Incorporation of n-3 Long-chain Polyunsaturated Fatty Acids into Duck Egg Yolks**

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ABSTRACT : The objective of this experiment was to determine the effects of different levels of refined cod liver oil (RCLO) on laying performance, n-3 polyunsaturated fatty acids composition (n-3 PUFAs) and the organoleptic evaluation of duck egg yolks. A total of 180 30 wk old laying Tsaiya ducks, at the beginning of peak production, were allotted into 6 treatments with 3 replicates each. Ducks were fed one of the 6 experimental diets, supplemented with 2% tallow (control) and graded levels of RCLO at 2, 3, 4, 5, and 6% to a corn-soybean diets, respectively, for 6 wks. All of the experimental diets were formulated to be both isocaloric and isonitrogenous. Feed and water were supplied *ad libitum* throughout the experimental period. The results indicated that the RCLO supplementation levels did not affect ($p>0.05$) egg production, egg mass, feed intake, feed efficiency or body weight change. Egg weight was the lightest when the ducks received the 6% RCLO diet. The eicosapentaenoic acid, docosahexaenoic acid, and total n-3 PUFAs contents in the yolks increased with increasing RCLO supplementation. The taste and general acceptability of the hard-boiled eggs were not significantly different among the treatments. However, a fishy flavor was much higher when ducks were fed diets supplemented with 5% and 6% RCLO diets. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 4 : 565-569)***

Key Words : Tsaiya Duck, Eicosapentaenoic Acid, Docosahexaenoic Acid, Fishy Flavor, Duck Egg

INTRODUCTION

The importance of long-chain n-3 polyunsaturated fatty acids (n-3 PUFAs), mainly eicosapentaenoic acid (C20:5n3, EPA) and docosahexaenoic acid (C22:6n3, DHA), in human nutrition is gaining considerable attention due to their role in various physiological processes (Salem et al., 1996). EPA and DHA are essential for normal growth and development and may play an important role in the prevention and treatment of coronary artery disease, hypertension, arthritis, inflammatory, and autoimmune disorders (Simopoulos, 1991). There is evidence of n-3 PUFA for its cardioprotective effect (Kinsella et al., 1990) and that these n-3 PUFAs are associated with a reduced incidence of coronary heart disease (Bang et al., 1980). The n-3 PUFAs inhibited arachidonic acid (C20:4n6, AA) metabolism and linoleic acid (C18:2n6, LA) conversion into AA (Land et al., 1973). The reduction in AA content and its metabolites, such as thromboxane A_2 in plasma, which had a potent proaggregatory effect on platelets, was suggested as one of the reason why n-3 PUFAs reduced the risk of coronary heart disease (Leaf and Weber, 1988). The n-3 PUFAs have been shown to have health promoting benefits in humans (Harris, 1989).

Fatty acids manipulation in egg yolks may provide a means to improve the quality of eggs as a healthy food. Numerous investigators have demonstrated that the fatty acids composition of egg yolks is readily modified by dietary fat. In 1934, Cruickshank conducted an investigation to study the dietary influences on yolk lipids and found that the fatty acid composition in the yolk could be altered by manipulating the dietary fat. Recently, the focus of studies exploring the means to alter the fatty acid composition in yolk has been the use of various dietary levels (3 to 10% of the diet) of fish oils in laying hen diets (Leskanich and Noble, 1997). Egg yolk enrichment is obtained with n-3 fatty acids resulting from fish oil inclusion in the diet. Dietary fish oil inclusion resulted in significant increases in the n-3 fatty acids such as DHA and EPA in egg yolks (Adams et al., 1989; Huang et al., 1990; Hargis et al., 1991; Hargis and Van Elswyk, 1993; Oh et al., 1994). Fish oils are rich sources of EPA and DHA (Barlow and Pike, 1977). However, they could cause a fishy flavor in the egg and meat if included at high levels (Leskanich and Noble, 1997). The optimal levels of fish oil inclusion in producing n-3 PUFAs-enriched eggs should be established.

The Tsaiya duck is the major laying duck in Taiwan and plays an important role in rural economy. Little information is available about the n-3 PUFAs content in duck egg yolks modified by dietary fish oil. Therefore, we conducted the present experiment to determine the effects of dietary supplementation of different levels of refined cod liver oil (RCLO) on the n-3 PUFA composition and its effect on the taste of duck eggs.

