Effects of dietary fats on egg quality and lipid parameters in serum and yolks of *Shan Partridge Duck*

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ABSTRACT The effects of different dietary fats with variable levels of polyunsaturated fatty acids (PUFAs) on egg quality of *Shan Partridge Duck*, serum, and yolk lipid parameters were examined in this study. A flock of 585 optimal produced ducks were selected and diets enriched with 0.5%, 1%, or 2% fish oil (F)/flaxseed oil (FL)/rapeseed oil (R)/tallow (T) plus basal diet were supplied through a 28-d period. Supplemental fat source and fat level had no effects on egg qualities. Proportions of yolk total cholesterol (TC), saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) were reduced ($P < 0.001$), while polyunsaturated fatty acids (PUFAs), ω-6 polyunsaturated fatty acids (n-6 PUFAs), ω-3 polyunsaturated fatty acids (n-3 PUFAs), Docosahexaenoic Acid (DHA), and Eicosapentaenoic Acid (EPA) were increased by fish oil, flaxseed oil, or rapeseed oil. Effects of supplementation increasing DHA and EPA were detected in F, FL, and R. Compared with C, fish oil significantly increased low-density lipoprotein cholesterol (LDL-C) in serum, flaxseed oil significantly reduced TC and increased very low-density lipoprotein cholesterol (VLDL-C), rapeseed oil significantly reduced TC and LDL-C in serum and increased VLDL-C, tallow significantly increased LDL-C. It is concluded that unsaturated fatty acids rich diets (fish oil, flaxseed oil, and rapeseed oil) might increase yolk PUFAs, reduce yolk cholesterol, and change serum lipid parameters without evident effect on egg qualities.

Key words: duck, supplemental fats, yolk cholesterol, yolk PUFAs, egg quality

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INTRODUCTION

Eggs are an important food source for balanced nutrition in humans. Polyunsaturated fatty acids (**PUFAs**) content is an important nutrition index of eggs. N-6 and n-3 PUFAs are essential fatty acids (**FAs**) for vertebrates. Linoleic Acid (**LA**) and Arachidonic Acid (**ARA**) are n-6 PUFAs, while Alpha-linolenic Acid (**ALA**), Eicosapentaenoic Acid (**EPA**), and Docosahexaenoic Acid (**DHA**) belong to n-3 PUFAs. N-6 and n-3 PUFAs play important parts in growth, development, and reproduction, because they are closely related to many diseases, such as inflammation (Eilati et al., 2013), mood disorders (Janssen and Kiliaan, 2014), neurodevelopment problems, cardiovascular disease, and cancer (Sharma et al., 2009).

Since the beginning of the Industrial Revolution, human dietary intake has drastically changed. Important changes in dietary fat quality are the increased intakes of certain saturated fatty acids (**SFAs**), LA, trans fatty acids, and reduced intakes of n-3 PUFAs (Muskiet, 2010). Only people in a few countries, such as Japan, Korea, the Philippines, Finland, Iceland, Norway, and Sweden reach the intake of 250 mg n-3 PUFAs per day (Sioen et al., 2009), which is suggested to be necessary by Kris-Etherton et al. (2009). The average intake in all other countries is below that amount. Recent studies suggest that dietary cholesterol has little incidence on blood cholesterol in normal subjects, as most subjects can effectively adapt to higher levels of cholesterol. Nevertheless, lowering cholesterol intake might reduce the risk of heart disease for a group of people who are highly responsive to dietary cholesterol changes (Kratz, 2005).

This eating pattern usually is characterized by high intake of SFAs and cholesterol, and a low intake of PUFAs, which has been consistently proved to have higher correlation with heart disease. Therefore, natural food

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such as a n-3 PUFAs supply source with lower cholesterol, like egg and meat, has drawn much attention nowadays (Bovet et al., 2007).

