Journal of Animal and Veterinary Advances 11 (6): 841-848, 2012
ISSN: 1680-5593

Alteration of Tissue Fatty Acid Composition and Concentration in Broilers by Diet Microalgae Oil

[1]Yan Li, [1]Chao Sun, [2]Peng Pu and [1]Renli Qi
[1]College of Animal Science and Technology, [2]College of Life Science,
Northwest A&F University, 712100 Yangling, Shaanxi, P.R. China

Abstract: This study investigated the effect of microalgae oil on tissue Fatty Acid (FA) profile of broilers. Through analysis, it was discovered that microalgae oil altered the FA composition and concentration during the development of different organs. In liver, microalgae oil increased the concentration of C22:6 (High Polyunsaturated Fatty Acids, ΣHUFA) but decreased the concentration of C18:1 (Monounsaturated Fatty Acids, ΣMUFA) during the initial growth period while at a later stage, there was a significant increase of C18:1 (ΣMUFA), C22:6 (ΣHUFA) and C20:4 (ΣHUFA) and a decrease of C18:2 (Polyunsaturated Fatty Acids, ΣPUFA). Moreover, after the withdrawal of microalgae oil, there was a continued effect on C22:6 for 1 week. Liver exhibited a similar change in concentration of FA as the breast, leg muscle and brain, especially at the later phase. Therefore, researchers can conclude that microalgae oil alters tissue FA composition and concentration during maturation which is an important period for sustained development and maintenance of FA. Unfortunately, pro-longed feeding of the broilers with microalgaee oil diets did not result in the accumulation of Docosahexaenoic Acid (DHA) in tissues.

Key words: Microalgae oil, broiler, tissue, fatty acid, unsaturated fatty acids, DHA

INTRODUCTION

As shown by huge amounts of assays in human as well as in animal models, n-3 PUFAs play an important role during the development and maintenance of different organs, primarily the brain and could be useful in the prevention of different pathologies, mainly the cardiovascular diseases and as proposed recently, some psychiatric, dermatological or rheumatological disorders. These beneficial biological effects of the n-3 Long-Chain Polyunsaturated Fatty Acids (LC-PUFA) can be attributed to the presence of Eicosapentaenoic Acid (EPA, C20:5 n-3), Docosapentaenoic Acid (DPA, C22:5 n-3) and DHA (22:6 n-3) which are all integral to the structure of LC-PUFA. By far, the most abundant fat sources in the human and animal diets today are of vegetable origin as well as products from terrestrial animal origin which are characterized by a large concentration of PUFAs of the n-6 series (Cherian, 2008; Simopoulos, 2000). EPA and DHA are mainly present in animals' products. The impact (qualitative and quantitative) of alterations in the lipid composition of animal foods on the nutritional value of derived products (in terms of EPA and DHA concentration) eaten by humans is more important in single-stomach animals than multi-stomach animals (due to their hydrogenating intestinal bacteria). The typical broiler diet today is corn and soybean-based and the added fat is mostly rendered fat and vegetable oils. If living in a natural environment, the natural diet of poultry would consist of seeds, plants and insects, etc. Green leaves have a surplus of the ω-3 fatty acid Alpha-Linolenic Acid (ALA) compared to the ω-6 fatty acid Linoleic Acid (LA). In most seeds and grain, LA dominates and the concentration of ALA is low. Some seeds have however, high levels of ALA, e.g., linseed and rapeseed. The natural diet of a hen would have a good balance between leaves and seeds and thus getting both ω-3 and ω-6 fatty acids.

