

RED PALM OIL IN LAYING DUCKS DIETS: EFFECTS ON PRODUCTIVE PERFORMANCE, EGG QUALITY, CONCENTRATIONS OF YOLK CAROTENOIDS

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ABSTRACT

Red palm oil (RPO) has high nutritional value but it has not been widely used as poultry feed material. The aim of this study was to examine the short-term effects of RPO as poultry feed material on laying performance, egg quality, egg yolk colour, content of carotenoids and fatty acid profile in the yolk. Eighty-four Khaki Campbell ducks (50 weeks of age) were studied for eight weeks to examine the effects of RPO on the above characteristics. Dietary treatments were as follows: i) Control group (contains 30 g kg⁻¹ soybean oil), ii) 10 g kg⁻¹ RPO (10 g kg⁻¹ RPO+20 g kg⁻¹ soybean oil), iii) 20 g kg⁻¹ RPO (20 g kg⁻¹ RPO+10 g kg⁻¹ soybean oil), and iv) 30 g kg⁻¹ RPO (contains 30 g kg⁻¹ RPO). RPO supplementation increased feed intake, improved the yolk egg colour, and increased the content of lutein, β -carotene and total carotenoids in egg yolk ($p < 0.05$). Dietary RPO could reduce serum triglyceride levels in laying ducks as well as total cholesterol (TC) and triglyceride (TG) levels in egg yolk. Our further studies also found that the saturated fatty acid (SFA) and monounsaturated fatty acid (MUFA) contents of duck egg yolks in experiment group were significantly higher than those in the control group ($p < 0.001$). Therefore, feeding of RPO can produce natural, healthy red-yolk eggs. The suitable RPO concentration is 20 g kg⁻¹.

Keywords: egg colour, egg quality, laying ducks, red palm oil, yolk carotenoids.

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INTRODUCTION

Eggs are accepted to be consumed by most people worldwide, either as such or as food ingredients (Fraeye *et al.*, 2012). Egg yolk colour is regarded as a major concern to consumers as it affects their purchasing behaviour (Fletcher, 1999), and the colour of yolk mainly depends on the content of

carotenoids and vitamin B₂ in egg yolk (Spasevski *et al.*, 2020).

However, laying birds are mostly incapable of synthesising carotenoids and carotenes have low deposition efficiency in egg yolks and thus, must obtain them through their diet (Hammershoj *et al.*, 2010). The intensity of yolk colour from normal feeding, such as corn-soya diet, is usually ranked seven from Roche's colour fan scale, whereas a scale of more than 10 is more preferable (Kijparkorn *et al.*, 2010). Currently, a majority of commercial eggs are produced by supplementing the fodder of ducks with commercial feed colourants, which is mainly canthaxanthin. It produces a deeper egg yolk colour than can be achieved by feeding with wheat, soya, barley or corn. However, consumers are

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always worried about the safety of the commercial feed colourants. According to European Food Safety Authority, because of the absence of data, canthaxanthin has been considered as an irritant to the skin and eyes, as a skin sensitiser and is hazardous when inhaled (FEEDAP, 2014). Besides, these products are banned in organic production, which rely mainly on yellow plant sources (Abiodun *et al.*, 2014).

Crude palm oil (CPO) comes from oil palms. After using a modified physical refining process (Go *et al.*, 2015; Keat *et al.*, 1991; Ooi *et al.*, 1993), a refined palm oil is produced. This refined palm oil retains most of the carotenoids and the vitamin E originally present in CPO, and is named red palm oil (RPO) (Keat *et al.*, 1991; Rauchova *et al.*, 2018). RPO gets its colour because it contains high concentrations of carotenoids, especially beta-carotene and alpha-carotene (Sundram *et al.*, 2003). RPO contains approximately 500 ppm carotene, 90% presence of α - and β -carotene. The vitamin E content is about 800 ppm, 70% of which is in the form of tocotrienols (mainly as α -, β - and δ -tocotrienols) (Andreu-Sevilla *et al.*, 2009; Rice and Burns, 2010). Other valuable minor components present in this oil are ubiquinones and phytosterols (Nagendran *et al.*, 2000).

RPO has 50% saturated fatty acid (SFA) (palmitic acid, C16:0), 40% monounsaturated fatty acid (MUFA) (oleic acid, C18:1) and 10% polyunsaturated fatty acid (PUFA) (linoleic acid, C18:2) (Ma, 2015). Excessive saturated fats increase the risk of cardiovascular diseases (CVD), while unsaturated fats are considered less harmful. Since RPO/palm oil (PO) has similar fatty acid profile and content, which contains approximately 50% of SFA, it has been reported that dietary PO induces platelet aggregation, which leads to an increase in venous thrombosis and increases serum triglyceride levels in rats (Mizurini *et al.*, 2011).

