Egg yolk omega-6 and omega-3 fatty acids modify tissue lipid components, antioxidant status, and ex vivo eicosanoid production in chick cardiac tissue

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ABSTRACT The effects of maternal n-6 and n-3 fatty acid (FA) supplementation on hatched chick tissue FA profile, antioxidant status, and ex vivo eicosanoid production by the cardiac tissue were investigated. Eggs with low, medium, and high levels of n-3 FA were obtained by feeding Cobb breeder hens were fed a corn-soybean meal-based diet containing 3.5% sunflower oil (low n-3), 1.75% sunflower oil plus 1.75% fish oil (medium n-3), or 3.5% fish oil (high n-3). Total n-3 FA in the yolk ranged from 1.8, 10.3, and 13.3% for low, medium, and high n-3 eggs, respectively ($P < 0.001$). Total long-chain (>20 C) n-6 FA in the egg yolk were 7.4, 2.1, and 1.3 for low n-3, medium n-3, and high n-3 eggs, respectively ($P < 0.001$). No differences were observed in total fat content of the eggs, which was 33.3, 31.6, and 31.9% for low n-3, medium n-3, and high n-3 eggs, respectively ($P > 0.05$). Hatchability for the low, medium, and high n-3 eggs was 89, 85, and 83%, respectively ($P > 0.05$). The total lipid content of chick liver, heart, brain, and lungs can be placed in the following descending order: liver > brain > heart > lung and was not affected by egg FA ($P > 0.05$). Total n-3 FA were higher in the tissues of medium and high n-3 chicks than in the tissue of low n-3 chicks ($P < 0.05$). There was no effect of egg FA on docosahexaenoic acid (22:6n-3) in the heart of low, medium, and high n-3 chicks ($P > 0.05$). There were no differences in total glutathione, glutathione peroxidase, glutathione reductase, or superoxide dismutase activities in the tissues of chicks from low n-3, medium n-3, and high n-3 eggs ($P > 0.05$). The medium n-3 and high n-3 chicks had lower catalase activity in the heart than did the low n-3 chicks ($P = 0.013$). The TBA reactive substances were significantly lower in the liver of high n-3 chicks than in that of low and medium n-3 chicks ($P < 0.05$). Heart tissue prostaglandin E_2 concentration was higher in low n-3 chicks than in those hatched from medium or high n-3 eggs ($P < 0.05$). Heart tissue thromboxane A_3 was lowest in low n-3 chicks ($P < 0.05$). There was no effect of yolk FA on ex vivo prostaglandin E_3 or thromboxane A_2 production in cardiac tissue ($P > 0.05$). These results indicate that modulating egg yolk n-3 FA enhances tissue n-3 FA and reduces proinflammatory cardiac eicosanoid production without affecting hatchability.

Key words: egg, omega-3 fatty acid, antioxidant enzyme, thiobarbituric acid reactive substance, prostaglandin

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INTRODUCTION

Egg yolk fatty acids (**FA**) are the major modifiable factor known to influence the lipid and FA composition of the developing chick (Cherian and Sim, 1991; Cherian et al., 1997). Among the different FA in egg, much work has been reported on yolk linoleic (18:2n-6) and α-linolenic (18:3n-3) due to their role in polyunsaturated FA (**PUFA**) synthesis during embryogenesis. Arachidonic acid (20:4n-6) is the major n-6 PUFA derived from linoleic acid and eicosapentaenoic acid (**EPA**, 20:5n-3) and docosapentaenoic acid (**DPA**, 22:5n-3) and docosahexaenoic acid (**DHA**, 22:6n-3) are the major n-3 PUFA derived from α-linolenic acid. Long-chain (>20 C) n-6 and n-3 PUFA are the precursors of eicosanoids. Eicosanoids derived from n-6 FA are proinflammatory [prostaglandin E_2 (**PGE_2**)] and prothrombotic (thromboxane A_2) and those derived from n-3 FA are anti or less proinflammatory and less prothrombotic [prostaglandin E_3 (**PGE_3**) and thromboxane A_3 (**TXA_3**); (Calder, 2006)]. Convincing evidence has been published that shows that alterations in egg yolk n-6 and n-3 FA composition brought about by maternal dietary lipid source result in significant changes in tissue n-6 and n-3 PUFA content, liver desaturase enzyme activity, immune responses, and PUFA-derived eicosanoid synthesis in progeny chicks (Cherian and Sim, 2001; Liu and Denbow, 2001; Ajuyah et al., 2003a,b; Wang et al., 2004; Hall et al., 2007). These results suggest a unique role of maternal (egg yolk) n-6 and n-3 FA in modulating lipid and eicosanoid metabolism and immune and inflammatory responses in the progeny.

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Altering the n-6 and n-3 FA content in egg yolk also increases the degree of unsaturation leading to lipid peroxidation in tissue and it may compromise the antioxidant status of progeny. The bird's antioxidant system includes enzymes (e.g., superoxide dismutase, glutathione peroxidase, glutathione reductase, and catalase) and molecules (e.g., glutathione, vitamin A and E, and carotenoids) (Surai, 1999). Hatching time is considered to be a period of high oxidative stress due to long-chain PUFA accretion in tissues (Noble and Cocchi, 1990; Cherian and Sim, 1992; Cherian et al., 1997), exposure to atmospheric oxygen, onset of pulmonary respiration, and sudden increase in rate of oxidative metabolism (Speake et al., 1998) and the hatchlings are expected to react with a compensatory induction of endogenous antioxidants. Tissue-specific differences in the antioxidant system during avian embryogenesis and the degree of lipid peroxidation have been reported previously (Surai et al., 1996, 1999). However, there is limited information regarding the effect of maternal (egg yolk) n-3 and n-6 FA enrichment on antioxidant status and lipid peroxidation in progeny.

