Effects of n-3 Polyunsaturated Fatty Acids-enriched Diet Supplemented with Different Levels of α-Tocopherol on Lipid Metabolism in Laying Tsaiya Ducks*

Tian-Fwu Chen[1] and Jenn-Chung Hsu**

Department of Animal Science, National Chung-Hsing University, 250 Kao-Kung Road. Taichung 402. Taiwan. ROC

ABSTRACT : The objective of this experiment was to determine the effects of n-3 polyunsaturated fatty acids (n-3 PUFAs)-enriched diet supplemented with different levels of α-tocopherol on the activities of hepatic lipogenic-related enzymes and the contents of liver and plasma lipid fractions in laying Tsaiya ducks. 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, containing 4% tallow (control), and 4% fish oil supplemented with graded levels of α-tocopheryl acetate (α-tocopherol) at 0, 100, 200, 300 and 400 mg/kg, respectively, for 6 wks. Feed and water were supplied *ad libitum* throughout the experimental period. The results indicated that the n-3 PUFAs-enriched diet supplemented with different levels of α-tocopherol did not affect ($p>0.05$) egg weight, feed intake, body weight change or liver and abdominal fat weights. Egg production, egg mass and feed efficiency significantly ($p<0.05$) improved as dietary α-tocopherol levels increased. The activities of hepatic lipogenic-related enzymes including acetyl-CoA carboxylase (EC 6. 2. 1. 3; ACC), glucose-6-phosphate dehydrogenase (EC 1. 1. 1. 49; G-6-PDH), ATP-citrate cleavage enzyme (EC 4. 1. 3. 8; CCE), NADP-malate dehydrogenase (EC 1.1.1.40; NADP-MDH) and fatty acid synthetase (FAS) were higher ($p<0.05$) in birds fed with the tallow diet than in those fed with fish oil diets and increased with increasing dietary α-tocopherol levels. None of the dietary treatments significantly affected the contents of triglyceride and total cholesterol in the liver, or total cholesterol, phospholipid and total lipid in the plasma. However, the contents of phospholipid and total lipid in the liver, and triglyceride in the plasma increased as dietary α-tocopherol levels increased. Increasing dietary α-tocopherol levels decreased the non-esterified fatty acid (NEFA) content in the plasma and trended to decrease the cholesterol contents in the egg yolk. The lipid metabolism of laying Tsaiya ducks was influenced not only by the dietary fat but also by the supplementation levels of α-tocopherol. ***(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 11 : 1562-1569)***

Key Words : Tsaiya Duck, n-3 PUFAs, α-Tocopherol, Lipid Metabolism

INTRODUCTION

The beneficial effects of the n-3 polyunsaturated fatty acids (n-3 PUFAs), mainly eicosapentaenoic acid (C20:5n-3, EPA) and docosahexaenoic acid (C22:6n-3, DHA), which are abundant in the fish oil, have been demonstrated by numerous reports (for a review see Leskanich and Noble, 1997; Mutia and uchida, 1999; Xia, et al., 2003), largely as a result of their modulating effects in disease states such as rheumatoid arthritis, systemic erythematous lupus, multiple sclerosis (BNF, 1992), and in reducing the risk of coronary heart disease (Kornhout et al., 1985; Herold and Kinsella, 1986). The n-3 PUFAs have been shown to have health promoting benefits in humans (Illingworth and Ullmann, 1990). Dietary fish oil inclusion resulted in significant increase in the n-3 PUFAs, in particular, the EPA and DHA contents in shell eggs (Hargis et al., 1991; Hargis and Van Elswyk, 1993; Oh et al., 1994; Chen and Hsu, 2003; Lien, et al., 2003). In a previous study, we demonstrated that n-3 PUFAs-enriched duck egg can be produced by supplementation with 4% refined cod liver oil (RCLO) to the duck diet without affecting the laying performance and organoleptic evaluation (Chen and Hsu, 2003). However, fish oils are highly unsaturated and readily susceptible to peroxidation when excessive consumption without added sufficient antioxidants (Fritche and Johnston, 1988; Qi and Sim, 1998). Several studies demonstrated that dietary n-3 PUFAs suppress hepatic lipogenic enzymes by inhibiting enzyme protein synthesis (Iritani, 1992; Clarke and Jump, 1993; An et al., 1995). Eder and Kirchgessner (1998) indicated that the lipid peroxidation products are involved in lipogenic enzymes suppression and the effects of dietary PUFAs on lipogenic enzymes suppression would be modified by the antioxidant state of the animal. It is well known that the oxidative phenomena can be prevented or limited by antioxidants such as vitamin E and β-carotene. Antioxidants inhibit the formation of free radicals in lipid peroxidation (Burton and Ingold, 1981; Hahm et al., 1993). Tocopherol, which has vitamin E activity, has been extensively used in foods as a natural antioxidant (Bauernfeind, 1997). Thus, incorporating tocopherol into poultry products would increase oxidative stability (Lin et al., 1989). Sklan (1983) indicated that tocopherol increased

* Contribution No. 1208 from Taiwan Livestock Research Institute, Council of Agriculture, Taiwan, ROC.

