Effects of rice bran on performance, egg quality, oxidative status, yolk fatty acid composition, and fatty acid metabolism-related gene expression in laying ducks

D. Ruan,[*] Y. C. Lin,[*,1] W. Chen,[*] S. Wang,[*] W. G. Xia,[*] A. M. Fouad,[*,†] and C. T. Zheng[*,1]

[*]*Institute of Animal Science, Guangdong Academy of Agricultural Science, Key Laboratory of Animal Nutrition and Feed Science (South China) of Ministry of Agriculture, State Key Laboratory of Livestock and Poultry Breeding, Guangdong Public Laboratory of Animal Breeding and Nutrition, Guangdong Key Laboratory of Animal Breeding and Nutrition, Guangzhou 510640, China; and* [†]*Department of Animal Production, Faculty of Agriculture, Cairo University, Giza 12613, Egypt*

ABSTRACT The study was designed to evaluate the effects of different dietary levels of rice bran (RB) in laying duck diets on performance, egg quality, oxidation status, egg yolk fatty acid composition, and hepatic expression of fatty acid metabolism-related genes. Longyan females (1080) with similar BW at 19 wk of age were randomly assigned to 6 dietary treatments, each consisting of 6 replicates of 30 birds. The basal diet (I) was a typical corn-soybean ration while the experimental diets (II to VI) substituted RB for corn and wheat bran and a small reduction of soybean meal. The level of substitution in diets (II to VI) was 6%, 12%, 18%, 24%, and 30%, respectively. The experiment lasted for 12 wks. Average egg weight and daily egg mass decreased linearly as the level of RB inclusion increased ($P < 0.001$) and feed conversion ratio linearly increased ($P < 0.001$). The proportions of C14:0 and C18:0 and total saturated fatty acids (SFA) in egg yolk linearly decreased with increasing RB, and many of the key polyunsaturated fatty acids (PUFA), like $C_{18:2\ n\text{-}6}$ and $C_{18:3\ n\text{-}3}$, linearly increased ($P < 0.001$), but not those of $C_{20:5\ n\text{-}3}$ and $C_{22:6\ n\text{-}3}$. There were linear decreases ($P < 0.001$) in hepatic abundance of *FAS* and *SREBP1* transcripts, with a substantial reduction to about 30% those of ducks fed the control diet; there were no treatment effects on productive performance, eggshell thickness, strength, Haugh unit, antioxidation status, and egg yolk cholesterol or triglyceride content ($P > 0.05$). In conclusion, the current study suggests that ducks from 19 to 31 wk could be fed diets with up to about 18% RB without effect on the number of eggs produced, egg quality, and oxidative status. Increasing amounts of RB linearly increased egg yolk concentrations of key fatty acids like $C_{18:2\ n\text{-}6}$ and $C_{18:3\ n\text{-}3}$ and decreased the hepatic abundance of *FAS* and *SREBP-1* transcripts.

Key words: rice bran, performance, egg quality, oxidative status, fatty acids

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INTRODUCTION

Rice bran (**RB**) is a major by-product from the milling process and is commonly a mixture of attached epidermal layer, rice germ, broken rice, and the aleurone layer of the endosperm. A total of 40 to 45 million tonnes of RB is produced annually, mainly in Southeast Asia. Rice bran is the most nutritious part of rice and is a good source of bioactive phytochemicals such as γ-oryzanol, tocopherols, and tocotrienols; these have hypolipidemic activity, anti-inflammatory activity, and inhibit cholesterol oxidation (Moongngarm et al., 2012). As a secondary energy feedstuff, RB could be a good alternative for replacing corn as an energy source, but there are potential problems limiting its use, especially from its content of anti-nutritional factors and its processing and storage. The lipid content of RB is high (15 to 23%), and 4 major fatty acids—palmitic (12 to 18%), oleic (40 to 50%), linoleic (30 to 35%), and linolenic (approximately 1%)—account for about 92% of the total (Malekian et al., 2000). A major restriction for widespread use of RB as an animal feed ingredient is its high susceptibility to rancidity during storage and loss of nutritional quality; up to 50% of the lipid in bran is degraded into free fatty acids within 6 wk after milling, because of oxidative changes. These post-milling changes may result in poor livestock acceptability and resultant growth depression, particularly in chicks (Gunawan and Tangendjaja, 1988). Current understanding and technological advances now allow better protection against these toxicity problems. Atapattu et al. (2013) concluded that butylated hydroxytoluene

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[1]Corresponding author: lyc0123@vip.tom.com (YCL) and zhengcht@163.com (CTZ)

Table 1. Composition and nutrient levels of the six diets (g/kg, as fed basis).