Cruickshank (1934) firstly proved that feeding laying hens with different dietary FAs could significantly change the egg FAs composition. Since then, different studies have proved that FAs transfer from the poultry feed to the poultry tissues can be easily accomplished (Lopez-Ferrer et al., 2001; Pappas et al., 2006; Hall et al., 2007). It was also demonstrated that PUFAs not only alter egg FAs composition, but also reduce yolk cholesterol in avian species (Liu et al., 2011; Deng et al., 2012). Fish oil is usually used to increase the content of PUFAs in meat or eggs, while flaxseed oil, rapeseed oil, and tallow are common fats supplied in the poultry industry. Compared to control diet, fish oil diet contains more EPA and DHA, flaxseed oil diet contains more ALA, rapeseed oil diet contains more oleic acid (**OA**), while tallow diet has lower level of EPA, DHA, ALA, and OA. Each kind of fat is typical and the aim of this work was to comprehensively study the effect of different dietary fats on egg quality in *Shan Partridge Duck*, and lipid parameters of serum and yolk as well. This work will provide some information for modifying the fatty acid profile of fat to produce eggs with high PUFAs and low cholesterol to meet human nutritional demands.

MATERIALS AND METHODS

Animals

Ducks were housed in the floor pens for 4 weeks under natural conditions of lighting, heating, and ventilation. Feed and water were provided for ad libitum consumption. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Institutional Animal Care and Use Committee (IACUC) for Zhejiang University. All surgery was performed according to recommendations proposed by European Commission, and all efforts were made to minimize suffering of animals.

Experiment Design

A flock of 585 *Shan Partridge Ducks* were randomly assigned to 5 groups, and fed with various diets with supplemental fats: basal diet (**C**); fish oil diet (**F**); flaxseed oil diet (**FL**); rapeseed oil diet (**R**); tallow diet (**T**). Each treatment group was further divided into 3 subgroups added various levels (various levels: 0.5%, 1%, and 2%) of fats on top. Control and all the subgroups were triplicated with 15 ducks in each pen. The ducks were fed with various sources and different levels of fats to a basal diet (Table 1), which met the requirements recommended by the National Research Council (Dale, 1994). Fatty acid contents of C and 2% treatments of each fat source are shown in Table 2. Three eggs per replicate were collected and measured on day 28 for egg quality. After assay for egg qualities, yolks per replicate were separated, pooled, and homogenized for the determination of fatty acids, total cholesterol (**TC**), triglyceride (**TG**), and crude fat. Blood samples were taken and pooled from three ducks per replicate of the control diet (C), 2% fish oil (2% F), 2% flaxseed oil (2% FL), 2% rapeseed oil (2% R), and 2% tallow (2% T) after yolk lipids analysis. Standing at room temperature for one hour, serum was isolated by centrifugation at 3,000 × *g* for 10 min and stored at −20°C until further analysis. All lipid parameters were measured by gas chromatography. No overt toxicity was observed for any treatment.

Table 1. Composition and main characteristics of the basal diet.

Ingredients	Content (g/kg)	Nutrient	Content (%)
Maize grain	400	Metabolizable energy	11.2[b]
Wheat	290	Crude protein	16.5
Soybean meal	120	Total phosphorus	0.70
Wheat bran	90	Total calcium	3.35
Monocalcium phosphate	12	Total lysine	0.79
Limestone powder	80	Total methionine	0.40
Salt	3	Ether extract	29.0
Premix[a]	5		

[a]Supplied per kg of diet: vitamin A 1,500 U, cholecalciferol 200 U, vitamin E (DL-α-tocopheryl acetate) 10 U, riboflavin 3.5 mg, pantothenic acid 10 mg, niacin 30 mg, cobalamin 10 μg, choline chloride 1,000 mg, biotin 0.15 mg, folic acid 0.5 mg, thiamine 1.5 mg, pyridoxine 3.0 mg, Fe 80 mg, Zn 40 mg, Mn 60 mg, I 0.18 mg, Cu 8 mg, Se 0.3 mg;
[b]Unit: MJ/kg.

Table 2. Fatty acid composition of five groups.