As hyperlipidemia is usually caused by a high-fat diet, palm oil, being partially saturated, has been suspected to be the cause of hyperlipidemia and CVD. However, studies have also shown that dietary RPO/PO does not increase subcutaneous fat and total fat in mice (Gouk *et al.*, 2013), therefore does not cause hyperlipidemia and increase the risk of CVD (Go *et al.*, 2015). Instead, it has a tendency to lower cholesterol and even has antithrombotic effects (Hornstra, 1987). Wilson *et al.* (2005) found that in comparison to other dietary oils rich in SFA 16:0, RPO/PO has been shown to reduce plasma cholesterol levels (Wilson *et al.*, 2005), explained by the sn-2 theory. The positions of fatty acid attachment on the glycerol backbone are referred to by stereospecific numbers, (sn) -1, -2 and -3. The attachment of a particular fatty acid to a particular position has an important effect on the fat/oil properties.

In PO, oleic acid is predominantly situated at the sn-2 position, while long-chain SFA is predominantly situated at the sn-1,3 position. The subcutaneous, visceral as well as total fat deposition in mice all had correlated negatively with the total SFA content at the sn-1, 3 positions, while no relationships were found for MUFA and PUFA. The present results show that the positional distribution of long-chain SFA exerts a more profound effect on body fat accretion than the total SFA content (Wilson *et al.*, 2005). RPO has been known as an excellent source of pro-vitamin A carotenoids for decades. However, the usage of RPO as poultry feed to increase egg yolk colour remains rarely researched. Most studies have focused on the effect of PO/RPO on lipid metabolism in poultry and on the oxidative stability of poultry eggs (Nawab *et al.*, 2019; Nyquist *et al.*, 2013).

Nyquist *et al.* (2013) replaced animal fat with PO and RPO had no negative effects on chicken muscle nutritional value with regard to fatty acid composition and RPO decreased plasma total cholesterol (TC), confirming the TC reducing effect of this dietary oil. A study showed that RPO could be used effectively to reduce egg lipid and cholesterol content and also to increase the linoleic acid content without altering their acceptability of the eggs from hens (Punita and Chaturvedi, 2000). Another experiment showed that yolk colour and oxidative stability of chicken eggs from Institute of Selection Animals (ISA) brown hens had markedly improved ($P<0.05$) with corresponding increase of CPO in the diets (Akter *et al.*, 2014). Cherian *et al.* (1996) drew similar conclusion that PO (oils at 3.5%) resulted in a significant ($P<0.05$) reduction in 2-thiobarbituric acid values in eggs compared with menhaden oil and flax oil ($P<0.05$), and it may suggest that dietary PO reduced lipid oxidation in egg yolk, which may be related to the rich carotenoid and/or vitamin E in CPO/PO (Cherian *et al.*, 1996).

RPO is a commercially available feedstuff, highly rich in carotenoids and vitamin E. Carotenoids and vitamin E have strong antioxidant properties and effects. Previous studies have suggested that natural red-yolk egg could be produced naturally by feeding poultry with carotenoid-rich fodder such as Lucerne (Karadas *et al.*, 2006), sano flower (Kijparkorn *et al.*, 2010). Supplying egg-laying ducks with RPO may affect the colour of its egg yolk. However, research on the usage of RPO as poultry feed to increase egg yolk colour is still lacking. Therefore, the objectives of this study were to determine the effectiveness of layer diet supplementation with RPO on: 1) productive performance and egg traits during eight-week period from ducks aged 50-58 weeks, 2) the change of the yolk colour after eight-week intervention, and 3) the change of egg yolk carotenoids concentrations after eight-week intervention.

MATERIALS AND METHODS

Chemicals

Petroleum ether, acetone and dichloromethane were of analytical grade, provided by Sinopharm; Methanol, acetonitrile (Merck, USA) and methyl tert-butyl ether (Sigma, USA) was chromatographic pure; β -carotene, lutein and zeaxanthin were obtained from Sigma. RPO used for the study was supplied by Malaysian Palm Oil Board (MPOB).

Animal Management and Treatments

The methods were carried out in accordance with the relevant guidelines and regulations concerning the use of animals in research. This study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Animal Experimental Ethical Inspection Form of Southeast University (20160501007) on 1 May 2016 (Nanjing, China).

A total of 84 Khaki Campbell ducks, aged 50 weeks and with similar body weights (2.41 ± 0.35 kg) were used for this study. Ducks were put at random into four treatment groups (three replicates and seven ducks per replicate). The experimental ducks were fed different diets for eight weeks. The ducks were fed four experimental diets: i) contains 30 g kg^{-1} soybean oil (control group), ii) 10 g kg^{-1} RPO + 20 g kg^{-1} soybean oil (10 g kg^{-1} RPO group), iii) 20 g kg^{-1} RPO + 10 g kg^{-1} soybean oil (20 g kg^{-1} RPO group), and iv) contains 30 g kg^{-1} RPO (30 g kg^{-1} RPO group). The diets (Table 1) were isocaloric and isonitrogenous containing 177.3 g kg^{-1} crude protein (CP) and 12.2 MJ of metabolisable energy (ME) kg^{-1} of diet. The diets were supplemented with a commercial feed premix (Huamu Technology Company Ltd., Wuhan, China). All diets were formed into pellets to reduce differences in feed physical form, to ensure the same quality and to prevent feed selection by ducks (Buchanan and Moritz, 2009). Water was provided *ad libitum* until the end. Food was offered twice daily, approximately at 7.00 am and 15.00 pm. The excrements were cleared every evening, with light hours maintained at 16 hr day^{-1} . It was ensured that natural ventilation and hygiene was maintained throughout the study. Vaccines and medication are provided under the supervision of a veterinarian.