** Corresponding Author: Jenn-Chung Hsu. Tel: +886-4-22850315, Fax: +886-4-22860265, E-mail: jchsu@dragon.nchu.edu.tw

[1] Department of Animal Farm, Taiwan Livestock Research Institute, Council of Agriculture, Hsinhua Tainan, 712, Taiwan, ROC.

Received September 15, 2003; Accepted June 12, 2004

Table 1. The composition of the experimental diets

Ingredients	4% TO	4% FO	4% FO+T 100	4% FO+T 200	4% FO+T 300	4% FO+T 400
Corn, yellow	48.50	48.50	48.50	48.50	48.50	48.50
Soybean meal, 43.5%	30.10	30.10	30.10	30.10	30.10	30.10
Fish meal, 65%	2.00	2.00	2.00	2.00	2.00	2.00
Wheat bran	6.50	6.50	6.50	6.50	6.50	6.50
Tallow	4.00	-	-	-	-	-
Fish oil	-	4.00	4.00	4.00	4.00	4.00
Limestone, pulverized	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 chloride, 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, %	19.00	19.02	19.02	19.02	19.02	19.02
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,%	18.46	19.07	18.61	19.05	18.68	18.53
Crude fat,%	6.96	6.99	7.08	6.95	7.05	6.96
Ca, %	3.07	3.16	3.17	3.19	2.99	3.03
Total phosphorus,%	0.66	0.67	0.69	0.70	0.72	0.73
α-tocopherol, mg/kg	36.00	33.00	137.00	226.00	331.00	447.00

TO: tallow, FO: fish oil, T: α-tocopheryl acetate.

[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).

the contents of triglyceride and cholesterol in the liver of chicks; *In vitro* fatty acid synthesis from lactate by liver homogenates was enhanced by tocopherol. However, the mechanisms by which tocopherol enhanced hepatic lipogenesis are not completely understood. No information is available about n-3 PUFAs-enriched diet supplemented with α-tocopherol on lipid metabolism in laying Tsaiya ducks. Therefore, we conducted this experiment to determine the effects of n-3 PUFAs-enriched diet supplemented with α-tocopherol on laying performance, activities of hepatic lipogenic-related enzymes and lipid traits in the liver and plasma of laying Tsaiya ducks.

MATERIALS AND METHODS

Animals and diets

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, containing 4% tallow (control), and 4% fish oil (refined cod liver oil) supplemented with graded levels of α-tocopheryl acetate (α-tocopherol) at 0, 100, 200, 300 and 400 mg/kg, respectively, for 6 wks. All of the experimental diets were formulated to be both isocaloric and isonitrogenous and analyzed for proximate constituents according to standard procedure (AOAC, 1984). The proximate composition and composition of the fatty acids in these experimental diets are presented in Table 1 and Table 2. 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, and eggs were collected and weighed daily throughout the experimental period. On the last day of the experiment, blood samples were taken from the wing vein of 6 ducks form each treatment. Plasma were later separated from each blood sample and then stored at -20°C pending analysis of various lipid fractions. The sampling and killing of ducks form each treatment was on a rotation basis. The ducks were subsequently decapitated, and the liver and abdominal fat were immediately removed and weighed. Liver samples were placed in ice-cold saline solution (0.9%) and subsequently homogenized for the analysis of the activities of lipogenic-related enzymes and the various lipid fractions.

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

Fatty acids	4% TO	4% FO	4% FO+T 100	4% FO+T 200	4% FO+T 300	4% FO+T 400
$C_{14:0}$	1.03	4.29	4.26	4.32	4.21	4.19
$C_{16:0}$	15.94	16.95	17.01	16.85	17.11	17.15
$C_{18:0}$	6.53	4.83	4.79	4.69	4.91	4.81
$C_{16:1\ n-9}$	1.54	5.58	5.57	5.68	5.71	5.77
$C_{18:1\ n-9}$	31.38	25.62	26.36	26.01	26.11	26.29
$C_{18:2\ n-6}$	21.68	19.15	19.24	19.29	19.33	19.40
$C_{20:4\ n-6}$	0.21	2.79	2.83	2.81	2.89	2.91
$C_{18:3\ n-3}$	0.22	2.16	2.19	2.23	2.29	2.34
$C_{20:5\ n-3}$ (EPA)	0.36	7.01	7.18	7.19	7.24	7.33
$C_{22:5\ n-3}$ (DPA)	0.12	1.70	1.73	1.76	1.81	1.83
$C_{22:6\ n-3}$ (DHA)	0.64	8.07	8.13	8.16	8.19	8.21
ΣSFA[2]	23.50	26.07	26.06	25.86	26.23	26.15
ΣMUFA[3]	32.92	31.20	31.93	31.69	31.82	32.06
ΣPUFA[4]	23.23	40.88	41.30	41.44	41.75	42.02
Σn-3[5]	1.34	18.94	19.23	19.34	19.53	19.71
Σn-6[6]	21.89	21.94	22.07	22.10	22.22	22.31
n3/n6	0.06	0.86	0.87	0.87	0.88	0.88
SFA/USFA[7]	0.42	0.36	0.36	0.35	0.36	0.35

TO: tallow, FO: fish oil, T: α-tocopheryl acetate.