Modern-day meat-type broiler chickens have fast growth rates and high feed conversion ratios and metabolic rates. These features promote an increased workload on the cardiovascular system, predisposing birds to metabolic disorders such as right ventricular failure, ascites syndrome, cardiac arrhythmias, cardiopulmonary disorders, and sudden death (Olkowski and Classen, 1998; Julian, 2005). A growing body of information supports the concept that inflammation is an important predisposing risk factor contributing to cardiovascular diseases such as heart failure and hypertension (Ghosh et al., 2007). The role of dietary FA in modulating cardiac health through alteration in eicosanoid synthesis in the heart has been reported in humans (Rana et al., 2007). Previously, we reported that thrombocytes from chicks hatched to hens fed a high n-3 FA diet produced higher leukotriene B_5 (less inflammatory) than did those from chicks hatched from hens fed a low n-3 FA diet (Hall et al., 2007). However, no information is available on the role of maternal (yolk) FA composition on eicosanoid production in the heart tissue of newly hatched chicks. In view of the roles of yolk FA in modulating lipid metabolism in the progeny, the present study was designed to determine the effects of yolk n-6 and n-3 FA on antioxidant status, lipid peroxidation, tissue PUFA profile (heart, lungs, liver, and brain), and ex vivo eicosanoid generation in the cardiac tissue of newly hatched chicks. These tissues were selected due to their respective roles in lipid assimilation (liver), oxidation (heart), functional long-chain PUFA incorporation (brain), and oxidative metabolism (lung). It is hypothesized that: 1) as n-3 PUFA in the maternal source (yolk) is increased, n-6 PUFA accretion in progeny tissue is decreased with a concomitant reduction in n-6 PUFA-derived eicosanoids and 2) the increase in n-3 PUFA in maternal source (yolk) will not affect the hatchability of fertile eggs and antioxidant status of hatchlings.

MATERIALS AND METHODS

Egg Enrichment of n-6 and n-3 FA, Incubation, and Chick Tissue Collection

A total of 270 eggs were collected from Cobb breeder hens (n = 24, 36 wk old) fed a corn-soybean diet containing either 3.5% fish oil (high n-3), a mixture of 1.75% sunflower oil and 1.75% fish oil (medium n-3), or 3.5% sunflower oil (low n-3). The hens were fed the experimental diets for 8 wk before egg collection. The breeder hen diets were isonitrogenous (16% CP) and isocaloric (2,866 kcal of ME). The FA composition of the breeder hen diet is shown in Table 1. The eggs were collected during a period of 7 d and kept in a cooler at 65°F (18.3°C). Eighteen eggs from each treatment were collected randomly and the yolks were separated. Three egg yolks were pooled to get a sample size of 6 per treatment. An aliquot of each yolk sample pool was taken for total lipid and FA analysis. The remaining 252 eggs were incubated at a dry bulb and wet bulb temperature of 37.5 and 29.4°C, respectively. At 18 d of incubation, the eggs were candled and infertile eggs were removed and counted. The eggs were transferred to hatch baskets and the hatch was pulled at 21.5 d. Hatched chicks from all treatments were counted and those eggs that did not hatch were removed from the hatcher and were also counted. Eighteen newly hatched chicks were randomly chosen from each treatment group, blood was collected from the jugular vein, and the chicks were killed. Chick tissue samples (heart, lung, brain, and liver) were quickly removed, snap-frozen with liquid nitrogen, and stored at −80°C until analysis. Tissue and blood samples (n = 3) from each treatment were pooled to obtain a sample size of 6 (n = 6) per treatment for analytical purposes. All protocols were approved by Oregon State University's Animal Care and Use Committee to ensure adherence to the Animal Care Guidelines.

Total Lipid and FA Analysis

Total lipids were extracted from feed, egg yolk, and chick tissues and plasma by the method of Folch et al. (1957). The mass of total lipid content was determined gravimetrically. Fatty acid methyl esters were prepared as reported earlier (Cherian et al., 2002). Fatty acid analysis was performed with an HP 6890 gas chromatograph (Hewlett-Packard Co., Wilmington, DE) equipped with an autosampler, flame ionization detector, and SP-2330 fused silica capillary column (30 mm × 0.25 mm i.d.). Samples (1 μL) were injected with helium as a carrier gas onto the column programmed for ramped oven temperatures (initial temperature was 110°C, held for 1 min, then ramped at 15°C/min to

Table 1. Fatty acid composition of the breeder hen diets

	Maternal diet[1]		
Fatty acid (% of total fatty acids)	Low n-3	Medium n-3	High n-3
Palmitic (16:0)	13.6	20.9	23.9
Stearic (18:0)	2.7	4.0	5.6
Linoleic (18:2n-6)	53.3	39.7	27.8
α-Linolenic (18:3n-3)	3.5	3.6	3.8
Arachidonic (20:4n-6)	0.0	0.0	0.8
Eicosapentaenoic (20:5n-3)	0.0	3.2	6.7
Docosapentaenoic (22:5n-3)	0.0	0.6	1.1
Docosahexaenoic (22:6n-3)	0.0	2.6	4.2
Total monounsaturated	26.9	25.4	26.1
Total saturated	16.3	24.9	29.5