The concentration of ω-3 fatty acids in animal products depends mostly on the fatty acid composition of the diet (Bou *et al*., 2005; Cherian and Sim, 1992; Jiang *et al*., 1991; Mach *et al*., 2006; Millet *et al*., 2006). The feed used by the modern poultry industry, however is mostly grain with a high ratio of ω-6 fatty acids compared to ω-3 fatty acids. This will induce a high concentration of the ω-6 fatty acid arachidonic acid (AA, 20:4 n-6) in the meat or egg product and less EPA (20:5 n-3), DPA (22:5 n-3) and DHA (22:6 n-3). In fact, several studies that sought ways to enrich poultry products with n-3 LC-PUFAs proved fish oil and

Corresponding Author: Chao Sun, College of Animal Science and Technology, Northwest A&F University, 32 Xinong Road, 712100 Yangling, Shaanxi, P.R. China

microalgae oil to be the most effective source for this purpose (Hargis and van Elswyk, 1993; Leskanich and Noble, 1997; Schiavone *et al*., 2007, 2004).

The consumer knows that eating fish is healthy but still the fish consumption is not increasing. It has also been predicted that there may be a shortage of fish and fish oil in the future. If broiler meat is produced to be as healthy as possible with a favorable fatty acid composition in broiler meat at about the same level as in fish (0.365-0.481 mg kg^{-1} in different fish species; turbot, tuna, swordfish and wolfish (Folch *et al*., 1957) and intake of broiler meat may improve the average human diet.

The purpose of this investigation was to study how DHA might affect tissue fatty acid composition and concentration in AA broilers and to produce broiler meat with high concentrations of ω-3 fatty acids as in fish. Therefore, chicken were fed on a diet supplemented with marine microalgae meal during the experiment period. The microalgae *Crypthecodinium cohnii* is a non-photosynthetic marine dinoflagellate rich in DHA (Mendes *et al*., 2009). Its effect on the percentage concentration of tissue FA in chicken was evaluated.

MATERIALS AND METHODS

Animals and diet formulation: Thirty six newly hatched AA chicks were weighed and distributed randomly to the experimental groups (3 replicates of 6 birds each). The chicks were fed corn soybean meal based starter. About 40 mg DHA kg^{-1} of starter was fed to the experimental groups. The trial lasted for 42 days and started after a pre-experimental period of 7 days during which the animals received the same treatment afterwards. The chicks were then fed control diet, after fed 2 weeks on DHA diet. Feed and water were supplied *ad libitum*. The chicks received 20 h light $days^{-1}$ throughout the experiment. Room temperature was kept at about 35°C when the birds were 1 day old and then gradually decreased by 1°C every 1 or 2 days to reach a final temperature of 25°C. Chicks were randomly assigned to one of 2 dietary programs (18 chicks per program 0 and 40 mg kg^{-1} DHA used, respectively in the two programs). The diets were mixed weekly and stored before use in a cold room (4°C) in airtight containers. The ingredients and chemical composition of the basal diet are shown in Table 1.

Sample collection: At the end of the experiment, 6 birds from each program (2 birds per replicate) were randomly selected. The birds were killed by decapitation and then the liver, breast muscle, thigh muscle, belly fat and brain tissue were harvested by a veterinary pathologist in the lab for lipid analysis.

Table 1: Composition and nutrient level of basal diets in broiler (%)

Composition	Brood diet	Finisher diet	Calculated composition	Brood diet	Finish diet
Corn	55.60	60.00	ME KJ	2862.00	3088.00
Maize oil	0.95	3.50	CP (%)	22.00	19.30
Fish meal (CP 60%)	2.00	2.00	CFat (%)	5.80	7.08
Soybean meal (CP43%)	34.80	28.00	Met (%)	0.48	0.53
Corn protein powder	3.30	3.00	Lys (%)	1.17	1.01
Lys	0.10	0.10	Salt (%)	0.33	0.37
Met	0.10	0.20	Ca (%)	0.91	0.99
Dicalcium phosphate	1.50	1.50	Total P (%)	0.68	0.62
Limestone	0.10	0.40	-	-	-
Salt	0.25	0.30	-	-	-
Vitamin-mineral premix*	1.00	1.00	-	-	-
Medical stone	0.30	0.00	-	-	-

*Supplied the following per kilogram of feed: iron 40 mg, zinc 70 mg, copper 6 mg, manganese 100 mg, iodine 0.5 mg, selenium 0.3 mg, mix vitamin 0.4 g, Cholinehydrochloride 2 g

