{xy}	8.50	0.04
Triglyceride (mg/dL)	1,126.2	1 011.3	1,165.6	1,198.3	10.22	0.28
Glucose (mg/dL)	189.7	191.6	191.0	191.1	6.89	0.32
Total protein (mg/dL)	6.06	5.61	5.42	5.86	0.63	0.45
AST (U/L)	187.5	176.4	188.3	169.1	12.69	0.43
ALT (U/L)	1.65	1.38	2.50	1.83	0.89	0.39

¹ Data are least squares means of 4 observations per treatments.

² Basal = basal diet; PRO = basal diet + 1 g/kg probiotics; FRB = basal diet + 10 g/kg *Lactobacillus*-fermented rice bran; FCR = basal diet + 10 g/kg *Lactobacillus*-fermented broken rice.

³ Pooled standard error of the means.

cholesterol because of the hydrophobic high molecular weight fraction produced by the enzymatic hydrolysis in rats. Xiao et al. (2003) reported that a dietary of milk products fermented by *Bifidobacterium longum* decreased the concentrations of total cholesterol and LDL-cholesterol in humans. Moreover, lactic acid bacteria have the capacity to assimilate and bind cholesterol (Hosono and Tonooka, 1995) which reduced serum total cholesterol due to inhibited absorption of bile acids in the intestine. Several mechanisms for cholesterol removal by probiotics have been proposed, such as assimilation of cholesterol into bacterial cell membranes (Kimoto et al., 2002), production of short-chain fatty acids (SCFAs) during the

growth of probiotics (Trautwein et al., 1998), and cholesterol conversion into coprostanol (Lye et al., 2010). These acids enter the small intestine, where they are absorbed and directed to the liver, and a decrease in bile acid recycling would ultimately result in a lowering of serum cholesterol concentration, because cholesterol is used for bile acid synthesis (St-Onge et al., 2000).

Egg yolk cholesterol was less ($P<0.05$) for FRB and FBR treatment groups than for the basal treatment. Recently, considerable attention has been paid to the potential of feed supplement in altering lipid metabolism. It was reported that supplementation of probiotics or natto in diets reduce chole-

Table 7. Egg cholesterol of laying hens fed the diet containing probiotics, fermented rice bran and fermented broken rice¹

Items	Dietary treatments ²				SEM ³	p-value
	Basal	PRO	FRB	FBR		
Egg cholesterol (mg/g)	15.94 ^a	14.94 ^{ab}	13.81 ^b	13.82 ^b	0.29	0.03

^{a,b} Values in a row with no common superscripts letter are significantly different ($P < 0.05$).

¹ Data are least squares means of 4 observations per treatments.

² Basal = basal diet; PRO = basal diet + 1 g/kg probiotics; FRB = basal diet + 10 g/kg *Lactobacillus*-fermented rice bran; FBR = basal diet + 10 g/kg *Lactobacillus*-fermented broken rice.

³ Pooled standard error of the means.

terol concentrations in egg yolk in laying hens (Mohan et al., 1995; Haddadin et al., 1996; Fujiwara et al., 2008). Our finding of the reduction in yolk cholesterol agrees with these results. 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 hydrolase activity in the intestine. Another reason for the decrease of cholesterol in probiotics-fed host, suggested by Fukushima and Nakano (1995), is that HMG-CoA reductase is the rate limiting enzyme for cholesterol synthesis and is regulated via a negative feedback mechanism mediated by sterols and non-sterol metabolites derived from mevalonate, the product of the reaction catalyzed by reductase.

CONCLUSION

The results of the current experiment indicate that the dietary supplementation of fermented rice bran or fermented half crushed rice improves the laying performance and reduces total cholesterol in the serum and cholesterol in egg yolk of Hy-Line Brown laying hens. It may be beneficial to supplement the diets of laying hens with fermented rice bran or fermented half crushed in the absence of probiotics.

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