[1]Low n-3, medium n-3, and high n-3 represent a corn-soybean meal-based maternal (breeder hen) diet supplemented with 3.5% sunflower oil, a mixture of 1.75% sunflower oil and 1.75% fish oil, or 3.5% fish oil, respectively. In addition, the maternal diet contained corn (55.1%), wheat middling (5.8%), soybean meal (21%), alfalfa (5.0%), limestone (6.7%), calcium phosphate (1.9%), salt (0.5%), and premix (0.5%). The premix composition included (per kg of feed): vitamin A, 12,500 IU; vitamin D_3, 4,000 IU; vitamin E, 25 IU; vitamin B_{12}, 0.014 mg; riboflavin, 8 mg; pantothenic acid, 12 mg; niacin, 40 mg; menadione, 2.5 mg; choline, 500 mg; thiamine, 1.75 mg; folic acid, 0.75 mg; pyridoxine, 2 mg; D-biotin, 0.15 mg; and ethoxyquin, 2.5 mg.

190°C and held for 55 min, then ramped at 5°C/min to 230°C and held for 5 min). Inlet and detector temperatures were both 220°C. Fatty acid methyl esters were identified by comparison with retention times of authentic standards (Nuchek Prep, Elysian, MN). Peak areas and percentages were calculated using Hewlett-Packard ChemStation software (Agilent Technologies Inc., Wilmington, DE). Fatty acid values were reported as percentages.

TBA Reactive Substances

Lipid peroxidation in tissues was measured as TBA reactive substances (**TBARS**) expressed in malondialdehyde equivalents as described by Cherian et al. (2007). Briefly, 2 g of tissues were minced and weighed into 50-mL test tubes, and 18 mL of 3.86% perchloric acid and butylated hydroxytoluene (50 μL in 4.5% ethanol) was added, after which the samples were homogenized. The homogenate was filtered and the filtrate was mixed with 20 m*M* TBA in distilled water and incubated in the dark at room temperature for 17 h. Absorbance was determined at 531 nm. The TBARS were expressed as milligrams of malondialdehyde per gram of sample.

Antioxidants Assays

Antioxidant concentration (total glutathione) and activities of glutathione peroxidase, glutathione reductase, superoxide dismutase, and catalase were measured in liver, heart, lung, and brain tissue. About 0.5 g of tissues was homogenized in 5 volumes of ice-cold PBS for 30 s. After centrifugation at 10, 000 × *g* for 30 min at 4°C, the supernatant was collected and stored at −80°C until assay. In the case of blood, only total glutathione was determined and was done on fresh blood samples collected.

Total Glutathione

Blood samples were homogenized in 1% picric acid and then centrifuged at 12,600 × *g* for 2 min. Total glutathione was determined according to Griffith (1985). Briefly, 0.7 mL of 0.3 m*M* NAD phosphate (**NADPH**) buffer, 0.1 mL of 6 *M* 5,5′-dithio-bis (2-nitrobenzoic acid) solution, and 0.15 mL of supernatant (diluted 1:20 with distilled water) were mixed and the absorbance was measured at 412 nm. A standard curve was made by substituting the supernatant for 1, 2, 3, and 4 nmol of glutathione, and appropriate amounts of water and total glutathione concentration in the sample were calculated and expressed in micromoles per gram of tissue.

Glutathione Peroxidase

Glutathione peroxidase activity was assayed using the method of Paglia and Valentine (1967). Briefly, 1.2 mL of a cofactor solution (0.25 m*M* NADPH, 0.5 U/mL of glutathione reductase, and 1.25 m*M* glutathione) and 0.20 mL of collected supernatant were mixed. The reaction was started by the addition of 0.1 mL of cumene hydroxide. The change in absorbance at 340 nm was measured. The supernatant of brain tissue was used undiluted, whereas that of heart, liver, and lung was diluted 1:10 with PBS. Glutathione peroxidase activity was expressed as units per gram of protein. One unit of glutathione peroxidase was defined as micromoles of NADPH oxidized per minute.

Glutathione Reductase

Glutathione reductase activity was determined using the method of Goldberg and Spooner (1983) as follows. Briefly, 1 mL of phosphate-EDTA buffer (0.11 *M* potassium and 0.55m*M* EDTA buffer, pH 7.2), 0.04 mL

of oxidized glutathione, and 0.04 mL of undiluted supernatant were mixed and the reaction was started by adding 0.02 mL of a cofactor solution (9.3 m*M* NADPH in 1% sodium bicarbonate). The change in absorbance at 340 nm was documented. Glutathione reductase activity values were expressed as units of activity (1 unit of activity = 1 μmol of NADPH oxidized per minute) per gram of protein.

Superoxide Dismutase

Superoxide dismutase was assayed using the method of Paoletti and Mocali (1990). In short, 0.8 mL of triethanolamine-diethanolamine-HCl buffer (0.1 *M* each), 0.04 mL of NADH (7.5 m*M*), 0.025 EDTA-$MnCl_2$ (0.1 *M* EDTA and 50 m*M* $MnCl_2$), and 0.1 mL of supernatant were mixed and the reaction was started with addition of 0.1 mL of mercaptoethanol (10 m*M*). The linear change in absorbance at 340 nm was measured. Superoxide dismutase activity was expressed as units per gram of protein.

