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Effects of Graded Levels of Rice Bran Oil on Laying Performance, Blood Parameters and Egg Yolk Cholesterol in Hy-Line Laying Hens

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ABSTRACT This study aimed to determine the effect of different dietary level of rice bran extract (RBO) on the laying performance, egg quality, blood parameter, cholesterol, and fatty acids in yolk of Hy-Line Laying hens. In all, 144 Hy-Line Brown laying hens (29 weeks old) were randomly allocated to one of 4 dietary treatments, with 4 replicates per treatment. A commercial basal diet was used and three additional diets were prepared by supplementing 2.5, 5.0 or 10.0 g/kg of RBO to the basal diet. The experimental diets were fed on an *ad libitum* basis to the bird during 8 weeks. Hen-day egg production increased (quadratic, $p<0.05$) with inclusion level of RBO, but feed intake, egg weight, and egg mass were not influenced by inclusion of level of RBO in diet. However, the supplementation of RBO did not have an effect on eggshell strength, eggshell thickness, egg yolk color, and HU during the feeding trial. There were no significant differences in the level of leukocyte. However, heterophil and lymphocytes decreased (quadratic, $p<0.01$) with inclusion level of RBO. H:L ratio tended to decrease (linear $p=0.08$) with inclusion level of RBO. As expected, increasing inclusion level of RBO in diets decreased (linear, $p<0.01$) the concentrations of total cholesterol in plasma. AST, ALT, glucose, and albumin were not affected by inclusion of RBO in diets. Egg yolk cholesterol increased (linear and quadratic, $p<0.05$) with inclusion level of RBO in diet. The results of this study indicate that dietary supplementation of RBO improves laying performance and decreased total cholesterol and egg yolk cholesterol levels in laying hens. Therefore, dietary RBO is considered a valuable functional ingredient to improve the performance of birds.

(Key words: laying hens, laying performance, rice bran oil, egg yolk cholesterol, total cholesterol)

INTRODUCTION

Interest in rice bran oil (RBO) has been growing from the health and nutritional aspects as well as its wide application as industrial oil. RBO as its natural state contains several constituents which would potentially provide benefits to health (Raghueam and Rukmini, 1995). The oil contains 4.2% unsaponifiable matter, which includes antioxidants and micronutrients, whereas all other oils have an unsapnifiable content of less than 1～2%. The RBO is rich in tocopherols and tocotrienols, ɣ-oryzanol, phytosterols, polyphenols and squalene (Sayre et al., 1987; Kahlon et al., 1996; Sugano and Tsuji, 1997).

Oryzanol is an antioxidant compound and is associated with decreasing plasma cholesterol (Yoshino et al., 1989), lowering cholesterol, decreasing cholesterol absorption (Gerhardt and Gallo, 1989) and decreasing platelet aggregation (Seetharamaiah et al., 1990). The beneficial effects of RBO have been recently been reported. The hypolipidemic effect RBO is not entirely explained by its fatty acid composition. RBO has a greater content of unsaponifiables (Sugano et al., 1997), which also lower cholesterol compared with most vegetable oils (Wilson et al., 2005). Moreover, RBO has a very good balance in its fatty acid composition i.e., mono-unsaturates to poly-unsaturates/saturates (Ghosh, 2007).

Previous studies on dietary supplementation of RBO in broilers have shown to improve growth performance (Anitha et al., 2006; Purushorthaman et al., 2006; Kang and Kim, 2016) and decreased cholesterol concentrations (Berger et al., 2005; Kang and Kim, 2016). Dietary supplementation of rice bran or RBO in mice or broilers has shown to enhance improved immune response (Henderson et al., 2012; Kang and Kim, 2016). However, data pertaining to the effects of dietary supplementation of RBO on the laying performance, egg quality, and egg yolk cholesterol of laying hens are limited. Therefore, the objective of the present study was to investigate the effect of dietary supplementation of RBO on the laying performance, egg quality, blood parameter, and

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egg yolk cholesterol of laying hens.

MATERIALS AND METHODS

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

1. Preparation of Rice Bran Extract

Rice bran harvested in 2015 was obtained from a rice processing complex (Seonghwan-eup, South Korea). RBO was extracted as described previously (Kang and Kim, 2016). The nutrient composition of RBO was analyzed in duplicate for crude fat (AOAC, 1990). The results are presented in Table 1.