[1] Expressed as percentage of total identified fatty acids.

[2] SFA: Saturated fatty acids (C14:0−C16:0−C18:0). [3] MUFA: Monounsaturated fatty acids (C16:1+C18:1).

[4] PUFA: Polyunsaturated fatty acids (C18:2−C18:3+C20:4+C20:5+C22:5−C22:6). [5] n-3: (C18:3−C20:5+C22:5+C22:6).

[6] n-6: (C18:2+C20:4). [7] USFA: Unsaturated fatty acids (C16:1+C18:1+C18:2+C18:3−C20:4−C20:5−C22:5+C22:6).

Preparation of liver homogenates

Each liver sample was homogenized in 0.25 M sucrose solution containing 1 mM EDTA-2 Na. Then the homogenates were centrifuged (Model 2 K-15, Sigma) at 10,000×g at 4°C for 10 min. The supernatants were centrifuged (Model XL 90, Backman) at 105,000×g at 4°C for 60 min and the resulting clear supernatants were used for assaying enzyme activities and total protein contents.

Enzyme assays

The activities of glucose-6-phosphate dehydrogenase (EC 1.1.1.49; G-6-PDH), NADP-malate dehydrogenase (EC 1.1.1.40; NADP-MDH), ATP-citrate cleavage enzyme (EC 4.1.3.8; CCE), acetyl-CoA carboxylase (EC 6.2.1.3; ACC) and fatty acid synthetase (FAS) were assayed according to the method of Lohr and Waller (1974), O'choa (1955), Takeda et al. (1963), Dakshinamurrti and Desjardins (1969) and Kumar et al. (1970), respectively. The protein contents of the liver supernatants used for enzyme assays were determined by the method of Lowry et al. (1951). Enzyme activities were expressed as *n* mole of substrate converted to product per minute per mg protein at 25 or 37°C.

Chemical analysis

The fatty acids composition of the experimental diets was determined by the method of Chen and Hsu (2003). Vitamin E contents in the experimental diets were determined by high performance liquid chromatography (HPLC) according to the method of Thompson and Hatina (1979) with some modification. Experimental diets were mixed with ethanol solution containing 1% pyrogallol then shake for 3 min and mixed with *n*-hexane to extracted tocopherols, and the extracted tocopherols were suspension with methanol. The extracted tocopherols were detected by fluorescence spectrophotometer (Model F-4500, Hitachi, Tokyo, Japan) (excitation wavelength: 285 nm, emission wavelength: 330 nm).

Lipid fractions analysis

The contents of total lipid in the plasma and in the liver were analyzed using the method of Bragdon (1960). Triglyceride, phospholipid and total cholesterol contents in the liver were determined by the method of Chen (1992). The concentrations of triglyceride, total cholesterol and non-esterified fatty acid (NEFA) in the plasma were measured enzymatically using an automatic analyzer (Model 7050, Hitachi, Tokyo, Japan) with a reagent kit (Wako, Japan). The concentrations of phospholipid in the plasma were measured by using spectrophotometer (Model U-2000, Hitachi, Tokyo, Japan) with commercially reagent kit (Audit diagnostics, Ireland). Yolk cholesterol contents were determined by the method of Dyer-Hurdon and Nnanna (1993).

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.

Table 3. Effects of n-3 polyunsaturated fatty acids-enriched diet supplemented with α-tocopherol on laying performance of laying Tsaiya ducks

Items	4% TO	4% FO	4% FO+T 100	4% FO+T 200	4% FO+T 300	4% FO+T 400	SEM
Egg production, %	81.83^{c}	83.49^{bc}	84.68^{bc}	85.92^{b}	86.25^{b}	92.46^{a}	0.91
Egg weight, g	65.70	65.20	65.66	65.51	65.70	65.04	0.21
Egg mass, g/bird/day	53.88^{b}	54.22^{b}	55.60^{ab}	56.29^{ab}	56.67^{ab}	60.70^{a}	1.55
Feed intake, g/bird/day	192.62	191.67	185.28	186.65	195.09	194.87	3.73
Feed efficiency, feed/egg	3.57^{a}	3.54^{ab}	3.33^{ab}	3.32^{ab}	3.44^{ab}	3.21^{b}	0.10
Body weight change, %	4.23	5.38	4.25	4.49	5.32	4.46	1.98
Liver weight, %	2.82	3.14	2.97	3.21	2.99	2.87	0.13
Abdominal fat weight, %	0.44	0.48	0.52	0.62	0.54	0.35	0.15

TO: tallow, FO: fish oil, T: α-tocopheryl acetate.