Catalase

Catalase activity was determined using the method of Aebi (1990). About 0.1 mL of supernatant was placed in a 15 × 75 mm test tube held on ice and the reaction was started sequentially at 15-s intervals by adding 1 mL of ice-cold 6 m*M* H_2O_2. After 3 min, the reaction was stopped in the same order in which it was started, by adding 0.2 mL of 6 *N* H_2SO_4. After adding 1.4 mL of 0.1 *M* $KMnO_4$ to each tube, the change in absorbance at 480 nm was recorded. A standard was prepared by adding 1.4 mL of 0.1 $KMnO_4$, 1.1 mL of 0.01 *M* potassium phosphate buffer (pH 7), and 0.2 mL of 6 *N* H_2SO_4 to a test tube. Catalase activity was expressed in units per milligram of protein.

Tissue Protein Determination

Total protein was determined as described by Lowry et al. (1951) using Folin reagent. The protein content was expressed as milligrams of protein per milliliter of supernatant.

Cardiac Tissue Preparation for Eicosanoid Extraction

Eicosanoids were extracted from heart tissue using a modification of the method by Powell (1980) and Murphy et al. (1999). About 0.5 g of heart tissue was minced and homogenized in 2 mL of PBS diluted 1:10 containing 1 m*M* EDTA and 10 μ*M* indomethacin and was incubated at 37°C for 45 min on a shaking water bath, after which the tubes were cooled on ice for 5 min. Three milliliters of methanol was added to the homogenate and mixed. After incubation at room temperature for 5 min, 15 mL of 20 m*M* acetic acid was added and the mixture was centrifuged at 1,500 × *g* for 10 min and the supernatant was applied to a 3-mL C_{18} solid phase extraction (**SPE**) column previously activated with 3 mL of methanol and equilibrated with 3 mL of 15% methanol in 20 m*M* acetic acid. The column was washed with 3 mL of 15% methanol in 20 m*M* acetic acid followed by another wash with 3 mL of water. The sample was eluted with 2 mL of methanol and evaporated under a stream of nitrogen, and the residue was dissolved in 1 mL of methanol.

Eicosanoid Separation

The eicosanoids were separated by HPLC (Shimadzu LC-2010AHT, Shimadzu Corp., Kyoto, Japan) using a 25 cm × 4.6 mm 5 μ*M* C_{18} SPE column at 40°C and a 1 mL/min isocratic mobile phase of 70% A (78.6% 1 m*M* acetic acid and 21.4% methanol) and 30% B (acetonitrile). An appropriate aliquot of the SPE elute was dried, dissolved in mobile phase A, filtered, and injected on the HPLC. Fractions containing the desired eicosanoids, determined previously by elution times of authentic standards, were collected and extracted with 2 mL of ethyl acetate. The extract was centrifuged at 1,500 × *g* for 10 min, after which the organic phase was collected and dried under nitrogen, and the residue was dissolved in 1 mL of ethanol. An appropriate volume of ethanol fraction was dried and dissolved in PBS containing 1% BSA and quantitated by ELISA.

Eicosanoid Quantification by ELISA

The eicosanoids were quantitated by ELISA using a procedure by Krämer et al. (1993). In short, high-binding 96-well enzyme immunoassay-RIA plates were coated with either PGE_2 or thromboxane B_2 (**TXB_2**) that had been conjugated with BSA by the carbodiimide method of Dray et al. (1982). The eicosanoid-BSA conjugates diluted in coating buffer (15 m*M* Na_2CO_3 and 35 m*M* $NaHCO_3$, pH 9.6), 1.25 and 0.125 μL/mL for PGE_2 and TXB_2 conjugate, respectively, were added at 0.2 mL/well to 96-well plates and incubated at 37°C for 2 h. The plates were washed 3 times with 0.3 mL/well of PBS containing 0.1% Tween 20 (**PBS-T**) and then blocked with 0.25 mL of PBS-BSA/well by incubating for 2 h at room temperature. After the plates were washed 3 times with PBS-T, PGE_2 and PGE_3 samples in PBS-BSA were added at 0.1 mL/well to PGE_2-BSA-coated plates and TXB_2 and TXB_3 samples to TXB_2-BSA-coated plates. Anti-PGE_2 (20 μL/mL) and anti-TXB_2 (50 μL/mL) antibodies (Cayman Chemical Co.) in PBS-BSA were added at 0.1 mL/well to the prostaglandin and thromboxane plates, respectively, followed by overnight incubation at 4°C. The plates were then washed as before and 0.2 mL of PBS-BSA containing 0.5 μL/mL of either anti-mouse or anti-rabbit IgG biotin conjugates was added to the prostaglandin or thromboxane plates, respectively, fol-

lowed by incubation for 2 h at room temperature on a rotary shaker. After washing, 0.2 mL of 1 μL of extravidin peroxidase conjugate/mL of PBS was added to the wells of both plates followed by incubation for 2 h at room temperature on a rotary shaker. After washing with 0.3 mL of PBS-T/well 4 times, the plates were stained by incubating with 0.2 mL/well of 2,2′-azinobis(3-ethylbenzthiazoline-6-sulphonic acid) (0.66 mg/mL) and H_2O_2 (0.33 μL/mL) in 42 m*M* citric acid and 58 m*M* Na_2HPO_4, pH 4.2, overnight at 4°C. The plates were read at 405 nm on a plate reader and unknowns quantitated against authentic standards.