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MATERIALS AND METHODS

Animals and diets

A total of 180 30-wk-old laying Tsaiya ducks, were allotted at the beginning of peak egg production into 6 treatments with 3 replicates each. Ducks were fed one of the 6 experimental diets, supplemented with 2% tallow (control) and graded levels of RCLO at 2, 3, 4, 5, and 6% to a corn-soybean diets for 6 wks. All of the experimental diets were formulated to be both isocaloric and isonitrogenous and analyzed for proximate constituents according to standard procedures (AOAC, 1984). The proximate composition and composition of the fatty acids in these experimental diets are presented in Table 1 and Table 2. The ducks were housed in individual cages (25×30×39 cm). Feed and water were supplied *ad libitum* during the experimental period.

General and analytical procedures

At the beginning and the end of the experimental period, all ducks were weighed individually. Feed consumption was recorded biweekly. Eggs were collected and weighed daily throughout the experimental period. On the 7th day of each week, the collected eggs were weighed and four yolks per replicate were separated from albumin, weighed, pooled, and blended. The lipids of experimental diets and the pooled yolks were extracted using chloroform:methanol (2:1, v/v) (Folch et al., 1957). The lipid extract was saponified and methyl esters were then prepared using borontrifluoride (Number 28.056, AOAC, 1984). Fatty acids were quantitated by gas chromatography (GL-Science 353B, Japan) using a column (183 cm×0.32 cm) packed with GP 10% SP 2330 (Supleco, Inc., U.S.A). Samples were chromatographed at 150°C to 240°C with a 2°C/min program temperature gradient and kept at 240°C for 10 min. The detector and injector temperature were kept at 250°C. The fatty acid peaks were identified by comparison with the retention times for fatty acid methyl ester standards.

Organoleptic evaluation

Twelve untrained volunteers were recruited for a taste panel. Eggs collected at 6th wk were hard-boiled for the organoleptic evaluation of taste, fishy flavor, and general acceptability at room temperature. A seven-point hedonic

Table 1. The composition of the experimental diets

Ingredients	Treatments[1]					
	2% TO	2% RCLO	3% RCLO	4% RCLO	5% RCLO	6% RCLO
Corn, yellow	56.20	55.00	52.00	48.50	46.00	42.70
Soybean meal, 44%	31.00	31.00	30.20	30.20	30.50	31.00
Fish meal, 65%	2.00					
Wheat bran	-	1.20	4.00	6.50	6.50	7.00
Red soil	-	-	-	-	1.20	2.50
Tallow	2.00	-	-	-	-	-
Fish oil	-	2.00	3.00	4.00	5.00	6.00
Limestone	6.20	6.20	6.20	6.20	6.20	6.20
Dicalcium phosphate	1.50	1.50	1.50	1.50	1.50	1.50
Iodized salt	0.40	0.40	0.40	0.40	0.40	0.40
DL-methionine	0.20	0.20	0.20	0.20	0.20	0.20
Choline, 50%	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin premix[a]	0.30	0.30	0.30	0.30	0.30	0.30
Mineral premix[b]	0.20	0.20	0.20	0.20	0.20	0.20
Total	100.00	100.00	100.00	100.00	100.00	100.00
Calculated value						
Crude protein, %	19.12	19.19	19.03	19.12	19.05	19.08
ME, kcal/kg	2,805	2,805	2,805	2,805	2,805	2,805
Calcium, %	3.00	3.00	3.00	3.00	3.00	3.00
Avail.phosphorus, %	0.46	0.46	0.46	0.46	0.46	0.46
Sulfur amino acid, %	0.76	0.76	0.76	0.76	0.76	0.76
Analyzed value						
Crude protein, %	18.85	19.05	18.74	18.88	18.78	18.93
Crude fat, %	5.12	6.96	6.84	7.12	8.14	8.58
Ca, %	3.01	3.26	2.97	3.07	2.97	3.03
Total phosphorus, %	0.65	0.69	0.68	0.71	0.73	0.70

[1] TO: Tallow; RCLO: Refined cod liver oil.

[a] Supplied per kg of diets: vitamin A, 10,000 IU; vitamin D_3, 1,000 IU; vitamin E, 25 IU; vitamin K, 3 mg; thiamin, 3 mg; riboflavin, 5 mg; pyridoxine, 3 mg; vitamin B_{12}, 0.03 mg; Ca-pantothenate, 10 mg; niacin, 50 mg; biotin, 0.1 mg; folic acid, 3 mg.

