Asian-Aust. J. Anim. Sci.
Vol. 19, No. 7 : 1047 - 1053
July 2006

www.ajas.info

Effects of Dietary Algal Docosahexaenoic Acid Oil Supplementation on Fatty Acid Deposition and Gene Expression in Laying Tsaiya Ducks*

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ABSTRACT : The current study was designed to determine the effects of dietary docosahexaenoic acid (DHA) on fatty acid deposition in egg yolk and various tissues of laying Tsaiya ducks, and on the mRNA concentrations of hepatic lipogenesis-related transcription factors. Thirty laying ducks were randomly assigned to three treatments with diets based on corn-soybean meal (ME: 2803 kcal/kg; CP: 17.1%; Ca: 3.4%) supplemented with 0% (control diet), 0.5% or 2% algal DHA oil. The DHA content in egg yolks of the ducks was elevated significantly ($p<0.01$) with the supplementation of dietary DHA. The DHA percentage of the total fatty acids in the egg yolk of laying ducks was 0.5%, 1.3% and 3.4% for 0%, 0.5% and 2% algal DHA oil treatments, respectively, for the 1st week, and 0.5%, 1.5% and 3.3% for the 2nd week. Therefore, algal DHA oil can be utilized by laying Tsaiya ducks to enhance the egg-yolk DHA content. The concentrations of triacylglycerol (TG) and cholesterol in plasma of laying Tsaiya ducks were not affected by dietary DHA treatments ($p>0.05$). The DHA concentration in plasma, liver, and skeletal muscle was increased with the addition of dietary algal DHA oil ($p<0.05$). The mRNA abundance of sterol regulatory element binding protein 1 (SREBP1) and SREBP2 in the livers of laying Tsaiya ducks was not affected by dietary DHA, suggesting that the expression of these transcription factors is tightly controlled and not sensitive to DHA treatments. (**Key Words :** Laying Tsaiya Ducks, Dietary Docosahexaenoic Acid, SREBP1, SREBP2, Fatty Acid Composition)

INTRODUCTION

Dietary fatty acids (FA) are essential to maintain normal physiological functions and can be incorporated into different tissues of animals. In pigs, changes in FA composition of liver, muscle and adipose tissue are achieved by feeding different dietary FA sources (Innis et al., 1996; Smith et al., 1996; Ding et al., 2003). In avian species, dietary FA can be deposited into egg yolks and into other tissues (Cruickshank, 1934; Donaldson, 1967; Ding and Lilburn, 1997). Cruickshank (1934) showed that the unsaturated, but not the saturated FA composition of egg yolks is modified by dietary FA. Feeding hens with fish oil increases n-3 polyunsaturated fatty acid (PUFA) levels in the egg yolk lipids, indicating that the FA composition of egg yolks can be changed by dietary fat sources (Navarro et al., 1972; Oh et al., 1988; van Elswyk et al., 1992).

Beneficial effects of n-3 PUFA, mainly docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), on human health have been reported. DHA plays an important role in reducing plasma triacylglycerol (TG) and cholesterol levels, preventing cardiovascular diseases, hypertension, platelet aggregation and arthritis in humans (Innis, 1992; Simopoulos, 2000). These findings have stimulated interest in enrichment of n-3 PUFA in animal feeds with fish oil, fish meal, algae, linseed, flaxseed or red crab meal supplementation to the diet (Navarro et al., 1972; Hargis et al., 1991; Nitsan et al., 1999; Schumann et al., 2000; López-Ferrer et al., 2001; Howe et al., 2002; Carrillo-Dominguez et al., 2005). High dietary DHA reduces the expression of a lipogenic transcription factor, sterol regulatory element binding protein 1 (SREBP1) in the liver of pigs (Hsu et al., 2004; Liu et al., 2005) and mice (Xu et al., 1999; Nakataki et al., 2003).

In the current study, we enriched egg-yolk, plasma, liver, and skeletal muscle DHA through dietary supplementation with algal DHA oil. Dietary DHA did not change the expression of liver lipogenesis-related transcription factors (SREBP1 and SREBP2), suggesting fatty acid and

* This work was supported by Council of Agriculture in Taiwan, ROC.