Statistical Analysis

The effect of high, medium, and low n-3 maternal lipid source (yolk) on chick tissue total lipids, PUFA content, antioxidant status, TBARS, and ex vivo eicosanoid production were analyzed by 1-way ANOVA using S-PLUS (MathSoft, 1988). Results were presented as mean ± SE. Percentage data underwent angular transformation (arc sine square root percentage transformation) before analysis. Means were compared using the Tukey method for multiple comparisons. Values were considered significant at $P < 0.05$.

RESULTS

Egg Yolk FA and Hatchability

The FA composition of maternal diet is shown in Table 1. Egg PUFA composition reflected the dietary source (Table 2). Docosahexaenoic acid (22:6n-3) was the major long-chain n-3 FA in the yolk and ranged from 1.5, 8.8, and 10.7% for low n-3, medium n-3, and high n-3 eggs, respectively ($P < 0.001$). The predominant n-6 long-chain PUFA in egg yolk was arachidonic acid (20:4n-6), constituting 4.6, 2.1, and 1.3 for low n-3, medium n-3, and high n-3 eggs, respectively. Other long-chain PUFA such as EPA (20:5n-3) and DPA (22:5n-3) were found in medium and high n-3 egg yolks. No differences were observed among the 3 diets in total fat content of eggs, which was 33.3, 31.6, and 31.9% for low n-3, medium n-3, and high n-3 eggs, respectively ($P > 0.05$). Hatchability for the low n-3, medium n-3, and high n-3 groups was 89, 85, and 83%, respectively. There was no association between dietary treatment and hatchability ($P = 0.14$).

Chick Tissue Total Lipids and FA

The total lipid and PUFA content in the tissues of newly hatched chicks is shown in Table 3. Yolk PUFA composition had no significant effect on the content of total lipids in the tissues of chicks hatched from low, medium, or high n-3 eggs. The total lipid content of tissues can be placed in the following descending order: liver > brain > heart > lung. However, the PUFA composition of tissues was significantly altered by the maternal (yolk) lipid source. The yolk content of n-3 PUFA (EPA, DPA, DHA) was associated with a significant increase in total n-3 PUFA (EPA+ DPA + DHA) in the heart, lung, liver, and brain. It is important to emphasize that the amounts of DHA (22:6n-3) were similar in the hearts of low, medium, and high n-3 chicks. The increase in n-3 PUFA in the tissues of medium and high n-3 chicks was associated with a concomitant decrease in arachidonic acid (20:4n-6) and other long-chain n-6 FA such as 22:4n-6 and 22:5n-6. Arachidonic acid (20:4n-6) constituted the major PUFA in the heart, lung, and liver. Docosahexaenoic acid (22:6n-3) was the major PUFA in the chick brain and was significantly higher ($P < 0.05$) in the brain tissue of high n-3 and medium n-3 chicks than that of the low n-3 chicks.

Effect of Maternal Diet on Antioxidant Status of Hatched Chicks

Total Glutathione. There were no significant differences in total glutathione concentration in blood and tissues ($P > 0.05$; data not shown). In terms of total glutathione concentration, chick tissues can be placed in the following descending order: blood > brain > liver > heart > lung.

Glutathione Peroxidase, Glutathione Reductase, Superoxide Dismutase, and Catalase. There was no difference in glutathione peroxidase, glutathione reductase, and superoxide dismutase activities in the tissues of chicks from low n-3, medium n-3, and high n-3 eggs. However, there was a significant difference in heart catalase activity between treatments. The cata-

Table 2. Fatty acid composition of eggs from broiler breeder hens fed low, medium, or high n-3 oils

	Fatty acid (%)											
Treatment[1]	16:0	16:1	18:0	18:1n-9	18:2n-6	18:3n-3	20:4n-6	20:5n-3	22:4n-6	22:5n-6	22:5n-3	22:6n-3
Low n-3	27.3[c]	1.9[c]	15.1[a]	26.3	19.9[a]	0.3	4.6[a]	0.0[b]	0.6[a]	2.2[a]	0.00[c]	1.5[c]
Medium n-3	29.6[b]	2.6[b]	12.7[b]	26.0	16.1[b]	0.4	2.1[b]	0.3[b]	0.0[b]	0.0[b]	0.8[b]	8.8[b]
High n-3	32.2[a]	4.1[a]	11.3[c]	27.0	10.0[c]	0.4	1.3[c]	0.9[a]	0.0[b]	0.0[b]	1.3[a]	10.7[a]
SE	0.34	0.18	0.19	0.55	0.28	0.02	0.09	0.04	0.01	0.05	0.04	0.30

[a–c]For each fatty acid, values with different superscripts are significantly different ($P < 0.05$).

[1]Low n-3, medium n-3, and high n-3 represent a corn-soybean meal-based maternal (breeder hen) diet supplemented with 3.5% sunflower oil, a mixture of 1.75% sunflower oil and 1.75% fish oil, or 3.5% fish oil, respectively. Values are means of 6 observations (n = 6).

lase activity in the heart was 0.38, 0.16, and 0.16 U/mg of protein for low, medium, and high n-3 chicks ($P = 0.013$). Catalase activity was not detectable in brain tissue.

Lipid Peroxidation in Chick Tissues

Thiobarbituric acid reactive substances were significantly lower in the liver tissue of high n-3 chicks than in the liver of those hatched from hens fed the medium n-3 diet ($P < 0.05$), but not significantly different from hens fed the low n-3 diet. The liver TBARS were 0.64, 0.85, and 0.57 mg of malondialdehyde/g for low, medium, and high n-3, respectively ($P < 0.05$). There were no significant differences in heart or lung TBARS levels among treatments (data not shown). Overall, lung tissue was more prone to lipid peroxidation followed by heart and liver tissue.