Performance and Egg Quality Parameters

All eggs were collected and egg production was calculated daily until the end of the experimental period. The weight, Haugh Unit and the yolk colour of each egg (10 per group) was measured weekly by EMT-5200 multi-function egg analyser (Robotmation, Japan). The albumen and egg

TABLE 1. INGREDIENT AND CHEMICAL COMPOSITION OF THE CONTROL GROUP'S DIET (g kg^{-1})

Ingredient	g kg^{-1}
Corn	610
Soybean meal	240
Wheat bran	40
Limestone power	50
Soybean oil	30
Fish meal	20
5% premix*	10
Nutrient**	
Crude protein	177.3
Lys	9.1
Met	3
Crude fat	58.9
Calcium	20.2
Total P	4.1
Metabolisable energy, MJ kg^{-1} of diet	12.2

Note: * The premix provided the following per kg of diets, retinol ≥ 60 KIU kg^{-1} , cholecalciferol ≥ 48 KIU kg^{-1} , tocopherol ≥ 320 IU, menadione ≥ 40 mg kg^{-1} , thiamin ≥ 11 KIU kg^{-1} , riboflavin ≥ 52 KIU mg kg^{-1} , vitamin B₆ ≥ 65 mg kg^{-1} , cobalamin ≥ 0.3 mg kg^{-1} , niacin ≥ 560 mg kg^{-1} , pantothenic acid ≥ 160 mg kg^{-1} , folic acid ≥ 16 mg kg^{-1} , biotin ≥ 1.4 mg kg^{-1} , choline chloride ≥ 9000 mg kg^{-1} , 20-500 mg kg^{-1} of Cu, 700-10 000 mg kg^{-1} of Fe, 800-2800 mg kg^{-1} of Zn, 900-2900 mg kg^{-1} of Mn, 27 mg kg^{-1} of I, 2-9.6 mg kg^{-1} of Se.

** Values were calculated from data provided by Feed Database in China (2013).

yolk were then separated and each egg yolk was stored at -20°C before further analysis. Each yolk was extracted and analysed in triplicates for each method. Feed consumption was registered weekly per group and feed conversion ratio was calculated as grams of feed per grams of egg. Duck mortality was recorded as it occurred. Data recording began seven days prior to the experimental period.

Extraction and Analysis of Diet and Yolk Total Carotenoids and Individual Carotenoids

Carotenoids from the extracted egg yolk and carotenoids standards were analysed by using a high performance liquid chromatography (HPLC) system. Commercial standards of relevant carotenoids were used for quantification by dissolving them in dichloromethane to the following concentrations ($\mu\text{g litre}^{-1}$): β -carotene 2.16, 3.24, 5.4, 10.8, 21.6, 32.4, 54 and 108 mg litre^{-1} , lutein 1, 3, 4, 6, 10, 20 and 30 mg litre^{-1} and zeaxanthin 1, 2, 3, 4, 5, 10, 15, 20 and 50 $\mu\text{g ml}^{-1}$. The solutions described above were filtered through a 0.45 μm polytetrafluoroethylene (PTFE) membrane into HPLC vials and subjected to HPLC analysis to draw standard curves.

Mixed 100 mg litre⁻¹ lutein 1 ml, 100 mg litre⁻¹ zeaxanthin 1 ml and 600 mg litre⁻¹ β-carotene 1 ml in volumetric flask and dichloromethane was added in the volumetric flask to 10 ml. The solution was then filtered through a 0.45 µm PTFE membrane into HPLC vials and subjected to HPLC analysis.

Egg yolk carotenoids concentration (µg g⁻¹ yolk) was analysed by HPLC. Yolk samples were analysed within six months after egg collection (Hargitai *et al.*, 2016), hence we did not expect a considerable reduction in carotenoids levels.

According to the Association of Official Analytical Chemists (AOAC) method, the spectroscopic determination of total carotenoids is equal to the spectroscopic determination of β-carotene (Islam and Schweigert, 2015). 1.08 mg of β-carotene standard was dissolved in dichloromethane (Concentrations 2.16, 3.24, 5.4, 10.8, 21.6, 32.4, 54 and 108 mg litre⁻¹) with optical density (OD) value at 450 nm. A standard curve line of best fit was then constructed to derive the relationship between OD value and carotenoid concentration.

Total carotenoid extractions were carried out using the methodology described by Paredes-Molina *et al.* (2016), with some slight modifications. The carotenoids composition of the test diets (Table 2) and the egg yolks were determined using HPLC analysis (Agilent Technologies Co. Ltd., China). Accordingly, 3 g of egg yolks (five per group)/ poultry feed was extracted in 15 ml of petroleum ether/acetone (2:1 v/v) in a Pyrex glass vial. Pyrex glass vials were sonicated using an ultrasonic bath (Kunshan Ultrasonic Instruments Co. Ltd., model KQ5200, China) for 5 min and then extracted for 60 min at room temperature (25°C). The supernatants were transferred to new vials and the extraction procedure was repeated three more times. The volume of supernatant was set to 50 ml, its absorbance was determined at 450 nm using an ultraviolet spectrophotometer (INESA Analytical Instrument Co. Ltd., model 752N, China). The total carotenoid content was calculated based on the standard curve line derived above.