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Received November 16, 2005; Accepted March 5, 2006

Table 1. Dietary composition

Ingredients (%)	Control	0.5% DHA oil	2% DHA oil
Yellow corn	61.27	61.27	61.27
Soybean meal (CP 44%)	26.65	26.65	26.65
Butter	2.0	1.5	0.0
DHA oil [a]	0.0	0.5	2.0
Dicalcium phosphate	1.11	1.11	1.11
Limestone (pulverized)	8.0	8.0	8.0
Salt (iodized)	0.3	0.3	0.3
Vitamin-premix [b]	0.3	0.3	0.3
Mineral-premix [c]	0.2	0.2	0.2
DL-methionine	0.17	0.17	0.17
Calculated values			
CP (%)	17.1	17.1	17.1
ME (kcal/kg)	2,803	2,803	2,803
Ca (%)	3.4	3.4	3.4

[a] DHA oil is extracted from alga and contains $C_{10:0,}$ 0.56%; $C_{12:0}$, 3.29%; $C_{14:0}$,11.33%; $C_{14:1}$, 0.18%; $C_{16:0}$, 10.55%; $C_{16:1}$, 2.12%; $C_{18:0}$, 0.98%; $C_{18:1n-9}$, 24.52% $C_{18:2n-6}$, 1.11%; $C_{18:3n-3}$, <0.1%; $C_{20:4n-6}$, <0.1%; $C_{20:5n-3}$, <0.1%; $C_{22:5n-3}$, 0.24%; $C_{22:6n-3,}$ 43.9% (Martek Bio).

[b] Supplied per kilogram of diet: Vit. A, 11,250 IU; Vit. D3, 1,200 IU; Vit. E, 37.5 IU; Vit. K, 2 mg; Vit. B1, 2.6 mg; Vit. B2, 8 mg; Vit. B6, 3 mg; Pantothenic acid, 15 mg; Niacin, 60 mg; Biotin, 0.2 mg; folic acid, 0.65 mg; Vit. B12, 0.013 mg.

[c] Supplied per kilogram of diet: Cu, 10 mg; Fe, 100 mg; Mn, 60 mg; Zn, 65 mg; Se, 0.15 mg.

cholesterol synthesis, important for egg-yolk production are not regulated by dietary DHA in laying ducks.

MATERIALS AND METHODS

Animals and diets

Thirty laying Tsaiya ducks were placed in individual cages and distributed randomly to three treatments. Corn-soybean meal based diets (ME: 2,803 kcal/kg; CP: 17.1%; Ca: 3.4%; Table 1) were supplemented with 0% (control diet), 0.5% or 2% algal DHA oil (Martek Bio). Vitamin E concentration was raised to 37.5 IU/kg of diet instead of the NRC recommendation level of 5 IU/kg (National Research Council, 1994) because the additional dietary DHA may compromise the antioxidant system of the laying Tsaiya ducks. The dietary FA compositions are listed in Table 2. After feeding the ducks with the control diet (2% butter) for one week, the birds were fed the experimental diets for 14 days. Egg production rate was determined for the first and second weeks. Egg weight and yolk weight were measured on the 7th and 14th day. Individual blood samples were obtained from the brachial vein (V. ulnaris) using EDTA as an anticoagulant. Plasma, dried egg yolk and diet samples were frozen at -80°C until analysis. Ducks were killed by cervical dislocation on the 14th day, and tissue samples (liver and skeletal muscle) were removed, and immediately frozen in liquid nitrogen to be stored at -80°C. The animal protocol used in the present experiment was approved by the Animal Care and Use Committee of the National Taiwan University.

Table 2. Fatty acid composition[1] of experimental diets

Fatty acid profile	Control (2% butter)	0.5% DHA oil	2% DHA oil
C16:0	18.3	19.5	21.6
C16:1	4.3	4.9	5.1
C18:0	8.9	8.0	8.0
C18:1	40.4	38.8	37.0
C18:2 n-6	22.1	20.8	21.8
C18:3 n-3	4.0	3.7	3.5
C20:4 n-6	1.6	1.4	1.2
C20:5 n-3	0.0	trace	trace
C22:6 n-3	0.0	2.0	6.3

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

Fatty acid analysis

Total lipids of diet, tissue, and yolk were extracted according to the procedure of Folch et al. (1957). Before the extraction, tissue and yolk samples were freeze-dried. One-tenth g of yolk, 1 g of diet, or 0.5 g of tissue sample powders was used to extract total lipids. Heptadecanoic acid (17:0; 1,000 nmol), as di-17:0 L-α-phosphatidylcholine (Sigma, St. Louis, MO) was added to the sample as an internal standard before extraction. Total plasma lipids were extracted with methanol: benzene (4:1 v/v) as modified by Kates (1986).