Lipid analysis: Chemical analysis of tissue lipids was done by extraction with chloroform: methanol (2:1, vol/vol) by the method of Folch *et al*. (1957). Fatty acid profiles of experimental organs were determined according to the methods of Cherian and Sim (1992). The fat extracted from each sample was methylated (Metcalfe and Schmitz, 1961) and the FAs were separated and identified using a gas chromatograph (Hitachi) and flame ionization detectors equipped with a Supelco SP- 2330 (3 m to 0.025 mm inside diameter) silica capillary column. The apparatus was programmed to an initial temperature of 150°C for 4 min, allowing increases of 1-3°C min^{-1} until a final temperature of 210°C was reached. The temperature of the injector and detector was 250°C. Hydrogen was used as the carrier gas. Calibration and identification of the peaks for the different FAs were obtained by comparing the retention time with that of a standard. Chromatography data system was used to integrate peak areas. Fatty acid values are expressed as weight percentages.

Statistical analysis: A one-way ANOVA of SPSS13.0 was done to analyze the effects of DHA on tissue FA concentration and composition. Variation observations within treatments were used as the error term. Significant differences among treatments were analyzed by Student-Newman-Keuls (SNK) multiple range test at ($p<0.05$).

RESULTS

Changes of fatty acids composition in liver: From Table 2 researchers can see, after feeding 2 weeks old chicken DHA for 2 weeks, no difference was observed in liver ΣSFA, ΣMUFA and ΣPUFA. C20:4 and C22:6 both increased following DHA intake ($p<0.01$). Thus, the

Table 2: The composition of fatty acids in liver and total fatty acids percentage