TABLE 2. THE CONTENT OF CAROTENOIDS OF LAYING DUCK'S DIETS (g kg⁻¹ forage)

Item	Control group	1%RPO group	2%RPO group	3%RPO group
Lutein	6 120	6 690	6 570	6 600
Zeaxanthin	470	510	960	750
β-carotene	9 810	13 450	17 750	24 850
Unknown	14 660	20 680	25 660	33 590
Total carotenoids	18 890	50 640	60 070	74 990

Note: RPO - red palm oil.

The sample extracts described above were filtered through a 0.45 µm PTFE membrane into HPLC vials and subjected to HPLC analysis. The contents of individual carotenoids in egg yolks were quantified on the basis of the corresponding retention times and standard curves.

An Agilent Technologies HPLC system with a variable wavelength detector (VWD) at 450 nm was used for analysis of carotenoids. A precolumn with inner dimensions of 10 mm × 4 mm was included before the column and held at room temperature. A column (YMC Europe GmbH, Dinslaken, Germany) with 5 µm C30 reverse phase material with inner dimensions of 250 mm × 4.6 mm was used for separation. Mobile phases A methanol: acetonitrile = 25:75; mobile phases B: MTBE; mobile phases C: H2O. Gradient elution was performed with 0~15 min: ~A 98%~70%, B 0~28%, C 2%; 15~20 min: A 70%~18%, B 28%~80%, C 2%; 20~22 min: A 18%, B 80%, C 2%; 22~23 min: A 18%~0, B 80%~100%, C 2%~0; 23~25 min: A 0~98%, B 100%~0, C 0~2%. Column temperature was at room temperature and flow rate was 1 ml min⁻¹. An 20 µl sample extracts and standard solutions were injected.

Extraction and Analysis of Yolk and Diet Fatty Acids

The fatty acid composition of the test diets (Table 3) and the egg yolks were determined using standard gas chromatography (Agilent Technologies Co. Ltd., America) techniques of the fatty acid methyl esters (AOAC, 1990), using C17:1 fatty acid (Nu-Chek Prep Inc., Elysian, Minnesota) as an internal standard. Fatty acids were extracted from the egg yolk according to the methods of Folch *et al.* (1957). The diet subsamples were also stored at -20°C before the fatty acids analysis (Couch *et al.*, 1970).

Determination of Total Cholesterol (TC) and Triglyceride (TG) in Egg Yolk and Serum

Blood samples of all ducks were drawn from the wing vein, at the beginning and the end of the experimental period. The content of TC and TG in the egg yolk and serum was detected with a kit (Nanjing Jiancheng Bioengineering Institute, China), and the absorbance was measured using a microplate reader (Bio Tek, USA), and the content was calculated according to the formula.

STATISTICAL ANALYSES

All data were analysed using one-way ANOVA using SPSS 17 .0 statistics software (SPSS, Chicago, Illinois, USA).

TABLE 3. FATTY ACID PROFILE OF LAYING DUCK'S DIETS

Fatty acids	Control group	1%RPO group	2%RPO group	3%RPO group
C16:0 (% ¹)	11.80	18.07	18.02	24.08
C18:0 (%)	3.21	3.27	3.36	3.45
C18:1 (%)	24.68	26.71	27.07	29.48
C18:2 n-6 (%)	52.95	49.69	44.81	36.95
C20:1 (%)	5.29	0.79	4.10	2.96
C22:0 (%)	0.47	0.03	0.32	0.32
C20:5 n-3 (%)	0.17	0.08	0.17	0.17
Others (%)	1.44	1.48	2.15	2.59
SFA (%)	16.26	22.46	23.07	29.85
MUFA (%)	30.11	27.65	31.45	32.64
PUFA (%)	53.63	49.88	45.48	37.51
n-3 PUFA (%)	0.19	0.20	0.20	0.20
n-6 PUFA (%)	53.34	49.65	45.21	37.19
Total (g/100 g feed ²)	5.9302	5.7350	5.6758	5.6197

Note: ¹ the percentage of individual fatty acids in total fatty acids; ² the mass of fatty acids contained in 100 g of feed; RPO - red palm oil; SFA - saturated fatty acid; MUFA - monounsaturated fatty acid; PUFA - polyunsaturated fatty acid.

RESULTS

Carotenoids and Fatty Acid Profile of the Diets

The individual carotenoids levels in each diet are as shown in Table 2. The total carotenoids in the control group diet was lower than that in the RPO group diets. The carotenoids in the RPO were mainly β -carotene, with small amounts of lutein. Therefore, the concentration of carotenoids was increased with the increase of RPO content in the experimental diets. The total carotenoids content in the 30 g kg⁻¹ RPO group was about 3.97 times than that of the control group.