Total lipids were then converted to fatty acid methyl esters (FAME) and separated by gas chromatography on a 30 m long by 0.25 mm ID with 0.20 um film thickness (SP-2380 capillary column; Supelco Inc., USA) with a Varian Star 3400cx gas chromatograph equipped with a hydrogen flame-ionization detector. Individual FA were identified by comparison to the retention times of standards (Nu Check Prep, Inc., Elysian, MN)

Plasma TG and total cholesterol analysis

The plasma TG and total cholesterol concentrations were determined spectrophotometrically using a commercial test kit (1.14856.0001 for TG; 1.14830.0001 for total cholesterol; Merck, Taipei, Taiwan).

RNA analysis

Total RNA was extracted from the frozen liver and muscle using the guanidinium- phenol - chloroform extraction method (Chomczynski and Sacchi, 1987); modifications were described previously (Hsu and Ding, 2003; Ying et al., 2003; Yang et al., 2004). The RNAs were separated by denatured electrophoresis, blotted to nylon membranes, and hybridized with radiolabeled cDNA probes in Ultrahyb (Ambion Inc., Austin, TX). Probes for Northern analysis were produced by PCR procedures described by Wang et al. (2004). The SREBP1 primer pair was generated from the chicken sequence whereas the SREBP2 primer

Table 3. Effect of dietary algal DHA on DHA levels in the plasma of laying Tsaiya ducks*

Time (week)	Plasma DHA level	
	1	2
	---------- % of total fatty acids ----------	
Control	1.1^{c}±0.55	1.6^{c}±0.79
0.5% DHA oil	3.2^{b}±1.47	4.6^{b}±1.95
2% DHA oil	8.0^{a}±2.36	9.8^{a}±3.05

* Mean±SD.
[a, b, c] Means within the same column with different superscripts differ significantly ($p<0.01$).

Table 4. Effect of dietary algal DHA on DHA levels in the livers and skeletal muscles of laying Tsaiya ducks

Tissue	Control	0.5% DHA oil	2% DHA oil
	--- % of total fatty acids (Mean±SD) ----		
Liver*	1.4^{c}±0.6	4.4^{b}±1.1	10.1^{a}±3.9
Skeletal muscle	0.8^{b}±0.3	1.6^{a}±0.8	2.0^{a}±0.7

* The data for liver DHA concentration was previous described by Ko et al. (2004).
[a, b, c] Means within the same row with different superscripts differ significantly ($p<0.01$).

pair was from the human sequence. The primer sequences are 5'-GCGCTACCGCTATCCATCA-3' and 5'-GGTCGGC ATCTCCATCACCT-3' for SREBP1 and 5'-TAATACG ACTCACTATAGCG-3' and 5'-TCAAGTCCTTCAGCCTC AAG-3' for SREBP2. These lipogenesis-related transcriptional factors were cloned from the liver of a Tsaiya duck (Yen et al., 2005), and the cDNA fragments were used to generate probes for Northern analysis. The SREBP1 cDNA fragment is 283 bp with 93% homology when compared with the chicken SREBP1 (GenBank, AJ310768). The SREBP2 cDNA fragment is 386 bp with 83% homology when compared with the chicken SREBP2 (GenBank, AJ310769). The 18S ribosomal RNA primer sequences and PCR conditions are indicated in Liu et al. (2005). Hybridization results were quantified by phosphor-image analysis. The densitometric value for an individual transcript in a sample lane was normalized to the densitometric value for the 18S ribosomal RNA in the same lane. Duplicate RNA samples for each tissue from each bird were analyzed.

Statistical analysis

All data were analyzed by analysis of variance using the general linear model procedures of the SAS Institute (SAS, 2001). The differences between means were detected with the Duncan's New Multiple Range Test. A P value≤0.05 was considered significant.

RESULTS

Production parameters

Egg production, egg weight, and yolk weight of the laying ducks were not affected by the algal DHA oil supplement in the diet ($p>0.05$). The egg production ranged from 87±18 to 98±5%. The egg weights ranged from 63.9±4.5 to 67.0±6.4 g. The average weight of a yolk was between 19.4±1.9 and 20.0±1.2 g.