[b] Supplied per kg of diets: Mn, 60 mg ($MnSO_4H_2O$); Zn, 60 mg (ZnO); Cu, 5 mg ($Cu_2SO_4 5H_2O$); Fe, 70 mg ($FeSO_4 7H_2O$); Se, 0.1 mg (Na_2SeO_3).

Table 2. The fatty acid composition of experimental diets (%[1])

Fatty acids	Treatments[2]					
	2% TO	2% RCLO	3% RCLO	4% RCLO	5% RCLO	6% RCLO
	%					
$C_{18:2\ n-6}$	40.81	31.39	28.59	25.98	23.50	20.56
$C_{20:4\ n-6}$	0.12	2.19	2.25	2.65	2.82	2.91
$C_{18:3\ n-3}$	1.08	1.51	1.65	1.49	1.90	1.68
$C_{20:5\ n-3}$ (EPA)	0.21	4.72	4.85	5.54	6.24	6.33
$C_{22:5\ n-3}$ (DPA)	0.01	1.09	1.21	1.41	1.61	1.71
$C_{22:6\ n-3}$ (DHA)	0.10	5.09	5.37	6.19	7.14	7.59
$\sum$n-3[3]	1.38	12.41	13.09	14.63	16.89	17.31
$\sum$n-6[4]	40.93	33.58	30.84	28.63	26.32	23.46
n3/n6	0.03	0.37	0.42	0.51	0.64	0.74

[1] Expressed as percentage of total identified fatty acids.
[2] TO: Tallow; RCLO: Refined cod liver oil.
[3] $\sum$n-3=($C_{18:3\ n-3}$ + $C_{20:5\ n-3}$+ $C_{22:5\ n-3}$+ $C_{22:6\ n-3}$)
[4] $\sum$n-6=($C_{18:2\ n-6}$+ $C_{20:4\ n-6}$)

score from 1 to 7, in which the highest numerical value represented the highest degree of taste preference and general acceptability, was used to rate the eggs. A fishy flavor score ranging from 1 to 7, which indicated no fishy flavor to strong fishy flavor, was used to evaluate flavor acceptability.

Statistical analysis

All data were analyzed by using the General Linear Model Procedures of SAS (SAS, 1988). Comparison of treatment means was based on a Duncan's multiple range test. A significance level of $p<0.05$ was applied in all cases.

RESULTS AND DISCUSSION

Experimental diets

The composition and chemical analysis of the experimental diets are shown in Table 1. Experimental diets were formulated to be both isocaloric and isonitrogenous and containing 2% tallow and graded levels of RCLO at 2, 3, 4, 5, and 6%. The fatty acid composition is presented in Table 2. The higher the dietary fish oil supplementation, the less the n-6 polyunsaturated fatty acid content and the greater the total n-3 polyunsaturated fatty acid content in the diets. Apparently, the fatty acid composition in the diets was readily modified by dietary fish oil.

Laying performance

Table 3 shows the RCLO supplementation effect on laying performance in laying Tsaiya ducks. The RCLO supplementation levels did not affect ($p>0.05$) egg production, egg mass, feed intake, feed efficiency or body weight change, probably reflecting the fact that all experimental diets were both isocaloric and isonitrogenous. These results were similar to the report by Oh et al. (1994) that hen diets isoenergetically supplemented with 5% fish oil for 8 wks did not adversely influence feed efficiency, body weight or egg production. However, the present results showed that egg weight was lightest when the ducks received the 6% RCLO diet. Van Elswyk et al. (1994) reported that the hypolipodemic effect of fish oil might have reduced the hepatic lipogenesis and lipid transport from blood into the developing ova. This phenomenon was found in this study in which ducks fed a 6% RCLO diet produced the lowest yolk weight. The lighter egg weight from the 6% RCLO diet might be due to a lower yolk weight. This result was consistent with reports by Hulan et al. (1988), Van Elswyk et al. (1991), Whitehead et al. (1993), and Van Elswyk et al. (1994).