Ingredients	I	II	III	IV	V	VI
Corn	560.9	522.0	482.8	443.7	404.4	365.4
Wheat bran	110.5	93.0	74.7	55.9	36.5	17.1
Soybean meal	220.3	217.1	215.0	213.3	212.5	211.0
Rice bran	0.0	60.0	120.0	180.0	240.0	300.0
L-Lysine·HCl	0.75	0.55	0.35	0.15	0.00	0.00
DL-Methionine	1.65	1.45	1.15	0.95	0.70	0.50
Limestone	81.7	81.7	81.8	81.8	81.9	82.0
Calcium hydrogen phosphate	11.2	11.2	11.2	11.2	11.0	11.0
Sodium chloride	3.0	3.0	3.0	3.0	3.0	3.0
Premix*	10.0	10.0	10.0	10.0	10.0	10.0
Total	1000	1000	1000	1000	1000	1000
Nutrient level						
AME (MJ/kg)	10.46	10.46	10.46	10.46	10.46	10.46
CP, %#	17.01	17.18	16.95	17.12	17.10	17.36
EE, %#	2.87	3.60	4.39	5.15	5.89	6.56
Ca, %	3.60	3.60	3.60	3.60	3.60	3.60
Total P, %	0.63	0.61	0.60	0.59	0.58	0.57
Available P, %	0.35	0.35	0.35	0.35	0.35	0.35
Total Lys, %	0.85	0.86	0.86	0.86	0.87	0.87
Total Met, %	0.40	0.40	0.40	0.40	0.40	0.40
Total Met+Cys, %	0.68	0.69	0.70	0.71	0.71	0.71
Total Thr, %	0.68	0.69	0.70	0.71	0.71	0.72
Total Trp, %	0.21	0.20	0.19	0.19	0.18	0.18

*Provided per kg of diet: VA 12000 IU, VD_3 2000 IU, VE 38 mg, VK_3 1.0 mg, VB_1 3.0 mg, VB_2 9.6 mg, VB_6 6.0 mg, VB_{12} 0.03 mg, chloride choline 500 mg, nicotinic acid 25 mg, D-pantothenic acid 28.5 mg, folic acid 0.6 mg, biotin 0.15 mg, Fe 50 mg, Cu 10 mg, Mn 90 mg, Zn 90 mg, I 0.5 mg, Se 0.4 mg.

#Measured values of CP and EE content. Other nutrient levels are calculated values.

effectively reduced rancidity of RB for about one mo whereas citric acid controls both lipolytic and oxidative rancidity for about 3 mo. There is a high content of phytate in RB (12.8 g/kg compared to 2.0 g/kg in corn) and non-starch polysaccharides (**NSP**), which are not digested by poultry and are mostly excreted. Laying hens tolerate higher dietary inclusions of RB than do broiler chickens (Warren and Farrell, 1990a,b). In layer diets, Majun and Payne (1977), Din and Sunde (1979a), and Balnave (1982) found that up to 45% RB can be used, but higher levels (60 or 74.7%) had adverse effects on egg production, eggshell thickness, and yolk color. Samli et al. (2006) found no adverse effects of up to 10% RB. Mulyantini et al. (2005) showed that AME of broiler diets with a high level (30%) of RB is improved by xylanase supplementation. In contrast with numerous studies in hens, essentially nothing is known about using RB for laying ducks. Ducks are efficient converters of agricultural by-products like seeds, grain, and grain by-products. The Longyan sheldrake, of economic importance in South China (>300 million birds), is an efficient egg producer, has medium size and adaptability, is disease resistant, tolerates crude feeds, and matures early. The present study has investigated the effect of dietary substitution with RB on performance, egg quality, fatty acid composition of yolk lipids, and hepatic expression of genes related to fatty acid metabolism in laying ducks.

MATERIALS AND METHODS

Experimental Design, Animals, and Housing

The study was approved by the Animal Care and Use Committee of Guangdong Academy of Agriculture Science. Longyan pullets (1080), of the same genetic background and of comparable BW at 19 wk of age, were randomly assigned to 6 dietary treatments, each with 6 replicates of 30 birds, and they were studied for 12 wk. The daily allowance of feed (average 160 g/bird), in 2 equal feedings at 07:00 and 15:00, was the maximum without their leaving refusals. Birds in the control group were fed a basal diet (I), and the others were fed diets (II to VI) where 6%, 12%, 18%, 24%, and 30%, respectively, RB was included, as partial substitution of corn and wheat bran and a small reduction of soybean meal, to maintain constant energy and CP, as shown in Table 1. Dietary nutrient levels were based on our previous results for Longyan pullets. To ensure freshness, RB was purchased locally from a mill (processed early rice harvested from Zhutian town of Nanxiong city in July, Guangdong, China) every 2 wk with known tested acid value (**AV**) and peroxide value (**PV**). The measured nutrient values of RB in the present study is presented in Table 2. Each new batch of diets was mixed and pelleted. The ducks were all housed in the same room, with incandescent lighting of 10 lx, providing 15 h of light and 9 h of dark.

Table 2. Measured composition of rice bran.