[a, b, c] Means within the same row with different superscripts differ significantly ($p<0.05$).

Table 4. Effects of n-3 polyunsaturated fatty acid-enriched diet supplemented with α-tocopherol on the activities of hepatic lipogenic-related enzymes of laying Tsaiya ducks

Items	4% TO	4% FO	4% FO+T 100	4% FO+T 200	4% FO+T 300	4% FO+T 400	SEM
	---------- *n* mole/min. mg protein ----------						
ACC	3.98^{a}	2.67^{c}	2.82^{c}	3.32^{b}	3.47^{b}	3.59^{b}	0.13
FAS	1.62^{a}	1.02^{c}	1.22^{bc}	1.39^{ab}	1.47^{ab}	1.41^{ab}	0.09
CCE	81.21^{a}	61.25^{d}	65.38^{cd}	71.52^{bc}	76.39^{ab}	78.53^{ab}	2.64
NADP-MDH	107.74^{a}	89.21^{b}	90.16^{b}	93.65^{b}	97.48^{b}	96.62^{b}	2.59
G-6-PDH	33.56^{a}	22.65^{d}	26.17^{bcd}	25.19^{cd}	29.83^{abc}	30.18^{ab}	1.56

TO: tallow, FO: fish oil, T: α-tocopheryl acetate.

ACC: acetyl-CoA carboxylase, FAS: fatty acid synthetase, CCE: ATP-citrate cleavage enzyme, NADP-MDH: NADP-malate dehydrogenase, G-6-PDH: glucose -6-phosphate dehydrogenase.

[a, b, c, d] Means within the same row with different superscripts differ significantly ($p<0.05$).

Table 5. Effects of n-3 polyunsaturated fatty acids-enriched diet supplemented with α-tocopherol on the contents of various lipid fractions in the liver of laying Tsaiya ducks

Items	4% TO	4% FO	4% FO+T 100	4% FO+T 200	4% FO+T 300	4% FO+T 400	SEM
	---------- mg/g ----------						
Triglyceride	13.19	12.24	12.54	12.83	13.06	12.98	0.54
Total cholesterol	3.84	3.77	3.81	3.92	4.02	3.97	0.17
Phospholipid	51.25^{ab}	47.36^{b}	48.49^{ab}	49.83^{ab}	50.77^{ab}	53.14^{a}	1.51
Total lipid	144.57^{a}	126.36^{c}	129.81^{bc}	136.84^{abc}	142.19^{ab}	140.27^{ab}	3.96

TO: tallow, FO: fish oil, T: α-tocopheryl acetate.

[a, b, c] Means within the same row with different superscripts differ significantly ($p<0.05$).

RESULTS

The mainly and nutritionally important compositions of n-3 and n-6 PUFAs in the experimental diets were altered by dietary fat sources used in the diets (Table 2). The linoleic acid (C18:2n-6) content was higher in the tallow diet than that in the fish oil diet. In contrast, the fish oil diet containing higher levels of α-linolenic acid (C18:3n-3), eicosapentaenoic acid (EPA, C20:5n-3), docosahexaenoic acid (DHA, C22:6n-3) and total n-3 PUFAs as compared to the tallow diet.

Table 3 shows the effects of n-3 PUFAs-enriched diet supplemented with α-tocopherol on laying performance of laying Tsaiya ducks. Dietary fat did not significantly influenced laying performance ($p>0.05$). The α-tocopherol supplementation significantly improved egg production, egg mass and feed efficiency ($p<0.05$). However, there were no significant differences ($p>0.05$) on egg weight, feed intake, body weight change, or liver and abdominal fat weights among dietary treatments.

The effects of n-3 PUFAs-enriched diet supplemented with α-tocopherol on the activities of hepatic lipogenic-related enzymes of laying Tsaiya ducks are shown in Table 4. Ducks fed on tallow diet had significantly higher ($p<0.05$) the activities of ACC, FAS, CCE, NADP-MDH and G-6-PDH in the liver compared to the fish oil diets, meanwhile the activities of these enzymes were increased with increasing α-tocopherol supplementation to fish oil diets.

Table 5 and Table 6 show the effects of n-3 PUFAs-enriched diet supplemented with α-tocopherol on the contents of various lipid fractions in the liver and in the plasma of laying Tsaiya ducks. None of the dietary treatments significantly affected the contents of triglyceride and total cholesterol in the liver, and total cholesterol, phospholipid and total lipid in the plasma ($p>0.05$).