FA	A3	B3	A5	B5	C5	D5	A6	C6	A7	C7	D7
C14:0	0.19±0.00	0.08±0.00	0.14±0.00	0.35±0.01	0.30±0.01	0.51±0.02	-	-	-	-	0.17±0.00
C16:0	19.98±0.30	19.84±0.27	18.14±0.36	25.17±0.35	22.60±0.33	20.17±0.35	17.66±0.12	18.33±0.20	14.1±0.150	19.72±0.17	24.61±0.30
C18:0	19.12±0.13	18.26±0.13	21.22±0.15	10.79±0.11	15.38±0.10	13.6±0.100	19.85±0.15	18.45±0.17	10.6±0.100	16.53±0.18	10.70±0.20
C20:0	0.22±0.00	0.24±0.00	0.22±0.00	0.30±0.00	0.41±0.02	0.30±0.00	0.22±0.00	-	1.26±0.04	0.52±0.01	0.54±0.01
C22:0	0.54±0.00	0.98±0.04	0.76±0.03	0.15±0.00	0.48±0.00	1.20±0.08	-	0.80±0.00	1.85±0.02	-	1.39±0.19
ΣSFA	40.05±0.67	39.39±0.56	40.47±0.81	36.76±0.62	39.17±0.64	35.78±0.53	37.73±0.58	37.58±0.57	27.81±0.43	38.77±0.45	47.40±0.75
C16:1	1.14±0.06	0.05±0.00	0.84±0.02	1.79±0.07	1.51±0.06	0.56±0.00	0.42±0.00	0.39±0.00	0.47±0.00	1.14±0.10	0.89±0.06
C18:1	14.83±0.19	3.02±0.17	12.14±0.47	42.31±0.62	29.54±0.56	26.83±0.34	8.81±0.14	10.27±0.25	28.78±0.62	16.13±0.35	24.12±0.40
ΣMUFA	15.97±0.20	3.07±0.17	12.98±0.77	44.10±0.85	31.05±0.85	27.39±0.34	9.22±0.14	10.66±0.25	29.25±0.62	17.27±0.46	25.01±0.60
C18:2	24.27±0.63	24.54±0.57	21.56±0.26	10.70±0.42	13.08±0.42	13.08±0.26	23.95±0.52	20.06±0.64	13.70±0.18	22.19±0.84	13.69±0.63
C18:3	0.43±0.00	0.07±0.00	0.25±0.00	0.08±0.00	0.15±0.00	0.21±0.00	0.38±0.00	-	0.56±0.01	0.24±0.00	1.26±0.13
C20:2	0.57±0.01	0.79±0.01	0.55±0.03	0.12±0.00	0.19±0.00	0.21±0.00	0.97±0.03	-	-	0.51±0.03	0.20±0.00
ΣPUFA	25.27±0.64	25.39±0.59	22.37±0.30	10.90±0.42	13.42±0.42	13.50±0.27	25.30±0.72	20.06±0.64	14.26±0.20	22.94±0.90	15.15±0.80
C20:3	0.52±0.00	0.69±0.01	0.89±0.02	0.48±0.00	0.61±0.00	0.90±0.02	0.54±0.00	0.75±0.01	0.46±0.00	1.04±0.03	0.38±0.00
C20:4	10.17±0.70	17.00±0.74	14.32±0.54	4.84±0.24	6.66±0.34	8.56±0.41	14.71±0.42	15.06±0.63	8.50±0.62	11.80±0.53	4.51±0.30
C20:5	0.51±0.00	0.54±0.00	0.36±0.00	0.07±0.00	0.14±0.00	0.06±0.00	0.71±0.00	0.95±0.01	3.89±0.10	0.97±0.02	0.06±0.00
C22:2	0.93±0.03	0.77±0.00	0.88±0.01	0.38±0.00	0.44±0.00	2.33±0.17	1.95±0.13	1.38±0.17	0.41±0.00	0.64±0.00	1.16±0.10