Table 5. Effect of 7-d dietary algal DHA treatment on fatty acid compositions in the egg yolk of laying Tsaiya ducks*

Fatty acid profile	Control	0.5% DHA oil	2% DHA oil
	---------- % of total fatty acids ----------		
C16:0	26.7±1.7	27.3±1.3	27.4±1.9
C16:1	3.3±0.8	3.1±0.3	3.1±0.4
C18:0	0.98±0.3	1.1±0.3	0.9±0.3
C18:1	50.3±2.4	50.0±3.2	48.2±1.7
C18:2 n-6	8.5±1.0	8.5±1.0	9.3±1.2
C18:3 n-3	0.3^{a}±0.1	0.4^{a}±0.1	0.4^{b} ±0.1
C20:4 n-6	3.1^{a}±0.3	2.9^{a}±0.4	2.4^{b}±0.5
C20:5 n-3	0.1±0.1	0.1±0.1	0.1±0.1
C22:6 n-3	0.5^{c}±0.2	1.3^{b}±0.4	3.4^{a}±1.4

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

Plasma TG and total cholesterol concentrations

Neither the plasma TG nor total cholesterol concentrations of laying ducks were affected by dietary algal DHA oil ($p>0.05$). The plasma TG concentration ranged from 94±22 to 116±27 mg/dl whereas the plasma cholesterol ranged from 919±499 to 1,342±395 mg/dl in the laying ducks.

Plasma DHA concentration

The plasma DHA concentration was significantly increased by one or two week dietary supplementation with algal DHA oil ($p<0.01$; Table 3).

Tissue DHA concentrations

The liver DHA concentration data were previously reported by Ko et al. (2004). It was significantly increased by the dietary algal DHA oil supplement after two weeks ($p<0.01$; Table 4). The muscle DHA concentration of the laying Tsaiya ducks was increased by dietary DHA ($p<0.01$; Table 4). The increase was smaller than that observed in the liver.

Yolk DHA concentration

The yolk DHA concentration averaged 0.5%, 1.4% and 3.4% of total fatty acids, for 0%, 0.5%, and 2% algal DHA oil supplement, respectively, for the 1st week, and 0.5%, 1.5%, and 3.3% of total fatty acids, respectively for the 2nd week ($p<0.01$; Tables 5 and 6). Furthermore, the yolk C20:4 were reduced with 2% algal DHA oil treatment. The concentration of C20:5 was low and not affected by treatment.

Table 6. Effect of 14-d dietary algal DHA treatment on fatty acid compositions in the egg yolk of laying Tsaiya ducks*

Fatty acid profile	Control	0.5% DHA oil	2% DHA oil
	---------- % of total fatty acids ----------		
C16:0	25.4±5.9	27.2±0.9	27.5±1.7
C16:1	3.2±0.9	3.2±0.7	3.4±0.7
C18:0	1.2±1.3	1.0±0.2	0.9±0.3
C18:1	51.4±4.5	48.9±1.6	48.3±2.1
C18:2 n-6	8.5±1.1	8.8±1.2	9.0±1.4
C18:3 n-3	0.3^{b}±0.1	0.4^{a}±0.1	0.4^{a}±0.1
C20:4 n-6	3.1^{a}±0.7	3.0^{a}±0.6	2.2^{b}±0.6
C20:5 n-3	0.1±0.1	0.1±0.1	0.1±0.1
C22:6 n-3	0.5^{c}±0.3	1.5^{b}±0.6	3.3^{a}±1.5

* Mean±SD.

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

The mRNA concentrations of SREBP1 and SREBP2

Neither the SREBP1 mRNA concentration nor the SREBP2 mRNA concentration in liver and muscle were affected by the dietary DHA treatment for two weeks ($p>0.05$; Figure1A; 1B).