Table 6. Effects of n-3 polyunsaturated fatty acid-enriched diet supplemented with α-tocopherol on the contents of various lipid fractions in the plasma of laying Tsaiya ducks

Items	4% TO	4% FO	4% FO+T 100	4% FO+T 200	4% FO+T 300	4% FO+T 400	SEM
	mg/dL						
Triglyceride	846[a]	799[b]	804[ab]	812[ab]	820[ab]	839[ab]	13.65
Total cholesterol	110.62	108.60	113.16	119.11	104.52	114.30	5.34
Phospholipid	544.38	517.73	541.14	540.79	537.41	551.60	11.73
Total lipid	1,598	1,550	1,571	1,579	1,590	1,584	25.39
	m Eq/L						
Non-esterified fatty acid	1.87[c]	2.36[a]	2.14[abc]	2.22[ab]	2.03[bc]	1.93[c]	0.09

TO: tallow, FO: fish oil, T: α-tocopheryl acetate.
[a, b, c] Means within the same row with different superscripts differ significantly ($p<0.05$).

Table 7. Effects of n-3 polyunsaturated fatty acid-enriched diets supplemented with α-tocopherol on yolk weight and cholesterol contents

Items	4% TO	4% FO	4% FO+T 100	4% FO+T 200	4% FO+T 300	4% FO+T 400	SEM
Yolk weight, g/egg	20.13	19.78	19.76	19.62	19.99	19.56	0.46
Yolk cholesterol							
mg/g	15.97[a]	15.62[ab]	15.54[bc]	15.49[bc]	15.34[bc]	15.18[c]	0.14
mg/yolk	321.48[a]	308.96[ab]	307.07[ab]	303.91[ab]	306.65[ab]	296.92[b]	6.25

TO: tallow, FO: fish oil, T: α-tocopheryl acetate.
[a, b, c] Means within the same row with different superscripts differ significantly ($p<0.05$).

However, the contents of phospholipid and total lipid in the liver, and triglyceride in the plasma were lower in the ducks fed with the fish oil diet than in those fed with the tallow diet; these parameters were increased by increasing α-tocopherol levels in the fish oil diet. In contrast, ducks fed with the fish oil diet had higher content of NEFA than those fed with the tallow diet ($p<0.05$), and the NEFA content decreased with increasing α-tocopherol levels in the fish oil diet.

Table 7 shows the effects of n-3 PUFAs-enriched diet supplemented with α-tocopherol on yolk weight and cholesterol content. There was no significant difference among the dietary treatments in yolk weight (g/egg) ($p>0.05$). However, ducks fed on tallow diet had higher yolk cholesterol compared to the 4% FO+T400 diet. Furthermore, the contents of total cholesterol (mg/g or mg/yolk) trended to decrease with increasing α-tocopherol levels in the fish oil diet.

DISCUSSION

The composition of fatty acids in the diet, in general, reflect dietary fat source. In our previous experiment (Chen and Hsu, 2003) showed that tallow contained more linoleic acid than fish oil, but fish oil contained higher levels of EPA, DHA and total n-3 PUFAs than tallow. Therefore, it is reasonable that the analyzed values of fatty acid compositions in the tallow and in the fish oil diets similar patterns as to those in tallow and fish oil (Table 2).

Dietary fat did not affect laying performance of laying Tsaiya ducks. It can be seen from ducks fed on 4% tallow or 4% FO diet in the present experiment. This phenomenon also found in our previous experiment (Chen and Hsu, 2003). Several researchers (Hargis et al., 1991; Meluzzi et al., 2000) have shown that the laying performance of hens was not significantly affected by the dietary fat. The present results are consistent with their findings. On the other hand, in the present experiment, the egg production, egg mass and feed efficiency were significantly improved by increasing dietary α-tocopherol levels (Table 3). This is similar to the findings of Bollengier-Lee et al. (1998) who indicated that egg mass and feed conversion efficiency were improved when hens were fed diet supplemented with vitamin E. The magnitude of the improvements of egg production were 11.16% and 8.97% higher from ducks fed on 4% FO+T400 diet compared to the 4% TO and 4% FO diets in the present experiment. The results are consistent with the findings of Bollengier-Lee et al. (1998) who indicated that the magnitude of the improvements of egg production were 8.2% from hens fed on a diet containing 500 mg vitamin E/kg. Fish oils are highly unsaturated and susceptible to peroxidation. Lipid peroxides may cause peroxidative stress and affected animal's production performance. Tocopherol is though to act as biological antioxidants by protecting polyunsaturated lipids against peroxidative stress and increasing synthesis of yolk lipids. Bollengier-Lee et al. (1998) indicated that the beneficial effect of α-tocopherol in egg production was associated with increased plasma concentrations of yolk precursors. It was speculated that α-tocopherol supplementation may enhanced synthesis of yolk precursors in the liver by protecting the liver from lipid peroxidation and damage to cell membranes. We also found the lipid peroxidation products in the liver was reduced in n-3 PUFAs-enriched diet supplemented with α-

tocopherol 400 mg/kg compared to n-3 PUFAs-enriched diet without supplemented with α-tocopherol to duck diet (Unpublished data). The present experiment also indicated that the contents of phospholipid and total lipid in the liver tended to increase as dietary α-tocopherol levels increased. There were no significant differences on egg weight, feed intake, body weight change, liver weight and abdominal fat weights among the different levels of α-tocopherol treatments in the present experiment. These results are in agreement with the findings of Bartov et al. (1991), who indicated that vitamin E did not affect final body weight, egg weight, or relative liver and abdominal fat weights of laying hens. Bollengier-Lee et al. (1998) also observed that the egg weight and food intake were unaffected by α-tocopherol treatment in hens subjected to heat stress.