Heart Tissue Ex Vivo Eicosanoid Production

Heart tissue PGE_2 concentration was significantly higher in low n-3 chicks than in those hatched from medium or high n-3 eggs ($P < 0.05$; Figure 1). Heart tissue TXA_3 was lowest in low n-3 chicks ($P < 0.05$). There was no effect of yolk PUFA composition on ex vivo PGE_3 or thromboxane A_2 production in cardiac tissue ($P > 0.05$).

DISCUSSION

The effect of maternal (yolk) n-6 and n-3 FA levels on the tissue PUFA profile, antioxidant status, lipid peroxidation, and ex vivo eicosanoid production by cardiac tissue in progeny is investigated. As reported earlier, egg n-6 and n-3 FA composition reflected the dietary source (Cherian and Sim, 1991; Cherian et al., 1997). No effect of yolk n-6 and n-3 FA on hatchability was no-

Table 3. Total lipids and polyunsaturated fatty acid (PUFA) content in tissues of chicks hatched from eggs containing low, medium, or high n-3 fatty acids

	Maternal diet[1]		
Lipids/fatty acid (%)	Low n-3	Medium n-3	High n-3
Heart			
ΣLipids	2.5 ± 0.07	2.6 ± 0.07	2.6 ± 0.07
20:4n-6	21.9 ± 1.08[a]	17.0 ± 1.08[b]	13.9 ± 1.08[b]
20:5n-3	0.0 ± 0.04[b]	0.7 ± 0.04[a]	1.0 ± 0.04[a]
22:4n-6	0.7 ± 0.14[a]	0.0 ± 0.14[b]	0.0 ± 0.14[b]
22:5n-6	1.7 ± 0.06[a]	0.0 ± 0.00[b]	0.0 ± 0.00[b]
22:5n-3	0.0 ± 0.02[b]	0.7 ± 0.02[a]	0.7 ± 0.02[a]
22:6n-3	3.0 ± 0.35	4.0 ± 0.35	3.5 ± 0.35
ΣPUFA n-3	3.0 ± 0.53[b]	5.4 ± 0.53[a]	5.2 ± 0.53[a]
ΣPUFA n-6	38.9 ± 1.07[a]	32.1 ± 1.07[b]	27.1 ± 1.07[b]
Lung			
ΣLipids	2.0 ± 0.11	2.3 ± 0.11	2.1 ± 0.11
20:4n-6	10.9 ± 0.69[a]	7.5 ± 0.69[b]	7.4 ± 0.69[b]
20:5n-3	0.0 ± 0.13[b]	0.9 ± 0.13[a]	1.5 ± 0.13[a]
22:4n-6	2.1 ± 0.12[a]	1.3 ± 0.12[b]	1.2 ± 0.12[b]
22:5n-6	1.1 ± 0.13[a]	0.0 ± 0.13[b]	0.0 ± 0.13[b]
22:5n-3	0.0 ± 0.13[b]	0.6 ± 0.13[a]	1.2 ± 0.13[a]
22:6n-3	2.3 ± 0.29[b]	4.0 ± 0.29[a]	4.7 ± 0.29[a]
ΣPUFA n-3	2.6 ± 0.51[b]	5.8 ± 0.51[a]	7.4 ± 0.51[a]
ΣPUFA n-6	25.1 ± 1.09[a]	18.7 ± 1.09[b]	17.7 ± 1.09[b]
Liver			
ΣLipids	13.5 ± 0.41	14.1 ± 0.41	12.9 ± 0.41
20:4n-6	10.0 ± 0.28[a]	7.1 ± 0.28[b]	5.6 ± 0.28[b]
20:5n-3	0.0 ± 0.07[b]	1.2 ± 0.07[a]	1.5 ± 0.07[a]
22:4n-6	0.3 ± 0.07[a]	0.0 ± 0.07[b]	0.1 ± 0.07[b]
22:5n-6	1.5 ± 0.07[a]	0.0 ± 0.07[b]	0.0 ± 0.07[b]
22:5n-3	0.0 ± 0.10[b]	0.2 ± 0.10[b]	0.6 ± 0.10[a]
22:6n-3	4.9 ± 0.31[b]	8.8 ± 0.31[a]	9.3 ± 0.31[a]
ΣPUFA n-3	4.9 ± 0.39[b]	10.1 ± 0.39[a]	11.4 ± 0.39[a]
ΣPUFA n-6	28.2 ± 0.50[a]	19.7 ± 0.50[b]	15.9 ± 0.50[b]
Brain			
ΣLipids	4.0 ± 0.10	4.0 ± 0.10	3.8 ± 0.11
20:4n-6	10.1 ± 0.23[a]	7.5 ± 0.23[b]	6.0 ± 0.23[b]
22:4n-6	2.5 ± 0.20[a]	1.2 ± 0.20[b]	0.22 ± 0.20[b]
22:5n-6	2.3 ± 0.30[a]	0.0 ± 0.00[b]	0.0 ± 0.00[b]
22:6n-3	14.9 ± 0.65[b]	17.9 ± 0.65[a]	18.3 ± 0.65[a]
ΣPUFA n-3	14.9 ± 0.65[b]	17.9 ± 0.65[a]	18.2 ± 0.65[a]
ΣPUFA n-6	17.9 ± 0.57[a]	11.5 ± 0.57[b]	9.1 ± 0.57[b]

[a,b]For each fatty acid, values with different superscripts are significantly different ($P < 0.05$).