C22:4	1.18±0.10	1.42±0.05	2.34±0.11	0.84±0.00	1.89±0.10	0.94±0.02	2.82±0.11	2.95±0.11	5.19±0.17	2.11±0.08	1.36±0.09
C22:5	0.63±0.00	0.46±0.00	0.54±0.00	0.15±0.00	0.30±0.00	0.87±0.01	0.67±0.00	0.87±0.02	2.67±0.13	0.77±0.02	0.53±0.00
C22:6	4.79±0.18	11.26±0.31	4.85±0.15	1.49±0.11	6.33±0.19	9.22±0.50	6.34±0.30	9.75±0.40	7.57±0.45	5.69±0.27	4.59±0.25
ΣHUFA	18.72±1.10	32.15±1.19	24.19±0.89	8.23±0.50	16.37±0.81	22.87±1.10	27.74±0.97	31.71±1.14	28.69±1.10	23.02±0.90	12.59±0.74

A stand for control group, B is added DHA in the early phase, C is added DHA in the later phase, D is longer term added; DHA (from 2-5 weeks old), the number 3 or others stand for chicken age

concentration of ΣHUFA in the experiment group is higher than in the control group ($p<0.05$). After withdrawing DHA for 1 week, there was no difference between the 2 groups (Table 2). After withdrawing DHA for 2 weeks, ΣSFA decreased ($p<0.05$). This may be due to the decrease of C18:0 ($p<0.05$). The increase of C18:1 may have contributed to the increase of ΣMUFA following DHA intake ($p<0.05$). When C18:2 decreased in the experimental group, ΣPUFA also decreased ($p<0.05$) with the decrease of C22:4 and C22:6, ΣHUFA also decreased ($p<0.05$).

Feeding 4 weeks old chickens DHA diet for 2 weeks caused no changes in the concentration of ΣSFA. A significant increase of ΣMUFA was observed following DHA intakes and this contributed to the increase of C18:1 ($p<0.05$). The decrease of C18:2 caused ΣPUFA to decrease ($p<0.05$). C22:6 increased following the decrease of C20:4 in the experimental group while ΣHUFA decreased ($p<0.05$). After withdrawing DHA for 1 week, there was a significant increase in ΣHUFA due to the increase of C22:6 following DHA intake ($p<0.05$). No other difference was observed in ΣSFA, ΣMUFA and ΣPUFA. After the withdrawal of DHA for 2 weeks, a decrease in ΣMUFA consequently triggered a decrease of C18:1 ($p<0.05$). ΣPUFA had a little increase when there was an increase of C18:2. ΣHUFA decreased following the decrease of C22:6 ($p<0.05$).