The present experiment indicated that the activities of lipogenic-related enzymes in the liver were altered when ducks were fed different sources of dietary fat and different levels of α-tocopherol among the fish oil diets. Laying ducks fed on fish oil diet had lower activities of ACC, FAS, CCE, NADP-MDH and G-6-PDH in the liver than fed on tallow diet. Diets rich in n-3 PUFAs were well known to suppress hepatic lipogenic enzymes compared to diets rich in saturated fatty acids (An et al., 1995; Eder and Kirchgessner, 1998). Several studies demonstrated that dietary n-3 PUFAs suppressed hepatic lipogenic enzymes by inhibiting enzyme protein synthesis (Iritani, 1992; Clarke and jump, 1993; Eder and Kirchgessner, 1998). An et al. (1995) also demonstrated that dietary linseed oil enriched with α-linolenic acid has a greater effect than safflower oil enriched with linoleic acid in reducing hepatic fatty acid synthesis. Clarke et al. (1990) examined the effect of n-3 PUFAs on FAS activity, and concluded that fish oil results in a decrease in the hepatic abundance of FAS and S14 mRNA. It is well know that fish oil contains higher level of n-3 PUFAs than tallow. On the other hand, it is mentioned that the fish oil diet contains higher levels of EPA and DHA. Thus, it is assumed that the lowering effect of activities of hepatic lipogenic-related enzymes in the fish oil treatments is attributed to the higher levels of dietary n-3 PUFAs. Furthermore, the activities of lipogenic-related enzymes in the liver were also affected by dietary α-tocopherol levels. The higher the dietary α-tocopherol levels, the higher the activities of lipogenic-related enzymes in the liver. It seems that the suppression effect of hepatic lipogenic-related enzymes can be reduced by supplementing α-tocopherol to n-3 PUFAs-enriched diet. Mikkelsen et al. (1993) pointed out that the PUFAs were susceptible to peroxidation and the lipid peroxidation products were involved in lipogenic enzymes suppression. In this respect, feeding diets enriched with antioxidants could reduce, and feeding diets with low levels of antioxidants enhanced the capability of PUFAs for lipogenic enzymes suppression. Hu et al. (1989) have demonstrated that the reduced *in vivo* lipid peroxidation by feeding diets rich in saturated and monounsaturated fatty acids compared to diets rich in PUFAs and by supplementing diets with high levels of vitamin E. We also found that the lipid peroxidation products in the liver was reduced in n-3 PUFAs-enriched diet supplemented with α-tocopherol 400 mg/kg compared to n-3 PUFAs-enriched diet without supplemented with α-tocopherol to duck diet (Unpublished data). Therefore, it is assumed that the lowering suppression of activities of hepatic lipogenic-related enzymes in the fish oil diets supplemented with α-tocopherol is attributed to the reduced lipid peroxidation products in duck liver. Accordingly, the contents of NEFA in the plasma of ducks decreased by increasing dietary α-tocopherol levels. The higher the contents of plasma NEFA, the lower the activities of lipogenic-related enzymes. Yeh and Leveille (1971) showed that the hepatic fatty acid synthesis was suppressed by NEFA and long chain acyl-CoA. Tanaka et al. (1983) also reported that a positive correlation between hepatic fatty acid synthesis and the activities of lipogenic-related enzymes in the liver of chicks. These phenomena could explain that dietary α-tocopherol levels increased the activities of hepatic lipogenic-related enzymes in the present experiment.

The present experiment indicated that the fish oil diets given to ducks significantly ($p<0.05$) decreased the contents of phospholipid and total lipid in the liver and triglyceride in the plasma in comparison with the tallow diet, and those compositions increased as dietary α-tocopherol levels increased. The lipid-lowering effect of n-3 PUFAs as a result of suppressed the activities of hepatic lipogenic enzymes and decreased hepatic lipogenesis. Eder and Kirchgessner (1998) indicated that the triglyceride concentration in liver and plasma reflected activities of hepatic lipogenic enzymes. Therefore, it seems reasonable that the observed alteration in the activities of lipogenic-related enzymes in the liver of laying ducks fed with the experimental diets, were associated with, or perhaps responsible for, the alteration in the liver and in the plasma lipid contents. The content of NEFA in the plasma was higher when birds fed with the fish oil diet than those fed with the tallow diet and it was decreased by increasing dietary α-tocopherol levels. The reason of this phenomenon may be attributed to the fish oil diets containing higher n-3 PUFAs and readily susceptible to peroxidation than the tallow diet, and α-tocopherol would increase oxidative stability in the fish oil diets.