Item	DM, %	CP, %	EE, %	Ca, %	P, %	Ash, %	CF, %	Phytate, %	NSP, %
Value	89.68	13.02	16.68	0.08	1.33	9.58	5.97	6.71	21.85

Tissue Sampling and Storage

After 12 wk of feeding, 2 birds were selected at random from each replicate, excluding obvious outliers in BW, for the treatment group. Heparinized blood was collected from the wing vein, centrifuged (1200 × *g*) at 4°C for 10 min and plasma was held at –20°C. The birds were then stunned and exsanguinated and samples of liver were collected, rinsed quickly with PBS, snap frozen in liquid nitrogen, and stored at –80°C.

Productive Performance

Feed intake was recorded daily on a per replicate basis. The numbers of total, broken, and shell-less eggs were recorded daily by replicate. Eggs produced were individually weighed and graded daily (European Economic Community, 1989). Egg production, egg weight, egg mass, ADFI, feed conversion ratio (**FCR**, g feed g^{-1} egg) were calculated daily on a per replicate basis, then presented as the averages for the complete 12-wk study period.

Egg Quality

Egg quality was measured on 4 eggs collected at random from each replicate each mo, and the average of the 24 eggs from each treatment was used. Yolk color, albumen height, and Haugh units were measured on the d of collection using an Egg Analyzer (model EA-01, ORKA Food Technology, Ramat HaSharon, Israel). The strength of the shell was determined on the vertical axis using an Egg Force Reader (model EFR-01, ORKA). Eggshell thickness was measured using a digital micrometer, and egg shape index (**SI**) was determined with a digital caliper and calculated with formula SI = width×100/length, the distance between the blunt and pointed ends for length, and the diameter at midlength for the width. Yolks were separated, weighed, and expressed as percentages of egg weight. The shells with membranes were weighed after drying at 105°C.

Biochemical Determinations

The plasma contents of malondialdehyde (**MDA**) and reduced glutathione (**GSH**) were measured with colorimetric assay kits (Nanjing Jiancheng Institute of Bioengineering, Nanjing, Jiangsu, P. R. China).

Forty milligrams of frozen liver were homogenized on ice in 4 mL of homogenization buffer (0.05 M Tris-HCl, pH 7.4, 1 mM EDTA, 0.25 M sucrose) with an Ultra-Turrax (T8, IKA-Labortechnik, Staufen, Germany) for 5 s at 13,500 rpm. The homogenate was centrifuged at 3,000 x *g* for 10 min at 4°C, and the supernatant was stored at –80°C. The activities of total superoxide dismutase (**T-SOD**), glutathione peroxidase (**GSH-PX**), and catalase (**CAT**) and contents of MDA were measured with kits (Nanjing Jiancheng Institute of Bioengineering).

All samples were measured in duplicate, at appropriate dilutions, to give activities of the enzymes in the linear range of standard curves constructed with pure enzymes. Protein content of supernatants was determined using the Coomassie Brilliant Blue G250 (Sigma Chemical, St. Louis, MO) with bovine serum albumin standards.

Lipid Analysis

The concentrations of triglycerides (**TG**) and total cholesterol (**TCH**) were measured with assay kits (Nanjing Jiancheng Institute of Bioengineering) on separated yolks from sampled eggs.

Total lipid was extracted from approximately 0.5 g separated egg yolk, weighed into a 50 mL test tube with 20 mL of chloroform:methanol (2:1, vol/vol), and was homogenized with a Polytron for 5 to 10 s at high speed (Folch et al., 1957). Butylated hydroxyanisole in 98% ethanol was added prior to homogenization. The homogenate was filtered through a Whatman 1 filter paper into a 100-mL graduated cylinder and 5 mL of 0.88% sodium chloride solution was added, stoppered, and mixed. After phase separation, the volume of lipid layer was recorded, and the top layer was completely siphoned off. Total lipids were converted to fatty acid methyl esters (**FAME**) using a mixture of boron-trifluoride, hexane, and methanol (35:20:45, vol/vol) (Metcalfe et al., 1961). The FAME were separated and quantified by an automated gas chromatograph equipped with an autosampler and flame ionization detector, using a 30 m × 0.32 mm inside diameter fused silica capillary column, as described by (Cherian and Sim, 1991). A Shimadzu EZChrom chromatography (2010 type) data system was used to integrate peak areas. The calibration and identification of fatty acid peaks were carried out by comparison with retention times of known authentic standards. Fatty acid composition is expressed as weight percentages.

RNA Extraction

Total RNA was isolated from the frozen tissues using Trizol reagent (Invitrogen, Carlsbad, CA, United States). After removal of any genomic DNA with DNase, RNA was dissolved at 1 $\mu g/\mu l$ and stored at –80°C.

Table 3. Primers for quantitative real-time PCR.