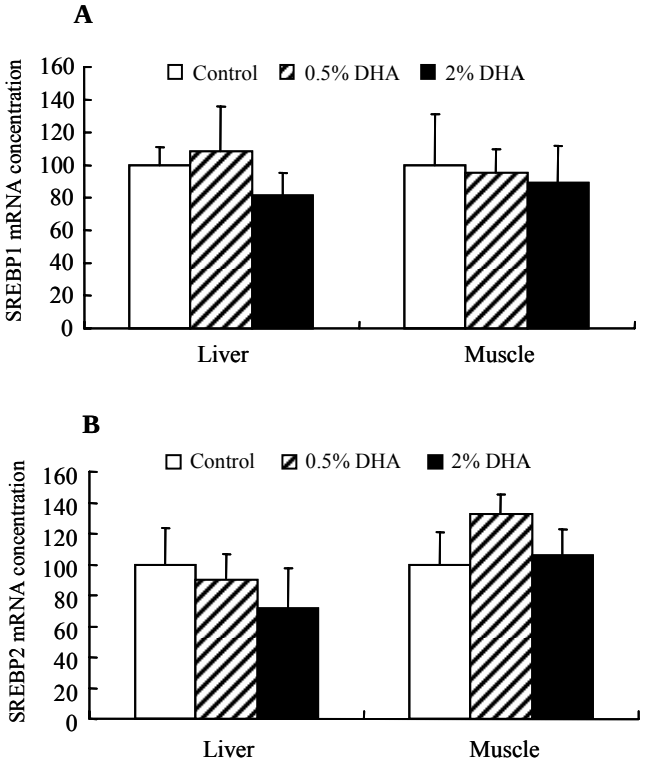

Figure 1. The effect of dietary algal DHA supplementation on the abundance of SREBP1 (A) and SREBP2 mRNA (B) in laying Tsaiya ducks. Tsaiya ducks were fed diets supplemented with 0%, 0.5% or 2% algal docosahexaenoic acid (DHA) oil for 14 days (10 ducks per group). At the day of sampling, ducks were killed 2 h after feeding. The SREBP1 and 18S rRNA were determined by Northern analysis. The SREBP1 mRNA abundances were depicted relative to the control. The mRNA values were normalized to 18S rRNA content with n = 10 replicates/treatment. There was no treatment effect detected ($p>0.05$).

DISCUSSION

Unsaturated FA concentrations in egg yolks are altered by dietary unsaturated FA in avian species (Cruickshank, 1934; Chen et al., 1965; Navarro et al., 1972). Fish meal, fish oil, linseed, flaxseed, and algae products have been incorporated into diets to increase the n-3 PUFA concentrations in chicken eggs and tissues. However, fishy odor is a concern when high amounts of fish oil are added to the diet (Nash et al., 1995). In the current study, we found that DHA concentrations in eggs, livers and muscles were increased whereas the production parameters of the laying ducks were not affected by dietary supplementation with algal DHA oil. The results on the production parameters in the current study are different from those of others that indicate the egg production, egg weight, and yolk size is reduced by dietary PUFA supplementation (Whitehead et al., 1993; Scheideler et al., 1994; van Elswyk et al., 1994). However, similar to the current study, other reports show no effect (Hargis et al., 1991; Ferrier et al., 1995; Meluzzi et al., 2000; Chen and Hsu, 2003; Cheng et al., 2004). We observed that changes in egg-yolk fatty acid composition had already taken place after on week of dietary DHA supplementation with no further change at two weeks (Table 5). Furthermore, several groups indicate that incorporation of dietary PUFA, including DHA, into egg-yolk lipids at 14 to 18 days of treatment (Meluzzi et al., 2000; Chen and Hsu, 2003; Cheng et al., 2004). Thus, two weeks was a sufficient time to feed the algal DHA. A longer time (12 weeks) of dietary PUFA supplementation also increases egg-yolk DHA (Watkins et al., 2003).

Nitsan et al. (1999) indicate that laying chickens fed with diets containing 1% algal meal had increased plasma DHA (30%). We found a greater increase (about three- to seven-fold) with the dietary supplementations of 0.5% or 2% algal DHA oil in laying ducks (Table 3) as well as in our previous study with laying hens (Cheng et al., 2004). Lopez-Ferrer et al. (2001) demonstrate that PUFA composition in the muscles of broilers was affected through dietary linoleic acid, linolenic acid or fish oil supplementation. The DHA concentration in the skeletal muscle and liver is increased by elevated dietary DHA in the current study, confirming data in the literature (Huang et al., 1990; Leskanich and Noble, 1997; Lopez-Ferrer et al., 2001; Schiavone et al., 2004). Other FA were either not affected or only slightly changed.

Dietary fish oil decreases plasma TG and cholesterol concentrations in mammals (Sanders and Hochland, 1983; Kromhout et al., 1985; Daviglus et al., 1997). Feeding the high DHA algae oil to laying ducks did not affect plasma

TG or cholesterol concentrations; similar to what is observed in laying chickens (Cheng et al., 2004). Physiologically, the de novo lipogenesis in the liver of laying birds is high in order to produce lipids for yolk deposition. Such function may be related to high estrogen concentrations in laying birds (Harms et al., 1972; Polin and Wolford, 1977; Dashti et al., 1983). The lipids generated in liver are transported into egg yolks by very low density lipoprotein (VLDL) and vitellogenin, both of which contain high TG and cholesterol (Walzem, 1996). If the efficiency of fat deposition in yolks was reduced, the egg production would be negatively affected. These observations suggest that in order to maintain egg production, the plasma TG and cholesterol concentrations in laying hens are not readily changed by dietary lipid composition as observed in mammals.