The present results indicated that the yolk cholesterol content (mg/g or mg/yolk) decreased as dietary α-tocopherol levels increased, but no same manner in the plasma cholesterol content was observed. Many studies have examined the relationship between egg yolk and

plasma cholesterol levels (See Hargis, 1988), but have found little or no correlation between the two. Although, it has been suggested that the decreased yolk cholesterol content (mg/g) parallel the decreased cholesterol concentration in the plasma of laying hens (Hsu et al., 1988), the present results do not concur with this finding.

CONCLUSION

N-3 PUFAs-enriched diet supplemented with α-tocopherol significantly improved the laying performance of laying Tsaiya ducks. The lipid metabolism of laying Tsaiya ducks was influenced not only by the dietary fat but also by the supplementation levels of α-tocopherol.

ACKNOWLEDGEMENT

The authors wish to thank the National Science Council of Taiwan, ROC, for the financial support of this project. The project number is NSC 89-2313-B-005-115.

REFERENCES

An, B. K., K. Tanaka and S. Ohtani. 1995. Effects of various n-3/n-6 fatty acid ratios in diet on lipid metabolism in growing chicks. Anim. Sci. Technol. (Jpn) 66:830-840.

AOAC. 1984. Official Methods of Analysis, 14th ed., Association of Official Analytical Chemists, Washington, DC.

Bartov, I., Y. Wiseman and E. Wax. 1991. Effects of high concentration of dietary vitamin E and ethoxyquin on the performance of laying hens. Br. Poult. Sci. 32:525-534.

Bauernfeind, J. C. 1997. The tocopherol content of food and influencing factors. CRC Crit. Rev. Food Sci. Nutr. 8:337-352.

Bollengier-Lee, M. A. Mitchell, D. B. Utomo, P. E. V. Williams and C. C. Whitehead. 1998. Influence of high dietary vitamin E supplementation on egg production and plasma characteristics in hens subjected to heat stress. Br. Poult. Sci. 39:106-112.

Bragdon, J. H. 1960. Method for determination of total serum lipids. In: Lipids and the steroid hormone in clinical medicine (Ed. F. W. Sundrman and F. W. Sunderman) pp. 9-15, Lippincott, Philadelphia.

British Nutrition Foundation (BNF). 1992. Unsaturated Fatty Acids Nutritional and Physiological Significance. The Report of the British Nutrition Foundation's Task Force, Chapman and Hall, Lodon. p. 221.

Burton, G. H. and K. U. Ingold. 1981. Autoxidation of biological molecules. 1. The antioxidant activity of vitamin E and related chain reaction of unsaturated lipids. Food Chem. 9:21-25.

Chen, T. F. 1992. Effects of dietary energy contents on hepatic lipogenic-related enzyme activities of White Roman geese. Master thesis, National Chung-Hsing University pp. 36-39. (in Chinese).

Chen, T. F. and J. C. Hsu. 2003. Incorporation of n-3 long-chain polyunsaturated fatty acids into duck egg yolk. Asian-Aust. J. Anim. Sci. 16:565-569.

Clarke, S. D. and D. B. Jump. 1993. Regulation of gene transcription by polyunsaturated fatty acids. Prog. Lipid Res. 32:139-149.

Clarke, S. D., M. K. Armstrong and D. B. Jump. 1990. Dietary polyunsaturated fats uniquely suppress rat liver fatty acid synthetase and S_{14} mRNA content. J. Nutr. 120:225-231.

Dakshinamurti, K. and P. R. Desjardins. 1969. Acetyl-CoA carboxylase from rat adipose tissue. Biochem. Biothys. Acta. 176:22-29.

Dyer-Hurdon, J. N. and I. A. Nnanna. 1993. Cholesterol content and functionality of plasma and granules fractionated from egg yolk. J. Food Sci. 58:1277-1281.

Eder, K. and M. Kirchgessner. 1998. The effect of dietary vitamin E supply and a moderately oxidized oil on activities of hepatic lipogenic enzymes in rats. Lipids 33:277-283.

Fritche, K. L. and P. V. Johnston. 1988. Rapid autoxidation of fish oil in diets without added antioxidants. J. Nutr. 118:421-426.

Hahm, T. S., D. L. King and D. B. Min. 1993. Food antioxidants. J. Foods Biotechnol. 2:1-18.

Hargis, P. S. 1988. Modifying egg yolk cholesterol - a review. World's Poult. Sci. J. 44:17-29.

Hargis, P. S., M. E. Van Elswyk and B. M. Hargis. 1991. Dietary modification of yolk lipid with menhaden oil. Poult. Sci. 70:874-883.

Hargis, P. S. and M. E. Van Elswyk. 1993. Manipulating the fatty acid composition of poultry meat and eggs for the health conscious consumer. World's Poult. Sci. J. 49:251-264.

Herold, P. M. and J. E. Kinsella. 1986. Fish oil consumption and decreased risk of cardiovascular disease: a comparison of findings from animal and human feeding trials. Am. J. Clin. Nutr. 43:566-589.