Gene[1]	GenBank accession	Primer sequences (5'-3')	Products (bp)	Annealing temperature (°C)
ACO	XM_005010178.1	F:CTTTTCATTTCGTGGGAGCC R:CAGGATGGAGTGAATGTGACG	235	58
PPARγ	EF546801.2	F:GCAGGAGCAGAACAAAGAGGT R:TCATCAGAGAAGCCAGGAGAGT	194	58
FAS	AY613443.1	F:CAGCGGCAGTTGGTCAGTT R:GGCTCTCTCTCACATTGGCAG	152	59
SREBP1	55793104	F:ACCGCTCATCCATCAACGA R:GGCTGAGGTTCTCCTGCTTC	156	59
APOA-1	XM_005009561.1	F:GCTGAGTACCAGGCCAAGGT R:GATGAAGCGGGTCTTGAGGT	123	59

[1]*ACO* = Acetyl-CoA oxidase; *PPARγ* = peroxisome proliferators-activated receptors γ; *FAS* = fatty acid synthase; *SREBP1* = sterol regulatory element binding protein 1; *APOA-1* = apolipoprotein A1.

Table 4. Effect of dietary rice bran substitution on performance of laying ducks at peak production.[1]

	Dietary rice bran level (%)							*P*-value	
Variable	0	6	12	18	24	30	SEM[2]	Linear	Quadratic
Egg production (%)	85.3	85.8	85.6	85.6	84.6	84.2	0.652		
Average egg weight (g)	62.9	61.9	61.5	61.4	61.3	60.7	0.292	<0.001	0.059
Egg mass (g/d)	53.8	53.1	52.6	52.4	51.7	51.2	0.394	<0.001	0.9
Feed conversion ratio (g feed g^{-1} egg)	3.01	3.04	3.08	3.09	3.13	3.16	0.024	<0.001	0.8
Broken rate (%)	0.02	0.02	0.09	0.04	0.04	0.06	0.020		
Abnormal rate (%)	0.01	0.04	0.03	0.08	0.02	0.03	0.026		

[1]Each value represents the mean of 6 replicates.
[2]Pooled standard error of mean.

RT-PCR

Total RNA (2.5 μg) was used to generate cDNA in a final volume of 25 μL according to the manufacturer's instructions (Promega, Madison, WI). PCR was performed in the presence of 1.5 mM $MgCl_2$, 200 μM dNTP mixtures, 1.5 IU Taq polymerase, and 10 pmol each of forward and reverse primers in a final volume of 50 μL. Primers were designed from GenBank sequences using Primer Premier 5.0 and obtained from Shanghai Sheng-Gong Biological Company (Shanghai, China), as shown in Table 3. Optimal PCR conditions consisted of an initial 5 min denaturation at 94°C; 35 cycles of 30 s at 94°C, 30 s annealing at X°C, and a 30 s extension at 72°C, with a final 10 min extension at 72°C. Aliquots of PCR products were evaluated by electrophoresis in 1.5% agarose gels, and excised products from the gels were sequenced to verify authenticity.

Real Time-PCR Analysis

Real time quantitative PCR (**qPCR**) was performed using the same primers to quantify mRNA content. Each 25 μL PCR mixture contained 12.5 μL 2X iQTM SYBR Green Supermix, 0.5 μL (10 mM) each primer, and 1 μL cDNA. Mixtures were incubated in an iCycler iQ Real-time Detection system (Bio-Rad, Hercules, CA) using 40 cycles (95°C for 15 s and X°C for 35 s). Quantification of the transcripts was performed using a standard curve with 10-fold serial dilutions of cDNA. A melting curve was constructed to verify that only a single PCR product was amplified. Samples were assayed in triplicate with standard deviations of threshold cycle (**CT**) values not exceeding 0.5. Data were normalized to *β-actin* mRNA and then expressed relative to the transcript abundance in birds fed the control diet (I).

Statistical Analysis

Replicate (n = 6) was taken as the experimental unit. Except where noted otherwise, 2 sampled birds per replicate were used. The effect of substituting RB in the diets was examined by one-way ANOVA procedure of SAS 9.1 (SAS Institute, 2004). Orthogonal polynomial contrasts were used to estimate the linear and quadratic effects of the increasing levels of RB, and probability level of 0.05 was applied to test significance. Data for each variable are presented as means, along with the SE for n = 6, based on the ANOVA error mean square.

RESULTS

Productive Performance

The results for performance of laying ducks are presented in Table 4. The average egg weight and daily egg mass decreased as linear responses to RB substitution ($P < 0.001$), and FCR linearly increased ($P < 0.001$). There were no significant effects on daily egg production, broken rate, and abnormal eggs due to treatment.