Fatty acid (g/100 g total fatty acids)	Diets				
	Control	2% F	2% FL	2% R	2% T
SFA	32.61	30.36	25.50	26.22	41.94
C14:0	5.32	5.80	3.88	3.44	5.52
C15:0	0.29	0.19	0.18	0.19	0.47
C16:0	21.23	18.57	15.81	16.07	24.11
C18:0	5.12	4.87	5.16	4.91	11.19
C20:0	0.65	0.94	0.46	1.13	0.48
C22:0	0	0	0	0.35	0.06
C24:0	0	0	0	0.12	0.10
MUFA	37.75	34.65	33.03	48.50	36.90
C16:1	5.23	6.06	3.50	3.53	4.01
C18:1	31.2	25.42	28.53	42.39	32.04
C20:1	1.32	1.23	0.96	2.50	0.84
C22:1	0	1.57	0.05	0.07	0
C24:1	0	0.37	0	0	0
PUFA	29.84	35.12	41.59	25.40	20.25
C18:2(n = 6)	18.77	12.73	18.67	17.42	12.93
C20:2	0.11	0.78	0.07	0.12	0.07
C20:3	2.32	1.48	1.51	1.49	1.48
C22:3(n = 6)	0	1.08	0	0	0
C20:4	0.46	0.69	0.38	0.29	0.25
C22:4	0	1.08	0	0	0
C18:4	0	0.84	0	0	0
C18:3(n = 3)	6.22	4.42	19.69	4.83	4.21
C20:5(n = 3)	0.98	6.14	0.64	0.63	0.60
C22:5	0.65	0.86	0.42	0.44	0.35
C22:6(n = 3)	0.33	5.01	0.21	0.20	0.18

Egg Quality Measurement

Egg quality is defined according to the following parameters: egg weight, egg shape index, shell thickness, shell strength, albumen height, yolk color, Haugh unit, and yolk ratio. All eggs and yolks were weighed individually to 0.01-g accuracy. Shape index was represented by the ratio of egg length to width. Shell strength was measured by EFG-0503 (Robotmation, Tokyo, Japan). Shell thickness was measured by ETG-1061 (Robotmation, Tokyo, Japan). Yolk color (Vuilleumier, 1969), egg albumen height (Heiman, 1936), and Haugh unit (HU = 100 log [H − (1.7 × $W^{0.37}$) + 7.6)]) (Haugh, 1937) were measured by EMT-5200 (Robotmation, Tokyo, Japan).

Oil and Yolk Lipid Analysis

Lipids in diets and pooled yolks were extracted using the chloroform/methanol (Lot. No. 20141120/Lot. No. 20140520, Shanghai Lingfeng chemical reagent co., LTD., Shanghai, China) procedure of Folch et al. (1957), and were measured through methylating to fatty acid methyl esters (**FAMEs**). FAMEs were prepared with boron trifluoride methanol (CFAC-61626-500ML#, ANPEL Scientific Instrument Co., Ltd., Shanghai, China) according to Morrissson and Smith (1964), and analyzed by gas chromatography (6890, Agilent Technologies, Santa Clara, CA) on an HP-5MS column (30 m × 0.25 mm × 0.2 μm, Agilent Technologies, Santa Clara, CA). As described, yolk total crude fat, TC, and TG were determined by cholesterol oxidase p-aminophenol method (E1015 for serum, E1016 for yolk, Applygen Technologies Inc., Beijing, China) and glycerol-3-phosphate oxidase p-aminophenol method (E1003 for serum, E1013-105 for yolk, Applygen Technologies Inc., Beijing, China), respectively.

Serum Lipid Parameter Analysis

Serum HDL-C, LDL-C, VLDL-C were directly determined by commercial kits (ml022447, ml133309, ml023074, Tongwei inductrial Co., Ltd., Shanghai, China) as described by Artiss et al. (1997).

Statistical Analysis

The mean of 3 ducks in each pen was used to derive performance data. The statistical package SPSS (Version 17.0) was used for data analysis. The covariate analysis with non-quantitative variable (fat source) and covariable (fat level) was applied to analyze the differences between groups. Slopes were plotted to test the effect of each fat source and level. Differences were considered statistically significant at the 5% level ($P < 0.05$). Data were transformed by neperian logarithm when necessary and back transformed data are presented.

RESULTS AND DISCUSSION

Egg Quality Measurement

All egg qualities were not affected by supplemental fat source, fat level, or an interactions between them (Table 3). This indicated that such levels of fish oil, flaxseed oil, rapeseed oil, or tallow do not have an adverse effect on egg quality of *Shan Partridge Ducks*. Similar results of shape index, shell thickness, shell strength, and Haugh unit were observed by Kirubakaran et al. (2011). However, according to the studies of Menge (1968) and Macmilan (1990), LA increased egg weight by promoting albumen weight, which is not consistent with our results of egg weight, yolk weight, and yolk ratio. Fish oil had no significant effect on yolk color, which conflicts with the report of Lu et al. (2009), who showed that 2% fish oil significantly increased yolk color of Shaoxing Ducks. The effects of supplemented ingredients on poultry egg quality varied dramatically with respect to their different source, composition, extraction, and processing, as well as animal species and age (Windisch et al., 2008; Koppenol et al., 2014).