[1]Low n-3, medium n-3, and high n-3 represent a corn-soybean meal-based maternal (breeder hen) diet supplemented with 3.5% sunflower oil, a mixture of 1.75% sunflower oil and 1.75% fish oil, or 3.5% fish oil, respectively. Values are means ± SE (n = 6).

ticed in the current study, which corroborates previous research (Cherian, 2008). However, Pappas et al. (2006) reported increased embryonic mortality in eggs laid by hens fed a 5.5% fish oil diet compared with hens fed diets with the same amount of soybean oil. In the current study, a 3.5% inclusion of fish oil was used, which may explain discrepancies with the work by Pappas et al. (2006). The egg yolk n-6 and n-3 FA composition did not affect total lipid content of heart, lung, liver, and brain of newly hatched chicks. However, alterations in n-3 and n-6 FA in the maternal reserves (yolk) led to significant changes in the tissue n-6 and n-3 PUFA status of chick liver, brain, and heart. This is not surprising because, from a nutritional standpoint, yolk FA are the major source of long-chain C20 and C22 PUFA to the developing chick and are incorporated into the tissue phospholipids serving as structural components. Changes in the tissue n-6 and n-3 PUFA composition brought about by maternal source as reported in the current study are in accordance with previous studies on the tissue FA composition of broiler chicks hatched from eggs varying in n-3 and n-6 FA (Cherian and Sim, 1991; Ajuyah et al., 2003a,b). The availability of yolk EPA (20:5n-3) and DPA (22:5n-3) is evidenced by the significant increase in EPA (20:5n-3) and DPA in the heart of medium and high n-3 chicks. However, the increase in EPA (20:5n-3) and DPA (22:5n-3) did not lead to any change in the DHA (22:6n-3), suggesting limited Δ^4-desaturase in chick heart. Although there was no effect of yolk n-3 FA on DHA content of chick heart, as a percentage of total n-3 long-chain PUFA, DHA (22:6n-3) was the major FA in the cardiac tissue of day-old chicks in all the treatments, constituting 100, 74.1, and 67% in low, medium, and high n-3 chicks, respectively, suggesting the importance of DHA in cardiac cell membrane lipids.

The amount and type of long-chain PUFA released in response to inflammatory stimuli depend on cell membrane phospholipid PUFA content. The availability of arachidonic acid (20:4n-6) and EPA (20:5n-3) in cardiac tissue of the chick is evidenced by changes in the ex vivo eicosanoid production observed. The higher levels of unesterified arachidonic acid in the low n-3 chicks led to a significant increase in ex vivo PGE_2 production by the cardiac homogenate. The substantial decrease in PGE_2 biosynthesis in the cardiac homogenate of medium and high n-3 chicks was consistent with the concentrations of arachidonic acid (20:4n-6), the precursor of PGE_2, which was over 22 and 36% less in the cardiac tissues of medium and high n-3 chicks when compared with that of low n-3 chicks. Similarly, the higher levels of EPA in the heart lipids of medium and high n-3 chicks may explain the increase in ex vivo TXA_3 production observed in the current study. Although EPA (20:5n-3) was higher in the cardiac tissue of medium and high n-3 chicks, no difference was observed in ex vivo PGE_3 production among the 3 treatments. Raisz et al. (1989) have reported that EPA (20:5n-3) was only one-tenth as effective for PGE_3 formation as arachidonic acid (20:4n-6) was for PGE_2 production. Arachidonic acid (20:4n-6) and EPA (20:5n-3) are esterified at the sn-2 position of phospholipids and after release by phospholipase A_2 are further metabolized by cyclooxygenases and lipooxygenase to different eicosanoids. In the cardiovascular system, arachidonic acid-derived eicosanoids are responsible for smooth muscle constriction, platelet aggregation, and decreasing thrombus formation. The alterations of n-6 and n-3 PUFA-derived eicosanoids observed in the current study demonstrate that the newly hatched chick has already developed a highly active phospholipase A_2 system capable of catalyzing eicosanoid formation. The role of EPA (20:5n-3) and DHA (22:6n-3) in reducing the beating rate of cardiac myocytes exposed to arrhythmogenic agents in vitro (Kang and Leaf, 1996) and in providing protection against ischemia-induced arrhythmia in pigs has been reported (Nair et al., 1997). Thus, the high cardiac concentration of arachidonic acid (20:4n-6) and absence of EPA (20:5n-3) in low n-3 chicks could contribute to a proinflammatory, proarrhythmic, and prothrombotic state. Previous studies have shown that addition of fish oil (rich n-3 FA source) to the maternal diet led to a significant reduction in ex vivo PGE_2 production by tibiae in Japanese quail (Liu and Denbow, 2001) and in leukotriene B_4 by thrombocytes in chickens (Hall et al., 2007). However, to our knowledge, the effect of