Egg Quality

Of the indices of egg quality (Table 5), only yolk color was affected by RB substitution; a linear decrease

Table 5. Effect of dietary rice bran substitution on egg quality of laying ducks at peak production.[1]

	Dietary rice bran level (%)							*P*-value	
Variable	0	6	12	18	24	30	SEM[2]	Linear	Quadratic
Eggshell thickness (mm)	0.35	0.36	0.34	0.36	0.34	0.35	0.005		
Eggshell strength (N)	37.9	38.8	38.7	40.7	42.2	41.2	2.251		
Egg shape index	73.4	74.0	73.5	73.3	72.2	72.9	0.784		
Haugh unit	81.2	80.4	76.8	78.9	80.0	81.8	2.156		
Yolk color	4.88	5.06	4.80	4.81	4.27	4.19	0.194	0.002	0.3
Yolk rate (%)	29.5	29.5	30.0	29.5	29.4	30.2	0.441		
Eggshell weight (g)	6.48	6.24	6.29	6.26	6.33	6.23	0.082		

[1]Each value represents the mean of 6 replicates.

[2]Pooled standard error of mean.

Table 6. Effect of dietary rice bran substitution on antioxidant indices in blood and liver of laying ducks at peak production.[1]

	Dietary rice bran level (%)							
Variable[2]	0	6	12	18	24	30	SEM[3]	*P*-value
Plasma								
GSH (mg/L)	5.62	4.82	4.34	4.88	4.37	5.82	1.022	0.2
MDA (nmol/mL)	3.86	3.87	4.31	3.82	4.29	3.91	0.547	0.9
Liver								
GSH-PX (U/mg prot)	27.3	27.8	28.0	27.3	27.8	28.6	1.127	0.9
T-SOD (U/mg prot)	1305	1308	1311	1319	1260	1299	43.41	0.9
CAT (U/mg prot)	8.35	9.13	8.80	8.75	8.22	7.27	1.135	0.7
MDA (nmol/mg prot)	0.83	0.79	0.86	0.86	0.78	0.73	0.086	0.7

[1]Each value represents the mean of 6 replicates.

[2]GSH = reduced glutathione; MDA = malondialdehyde; GSH-PX = glutathione peroxidase; T-SOD = total superoxide dismutase; CAT = catalase.

[3]Pooled standard error of mean.

occurred ($P = 0.002$). There was no effect on eggshell thickness, egg SI, eggshell strength, eggshell weight, Haugh unit, and yolk proportion.

Biochemical Analyses

As shown in Table 6, there were no significant effects of RB substitution on plasma contents of GSH or MDA, nor hepatic activities of GSH-PX, T-SOD, or CAT.

Fatty Acid Composition of Eggs and Hepatic Expression of Genes Related to Fatty Acid Metabolism

The fatty acid composition of egg yolk lipids is provided in Table 7. The proportions of $C_{14:0}$ and $C_{18:0}$ and total saturated fatty acids (**SFA**) decreased linearly with increasing RB substitution, and a quadratic effect existed for $C_{18:0}$ where the highest content occurred with 12% RB. The contents of many of the polyunsaturated fatty acids (**PUFA**) in egg yolk linearly increased ($P < 0.001$) but not those of $C_{20:5\ n\text{-}3}$ and $C_{22:6\ n\text{-}3}$. This increase with RB substitution also occurred in total n-6 fatty acids and a smaller increase in n-3 fatty acids, but n-6/n-3 was unaffected. There were no treatment effects on egg yolk contents of TCH or TG.

When effects of increasing dietary RB on hepatic gene expression were examined (Table 8), the only significant changes were linear ($P < 0.001$) decreases in relative abundance of *FAS* and *SREBP1* transcripts; expression of the other genes examined was unaffected by the diets.

DISCUSSION

Effect of Dietary Rice Bran Substitution on Performance and Egg Quality of Laying Ducks at Peak Production

The present study demonstrated a negative effect of increasing dietary content of RB on performance, as measured by average egg weight, egg mass, and FCR in laying ducks. The extent of these changes was considerable. For example, as the level of RB increased in the diet, egg weight decreased progressively, up to over 2 g when the inclusion level reached 30%. The only effect on egg quality was the decrease in yolk color with the highest levels of dietary RB. Samli et al. (2006) indicated that RB could be included up to 10% without any adverse effect on laying performance, egg quality, and the digestive organs, while 25% was acceptable in another study (Haghnazar and Rezaei, 2004). Dadang (2006) revealed that more than 15% RB in the diet of laying hens (21 to 53 wk of age) had poorer FCR and egg production. Ukil (1999) suggested that RB could be used at up to 30% in growing birds' diet while Gallinger

Table 7. Effect of dietary rice bran substitution on triglyceride and total cholesterol content and fatty acid composition in egg yolk of laying ducks at peak production.[1]