The fatty acid profile in the control and experimental diet are as shown in Table 3. The total fatty acid content of the feeds in each group was

similar (5.62 to 5.93 g/100 g feed). Adding RPO to the feed can significantly increase the content of SFA and MUFA such as palmitic acid, stearic acid and oleic acid in the feed, while the content of PUFA such as linoleic acid is significantly reduced, and the content of n-3 PUFA in each group is similar. The content of n-6 PUFA decreases with the increase of RPO content.

Effects of RPO on the Productive Performance

As shown in Table 4, the average daily feed intake and laying rate of feed treatment groups of 10 g kg⁻¹ and 20 g kg⁻¹ were significantly higher than the control group ($P < 0.05$). Furthermore, the laying rate of group with RPO at 30 g kg⁻¹ was significantly lower than the groups with RPO at 10 g kg⁻¹ and 20 g kg⁻¹ ($P < 0.05$). However, there was no significant difference observed for the feed conversion rate in the RPO groups compared with the control group ($P > 0.05$) (Table 4).

Effects of RPO on Egg Quality of Ducks

All levels of RPO meal used in this study had no effect on average egg weight, albumen height and Haugh unit ($P > 0.05$) (Table 5). However, there was a significant difference in egg yolk colour of RPO groups compared with the control group ($P < 0.05$). There was a dose-response relationship between egg yolk colour and RPO concentration in the feed. Yolk colour changed significantly ($P < 0.001$) with the RPO concentration ($P < 0.05$), and the eggs of RPO at 30 g kg⁻¹ group had the highest colour concentration of egg yolk.

Carotenoids Levels in Egg Yolk

Lutein, β -carotene and total carotenoids in the egg yolk changed significantly ($P < 0.05$) after feeding RPO (Table 6). After eight weeks of RPO intervention, the content of lutein and total carotenoids in the group with RPO at 30 g kg⁻¹ was significantly higher than those in the control group and groups with RPO at 10 g kg⁻¹ and 20 g kg⁻¹ ($P < 0.05$). After eight weeks of intervention, the content of β -carotene in

TABLE 4. EFFECTS OF RPO SUPPLEMENTATION TO LAYING DUCKS ON WEIGHT AND PRODUCTIVE PERFORMANCE (mean \pm SD)

	Control group	1% RPO group	2% RPO group	3% RPO group	F	P
Average daily feed intake (g/bird/day)	164.90 \pm 18.33 ^a	264.75 \pm 15.16 ^b	255.82 \pm 1.86 ^b	244.85 \pm 32.15 ^b	15.738	0.001
Laying rate (%)	71.11 \pm 6.94 ^{ab}	80.70 \pm 3.04 ^b	80.70 \pm 3.04 ^b	63.49 \pm 7.27 ^a	6.981	0.013
Feed conversion rate (g feed/g egg)	3.76 \pm 0.97	4.26 \pm 0.32	4.14 \pm 0.71	5.04 \pm 0.49	1.931	0.203

Note: ^{a,b} In the same row, values with the different symbol superscripts mean significant differences ($P < 0.05$), while with the same or no symbol superscripts mean no significant differences ($P > 0.05$); RPO - red palm oil; F - statistical value; P - intragroup comparison for changes in the parameters.

TABLE 5. EFFECTS OF RPO ON EGG QUALITY OF LAYING DUCKS AFTER FEEDING EIGHT WEEKS (mean ± SD) (n=10)

	Item	Control group	1% RPO group	2% RPO group	3% RPO group	F	P
Baseline	Average egg weight /g	77.28 ± 4.82	80.14 ± 6.22	78.97 ± 6.76	79.08 ± 5.82	0.395	0.757
	Albumen height /mm	7.46 ± 1.12	7.26 ± 0.84	7.06 ± 1.57	6.76 ± 1.72	0.484	0.695
	Yolk colour score	9.09 ± 1.21	9.08 ± 0.69	8.95 ± 0.84	9.34 ± 0.55	0.359	0.783
	Haugh unit	81.22 ± 7.55	79.26 ± 6.13	77.25 ± 11.40	79.31 ± 6.47	0.391	0.761
Eight weeks	Average egg weight /g	71.20 ± 8.75	72.57 ± 5.29	72.44 ± 4.21	74.90 ± 5.55	0.623	0.605
	Albumen height /mm	7.49 ± 0.61	8.15 ± 1.29	7.11 ± 0.88	8.38 ± 1.56	2.627	0.065
	Yolk colour score	9.02 ± 0.68 ^a	9.83 ± 0.48 ^b	10.22 ± 0.77 ^{b,c}	10.60 ± 0.24 ^c	12.803	<0.001
	Haugh unit	83.54 ± 3.92	86.52 ± 6.65	80.38 ± 6.73	85.06 ± 7.86	1.669	0.191

Note: ^{a,b} In the same row, values with the different symbol superscripts mean significant differences ($P<0.05$), while with the same or no symbol superscripts mean no significant differences ($P>0.05$); RPO - red palm oil; F - statistical value; P - intragroup comparison for changes in the parameters.