Yolk TC, TG, and Crude Fat Analysis

Supplemental fat source and interaction between fat source and fat level were detected for yolk cholesterol but not for yolk triglyceride or crude lipids. For yolk cholesterol, fat source was more significant ($P \leq 0.001$) than the interaction ($P = 0.040$) and therefore, main effects are discussed independently of the potential existing interaction (Jimenez-Moreno et al., 2016). Yolk cholesterol in level of F, FL, and R groups were lower ($P < 0.001$) than C and level of T groups, and the effect of level is significant for fish oil, flaxseed oil, and tallow (Table 4 and Figure 1). The total lipid composition of C conformed to a previous study (Sinanoglou et al., 2011), which suggested the reliability of our data. Three sources of fat might reduce yolk cholesterol through inhibiting the activity of 3-hydroxy-3-methyl glutaryl coenzyme A reductase, a key enzyme in cholesterol synthesis (Choi et al., 1989). No changes were detected in yolk TG and crude fat in all treatments, which is in agreement with Irandoust et al. (2015), while in conflict with Hodzic et al. (2008). Concentration effects were observed for reducing yolk cholesterol in fish oil and flaxseed oil, which is in accordance with previous study (Basmacioglu et al., 2004). The results indicated that fish oil, flaxseed oil, and rapeseed oil could be used as natural feed supplements to produce low-cholesterol duck egg for humans.

Yolk Fatty Acid Proportion Analysis

Supplemental fat source had significant effects on all fatty acids contents (Table 5). Compared with C, level of FL, R, F, and T had lower ($P < 0.001$)

Table 3. Influence of supplemental fat source and level on egg qualities.

Oil kind	Oil level (%)	Egg weight(g)	Egg shape index (%)	Shell strength (kg.f)	Shell thickness (mm)	Albumen height (mm)	Yolk color (level)	Haugh unit	Yolk weight (g)	Yolk ratio (%)
C[a]	0	74.03	1.33	4.34	0.43	6.60	12.38	73.64	24.56	0.34
F[a]	0.5	71.65	1.35	3.97	0.40	5.88	12.46	73.06	22.95	0.32
	1	73.16	1.35	3.39	0.41	5.83	12.13	70.52	24.78	0.33
	2	72.24	1.35	4.11	0.43	6.18	11.39	73.48	23.43	0.33
FL[a]	0.5	71.58	1.35	3.88	0.41	4.74	12.70	61.16	23.16	0.32
	1	71.70	1.37	4.46	0.43	5.23	12.51	64.83	22.54	0.32
	2	74.97	1.32	4.12	0.44	6.83	12.16	73.12	23.16	0.31
R[a]	0.5	74.59	1.35	3.98	0.40	6.74	12.39	75.91	24.11	0.32
	1	72.20	1.34	3.98	0.40	6.37	12.61	73.03	22.73	0.32
	2	70.58	1.31	3.77	0.41	6.16	12.66	72.89	22.79	0.33
T[a]	0.5	71.09	1.35	3.72	0.41	5.67	12.38	67.23	22.88	0.32
	1	71.70	1.38	4.22	0.41	5.20	12.29	63.37	23.87	0.33
	2	72.32	1.38	3.62	0.39	6.36	12.71	74.68	22.91	0.32
SEM		0.411	0.005	0.075	0.004	0.143	0.577	1.090	0.181	0.002
Source	C[a]	74.03	1.33	4.34	0.43	6.60	12.38	73.64	24.56	0.34
	F[b]	72.35	1.35	3.83	0.41	5.96	13.11	72.35	23.72	0.020
	FL[b]	72.75	1.35	4.16	0.43	5.60	14.68	66.37	22.95	0.016
	R[b]	72.46	1.33	3.91	0.40	6.42	12.55	73.94	223.21	0.016
	T[b]	71.70	1.37	3.85	0.40	5.74	12.46	68.43	23.22	0.016
Level	0[a]	74.03	1.33	4.34	0.43	6.60	12.38	73.64	24.56	0.34
	0.5[c]	72.23	1.35	3.89	0.41	5.66	14.98	69.34	23.28	0.32
	1[c]	72.19	1.36	4.01	0.41	6.38	12.39	67.94	23.48	0.33
	2[c]	72.53	1.34	3.90	0.42	6.60	12.23	76.54	23.07	0.32
					P-value					
Source		0.12	0.053	0.64	0.075	0.35	0.51	0.81	0.43	0.22
Level		0.77	0.61	0.94	0.17	0.079	0.060	0.12	0.66	0.49
Source × level		0.089	0.29	0.84	0.28	0.061	0.19	0.20	0.54	0.62

[a]Each value represents the mean of 3 replicate pens.
[b]Each value represents the mean of 9 replicate pens.
[c]Each value represents the mean of 12 replicate pens.