Cholesterol and FA synthesis in birds is primarily in the liver (Leveille et al., 1968; 1975). Fatty acid synthase (FAS) and 3-hydroxyl-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase) are the two key enzymes involved in lipid synthesis (Back et al., 1986; Semenkovich, 1997; Horton et al., 1998; Gondret et al., 2001). These enzymes are regulated by two lipogenic transcription factors–SREBP1 and SREBP2, respectively. Recent data show that the dietary long-chain PUFA (from fish oil or high DHA algae) decrease the SREBP1 mRNA abundance in rodent and pig livers (Xu et al., 1999; Yahagi et al., 1999; Hsu et al., 2004). Several days of feeding fish oil causes a reduction in mouse hepatic SREBP1 mRNA (Nakatani et al., 2003). In contrast to observations in mammals, there was no effect of dietary DHA on the expression of these transcription factors in the liver and skeletal muscle of laying ducks after two weeks feeding with the DHA oil (Figure 1). This observation is similar to that in laying hens (Cheng et al., 2004). A longer time of feeding might decrease the SREBPs, but the evidence from mammals suggests these transcription factors rapidly respond to PUFA. In the laying duck dietary DHA is incorporated into liver and muscle, but the SREBP transcription factors are not responsive. The necessity to produce egg-yolk lipids appears to negate the regulation of the SREBPs by dietary fatty acids in laying birds.

Taken together, although dietary algal DHA oil did not change the hepatic SREBP1 and SREBP2 mRNA in the laying ducks, the enrichment of DHA in the egg yolk will increase the value of the egg products for human consumption.

REFERENCES

Back, D. W., M. J. Goldman, J. E. Fisch, R. S. Ochs and A. G. Goodridge. 1986. The fatty acid synthase gene in avian liver. Two mRNAs are expressed and regulated in parallel by feeding, primarily at the level of transcription. J. Biol. Chem. 261:4190-4197.

Carrillo-Dominguez, S., M. E. Carranco-Jauregui, R. M. Castillo-Dominguez, M. I. Castro-Gonzalez, E. Avila-Gonzalez and F. Perez-Gil. 2005. Cholesterol and n-3 and n-6 fatty acid content in eggs from laying hens fed with red crab meal (Pleuroncodes planipes). Poult. Sci. 84:167-172.

Chen, P. H., R. H. Common, N. Nikolaiczuk and H. F. MacRae. 1965. Some effects of added dietary fats on the lipid composition of hens egg yolk. J. Food Sci. 30:838-845.

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

Cheng, C. H., T. F. Shen, W. L. Chen and S. T. Ding. 2004. Effects of dietary algal docosahexaenoic acid oil supplementation on fatty acid deposition and gene expression in laying Leghorn hens. J. Agric. Sci. 142:683-690.

Chomczynski, P. and N. Sacchi. 1987. Single-step method of rna isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem. 162:156-159.

Cruickshank, E. M. 1934. Studies in fat metabolism in the fowl. 1. The composition of the egg fat and depot fat of the fowl as affected by the ingestion of large amount of different fats. Biochem. J. 28:965-977.

Daviglus, M. L., J. Stamler, A. J. Orencia, A. R. Dyer, K. Liu, P. Greenland, M. K. Walsh, D. Morris and R. B. Shekelle. 1997. Fish consumption and the 30-year risk of fatal myocardial infarction. N. Engl. J. Med. 336:1046-1053.

Dashti, N., J. L. Kelly, R. H. Thayer and J. A. Ontko. 1983. Concurrent inductions of avian hepatic lipogenesis, plasma lipids, and plasma apolipoprotein B by estrogen. J. Lipid Res. 24:368-379.

Ding, S. T. and M. S. Lilburn. 1997. Inclusion of coconut oil in diets for turkey breeders and its effects on embryonic yolk and liver fatty acids. Poult. Sci. 76:1714-1721.

Ding, S. T., A. Lapillonne, W. C. Heird and H. J. Mersmann. 2003. Dietary fat has minimal effects on fatty acid metabolism transcript concentrations in pigs. J. Anim. Sci. 81:423-431.

Donaldson, W. E. 1967. Lipid composition of chick embryo and yolk as affected by stage of incubation and maternal diet. Poult. Sci. 46: 693-697.

Ferrier, les K., L. J. Caston, S. Leeson, J. Squires, B. J. Weaver and B. J. Holub. 1995. α-Linolenic acid- and docosahexaenoic acid-enriched eggs from hens fed flaxseed : influence on blood lipids and platelet phospholipids fatty acids in humans. Am. J. Clin. Nutr. 62:81-86.

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

Gondret, F., P. Ferre and I. Dugail. 2001. ADD-1/SREBP-1 is a major determinant of tissue differential lipogenic capacity in mammalian and avian species. J. Lipid Res. 42:106-113.

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.

Harms, R. H., C. F. Simpson and B. L. Damron. 1972. Some new observations on "fatty liver syndrome" in laying hens. Avian Dis. 16:1042-1046.