TABLE 6. EFFECTS OF RPO SUPPLEMENTATION TO LAYING DUCKS ON CAROTENOIDS LEVELS IN EGG YOLK (mean ± SD) (n=5)

	Carotenoids	Control group	1% RPO group	2% RPO group	3% RPO group	F	P
Baseline	Lutein ($\mu\text{g g}^{-1}$ yolk)	12.69 ± 0.28	12.35 ± 0.74	12.47 ± 0.54	12.33 ± 0.90	0.267	0.848
	Zeaxanthin ($\mu\text{g g}^{-1}$ yolk)	9.01 ± 1.09	9.17 ± 2.00	8.49 ± 1.05	9.03 ± 1.25	0.225	0.878
	β -carotene ($\mu\text{g g}^{-1}$ yolk)	8.02 ± 0.09	7.97 ± 0.07	8.00 ± 0.11	8.03 ± 0.08	0.481	0.700
	Total carotenoids ($\mu\text{g g}^{-1}$ yolk)	34.70 ± 1.85	34.90 ± 4.01	37.32 ± 2.24	37.60 ± 0.92	1.884	0.173
After eight weeks	Lutein ($\mu\text{g g}^{-1}$ yolk)	12.98 ± 0.47 ^a	12.25 ± 0.92 ^a	13.16 ± 0.95 ^a	28.09 ± 6.71 ^b	23.515	<0.001
	Zeaxanthin ($\mu\text{g g}^{-1}$ yolk)	9.79 ± 1.26	9.62 ± 1.92	10.43 ± 1.22	10.09 ± 2.11	0.224	0.878
	β -carotene ($\mu\text{g g}^{-1}$ yolk)	7.94 ± 0.07 ^a	8.13 ± 0.17 ^a	8.86 ± 0.24 ^b	9.97 ± 0.93 ^c	18.609	<0.001
	Total carotenoids ($\mu\text{g g}^{-1}$ yolk)	36.61 ± 2.90 ^a	40.46 ± 9.44 ^a	41.53 ± 1.75 ^a	58.22 ± 9.02 ^b	9.051	0.001

Note: ^{a,b,c} In the same row, values with the different symbol superscripts mean significant differences ($P<0.05$), while with the same or no symbol superscripts mean no significant differences ($P>0.05$); RPO - red palm oil; F - statistical value; P - intragroup comparison for changes in the parameters.

the yolk had gradually increased with the increase of RPO content in the diet ($P<0.05$). The content of β -carotene in the groups with RPO at 20 g kg^{-1} and 30 g kg^{-1} was significantly higher than that in the control group. In the RPO group ($P<0.05$), the content of β -carotene in yolk was highest in the group with RPO at 30 g kg^{-1} .

TC and TG Content of Serum and Eggs

RPO had a significant effect on serum TG, yolk TC and yolk TG contents in duck eggs ($P<0.05$). The serum TG, yolk TC and yolk TG contents in RPO groups were significantly lower than those in the control group, but there was no significant difference among the three RPO groups. The effect of RPO on serum TC in laying ducks was not significant ($P>0.05$) (Table 7).

Fatty Acids Levels in Egg Yolk

Main fatty acids of egg yolk in the control group are linoleic acid and palmitic acid, while the main

fatty acids in egg yolk of the RPO groups, are oleic acid and palmitic acid. After eight weeks of RPO feeding, the contents of SFA and MUFA in the RPO groups were higher than those in the control group ($P<0.001$), and the content of MUFA in the egg yolk had also gradually increased with the increase of RPO content in the feed. The content of PUFA in the egg yolk of each group had decreased with the increase of RPO content in the feed ($P<0.001$). The total fatty acid content of yolk in each RPO group was slightly lower than that in the control group but the difference was not statistically significant ($P>0.05$) (Table 8).

DISCUSSION

Effects of RPO on the Productive Performance and Egg Quality of Ducks

This study found that the feed intake of RPO groups were higher than that of the control group ($P<0.05$). The egg laying rate of each RPO groups had no significant difference compared with the

TABLE 7. EFFECTS OF RED PALM OIL SUPPLEMENTATION TO LAYING DUCKS ON SERUM AND EGG YOLK LIPID INDICES (mean \pm SD) (n=12)

	Item	Control group	1% RPO group	2% RPO group	3% RPO group	F	P
Baseline	Serum TC/ (mmol litre ⁻¹)	4.63 \pm 1.78	4.43 \pm 1.90	3.96 \pm 1.17	4.10 \pm 1.07	0.456	0.714
	Serum TG/(mmol litre ⁻¹)	19.81 \pm 5.56	18.78 \pm 4.24	17.13 \pm 3.35	18.78 \pm 4.48	0.688	0.564
	Yolk TC/ (mmol g ⁻¹ yolk)	6.75 \pm 3.31	6.78 \pm 3.12	7.84 \pm 2.50	7.69 \pm 3.21	0.359	0.783
	Yolk TG/ (mmol g ⁻¹ yolk)	41.65 \pm 10.14	41.71 \pm 8.70	44.74 \pm 8.95	42.29 \pm 7.99	0.264	0.851
After eight weeks	Serum TC/ (mmol litre ⁻¹)	4.61 \pm 1.85	6.02 \pm 1.78	5.46 \pm 2.00	3.86 \pm 2.03	2.800	0.052
	Serum TG/ (mmol litre ⁻¹)	17.07 \pm 5.82 ^a	9.86 \pm 8.31 ^b	7.14 \pm 5.87 ^b	6.45 \pm 4.48 ^b	6.378	0.001
	Yolk TC/ (mmol g ⁻¹ yolk)	7.78 \pm 2.54 ^a	4.74 \pm 2.67 ^b	4.76 \pm 1.62 ^b	5.36 \pm 0.85 ^b	4.952	0.006
	Yolk TG/ (mmol g ⁻¹ yolk)	44.10 \pm 9.69 ^a	32.76 \pm 7.17 ^b	28.78 \pm 7.95 ^b	31.50 \pm 6.73 ^b	7.016	0.001