Long-chain n-3 polyunsaturated fatty acids of egg yolks

The effects of RCLO supplementation on the EPA, DHA and total n-3 fatty acids content incorporated into the egg yolks are shown in Table 4. The EPA, DHA and total n-3 fatty acids content in egg yolks increased when the RCLO supplementation level increased. The yolk EPA content was significantly higher ($p<0.05$) in the 4% to 6% RCLO diets than in the control and 2% RCLO diets at 1st wk. Ducks fed on diets supplemented with 2% to 6% RCLO had significantly higher ($p<0.05$) yolk EPA, DHA, and total n-3 fatty acid content compared to the control diet from 2nd to

Table 3. Effects of supplementation of refined cod liver oil on laying performance in laying Tsaiya ducks

Items	Treatments[1]						SEM
	2% TO	2% RCLO	3% RCLO	4% RCLO	5% RCLO	6% RCLO	
Egg production, %	86.11	87.68	85.63	85.15	87.78	85.52	0.83
Egg weight, g	61.62[ab]	61.64[ab]	61.51[ab]	61.92[a]	61.67[ab]	60.98[b]	0.23
Egg mass, g/bird/day	53.06	54.05	52.67	52.72	54.13	52.09	2.07
Feed intake, g/day/bird	189.62	196.67	189.28	186.65	185.09	185.87	8.11
Feed efficiency, feed/egg	3.57	3.64	3.59	3.54	3.42	3.57	0.19
Body weight change, %	8.73	7.45	8.93	10.06	9.15	9.96	1.51
Yolk weight, g/egg	20.33	20.27	20.18	20.25	20.41	19.94	0.41

[1] TO: Tallow; RCLO: Refined cod liver oil.
[a,b] Means at the same row the without same superscript are significantly different ($p<0.05$).

Table 4. Effects of supplementation of refined cod liver oil on the contents of EPA, DHA and total n-3 fatty acids incorporation into egg yolks

Weeks	Treatments[1]						SEM
	2% TO	2% RCLO	3% RCLO	4% RCLO	5% RCLO	6% RCLO	
				EPA (%*)			
0	0.09	0.11	0.11	0.12	0.12	0.11	0.02
1	0.06^{b}	0.15^{b}	0.63^{ab}	1.01^{a}	1.08^{a}	1.29^{a}	0.22
2	0.07^{c}	0.39^{b}	0.45^{b}	0.47^{b}	0.60^{a}	0.69^{a}	0.04
3	0.04^{d}	0.38^{c}	0.42^{c}	0.55^{b}	0.59^{b}	0.72^{a}	0.03
4	0.12^{d}	0.33^{c}	0.42^{bc}	0.50^{b}	0.68^{a}	0.69^{a}	0.05
5	0.05^{d}	0.29^{c}	0.32^{c}	0.53^{b}	0.76^{a}	0.71^{a}	0.04
6	0.04^{f}	0.25^{e}	0.38^{d}	0.53^{c}	0.67^{a}	0.84^{a}	0.03
				DHA (%*)			
0	0.54	0.62	0.70	0.57	0.59	0.62	0.16
1	0.35^{d}	1.87^{c}	2.23^{bc}	2.43^{bc}	2.68^{ab}	3.18^{a}	0.21
2	0.51^{c}	3.79^{b}	4.34^{ab}	4.55^{a}	4.62^{a}	4.69^{a}	0.22
3	0.49^{e}	3.75^{d}	4.03^{cd}	4.50^{bc}	4.88^{ab}	5.44^{a}	0.22
4	0.56^{d}	3.50^{c}	4.14^{bc}	4.63^{ab}	5.41^{a}	5.49^{a}	0.29
5	0.49^{d}	2.66^{c}	3.61^{b}	4.42^{b}	5.52^{a}	5.79^{a}	0.28
6	0.51^{e}	2.72^{d}	4.31^{c}	4.88^{bc}	5.53^{ab}	5.70^{a}	0.23
				Total n-3 fatty acids** (%*)			
0	1.37	1.41	1.49	1.39	1.42	1.34	0.23
1	1.04^{d}	2.85^{c}	3.80^{b}	4.24^{ab}	4.55^{ab}	5.03^{a}	0.31
2	1.12^{c}	5.01^{b}	5.74^{ab}	6.21^{a}	6.30^{a}	6.45^{a}	0.26
3	0.93^{d}	5.03^{c}	5.39^{c}	6.18^{b}	6.58^{b}	7.41^{a}	0.27
4	1.19^{e}	4.66^{cd}	5.55^{cd}	6.09^{bc}	7.19^{ab}	7.30^{a}	0.38
5	1.03^{d}	3.69^{c}	4.86^{b}	6.61^{a}	7.48^{a}	7.74^{a}	0.38
6	1.02^{e}	3.75^{d}	5.59^{c}	6.54^{b}	7.57^{a}	7.66^{a}	0.27

[1] TO: Tallow; RCLO: Refined cod liver oil.