	Dietary rice bran level (%)							*P*-value	
Variable[2]	0	6	12	18	24	30	SEM[3]	Linear	Quadratic
TCH (mg/g)	28.8	30.7	26.1	28.5	26.0	26.4	1.640		
TG (mg/g)	301	297	296	304	295	299	7.194		
Fat content	31.6	32.1	33.3	32.0	33.2	32.6	0.006	0.2	0.01
$C_{14:0}$	0.35	0.34	0.34	0.29	0.27	0.28	0.016	<0.001	0.9
$C_{16:0}$	24.5	24.2	23.9	23.3	23.3	23.1	0.364		
$C_{18:0}$	4.74	4.71	4.85	4.55	4.65	4.18	0.113	0.04	0.007
Total SFA	29.5	29.2	29.1	28.3	28.2	27.6	0.417	<0.001	0.7
$C_{16:1}$	2.89	2.66	2.36	2.20	1.96	1.85	0.129	<0.001	0.5
$C_{18:1}$	51.3	51.2	51.1	50.7	50.2	50.2	0.810		
$C_{20:1}$	0.31	0.33	0.29	0.29	0.32	0.26	0.018		
Total MUFA	54.5	54.2	53.8	53.1	52.5	52.3	0.852		
$C_{18:2\ n-6}$	8.18	8.74	9.29	9.55	10.8	12.0	0.528	<0.001	0.2
$C_{18:3\ n-3}$	0.28	0.29	0.31	0.31	0.37	0.42	0.023	<0.001	0.058
$C_{20:2\ n-6}$	0.17	0.16	0.18	0.19	0.22	0.23	0.015	0.001	0.3
$C_{20:3\ n-3}$	0.20	0.17	0.17	0.20	0.23	0.22	0.027		
$C_{20:4\ n-6}$	1.14	1.15	1.23	1.35	1.39	1.45	0.073	<0.001	0.7
$C_{20:5\ n-3}$	0.12	0.13	0.11	0.11	0.13	0.13	0.008		
$C_{22:6\ n-3}$	0.12	0.14	0.16	0.17	0.17	0.17	0.015		
Total PUFA	10.2	10.8	11.4	12.0	13.3	14.6	0.618	<0.001	0.2
n-6	9.49	10.0	10.7	11.2	12.4	13.7	0.576	<0.001	0.2
n-3	0.72	0.72	0.74	0.79	0.89	0.93	0.049	<0.001	0.2
n-6/n-3	13.3	14.0	14.6	14.2	14.0	14.8	0.514		

[1]Each value represents the mean of 6 replicates.

[2]TG = triglycerides; TCH = total cholesterol; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

[3]Pooled standard error of mean.

Table 8. Effects of dietary rice bran level on relative hepatic expression of genes related to lipid synthesis and metabolism in laying ducks at peak production.[1]

	Dietary rice bran level (%)							*P*-value	
Gene[2]	0	6	12	18	24	30	SEM[3]	Linear	Quadratic
ACO	1.00	0.89	0.90	0.86	0.91	0.63	0.137		
PPARγ	1.06	0.95	0.86	0.93	0.94	0.88	0.111		
FAS	0.99	0.67	0.69	0.57	0.46	0.27	0.111	<0.001	0.7
SREBP1	0.98	0.93	0.74	0.60	0.60	0.38	0.096	<0.001	0.4
APOA-1	1.08	1.03	1.08	1.24	1.29	0.88	0.206		

[1]Each value represents the mean of 6 replicates.

[2]*ACO* = Acetyl-CoA oxidase; *PPARγ* = peroxisome proliferators-activated receptors γ; *FAS* = fatty acid synthase; *SREBP1* = sterol regulatory element binding protein 1; *APOA-1* = apolipoprotein A1.

[3]Pooled standard error of mean.

et al. (2004) showed that more than 10% has an adverse effect on broiler performance from one to 21 d of age. The literature concerning the effect of various levels of RB on the performance of laying hens is conflicting. This may be due to increased dietary fiber content, and laying hens can tolerate higher dietary inclusions of RB than broiler chickens.

Rice bran contains a high level of phytate and NSP (6.71 and 21.85%, respectively), which are not digested by poultry and have a negative impact on protein digestibility and energy utilization, partly due to inhibition of digestive enzymes, including pepsin and trypsin (Kies et al., 2001). Haghnazar and Rezaei (2004) reported that egg weight increased because of the increased linoleic acid content in feed in diets supplemented with RB. Farrell (1994) showed that inclusion of > 20% RB in chicken diets frequently depressed growth, but that higher levels were tolerated by ducklings. The negative effects shown here are consistent with reasonably good tolerance of dietary RB by highly productive laying ducks.

Effect of Dietary Rice Bran Substitution on Antioxidant Capacity of Laying Ducks at Peak Production