The 2 weeks old chickens that were fed DHA diet for 4 weeks had a significant increase in ΣMUFA following the increase of C18:1 ($p<0.05$). A decrease in C18:2 caused a decrease in ΣPUFA ($p<0.05$). ΣHUFA was unaffected by the increase of C22:6 and the decrease of C20:4. After withdrawing DHA for 1 week, no difference was noted in the concentration of FA (Table 2). After the withdrawal of DHA for 2 weeks, an increase of C18:0 caused a significant increase of ΣSFA ($p<0.05$). As a result, ΣHUFA significantly decreased ($p<0.05$).

Changes of fatty acids composition in belly: From Table 3 we can see that the 2 weeks old chickens fed DHA diet for 2 weeks had no differences in belly fat (Table 3). After withdrawing DHA for 1 week, ΣMUFA decreased as a result of the decrease of C18:1 following DHA intake ($p<0.05$). Thereafter, C18:2 increased in the experimental group which brought about an increase of ΣPUFA ($p<0.05$). The decrease of C22:4 and C22:6 triggered the decrease of ΣHUFA ($p<0.05$). After the withdrawal of DHA for 2 weeks, no difference was detected between the 2 groups (Table 3).

Feeding 4 weeks old chickens DHA for 2 weeks led to an increase of C16:0 and C18:1 which caused an increase of ΣSFA and ΣMUFA, respectively ($p<0.05$). A slight increase of ΣHUFA was due to the increase of C22:6 following DHA intake. After withdrawing DHA for 1 week, a significant increase in ΣMUFA was detected due to the increase of C18:1 following DHA treatment ($p<0.05$). A decrease of ΣPUFA was brought about by the decrease of C18:2 ($p<0.05$). No other difference was observed in ΣSFA and ΣHUFA. After the withdrawal of DHA for 2 weeks, no difference was observed in the concentration

Table 3: The composition of fatty acids in berry fat and total fatty acids percentage

FA	A4	B4	A5	C5	D5	A6	C6	sD6
C14:0	0.55±0.00	0.73±0.01	0.56±0.00	0.71±0.01	0.66±0.01	0.57±0.00	0.68±0.01	0.76±0.01
C16:0	22.12±0.65	23.96±0.64	21.55±0.62	24.39±0.72	19.13±0.54	22.55±0.57	24.00±0.62	22.51±0.63
C18:0	1.44±0.02	1.63±0.01	3.22±0.03	3.92±0.03	1.34±0.01	3.92±0.03	3.61±0.02	1.23±0.01
C20:0	0.10±0.00	0.28±0.00	0.29±0.00	0.32±0.00	0.18±0.00	0.28±0.00	0.29±0.00	0.25±0.00
ΣSFA	24.20±0.70	26.60±0.67	25.62±0.67	29.34±0.80	21.30±0.60	27.32±0.70	28.58±0.70	24.75±0.73
C16:1	4.85±0.09	3.76±0.09	4.31±0.10	5.15±0.10	2.26±0.06	3.41±0.08	5.56±0.10	2.99±0.07
C18:1	54.75±1.30	45.70±1.20	37.06±1.13	39.68±1.31	45.42±1.37	35.20±1.18	41.59±1.20	45.42±1.25
C20:1	0.18±0.00	0.05±0.00	0.17±0.00	0.13±0.00	0.16±0.00	0.23±0.00	0.08±0.00	0.09±0.00
ΣMUFA	59.78±1.40	49.51±1.30	41.54±1.27	44.97±1.42	47.83±1.40	38.83±1.30	47.23±1.29	48.51±1.30
C18:2	14.87±0.38	22.37±0.41	30.49±0.94	23.65±0.38	27.32±0.57	31.65±0.93	22.86±0.74	25.74±0.80
C18:3	0.40±0.00	0.96±0.07	1.19±0.09	0.91±0.06	0.88±0.04	1.19±0.08	0.88±0.04	0.80±0.03
C20:2	0.14±0.00	0.07±0.00	0.18±0.00	0.11±0.00	0.12±0.00	0.14±0.00	0.08±0.00	0.08±0.00
ΣPUFA	15.41±0.38	23.40±0.50	31.85±1.00	24.66±0.45	28.32±0.60	32.97±0.97	23.82±0.82	26.62±0.85
C20:3	0.14±0.00	0.15±0.00	0.34±0.00	0.34±0.00	0.45±0.00	0.35±0.00	0.19±0.00	0.21±0.00
C20:4	0.14±0.00	0.05±0.00	0.11±0.00	0.08±0.00	0.16±0.00	0.11±0.00	0.05±0.00	0.13±0.00
C20:5	0.09±0.00	0.06±0.00	0.03±0.00	0.13±0.00	0.14±0.00	0.04±0.00	0.05±0.00	0.13±0.00
C22:4	0.11±0.00	0.06±0.00	0.09±0.00	0.13±0.00	0.39±0.00	0.15±0.00	0.02±0.00	0.13±0.00
C22:5	0.12±0.00	0.05±0.00	0.23±0.00	0.09±0.00	0.20±0.00	0.07±0.00	0.04±0.00	0.14±0.00
C22:6	0.03±0.00	0.13±0.00	0.18±0.00	0.28±0.00	1.21±0.06	0.14±0.00	0.02±0.00	0.14±0.00
ΣHUFA	0.62±0.00	0.50±0.00	0.99±0.00	1.04±0.00	2.54±0.06	0.87±0.00	0.37±0.00	0.89±0.00