2. Birds and Experimental Design

A total 144, 29-week-old Hy-Line Brown laying hens were randomly allotted to 1 of 4 dietary treatments. Each treatment has 4 replicates and 3 adjacent cages, 3 hen per cage, considered a replicate (36 cm × 40 cm × 42 cm = width × length × height). A commercial type basal diet was formulated to meet or exceed nutrient recommendations of the National Research Council (NRC, 1994) for laying hens (Table 2). Three additional diets were prepared by supplementing 2.5, 5.0 or 10.0 g/kg of RBO to the basal diet at the expense of corn. The experimental period lasted 8 weeks. During the experiment, hens were provided with feed and water *ad libitum* and were exposed to a 16h:8h light:dark lighting schedule. The temperature and humidity of the laying house was maintained at 18±3℃ and 65～70%, respectively, during the experiment.

Table 1. Analyzed fatty acids composition of RBO used in this experiment (as is basis)

Composition		RBO[a]
Crude fat (g/kg)		305.1
Fatty acid composition (g/kg)	Myristic acid (C14:0)	3.6
	Palmitic acid (C16:0)	179.6
	Palmitoletic acid (C16:1n7)	2.1
	Stearic acid (C18:0)	0.6
	Vaccenic acid (C18:1n7)	13.1
	Oleic acid (C18:1n9)	422.5
	Linoleic acid (C18:2n6)	378.5
	Total	1,000.0
Saturated fatty acid		183.8
Unsaturated fatty acid		816.2
Mono unsaturated fatty acid		433.7
Poly unsaturated fatty acid		378.5

[a] Nutrient composition was analyzed for crude fat (AOAC, 1990).

3. Laying Performance

Hen-day egg production rate and egg weight were recorded daily. Feed intake (FI) and feed conversion ratio (FCR) were recorded weekly. Egg mass (EM) was calculated as follows (Hayat et al., 2009).

Egg mass = (Weekly number of eggs in replicate × Average egg weight)

4. Determination of Egg Quality Parameter

Ten eggs per replicate were randomly collected at the end of the each 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/cm^2). Eggshell thickness is defined as the mean value of measurements at 3 different locations on the egg (aircell, equator, and sharpend) and was measured with a dial pipe gauge (model 7360, Mitutoyo Co. Ltd., Kawasaki, Japan) and calculated using the following formula (Yannakopouls and Tserveni-Gousi, 1986).

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, Walthman, MA, USA) with the following formula described by Eisen et al. (1962):

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

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

Items		0	2.5	5.0	10.0
Ingredients (g/kg)	Corn	595.3	592.8	588.3	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
	D,L-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[a]	2.0	2.0	2.0	2.0
	Rice bran oil	0.0	2.5	5.0	10.0
	Total	1,000.0	1,000.0	1,000.0	1,000.0
Energy and nutrient contents[b]	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

[a] Provided per kilogram of the complete diet: vitamin A (from vitamin A acetate), 12,500 IU; vitamin D_3, 2,500 IU; vitamin E (from DL-α-tocopheryl acetate), 20 IU; vitamin K_3, 2 mg; vitamin B_2, 5 mg; vitamin B_6, 3 mg; vitamin B_{12}, 18 μg; calcium pantothenate, 8 mg; folic acid, 1 mg; biotin, 50 μg; niacin, 24 mg; Zn (as ZnO), 60 mg; Mn (as $MnSO_2 \cdot H_2O$), 50 mg; Fe (as $FeSO_4 \cdot 7H_2O$), 50 mg; Cu (as $CuSO_4 \cdot 5H_2O$), 6 mg; Co (as $CoCO_3$), 250 μg; I [as Ca $(IO_3)_2 \cdot H_2O$], 1 mg; Se (as Na_2SeO_3), 150 μg.
[b] Nutrient contents in all diet were calculated.

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

5. Haematological Analysis

At the end of the 8 week feeding trial, 8 birds/treatments with a body weight close to the average were selected to be euthanized by cervical dislocation. Immediately after death, a 5-mL blood sample was collected from jugular vein of each bird using EDTA-treated vacutainer tubes and non EDTA-treated vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ, USA). The whole blood samples were kept on ice and provided for immediate analysis of hematology. Leukocytes (white blood cells, heterophils, lymphocytes, monocytes, eoisnophils, and basophils) of blood samples were analyzed using Hemavet Multispecies Hematology 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℃, were stored at −15℃. Total cholesterol, triglyceride, aspirate aminotransperase (AST), alanine aminotransperase (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 performed 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 Libermann-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 ANOVA according to completely randomized design using the PROC MIXED procedure of SAS (SAS Institute Inc., Cary, NC). Outlier data were identified according to Steel et al. (1997) using the UNIVARIATE procedure of SAS, but no outliers was detected. The battery cage was an experimental unit for growth performance data, whereas the individual bird was an experimental unit for blood parameter, serum enzyme activities and immune response data. Dietary treatment was fixed effect in all statistical models. The LSMEANS procedure was used to calculate mean values. The orthogonal polynomial contrast test was performed to determine linear and quadratic effects of increasing inclusion level of RBO in diets on each measurement. Significance and tendency for statistical tests were set at $P<0.05$ and $0.05 \leq P \leq 0.10$, respectively.