Hsu, J. C., K. Tanaka, S. Ohtani and C. M. Collado. 1988. Effects of pantethine supplementation to diets on hepatic lipogenesis of laying hens exposed to a high environmental temperature. Jpn. J. Zootech. Sci. 59:1059-1072.

Hu, M. L., E. N. Frankel, B. E. Leibovitz and A. L. Tappel. 1989. Effect of dietary lipids and vitamin E on *in vitro* lipid peroxidation in rat liver and kidney homogenenates. J. Nutr. 119:1574-1582.

Illingworth, D. R. and D. Ullmann. 1990. Effects of omega-3 fatty acids on risk factors for cardiovascular disease. Ch. 2. In omega-3 Fatty Acids in Health and Disease (Ed. R. S. Lees and M. Karel), p. 39. Marces Dekker, Inc., New York.

Iritani, N. 1992. Nutritional and Hormonal Regulation of lipogenic-enzyme gene expression in rat liver. Eur. J. Biochem. 205:433-442.

Kornhout, D., E. B. Bosschieter and C. D. L. Coulander. 1985. Inverse relationship between fish consumption and 20 year mortality from coronary heart disease. N. Engl. J. Med. 312:1205-1209.

Kumar, S., J. A. Dorsey, R. A. Muesing and J. W. Porter. 1970. Comparative studies of the pigeon liver fatty acid synthetase complex and its subunit. J. Biol. Chem. 245:4732-4744.

Leskanich, C. O. and R. C. Noble. 1997. Manipulation of the n-3 Polyunsaturated fatty acid composition of avian eggs and meat. World's Poult. Sci. J. 53:155-183.

Lien, T. F., C. P. Wu and J. J. Lu. 2003. Effects of cod liver oil and chromium Picollinate Supplements on the serum traits, egg yolk fatty acids and cholesterol contents in laying hens. Asian-

Aust. J. Anim. Sci. 16:1177-1181.

Lin, C. F., J. I. Gray, A. Asghar, D. J. Buckley, A. M. Booren and C. J. Flegal. 1989. Effcet of dietary oils and α-tocopherol supplementation on lipid composition and stability of broiler meat. J. Food Sci. 54:1457-1461.

Lohr, G. H. and H. D. Waller. 1974. Method of enzymatic analysis. Academic Press. New York and London, Vol. 2:636.

Lowry, O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall. 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193:265-275.

Meluzzi, A., F. Sirri, G. Manfreda, N. Tallarico and A. Franchini. 2000. Effects of dietary vitamin E on the quality of table eggs enriched with n-3 long-chain fatty acids. Poult. Sci. 79:539-545.

Mikkelsen, L., H. S. Henson, N. Grunnet and J. Dich. 1993. Inhibition of fatty acid synthesis in rat hepatocytes by exogenous polyunsaturated faty acids is caused by lipid peroxidation. Biochem. Biophys. Acta 1166:99-104.

Mutia, R. and S. Uchida. 1999. Effects of feeding winged bean oil on cholesterol and lipid contents in egg and liver, and fatty acid composition of egg in Japanese Quail. Asian-Aust. J. Anim. Sci. 12:376-380.

O'choa, S. 1955. Malic enzyme. In: Method enzymology. Vol. 1:739. Academic Press. Inc., New York.

Oh, S. Y., C. H. Lin, J. Ryue and D. E. Bell. 1994. Eggs enriched with omega-3 fatty acids as a wholesome food. J. Applied Nutr. 46:14-25.

Qi, G. H. and J. S. Sim. 1998. Natural tocopherol Enrichment and its effect in n-3 fatty acid modified chicken eggs. J. Agric. Food Chen. 46:1920-1926.

SAS. 1988. User' s Guide: Statistics, SAS Inst., Inc., Carry, NC.

Sklan, D. 1983. Effect of high vitamin A or tocopherol intake on hepatic lipid metabolism and intestinal absorption and secretion of lipids and bile acids in the chick. Br. J. Nutr. 50:409-416.

Takeda, Y., F. Sazuki and H. Inoue. 1963. Citrate cleavage enzyme, In; Method enzymology. Vol. 5:154. Academic Press. Inc. New York.

Tanaka, K., S. Ohtani and K. Shigeno. 1983. Effect of increasing dietary energy on hepatic lipogenesis in growing chicks. I: increasing energy by carbohydrate supplementation. Poult. Sci. 62:445-451.

Thompson, J. N. and G. Hatina. 1979. Determination of tocopherols and tocotrienols in foods and tissues by high performance liquid chromatography. J. Liquid Chromatography 2:327-344.

Xia, Z. G., Y. M. Guo, S. Y. Chen and J. M. Yuan. 2003. Effects of dietary polyunsaturated fatty acids on antibody production and lymphocyte proliferation of laying hens. Asian-Aust. J. Anim. Sci. 16:1320-1325.

Yeh, Y. Y. and G. A. Leveille. 1971. Studies on the relation-ship between lipogenesis and the level of coenzyme A derivatives, lactate and pyruvate in chick liver. J. Nutr. 101:911-918.