Table 4. Influence of supplemental fat source and level on yolk cholesterol, yolk triglyceride, and yolk crude fat (n = 3).

Oil kind	Oil level (%)	Yolk cholesterol level (mmol/L)	Yolk triglyceride level (mmol/L)	Yolk crude lipids (mmol/L)
C[1]	0	16.16	753.7	30.52
F[1]	0.5	14.65	746.7	31.39
	1	14.16	753.4	31.68
	2	14.11	751.4	31.90
FL[1]	0.5	14.64	747.5	31.49
	1	14.41	751.3	31.54
	2	14.13	753.1	31.91
R[1]	0.5	14.50	754.6	31.43
	1	14.63	757.3	31.68
	2	14.50	760.7	31.90
T[1]	0.5	16.05	764.3	31.31
	1	16.11	769.7	32.00
	2	16.40	772.7	32.28
SEM		0.14	1.8	0.21
Source	C[1]	16.16[1]	753.7	30.52
	F[2]	14.31[2]	750.5	31.66
	FL[2]	14.39[2]	750.6	31.65
	R[2]	14.54[2]	757.5	31.67
	T[2]	16.22[1]	768.9	31.86
Level	0[1]	16.16	753.7	30.52
	0.5[3]	14.96	753.3	31.41
	1[3]	14.83	757.9	31.73
	2[3]	14.80	759.5	32.00
			P-value	
Source		<0.001	0.19	0.97
Level		0.11	0.10	0.30
Source × Level		0.040	0.99	0.98

Means with different superscripts within the same column differ significantly ($P < 0.05$).
[1]Each value represents the mean of 3 replicate pens.
[2]Each value represents the mean of 9 replicate pens.
[3]Each value represents the mean of 12 replicate pens.

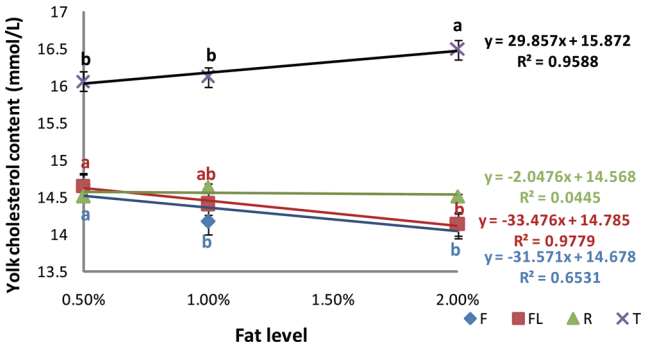

Figure 1. Regression analyses of fat level and the content of yolk cholesterol in different groups. Level effects were detected in F, FL, and T. Yolk cholesterol content increased in T as fat level increased, while reduced in F and FL. Different letters indicate significant differences ($P < 0.05$).

proportions of yolk SFAs, level of F, FL, and R had higher ($P < 0.001$) proportions of yolk PUFAs, including both n-6 PUFAs and n-3 PUFAs. The predominant increases induced by F, FL, R were long-chain PUFAs (EPA and DHA), ALA, and LA, respectively. N-3 PUFAs (EPA and DHA) in F or FL were highly significant higher and n-6/n-3 was highly significantly lower than other groups. Nevertheless, level of T showed no significant difference to C in these indexes. Significant effects of fat level were observed in n-3 PUFAs, ALA, EPA, and DHA. Regression analyses of fat levels are plotted in Figure 2. Interactions between fat source and fat level were also detected in some indexes, but it can be

Table 5. Influence of supplemental fat source and level on yolk fatty acid composition and proportion (g/100 g total fatty acids).