Oil in brown rice is stable, but after the bran layer is removed, bran lipids in the aleurone are exposed to lipases from disrupted cells. The lipoxidase in RB rapidly oxidizes PUFA such as linoleic acid, linolenic acid, and arachidonic acid, using environmental oxygen to produce lipid peroxides, aldehydes, carboxylic acids, and abundant free radicals with increased

rancidity (Malekian et al., 2000). Rice bran does contain tocopherols, tocotrienol, γ-oryzanol, and other active substances with both antioxidant and oxygen free radicals scavenging function (Godber and Wells, 1994; Moldenhauer et al., 2003; Nam et al., 2005). In the experiment, the measured AV of RB was 7.21 to 10.58 KOH mg/g, and PV was 1.36 to 2.01 meq/kg, which was assured freshness. Consistent with an earlier finding (Ruan et al., 2013) in which 2.5% and 4% crude RB oil in starter and grower feed for broilers did not affect antioxidant capacity of muscle and liver, the results obtained here with ducks showed no effect of RB on plasma or hepatic indices of redox status. It has recently been shown that RB possesses health-promoting phytochemicals that have strong antioxidant activities. Kang et al. (2012) reported that addition of RB to a high-fat mouse diet counteracted the decline in the activities of GSH-PX and CAT enzymes, and induced activity of glutathione reductase. The addition in this study of up to 30% RB in typical diets for laying ducks did not produce a detected effect on the birds' redox status.

Effect of Dietary Rice Bran Substitution on Fatty Acid Composition of Yolk and Hepatic Expression of Genes Related to Fatty Acid Metabolism in Laying Ducks at Peak Production

Dietary fatty acid composition is the most important factor influencing the fatty acid composition of broiler meat and hen eggs (Cortinas et al., 2004). Ruan et al. (2013) showed that feeding RB oil to broilers increased the PUFA content in breast muscle. Increasing dietary content of RB in the ducks here increased egg yolk concentrations of important fatty acids like $C_{18:2\ n\text{-}6}$ and $C_{18:3\ n\text{-}3}$, both of which are major fatty acids in RB.

Because of the importance in birds of hepatic fatty acid synthesis, along with dietary provision, hepatic transcript abundance of selected genes relevant to overall lipogenesis was examined. In the ducks fed increasing amounts of RB, there was substantial reduction in relative abundance of *FAS* and *SREBP1* transcripts, to about 30% those of ducks fed the control diet. The multifunctional enzyme fatty acid synthase is critical in the synthesis of fatty acids, and its gene expression correlates with body fat content in animals while acetyl-CoA carboxylase is rate-limiting. Clarke et al. (1990) found that unsaturated fatty acids can inhibit the activity of FAS, and the inhibition is related to the content, degree of unsaturation, chain length, and position of double bonds. Polyunsaturated fatty acids also inhibit *FAS* expression. Dietary PUFA of the n-6 and n-3 families uniquely suppress the expression of lipogenic genes while concomitantly inducing the expression of genes encoding enzymes of fatty acid oxidation (Gossett et al., 1996; Nakamura et al., 2000). The fatty acid composition of RB likely accounts for the effects on hepatic *FAS* expression seen in ducks in the current study. Also affected by RB in the diets, *SREBP1* encoding sterol regulatory element-binding protein (**SREBP**) is an important transcription factor regulating fatty acid and cholesterol metabolism. Expression of *SREBP-1* is suppressed by PUFA as is maturation of the transcription factor protein resulting in suppression of its target genes including *FAS* and the acyl-transferase *GPAT*, resulting in reduced fatty acid and triglyceride synthesis (Kim et al., 2002). The changes in transcript abundance, detected here in the liver, were of greater magnitude than the non-significant changes in egg yolk TG content.

In conclusion, the current study suggests that ducks from 19 to 31 wk could be fed diets with up to about 18% RB, and the number of eggs produced, egg quality, and oxidative status were not affected. Increasing amounts of RB linearly increased egg yolk concentrations of key fatty acids like $C_{18:2\ n\text{-}6}$ and $C_{18:3\ n\text{-}3}$ and decreased the hepatic abundance of *FAS* and *SREBP-1* transcripts.

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REFERENCES

Atapattu, N., K. P. Wickramasinghe, T. Serasinghe, and S. P. Gunarathne. 2013. Effects of citric acid and butylated hydroxytoluene alone or in combination on prevention of rancidity of rice bran during storage. J. Rice Res. 1:114.

Balnave, D. 1982. Egg weight and production responses of laying hens fed rice pollard. J. Sci. Food. Agric. 33:231–236.

Cherian, G., and J. S. Sim. 1991. Effect of feeding full fat flax and canola seeds to laying hens on the fatty acid composition of egg, embryos and newly hatched chicks. Poult. Sci. 70:917–922.

Clarke, S. D., M. K. Armstrong, and D. B. Jump. 1990. Nutritional control of rat liver fatty acid synthase and *S14* mRNA abundance. J. Nutr. 120:218–224.

Cortinas, L., C. Villaverde, J. Galobart, M. D. Baucells, R. Codony, and A. C. Barroeta. 2004. Fatty acid content in chicken thigh and breast as affected by dietary polyunsaturation level. Poult. Sci. 83:1155–1164.

Dadang, R. A. 2006. Effects of rice bran and phytase supplementation on egg laying performance and egg quality of laying hens. MD. Thesis. Universiti Putra Malaysia, Serdang. Malaysia.

Din, M. G., and M. L. Sunde. 1979a. Effect of feeding plant by-products diets on growth and egg production. Poult. Sci. 58:1274–1283.