* Expressed as percentage of total identified fatty acids.

** Total n-3 fatty acid=($C_{18:3\,n-3}$–$C_{20:5\,n-3}$+$C_{22:5\,n-3}$–$C_{22:6\,n-3}$)

^{a,b,c,d,e,f} Means at the same row without the same superscript are significantly different ($p<0.05$).

6th wks of the experiment. At 6th wk, the EPA, DHA and total n-3 fatty acid content in the yolks were higher by factors of 3.13, 5.33 and 3.68, 4.75, 8.45 and 5.48, 6.63, 9.57 and 6.41, 8.38, 10.84 and 7.42, 10.50, 11.18 and 7.51 from respectively, ducks given the 2%, 3%, 4%, 5%, and 6% RCLO diets compared to the control diet. Preliminary results of the effects of various dietary levels (e.g., 3 to 10% of the diet) of fish oil on the fatty acid composition of the hens' egg yolk have been reported by a number of investigators (Yu and Sim, 1987; Hulan, 1988; Oh et al., 1988; Adams et al., 1989; Van Elswyk et al., 1992; Oh et al., 1994). Yu and Sim (1987) incorporated graded levels of Pacific salmon oil blended with animal tallow into a standard laying diet to a total dietary level of 8% fat resulting in an increase in EPA, DHA, and total n-3 fatty acids in the yolk. When hens were fed diets supplemented with 3% menhaden oil, the EPA and DHA contents were significantly increased in the yolks at 1st and 2th wks of the trial (Hargis et al., 1991). Hargis et al. (1991) and Huang et al. (1990) demonstrated substantial enrichment of n-3 PUFAs in eggs from hens given diets containing 3 to 5% menhaden oil. These results were consistent with the results from this study. Eggs enriched with n-3 PUFAs, especially in EPA and DHA, could be another healthy food in addition to typical marine sources.

Organoleptic evaluation

The seven-point hedonic score was used for the organoleptic evaluation in this experiment. The taste preference and general acceptability ranged from 1 to 7, with 1 representing the lowest degree of preference. Fishy flavor scores ranged from 1 to 7, which indicated no fishy flavor to strong fishy flavor. There were no significant differences in taste and general acceptability among the treatments in this experiment (Table 5). We found that fishy flavor was much higher ($p<0.05$) in the 5% and 6% RCLO diets compared to the control and 2% RCLO diets. Apparently, RCLO supplementation up to 4% was acceptable in flavor in producing n-3 fatty acids-enriched eggs in this study. This result was consistent with the finding by Leskanich and Noble (1997) who indicated that inclusion of high levels of fish oil into hen diets may cause a fishy flavor. Significant off-flavors were also reported for poultry meat and eggs that have been enriched with n-3 long-chain PUFAs (Hargis and Van Elswyk, 1993). Van Elswyk et al. (1992) found that eggs from hens on a diet with 30 g menhaden oil/kg were given different sensory evaluations from commercial eggs. Inclusion of 6% fish oil has been reported to impart an off-flavor in n-3 fatty acid-enriched eggs (Adams et al., 1989). Inclusion levels of fish oil and sensory quality should be considered in producing n-3 fatty acids-enriched eggs.

Table 5. Effects supplementation with refined cod liver oil on organoleptic evaluation of hard-boiled egg

Items	Treatments[1]						SEM
	2% TO	2% RCLO	3% RCLO	4% RCLO	5% RCLO	6% RCLO	
Taste*	5.17	4.50	4.75	4.25	5.00	5.00	0.29
Fishy flavor**	3.33^{b}	3.25^{b}	3.58^{ab}	4.17^{ab}	4.67^{a}	4.75^{a}	0.41
General acceptability*	4.25	4.75	4.58	3.95	3.75	3.75	0.39

[1] TO: Tallow; RCLO: Refined cod liver oil.

* A score of 1 indicated very low desirability in taste and general acceptability and a score of 7 indicated very high desirability in taste and general acceptability.

** A score of 1 indicated no fishy flavor and a score of 7 indicated strong fishy flavor.

^{a,b} Means at the same row without the same superscript are significantly different ($p<0.05$).

CONCLUSION

n-3 PUFAs-enriched duck egg can be produced by supplementation with 4% RCLO to the duck diet without affecting laying performance and organoleptic evaluation. These eggs may serve as viable dietary alternatives to fish, fish products or hen eggs to provide significant amounts of n-3 PUFAs in our daily diet.

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