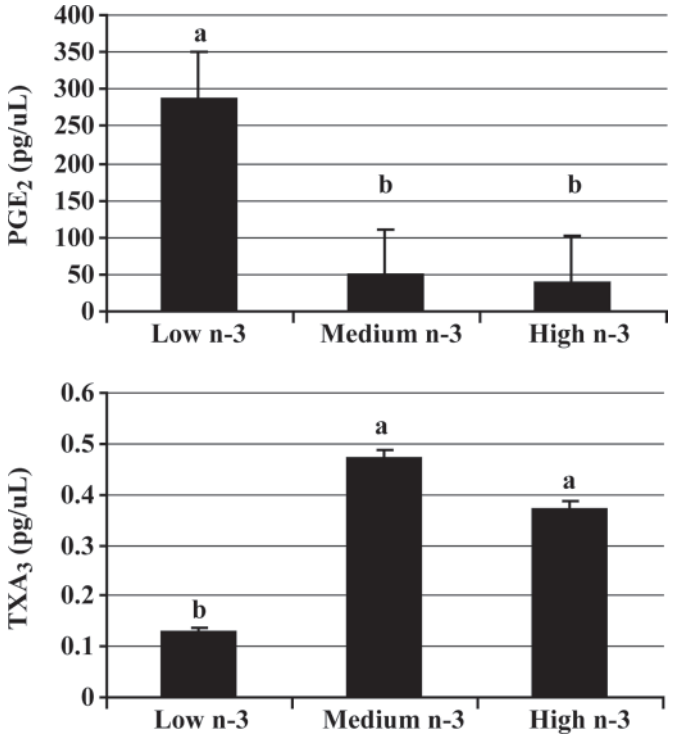

Figure 1. Ex vivo prostaglandin E_2 (PGE_2) and thromboxane A_3 (TXA_3) production in the cardiac homogenate of day-old chicks from broiler breeder hens fed low n-3, medium n-3, and high n-3 diets. Low n-3, medium n-3, and high n-3 represent a basal corn-soybean breeder hen diet supplemented with 3.5% sunflower oil, a mixture of 1.75% sunflower oil and 1.75% fish oil, or 3.5% fish oil, respectively. Values are means of 6 observations (n = 6). [a,b]Indicate significant differences ($P < 0.05$).

maternal diet on cardiac eicosanoid production in the progeny of avians has not been reported. Considering the role of inflammation as an important risk factor contributing to cardiovascular diseases (Ghosh et al., 2007; Rana et al., 2007), the role of maternal diet in modulating eicosanoid formation and progeny cardiac health needs to be investigated.

In the present study, total glutathione and the antioxidant activities of enzymes such as glutathione peroxidase, glutathione reductase, and superoxide dismutase in the newly hatched chick were not affected by yolk PUFA reserves. At hatching, tissue phospholipids contain large amounts of PUFA (Cherian and Sim, 1993; Noble and Speake, 1997). In the present study, tissue total PUFA was either similar or lower in medium n-3 and high n-3 as compared with low n-3 chicks. In the lung tissue, there were no differences in percentage of total PUFA, but in the brain, heart, and liver tissues, total n-6 + n-3 PUFA was significantly lower in the high n-3 as opposed to low n-3 chicks. Therefore, it is possible that the high levels of n-3 PUFA in medium and high n-3 chicks were not capable of producing differences in the antioxidant enzymatic activities in the tissues studied. However, maternal n-3 PUFA led to a decreased catalase activity in the heart of high n-3 and medium n-3 chicks compared with low n-3 chicks. Normally, catalase does the same job as glutathione peroxidase in destroying H_2O_2 produced during cell metabolism, once superoxide dismutase has converted O_2-derived toxic molecules into H_2O_2. Catalase activity was moderately correlated to the long-chain n-6:n-3 FA ratio ($r = 0.61$) in the heart. It may be that as the long-chain n-6:n-3 FA ratio increases, catalase activity decreases in the heart, suggesting an increased need for antioxidants. Inclusion of vitamin E in the hen diet has been reported to enhance catalase activity in the liver of chicks (Lin et al., 2005). These authors suggested that elevation of catalase activity in chicks could be an indication of increased antioxidant protection of the tissue. Notably, no evidence of increased lipid peroxidation measured as TBARS was observed in the cardiac or lung tissue. It is not clear that a significant decrease in liver TBARS of high n-3 chicks is anyway related to antioxidant status because antioxidant enzyme activities were not affected. In this regard, maternal supplementation of vitamin E has been reported to reduce lipid peroxidation (malondialdehyde) levels in the plasma and brain tissue of chicks (Lin et al., 2005). Alternatively, the increase in liver TBARS observed in low and medium n-3 chicks did not affect antioxidant enzyme activities or hatchability.

In summary, data from this experiment confirm previous research that maternal supplementation of n-6 and n-3 FA alters egg yolk and hatched tissue PUFA profile. In addition, using fertile eggs differing in n-6 and n-3 PUFA but not total fat, it is demonstrated that egg yolk n-6 and n-3 PUFA reserves do influence the ex vivo production of proinflammatory and less prothromobotic eicosanoids in the cardiac tissue of broiler chicks. Considering the role of n-6 PUFA-derived eicosanoids in the pathobiology of various disease conditions, results from the present study and those reported earlier (Hall et al., 2007) on the role of maternal diet in modulating eicosanoid production in the hatched chick may have practical applications. Under commercial conditions, mortality during the first 2 wk of growth is around 5% and this remains a problem for the broiler industry. In addition, metabolic disorders and heart-related conditions are the major cause of mortalities and morbidities in fast-growing broiler birds and have been reported in birds as early as 3 d of age (Gardiner et al., 1988). Therefore, the amounts and balance of n-6 and n-3 PUFA in the maternal diet and yolk reserves will affect the eicosanoid precursor pool and thereby modulate eicosanoid-controlled functions, with effects on cardiovascular disease and metabolic disorders in broiler birds.

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