Note: ^{a,b} In the same row, values with the different symbol superscripts mean significant differences ($P < 0.05$), while with the same or no symbol superscripts mean no significant differences ($P > 0.05$); RPO - red palm oil; TC - total cholesterol; TG - triglyceride; F - statistical value; P - intragroup comparison for changes in the parameters.

TABLE 8. EFFECT OF DIETARY OIL ON THE FATTY ACID IN EGG YOLK AFTER FEEDING RPO (percentage of total fatty acids)

Fatty acids	Control group	1% RPO group	2% RPO group	3% RPO group	F	P
C16:0	21.55 \pm 0.35 ^a	23.94 \pm 0.94 ^{b,c}	23.14 \pm 0.23 ^b	24.61 \pm 0.24 ^c	27.830	<0.001
C16:1	1.94 \pm 0.21 ^a	2.19 \pm 0.43 ^{a,b}	2.27 \pm 0.06 ^{a,b}	2.51 \pm 0.20 ^b	3.519	0.043
C18:0	0.66 \pm 0.14 ^a	1.47 \pm 0.10 ^b	1.25 \pm 0.71 ^b	1.32 \pm 0.15 ^b	7.070	0.004
C18:1	5.97 \pm 0.48 ^a	51.25 \pm 1.59 ^b	53.09 \pm 2.17 ^{b,c}	54.01 \pm 0.95 ^c	1 533.562	<0.001
C18:2 n-6	60.64 \pm 0.58 ^a	11.58 \pm 0.38 ^b	12.17 \pm 2.93 ^b	9.38 \pm 0.79 ^c	1 939.649	<0.001
C18:3 n-6	0.22 \pm 0.04 ^a	0.23 \pm 0.02 ^a	0.18 \pm 0.01 ^b	0.17 \pm 0.01 ^b	6.449	0.006
C18:3 n-3	1.05 \pm 0.05	0.53 \pm 0.35	0.66 \pm 0.56	0.42 \pm 0.34	3.275	0.053
C20:2	0.34 \pm 0.04 ^a	0.24 \pm 0.02 ^b	0.21 \pm 0.05 ^b	0.22 \pm 0.02 ^b	16.573	<0.001
C20:3 n-6	0.43 \pm 0.05 ^a	0.35 \pm 0.04 ^{a,b}	0.29 \pm 0.11 ^b	0.38 \pm 0.02 ^{a,b}	4.693	0.018
C20:4 n-6	4.71 \pm 0.14	5.13 \pm 0.41	3.97 \pm 1.28	4.35 \pm 0.46	2.819	0.077
C24:1	0.51 \pm 0.11	0.59 \pm 0.08	0.46 \pm 0.04	0.45 \pm 0.03	3.164	0.058
C22:6 n-3	1.00 \pm 0.16 ^a	0.94 \pm 0.15 ^a	1.00 \pm 0.32 ^a	0.61 \pm 0.07 ^b	5.611	0.010
SFA	23.05 \pm 0.23 ^a	26.30 \pm 1.06 ^c	25.16 \pm 0.71 ^b	26.88 \pm 0.36 ^c	32.201	<0.001
MUFA	8.58 \pm 0.35 ^a	54.63 \pm 1.02 ^b	56.90 \pm 2.38 ^c	57.51 \pm 0.68 ^c	2 162.841	<0.001
PUFA	68.38 \pm 0.54 ^a	19.07 \pm 0.59 ^b	17.94 \pm 1.87 ^b	15.61 \pm 0.66 ^c	3 895.144	<0.001
n-3 PUFA	2.26 \pm 0.12 ^a	1.76 \pm 0.46 ^b	1.85 \pm 0.75 ^b	1.24 \pm 0.33 ^b	4.943	0.015
n-6 PUFA	66.00 \pm 0.63 ^a	17.30 \pm 0.19 ^b	16.63 \pm 1.64 ^b	14.31 \pm 0.62 ^c	4 889.944	<0.001
Others	0.69 \pm 0.04 ^a	1.21 \pm 0.64 ^{a,b}	1.68 \pm 0.04 ^b	1.16 \pm 0.49 ^{a,b}	3.427	0.047
Total	45.70 \pm 1.01	34.84 \pm 8.09	35.95 \pm 6.26	44.34 \pm 3.24	4.538	0.059

Note: ^{a,b,c} In the same row, values with the different symbol superscripts mean significant differences ($P < 0.05$), while with the same or no symbol superscripts mean no significant differences ($P > 0.05$); RPO - red palm oil; SFA - saturated fatty acid, MUFA - monounsaturated fatty acid; PUFA - polyunsaturated fatty acid; F - statistical value; P - intragroup comparison for changes in the parameters.