RESULTS AND DISCUSSION

Hen-day egg production increased (quadratic, $p<0.05$) with inclusion level of RBO, but feed intake, egg weight, and egg mass were not influenced by inclusion of level of RBO in diet (Table 3). Dietary supplementation of rice bran or RBO has been reported to improve BW gain in broiler chickens and hen-day egg production in laying hens (Murugesan, 1997; Purshothaman et al., 2000; Kang and Kim et al., 2016). It is suggested that positive effects of dietary RBO on bird's performance may result from its high concentrations of oryzanols, tocopherols, vitamin E, ferulic acid, phytic acid, lecithin, and inositol (Sharma and Rukmini, 1986; Jo and Choi, 2010). Resulted best performance in RBO may be associated with synergistic effects of fatty acids contents of this oil (Rahimi et al., 2011; Baiao and Lara, 2005). Addition of oils in broiler diet may result in not only improvement in absorption and digestion of lipoproteins and essential fatty acids and less heat increment compared to carbohydrates and proteins (Grobas et al., 2001; Bhonzack et al., 2002). In addition to the advantages of the use of oils in broiler diets, reduction in passage rate of the digesta through the gastrointestinal tract may also be contributed to the better nutrient absorption and utilization as well (Lastshaw, 2008), which allow more efficient use of nutrient from diets. RBO is rich in linoleic acid and tocophe-

Table 3. Laying performance of laying hens fed the diet containing RBO[a]

Items		RBO (g/kg)[b]				Pooled SEM[c]	*p*-value	
		0.0	2.5	5.0	10.0		Linear	Quadratic
Laying performance	Hen-day egg production (%)	87.30	88.30	88.08	87.92	0.248	0.172	0.041
	Feed intake (g)	120.40	118.50	117.80	119.00	1.230	0.411	0.232
	Egg weight (g)	57.38	57.31	57.79	57.06	0.701	0.883	0.651
	Egg mass	50.00	50.60	51.00	50.40	0.590	0.482	0.312
	Feed conversion ratio	2.41	2.34	2.31	2.36	0.034	0.251	0.091

[a] Data are least squares means of 4 observations per treatment.
[b] Basal diet was supplemented with RBO at 2.5, 5.0 or 10.0 g/kg.
[c] Pooled standard error of the mean.

rols. The beneficial effects of essential fatty acid on egg weight and egg production have been documented (March, 1990). Due to limited experiments on RBO in laying hens, further studies are needed to be conducted.

However, the supplementation of RBO did not have an effect on eggshell strength, eggshell thickness, egg yolk color, and HU during the feeding trial (Table 4). The lack of adequate data on the influence of inclusion level of RBO of laying hens in egg quality in 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. However, heterophil and lymphocytes decreased (quadratic, $p<0.01$) with inclusion level of RBO. H:L ratio tended to decrease (linear $p=0.08$) with inclusion level of RBO. For example, leukocytes are known to 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 chemicals can cause changes in both granulocytic white blood cells (Lucas and Jamroz, 1961). The lack of adequate data on the influence of dietary inclusion level of RBO of laying hens in altering blood parameters in poultry requires further research.

As expected, increasing inclusion level of RBO in diets decreased (linear, $p<0.01$) the concentrations of total cholesterol in plasma (Table 6). AST, ALT, glucose, and albumin

Table 4. Egg quality of laying hens fed the diet containing RBO[a]

Items		RBO (g/kg)[b]				Pooled SEM[c]	*p*-value	
		0.0	2.5	5.0	10.0		Linear	Quadratic
Egg quality	Eggshell strength (kg/cm^2)	4.42	4.12	4.14	4.10	19.372	0.322	0.370
	Eggshell thickness (μm)	408.00	403.00	403.00	414.00	6.710	0.551	0.182
	Egg yolk color	6.40	6.60	6.60	6.40	0.230	0.852	0.494
	Haugh unit	92.90	91.50	91.50	90.90	1.670	0.422	0.831

[a] Data are least squares means of 40 observations per treatment.
[b] Basal diet was supplemented with RBO at 2.5, 5.0 or 10.0 g/kg.
[c] Pooled standard error of the mean.