Oil kind	Oil level	SFA	MUFA	PUFA	n-6 PUFA	C18:2 (n = 6)	C20:4 (n = 6)	n-3 PUFAs	C18:3 (n = 3)	C20:5 (n = 3)	C22:6 (n = 3)	n-6/ n-3
C[1]	0	36.28	47.18	16.54	15.89	13.17	2.32	0.65	0.32	0.05	0.27	23.96
F[1]	0.5	34.28	38.97	21.04	17.70	15.39	2.31	2.81	0.79	0.63	1.39	6.30
	1	34.33	38.62	21.19	17.69	15.03	2.66	2.96	0.81	0.67	1.48	5.97
	2	32.79	39.44	21.73	18.11	15.26	2.85	3.04	0.76	0.72	1.56	5.98
FL[1]	0.5	31.60	40.85	24.47	20.95	18.40	2.55	2.99	1.27	0.49	1.23	7.02
	1	30.87	41.50	24.37	20.89	18.34	2.58	3.14	1.41	0.45	1.28	6.64
	2	30.03	44.80	24.99	21.30	18.66	2.64	3.32	1.44	0.52	1.36	6.45
R[1]	0.5	30.38	45.44	23.62	21.14	19.07	2.07	1.88	0.90	0.17	0.81	11.23
	1	30.03	45.36	23.93	21.48	19.31	2.17	2.14	0.99	0.21	0.94	10.05
	2	29.90	44.38	25.12	22.49	20.23	2.34	2.23	1.04	0.24	0.94	10.12
T[1]	0.5	33.90	49.90	15.93	14.74	12.98	1.76	0.73	0.31	0.02	0.39	20.72
	1	34.42	49.18	16.10	14.95	12.93	2.03	0.71	0.34	0.03	0.35	20.98
	2	35.24	47.68	16.73	15.54	13.08	2.46	0.72	0.32	0.02	0.38	22.55
SEM		0.373	0.629	0.573	0.448	0.436	0.054	0.165	0.065	0.041	0.075	1.137
Source	C[1]	36.28^{a}	46.94^{a,b}	16.54^{c}	15.49^{c}	13.17^{d}	2.32^{a,b}	0.65^{c}	0.32^{d}	0.05^{d}	0.27^{d}	23.96^{a}
	F[2]	33.80^{b}	39.01^{d}	21.32^{b}	17.83^{b}	15.23^{c}	2.61^{a}	2.94^{a}	0.79^{c}	0.67^{a}	1.48^{a}	6.08^{c}
	FL[2]	30.83^{c}	42.38^{c}	24.61^{a}	21.05^{a}	18.47^{b}	2.59^{a}	3.15^{a}	1.37^{a}	0.48^{b}	1.18^{b}	6.70^{c}
	R[2]	30.16^{c}	45.06^{b}	24.22^{a}	21.70^{a}	19.54^{a}	2.19^{a,b}	2.08^{b}	0.99^{b}	0.21^{c}	0.89^{c}	10.47^{b}
	T[2]	34.52^{b}	48.92^{a}	16.26^{c}	15.08^{c}	13.00^{d}	2.08^{b}	0.72^{c}	0.32^{d}	0.02^{d}	0.37^{d}	21.42^{a}
Level	0[1]	36.28	46.94	16.54^{b}	15.49^{c}	13.17^{c}	2.32a^{b}	0.65^{b}	0.32^{b}	0.05^{c}	0.27^{c}	23.96
	0.5[3]	33.52	48.05	18.40^{b}	16.59^{b,c}	14.52^{b,c}	2.08^{b}	1.01^{b}	0.47^{b}	0.06^{c}	0.48^{c}	18.87
	1[3]	30.59	43.02	24.47^{a}	21.45^{a}	19.07^{a}	2.41^{a,b}	2.62^{a}	1.18^{a}	0.35^{b}	1.01^{b}	8.46
	2[3]	32.86	40.46	22.24^{a}	18.70^{b}	16.08^{b}	2.62^{a}	3.03^{a}	0.95^{a}	0.63^{a}	1.45^{a}	6.17
						P-value						
Source		<0.001	<0.001	<0.001	<0.001	<0.001	0.042	<0.001	<0.001	<0.001	<0.001	<0.001
Level		0.14	0.60	<0.001	0.001	0.037	<0.001	<0.001	0.040	<0.001	0.004	0.95
Source × Level		0.047	0.022	0.44	0.30	0.037	0.033	0.042	0.093	0.016	0.059	0.50

Means with different superscripts within the same column differ significantly ($P < 0.05$).
[1]Each value represents the mean of 3 replicate pens.
[2]Each value represents the mean of 9 replicate pens.
[3]Each value represents the mean of 12 replicate pens.

independently discussed as the effects were much smaller than fat source or fat level.