Farrell, D. J. 1994. Utilization of rice bran in diets for domestic fowl and ducklings. World's Poult. Sci. J. 50:115–131.

Folch, J., M. Lees, and G. H. Sloane-Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226:497–509.

Gallinger, C. I., D. M. Suárez, and A. Irazusta. 2004. Effects of rice bran inclusion on performance and bone mineralization in broiler chicks. J. Appl. Poult. Res. 13:183–190.

Godber, J. S., and J. H. Wells. 1994. Rice bran: as a viable source of high value chemicals. Louisiana Agric. 37:13–17.

Gossett, R. E., A. A. Frolov, and J. B. Roths. 1996. Acyl-CoA binding proteins: multiplicity and function. Lipids. 31:895–918.

Gunawan, B., and B. Tangendjaja. 1988. The effect of long storage of rice bran for growing broiler chickens. Ilmu dan Peternakan. 3:135–138.

Haghnazar, A., and M. Rezaei. 2004. To determine the metabolizable energy of rice bran and the use of it in layer ration. XII. World's Poultry, Congo Istanbul: Turkey, pp. 8–13.

Kang, M. Y., S. M. Kim, C. W. Rico, and S. C. Lee. 2012. Hypolipdemic and antioxidative effects of rice bran and phytic acid in high fat-fed mice. Food Sci. Biotechnol. 21:123–128.

Kies, A., K. V. Hemert, and W. C. Sauer. 2001. Effects of phytase on protein and amino acid digestibility and energy utilization. World's Poult. Sci. J. 57:109–129.

Kim, H., M. Miyazaki, and W. C. Man. 2002. Sterol regulatory element-binding proteins (SREBPs) as regulators of lipid metabolism. Annals of the New York Academy of Sciences. 967:34–42.

Majun, G. K., and C. G. Payne. 1977. Autoclave rice bran in layers' diets. Br. Poult. Sci. 18:201–203.

Malekian, F., R. M. Rao, W. Prinyawiwatkul, W. E. Marshall, M. Windhauser, and M. Ahmedna. 2000. Lipsae and lipoxigenase activity, functionality, and nutrient losses in rice bran during storage. Lousiana State University Agriculture Centre, USA.

Metcalfe, L. D., A. Smitz, and J. B. Pelka. 1961. The rapid preparation of fatty acid esters for gas chromatography. Anal. Chem. 33:363–364.

Moldenhauer, K. A., E. T. Champagne, D. R. McCaskill, and H. Guraya. 2003. Functional products from rice. Pages 71–89 in Functional Foods, G. Mazza ed.

Moongngarm, A., N. Daomukda, and S. Khumpika. 2012. Chemical compositions, phytochemicals, and antioxidant capacity of rice bran, rice bran layer, and rice germ. APCBEE Procedia. 2:73–79.

Mulyantini, N. G. A., M. Choct, X. Li, and U. R. Lole. 2005. The effect of xylanase, phytase and lipase supplementation on the performance of broiler chickens fed a diet with a high level of rice bran. Aust. Poult. Sci. Sypm. 17:305–307.

Nakamura, M. T., H. P. Cho, and S. D. Clarke. 2000. Regulation of hepatic Δ-6 desaturase expression and its role in the polyunsaturafed fatty acid inhibition of fatty acid synthase gene expression in mice. J. Nutr. 130:1561–1565.

Nam, S. H., S. P. Choi, M. Y. Kang, N. Kozukue, and M. Friedman. 2005. Antioxidative, antimutagenic, and anticarinogenic activities of rice bran extracts in chemical and cell assays. J. Agric. Food Chem. 53:816–822.

Ruan, J. J., H. L. Huan, J. S. Yan, Y. Zhao, Y. F. Du, G. H. Tian, D. H. Jia, Y. F. Xue, and W. R. Zhou. 2013. Crude rice bran oil: effects on meat quality, fatty acid composition and antioxidant capacity of broilers. Chin. J. Anim. Nutr. 25:1976–1988.

Samli, H. E., N. Senkoylu, H. Akyurek, and A. Agma. 2006. Using rice bran in laying hen diets. J. Cent. Eur. Agric. 7:135–140.

SAS Institute. 2004. SAS User's Guide: Statistics. Version 9.1. SAS Institute Inc., Cary, NC.

Ukil, M. A. 1999. Rice bran for broiler chicken (Finisher) ration and utilization of the phytate phosphorus. PhD. Thesis. Universiti Putra Malaysia, Serdang. Malaysia.

Warren, B. E., and D. J. Farrell. 1990a. The nutritive value of full-fat and defatted Australian Rice Bran. I. Chemical Composition. Anim. Feed Sci. Tech. 27:219–228.

Warren, B. E., and D. J. Farrell. 1990b. The nutritive value of full-fat and defatted Australian Rice Bran. II. Growth studies with chickens, rats and pigs. Anim. Feed Sci. Tech. 27:229–246.