Table 5. Blood parameter of laying hens fed the diet containing RBO[a]

Items		RBO (g/kg)[b]				Pooled SEM[c]	*p*-value	
		0	2.5	5.0	10.0		Linear	Quadratic
Leukocytes[d]	WBC (K/μL)	19.43	19.40	19.66	20.74	0.562	0.212	0.131
	HE (K/μL)	3.92	2.73	2.89	3.19	0.232	0.071	<0.01
	LY (K/μL)	4.38	3.20	3.31	3.98	0.221	0.281	<0.01
	SI (HE:LY)	0.89	0.86	0.87	0.80	0.032	0.081	0.590
	MO (K/μL)	0.19	0.15	0.26	0.22	0.033	0.192	0.532
	EO (K/μL)	0.22	0.10	0.15	0.12	0.034	0.141	0.212
	BA (K/μL)	0.06	0.04	0.05	0.05	0.011	0.782	0.571

[a] Data are least squares means of 6 observations per treatment.
[b] Basal diet was supplemented with RBO at 2.5, 5.0, or 10.0 g/kg.
[c] Pooled standard error of the mean.
[d] Leukocytes: WBC = white blood cells; HE = heterophils; LY = lymphocytes; SI = heterophil : lymphocytes; MO = monocytes; EO = eosinophils; BA = basophils.

were not affected by inclusion of RBO in diets. Anitha et al. (2006) reported that dietary supplementation of RBO decreased the concentrations of total serum cholesterol in broilers. Several studies on humans and animals (Sharma and Rukmini, 1986; Nicolosi et al., 1991; Kahlon et al., 1992; Hegsted and Windhauser, 1993; Kang and Kim, 2016) showed that RBO lowered the level of low-density lipoprotein cholesterol and total serum cholesterol or enhanced the conversion of cholesterol to fecal bile acids and sterols. Serum (total cholesterol, beta lipoprotein, and LDL cholesterol) decreased significantly (Moriyama et al., 2002). It was reported that oryzanol was at least partly responsible for the cholesterol lowering action of RBO and is associated with the reduction in aortic fatty streak formation (Rong et al., 1997). γ-oryzanol can also lower the plasma cholesterol level (Nestel, 1990). Although the mechanism underlying this effect is not apparent the presence of oryzanol and tocopherols in the rice bran is thought to be responsible for this favorable effect. Nutritional and biochemical aspects of the hypolipidemic action of RBO have been reviewed by Rukmini and Raghuram (1991).

Egg yolk cholesterol increased (linear and quadratic, $p < 0.05$) with inclusion level of RBO in diet (Table 7). Consumption of poly-unsaturated fatty acid has been reported to reduce the risk of atherosclerosis and heart stroke. Mono and poly unsaturated fats may lower blood cholesterol levels when they replace saturated fat in the diet. Howell et al. (1997) investigated the relationship between diet and blood cholesterol levels and found that saturated fat in the diet, but not dietary cholesterol, influences blood cholesterol levels. Modification of egg yolk cholesterol and fatty acid contents requires better understanding of the factors that influenced the deposition of cholesterol and fatty acids in egg yolk (Shafey et al., 2003). Cholesterol concentrations of egg yolk vary depending on dietary manipulation and pharmacological agents as well as production level of the bird (Grobas et al., 2001; Shafey et al., 2003; Cabrera et al., 2005).

The results of this study indicate that dietary supplementation of RBO improves laying performance and decreased

Table 6. Serum enzyme activities of laying hens fed the diet containing RBO[a]

Items	RBO (g/kg)[b]				Pooled SEM[c]	*p*-value	
	0	2.5	5.0	10.0		Linear	Quadratic
AST(U/L)	259.5	296.6	311.4	293.4	25.76	0.332	0.311
ALT (U/L)	3.22	6.44	4.05	5.11	1.013	0.482	0.311
Total cholesterol (mg/dL)	223.6	185.4	180.7	169.5	4.77	<0.01	0.032
Triglyceride	1,671.1	1,263.4	1,611.6	1,450.8	104.11	0.511	0.262
Glucose (mg/dL)	153.8	151.1	171.7	137.5	12.253	0.642	0.262
Total protein (g/dL)	6.56	6.29	6.78	6.80	0.313	0.391	0.642
Calcium (mg/dL)	25.1	23.4	25.3	23.4	1.30	0.581	0.942

[a] Data are least squares means of 6 observations per treatment.
[b] Basal diet was supplemented with RBO at 2.5, 5.0, or 10.0 g/kg.
[c] Pooled standard error of the mean.

Table 7. Cholesterol in egg yolk of laying hens fed the diet containing RBO[a]

Items	RBO (g/kg)[b]				Pooled SEM[c]	*p*-value	
	0	2.5	5.0	10.0		Linear	Quadratic
Egg yolk cholesterol (mg/dL)	13.89	13.30	13.57	10.78	0.358	<0.01	0.042

[a] Data are least squares means of 6 observations per treatments.
[b] Basal diet was supplemented with RBO at 2.5, 5.0, or 10.0 g/kg.
[c] Pooled standard error of the mean.

total cholesterol and egg yolk cholesterol levels in laying hens. Therefore, dietary RBO is considered a valuable functional ingredient to improve the performance of birds.

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