Yolk fatty acid composition analysis indicated that unsaturated fatty acids (**UFAs**) diets (F, FL, R) higher than a certain level can increase the levels of PUFAs (both n-6 PUFAs and n-3 PUFAs) in yolk. This was in agreement with the findings that supplementation of UFAs increases the concentration of the same UFAs in yolks (Cherian et al., 1996; Antruejo et al., 2011). Koppenol et al. (2014) found the ratio of DHA to EPA in yolk is closely related to the ratio in diets, which was supported by our study. However, our results also indicated the conversion of DHA and EPA from shorter fatty acids as there were higher ratios of long-chain PUFAs in yolk of FL and R than in relevant diets. This is in agreement with the hypothesis that long-chain PUFA, such as DHA and EPA, can be synthesized from C18 PUFAs by fatty acyl desaturases and elongases (Pereira et al., 2003; Leonard et al., 2004).

Compared with the C and T groups, the n-6/n-3 PUFAs value decreased dramatically in level of F, FL, and R groups. However, it is still possible to further reduce the n-6/n-3 ratio to the value of 1 to 4 according to the suggestion of Basmacioglu et al. (2004). As our fat supplementation only lasted for 4 weeks, the limited feeding period might account for the higher ratio. In general, FL performed best in promoting ALA deposition in yolk, while F is very good at increase yolk EPA and DHA.

Serum Lipid Parameter Analysis

Values of 2% F, 2% FL, 2% R, and 2% T were chosen for serum lipid analysis, because these groups performed better in most important yolk lipids analysis. Serum lipid parameter results are shown in Table 6. Among all groups, 2% T has the highest TG, TC, HDL-C, and LDL-C. 2% FL and 2% R reduced TG ($P < 0.05$), 2% R reduced TG and LDL-C, meanwhile, increased VLDL-C. Other parameters had no significant differences.

Diets containing UFAs have been reported to reduce serum TC, TG, and LDL-C, but increase serum HDL-C (Sunitha et al., 1997; Ortiz-Munoz et al., 2009; Liu et al., 2011). Our results showed that serum TG was reduced by FL and R, which are rich in UFAs. Serum TG, TC, HDL-C, and LDL-C were increased by T, which is rich in SFAs. It is well known that high blood TG and TC are the major risk factors for heart disease. Our study demonstrated the beneficial effects of UFAs and potential risks of SFAs intake on serum TG and TC levels. VLDL and LDL are different types of lipoprotein that mostly transport fatty acids and cholesterol through blood circulation. VLDL-C and LDL-C are considered as "bad" types of cholesterol. Our study showed LDL-C could be increased by T, and reduced by R, while all the oils could enhance VLDL-C. Previous studies (Schneider et al., 1990; Bujo et al., 1994; Elkin et al., 1999; Walzem et al., 1999) demonstrated that