Horton, J. D., I. Shimomura, M. S. Brown, R. E. Hammer, J. L. Goldstein and H. Shimano. 1998. Activation of cholesterol synthesis in preference to fatty acid synthesis in liver and

adipose tissue of transgenic mice overproducing sterol regulatory element-binding protein-2. J. Clin. Invest. 101:2331-2339.

Howe, P. R., J. A. Downing, B. F. Grenyer, E. M. Grigonis-Deane and W. L. Bryden. 2002. Tuna fishmeal as a source of DHA for n-3 PUFA enrichment of pork, chicken, and egg. Lipids. 37:1067-1076.

Hsu, J. M. and S. T. Ding. 2003. Effect of polyunsaturated fatty acids on the expression of transcription factor adipocyte determination and differentiation-dependent factor 1 and of lipogenic and fatty acid oxidation enzymes in porcine differentiating adipocytes. Br. J. Nutr. 90:507-513.

Hsu, J. M., P. H. Wang, B. H. Liu and S. T. Ding. 2004. The effect of dietary docosahexaenoic acid on the expression of porcine lipid metabolism related genes. J. Anim. Sci. 83:683-689.

Huang, Z. B., H. Leibovitz, C. M. Lee and R. Millar. 1990. Effect of dietary fish oil on ω-3 fatty acid levels in chicken eggs and thigh flesh. J. Agric. Food Chem. 38:743-747.

Innis, S. M. 1992. N-3 fatty acid requirements of the newborn. Lipids 27:879-885.

Innis, S. M., R. Dyer, P. T. Quinlan and D. Diersen-Schade. 1996. Dietary triacylglycerol structure and saturated fat alter plasma and tissue fatty acids in piglets. Lipids 31:497-505.

Kates, M. 1986. Techniques of lipidology. Isolation, analysis, and identification of lipids, pp. 123-128. New York, USA : Elsevier.

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

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

Leveille, G. A., E. K. O'Hea and K. Chakrabarty. 1968. *In vivo* lipogenesis in the domestic chicken. Proc. Soc. Exp. Biol. Med. 128:398-401.

Leveille, G. A., D. R. Romsos, Y. Y. Yeh and E. K. O'Hea. 1975. Lipid biosynthesis in the chick. A consideration of site of synthesis, influence of diet and possible regulatory mechanisms. Poult. Sci. 54:1075-1093.

Liu, B. H., Y. C. Wong, W. M. Cheng, T. F. Shen and S. T. Ding. 2005. The effects of docosahexaenoic acid oil and soybean oil on the expression of lipid metabolism related mRNA in pigs. Asian-Aust. J. Anim. Sci. 18:1451-1456.

López-Ferrer, S., M. D. Baucells, A. C. Barroeta, J. Galobart and M. A Grashorn. 2001. n-3 enrichment of chicken meat. 2. Use of precursors of long-chain polyunsaturated fatty acids: Linseed oil. Poult. Sci. 80:753-761.

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.

Nakatani, T., H. J. Kim, Y. Kaburagi, K. Yasuda and O. Ezaki. 2003. A low fish oil inhibits SREBP-1 proteolytic cascade, while a high-fish-oil feeding decreases SREBP-1 mRNA in mice liver: relationship to anti-obesity. J. Lipid Res. 44:369-379.

Nash, D. M., R. M. G. Hamilton and H. W. Hulan. 1995. The effect of dietary herring meal on the omega-3 fatty acid content of plasma and egg yolk lipids of laying hens. Can. J. Anim. Sci. 75:247-253.

National Research Council. 1994. Nutrient requirements of poultry. 9th rev. ed. Washington, D.C., USA: National Academies Press.

Navarro, J. G., J. C. Saaverder, F. B. Borie and M. M. Caiozzi. 1972. Influence of dietary fish meal on egg fatty acid composition. J. Sci. Food Agric. 23:1287-1292.

Nitsan, Z., S. Mokady and A. Sukenik. 1999. Enrichment of poultry products with ω3 fatty acids by dietary supplementation with the alga Nannochloropsis and mantur oil. J. Agric. Food Chem. 47:5127-5132.

Oh, S. Y., J. Ryue, J. Hsieh and D. E. Bell. 1988. Effects of dietary eggs enriched in omega-3 fatty acids on plasma cholesterol, lipoprotein composition and blood pressure in human. FASEB J. 2: A425-451.

Polin, D. and J. H. Wolford. 1977. Role of estrogen as a cause of fatty liver hemorrhagic syndrome. J. Nutr. 107:873-886.