control group ($P > 0.05$) but the egg laying rate of the group with RPO at 30 g kg⁻¹ was significantly lower than that in the groups with RPO at 10 g kg⁻¹ and 20 g kg⁻¹ ($P < 0.05$). It is suggested that short-term RPO and soybean oil intake had no effect on egg laying

rate of the ducks ($P > 0.05$) but rather the intervention of high-dose (30 g kg⁻¹) RPO may reduce egg laying rate ($P < 0.05$). This suggests that there is a limit to using RPO, which should be paid attention in practicality to derive the maximum quality of the

product and reap economic benefits. This is likely because RPO in itself has a special odour and colour which did not change drastically when a low dose is added to the feed. However, the content of nutrients such as carotenoids and vitamin E in the feed could be increased, thereby increasing the yield. When the ratio of addition is increased, the feed odour and colour can also change drastically, thereby affecting the feed intake, digestion and utilisation of laying ducks. However, studies have shown that feeding Cambrian duck with soybean oil (2%) or PO (2%) did not affect the growth and development of ducks (weight) ($P>0.1$), nor does it affect the feed intake of the ducks ($P>0.1$) (Zosangpui *et al.*, 2015).

All levels of RPO meal used in this study had no effect on the average egg weight, albumen height and Haugh unit ($P>0.05$). It is suggested that short-term RPO intake had no effect on egg quality of laying ducks compared with soybean oil ($P>0.05$).

Effects of RPO on the Egg Yolk Colour and Carotenoids Levels

After eight weeks of RPO intervention, there was a significant difference in egg yolk colour of RPO groups compared with the control group ($P<0.05$). There was a dose-response relationship between egg yolk colour and RPO concentration in the feed. Yolk colour had changed significantly ($P<0.001$) with the RPO concentration ($P<0.05$), and the eggs of the group with RPO at 30 g kg^{-1} had the highest colour of egg yolk. The results of this study suggest that adding RPO to feed can significantly improve the colour of egg yolk, and the level of yolk colour showed a significant upward trend with the increase of RPO in the diet. Further research found that RPO can increase the content of lutein, β -carotene and total carotenoids in egg yolk, with varying degrees ($P<0.05$), and the level of lutein, β -carotene and total carotenoids had increased with the increase of RPO in the diet. Since there was no zeaxanthin in RPO, there was no significant difference in yolk zeaxanthin content between the groups. This is consistent with the composition and content of carotenoids in RPO.

The traditional raw materials used in poultry feed usually do not provide sufficient carotenoids to achieve the yolk pigmentation demanded by consumers, so colour additives are included commonly in the form of carotenoid-rich dried plant extracts. Many studies focus on how to improve the colour of eggs to reap higher benefits. For example, Moreno *et al.* (2020) used carotenoid-enriched maize, while Titcomb *et al.* (2019) used carrot leaves and marigold fortification. RPO is rich in carotenoids, and the carotenoids content in the diet of each RPO group is also significantly higher than that of the control group. From this study, the carotenoids in RPO can be effectively absorbed and

converted into egg yolk by ducks and effectively improve the colour of egg yolk.

Effect of RPO on Serum Lipid Level and Egg Yolk Lipid Content

Research has shown that the lipid composition and fatty acid profile of an egg can be influenced by dietary manipulation (Ouyang *et al.*, 2004). After eight weeks of RPO intervention, serum TG, yolk TC and yolk TG level in each RPO groups were significantly lower than those in control group, which may be related to the sn-2 hypothesis, and may also be related to the rich tocotrienols and carotenoids in RPO. Studies have shown that tocotrienols can reduce the activity of HMG Co-A reductase (a rate-limiting enzyme in the synthesis of cholesterol in liver cells) and act as oxysterols to lower cholesterol level (Pearce *et al.*, 1992). Tocotrienol in PO is more efficient to transfer to the egg yolk than other oils (barley oil) (Walde *et al.*, 2014).

Further research found that because RPO contains approximately 44.13% SFA (mainly palmitic acid, 36.82%) and 43.36% MUFA (mainly oleic acid, 43.08%), the content of SFA (palmitic acid) and MUFA (oleic acid) in the egg yolk increased significantly with the increase of RPO in diets. Due to RPO has a low contain of PUFA, approximately 12.49%, dietary RPO had decreased the content of PUFA in egg yolk ($P<0.05$).

CONCLUSION

Eggs are regarded as one of the nature's most wholesome foods because they contain high-quality protein and lipids as well as essential and non-essential minerals and vitamins. Studies have shown that the bioavailability of lutein in eggs is high, which may be related to the fat contained in the egg yolk. Adding appropriate amount of RPO (10 g kg^{-1} and 20 g kg^{-1} in this article) may have the potential to increase the laying rate, bring the possibility of increasing production and increase people's income. The results of this study showed that dietary RPO can significantly increase egg yolk colour, increase carotenoids content in egg yolk and reduce certain lipid indicators, which could be more attractive to customers. In summary, RPO as a green and safe feed colourant deserves further development and promotion.

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