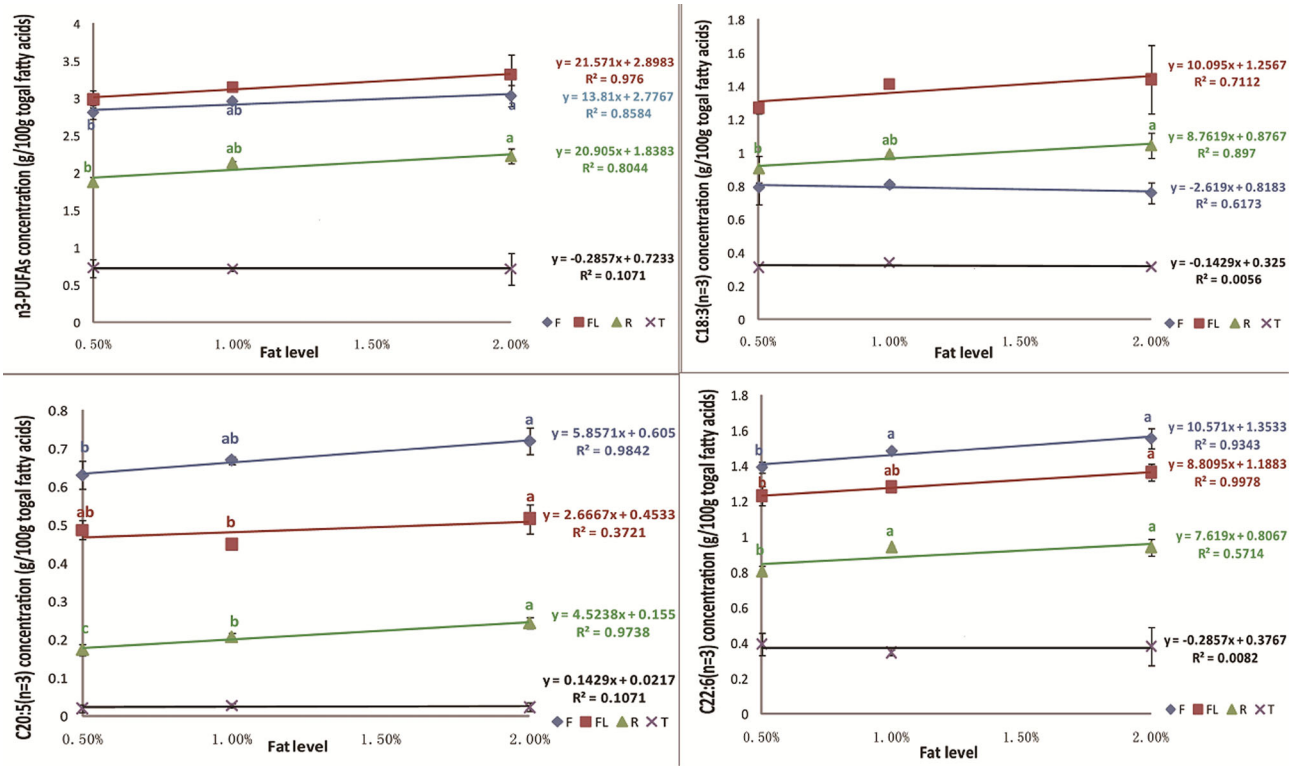

Figure 2. Regression analyses of fat level and the concentration of fatty acids in different groups. A-D denote the contents of n-3 PUFAs, C18:3 (n-3), C20:5 (n-3), and C22:6 (n-3) in four treatment groups. Different letters indicate significant differences ($P < 0.05$).

Table 6. Influence of supplemental fat source and level on lipid parameters of serum (n = 3).

Parameters	C	2% F	2% FL	2% R	2% T	SEM	*P*-value
TG(mmol/L)	1.51 ± 0.04^{a,b}	1.48 ± 0.08^{b}	1.31 ± 0.07^{c}	1.35 ± 0.05^{c}	1.66 ± 0.16^{a}	0.15	<0.001
TC(mmol/L)	5.74 ± 0.31^{a,b}	5.59 ± 0.06^{b}	5.61 ± 0.13^{b}	5.52 ± 0.20^{b}	5.92 ± 0.16^{a}	0.04	0.011
HDL-C(mmol/L)	1.06 ± 0.11^{a,b}	1.11 ± 0.08^{a}	0.99 ± 0.07^{b}	1.11 ± 0.04^{a}	1.09 ± 0.07^{a}	0.02	0.058
LDL-C (mmol/L)	2.12 ± 0.05^{b}	2.11 ± 0.04^{b}	2.07 ± 0.03^{b}	1.85 ± 0.06^{c}	2.24 ± 0.07^{a}	0.03	<0.001
VLDL-C (mmol/L)	1.05 ± 0.03^{c}	1.11 ± 0.06^{b}	1.17 ± 0.05^{b}	1.42 ± 0.07^{a}	1.11 ± 0.05^{b,c}	0.03	<0.001

Means with different superscripts within the same column differ significantly ($P < 0.05$).

UFAs supplemented in diets can interfere the reception of VLDL through inhibiting oocyte vitellogenesis receptor in follicles. HDL-C is known as the "good" cholesterol. HDL removes cholesterol from bloodstream and carries it back to the liver for recycling. Regretfully, no obvious effect of the oils was detected on serum HDL-C levels.

CONCLUSIONS

Three dietary oils (F, FL, and R) enriched with PUFAs could promote lipid parameters of serum and yolk of *Shan Partridge Duck* with little negative effects on egg quality. These oils might be able to serve as natural feed additive to produce high-PUFA and low-cholesterol eggs for better health in humans.

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