Sanders, T. A. B. and M. C. Hochland. 1983. A comparison of the influence on plasma lipids and platelet function of supplements of ω-3 and ω-6 polyunsaturated fatty acids. Br. J. Nutr. 50:521-529.

SAS Institute, 2001. SAS User's Guide : Statistics. Cary NC: SAS Institute Inc.

Scheideler, S. E., G. Froning and S. Cuppett. 1994. Effect of dietary flaxseed and fish oil on egg components, sensory analysis and oxidation products. Poult. Sci. 73 (Suppl. 1):118. (Abstr.)

Schiavone, A., I. Romboli, R. Chiarini and M. Marzoni. 2004. Influence of dietary lipid source and strain of fatty acid composition of Muscovy duck meat. J. Anim. Physiol. Anim. Nutr. 88:88-93.

Schumann, B. E., E. J. Squires and S. Leeson. 2000. Effect of dietary flaxseed, flax oil and n-3 fatty acid supplement on hepatic and plasma characteristics relevant to fatty liver haemorrhagic syndrome in laying hens. Br. Poult. Sci. 41:465-472.

Semenkovich, C. F. 1997. Regulation of fatty acid synthase (FAS). Prog. Lipid Res. 36:43-53.

Simopoulos, A. P. 2000. Human requirement for n-3 polyunsaturated fatty acids. Poult. Sci. 79:961-970.

Smith, D. R., D. A. Knabe, H. R. Cross and S. B. Smith. 1996. A diet containing myristoleic plus palmitoleic acids elevates plasma cholesterol in young growing swine. Lipids 31:849-858.

Van Elswyk, M. E., A. R. Sams and P. S. Hargis. 1992. Composition, functionality and sensory evaluation of eggs from hens fed dietary menhaden oil. J. Food Sci. 57:342-344.

Van Elswyk, M. E., B. M. Hargis, J. D. Williams and P. S. Hargis. 1994. Dietary menhaden oil contributes to hepatic lipidosis in laying hens. Poult. Sci. 73:653-662.

Wang, P. H., B. H. Liu, Y. H. Ko, Y. C. Li and S. T. Ding. 2004. The expression of porcine adiponectin and stearoyl coenzyme A desaturase genes in differentiating adipocytes. Asian-Aust. J. Anim. Sci. 17:588-593.

Walzem, R. L. 1996. Lipoproteins and the laying hen: form follows function. Poultry and Avian Biology Reviews 7:31-64.

Watkins, B. A., S. Feng, A. K. Strom, A. A. DeVitt, L. Yu and Y. Li. 2003. Conjugated linoleic acids alter the fatty acid composition and physical properties of egg yolk and albumen. J. Agric. Food Chem. 51:6870-6876.

Whitehead, C. C., A. S. Bowman and H. D. Griffin. 1993.

Regulation of plasma oestrogen by dietary fats in the laying hen: relationships with egg weight. Br. Poult. Sci. 34:999-1010.

Xu, J., M. T. Nakamura, H. P. Cho and S. D. Clarke. 1999. Sterol regulatory element binding protein-1 expression is suppressed by dietary polyunsaturated fatty acids. J. Biol. Chem. 274:23577-23583.

Yahagi, N., H. Shimano, A. H. Hasty, M. Amemiya-Kudo, H. Okazaki, Y. Tamura, Y. Iizuka, F. Shionoiri, K. Ohashi, J. Osuga, K. Harada, T. Gotoda, R. Nagai, S. Ishibashi and N. Yamada. 1999. A crucial role of sterol regulatory element binding protein-1 in the regulation of lipogenic gene expression by polyunsaturated fatty acids. J. Biol. Chem. 274: 35840-35844.

Yen, C. F., Y. N. Jiang, T. F. Shen, I. M. Wong, C. C. Chen, K. C. Chen, W. C. Chang, Y. K. Tsao and S. T. Ding. 2005. Cloning and expression of the genes associated with lipid metabolism in Tsaiya ducks. Poult. Sci. 84:67-74.

Ying, C., M. A. Chan, W. T. K. Cheng and W. F. Hong. 2003. Co-expression and sequence determination of estrogen receptor variant messenger RNS in swine uterus. Asian-Aust. J. Anim. Sci. 16:1716-1721.

Yang, C. C., H. S. Chang, C. J. Lin, C. C. Hsu, J. I. Cheng, L. Hwu, and W. T. K. Cheng. 2004. Cock spermatozoa serve as the gene vector for generation of transgenic chicken (*Gallus gallus*). Asian-Aust. J. Anim. Sci. 17:885-891.