Table 4: The composition of fatty acids in breast muscle and total fatty acids percentage

FA	A4	B4	A5	B5	C5	D5	A6	C6	D6	A7	C7	D7
C14:0	1.07±0.02	0.46±0.00	0.51±0.00	0.44±0.00	0.77±0.01	0.77±0.00	0.48±0.00	0.59±0.00	0.70±0.00	0.57±0.00	0.67±0.00	0.79±0.01
C16:0	24.29±0.56	23.51±0.51	21.28±0.37	23.59±0.47	25.07±0.52	30.13±0.52	32.65±0.57	21.90±0.47	29.60±0.51	27.10±0.49	23.20±0.42	21.89±0.26
C18:0	1.11±0.02	1.47±0.02	0.64±0.00	1.33±0.03	0.55±0.00	1.10±0.03	0.68±0.00	0.72±0.01	0.51±0.00	1.24±0.03	0.63±0.00	0.93±0.01
C20:0	0.10±0.00	0.17±0.00	0.15±0.00	0.07±0.00	0.33±0.00	0.38±0.00	0.15±0.00	0.31±0.00	0.51±0.00	0.17±0.00	0.13±0.00	0.07±0.00
ΣSFA	26.57±0.60	25.60±0.56	22.58±0.37	25.44±0.51	26.72±0.50	32.39±0.50	33.96±0.57	23.52±0.49	31.33±0.51	29.07±0.51	24.63±0.42	23.68±0.28
C16:1	2.31±0.05	0.54±0.00	1.25±0.03	1.42±0.02	0.96±0.01	0.72±0.00	0.02±0.00	0.45±0.00	0.51±0.00	0.21±0.00	1.80±0.05	0.67±0.00
C18:1	51.54±0.73	34.25±0.57	43.98±0.37	40.78±0.48	45.63±0.46	40.86±0.44	31.17±0.38	43.24±0.40	43.47±0.55	31.70±0.40	42.61±0.61	33.78±0.60
C20:1	0.12±0.00	0.11±0.00	0.13±0.00	0.45±0.00	0.10±0.00	0.22±0.00	0.18±0.00	0.21±0.00	0.16±0.00	0.24±0.00	0.03±0.00	0.37±0.00
ΣMUFA	53.97±0.74	34.90±0.57	45.36±0.41	42.64±0.50	46.69±0.47	41.81±0.44	31.38±0.38	43.90±0.40	44.01±0.55	32.15±0.40	44.43±0.63	34.82±0.60
C18:2	15.57±0.24	27.92±0.28	24.47±0.26	24.09±0.41	21.24±0.36	19.79±0.33	30.08±0.52	24.85±0.42	19.6±0.25	28.55±0.27	28.17±0.37	24.22±0.34
C18:3	2.15±0.05	1.44±0.04	2.51±0.04	6.27±0.05	0.99±0.01	0.07±0.00	1.38±0.02	1.56±0.02	2.53±0.03	2.13±0.02	1.14±0.01	4.35±0.02
C20:2	0.08±0.00	0.35±0.00	0.20±0.00	0.23±0.00	0.18±0.00	0.76±0.00	0.16±0.00	0.24±0.00	0.07±0.00	0.27±0.00	0.12±0.00	0.12±0.00
ΣPUFA	17.81±0.28	29.71±0.30	27.18±0.30	30.59±0.42	22.41±0.38	20.61±0.33	31.62±0.54	26.64±0.44	22.20±0.28	30.95±0.29	29.42±0.38	28.69±0.36
C20:3	0.14±0.00	0.42±0.00	2.21±0.03	0.45±0.00	1.50±0.02	0.20±0.00	1.41±0.02	2.86±0.04	0.14±0.00	3.97±0.08	0.30±0.00	0.21±0.00
C20:4	0.43±0.00	0.68±0.00	0.07±0.00	0.67±0.00	0.34±0.00	1.46±0.03	0.09±0.00	0.30±0.00	1.12±0.02	0.07±0.00	0.29±0.00	0.99±0.01
C20:5	0.29±0.00	0.08±0.00	0.06±0.00	0.07±0.00	0.17±0.00	0.34±0.00	0.02±0.00	0.24±0.00	0.20±0.00	0.17±0.00	0.03±0.00	0.16±0.00
C22:4	0.18±0.00	0.64±0.00	0.97±0.01	0.01±0.00	0.30±0.00	0.19±0.00	0.69±0.00	0.55±0.00	0.05±0.00	1.22±0.02	0.16±0.00	0.28±0.00
C22:5	0.46±0.00	1.06±0.02	0.61±0.00	0.02±0.00	0.34±0.00	0.23±0.00	0.43±0.00	0.47±0.00	0.27±0.00	0.99±0.01	0.37±0.00	0.68±0.00
C22:6	0.15±0.00	3.17±0.03	0.95±0.02	0.12±0.00	1.54±0.01	2.79±0.03	0.42±0.00	1.53±0.03	0.67±0.00	1.41±0.02	0.37±0.00	0.51±0.00
ΣHUFA	1.67±0.00	6.04±0.04	4.87±0.05	1.34±0.00	4.18±0.03	5.19±0.05	3.05±0.02	5.95±0.07	2.45±0.02	7.82±0.10	1.51±0.00	2.83±0.01

of FA (Table 3). The 2 weeks old chickens that were fed DHA for 4 weeks had a significant increase in ΣMUFA which resulted from an increase of C18:1 ($p<0.05$). Decreases in ΣSFA and ΣPUFA were detected due to the decrease in C16:0 and C18:2 ($p<0.05$). The increase of ΣHUFA was as a result of the increase of C22:6 ($p<0.05$). After withdrawing DHA for 1 week, a significant increase in ΣMUFA was triggered by an increase of C18:1. No difference was noted in other FA constitutions ($p<0.05$). After withdrawing DHA for 2 weeks, no difference was noted in the concentration of FA (Table 3).

Changes of fatty acids composition in breast muscle: From Table 4 researchers can see that the 2 weeks old chickens that were fed DHA for 2 weeks had no difference in concentration in breast muscle between the two groups (Table 4). The withdrawal of DHA for 1 week resulted in a decrease of C18:1 which led to a significant decrease of ΣMUFA ($p<0.05$). A significant increase of ΣPUFA and ΣHUFA was due to the increase of C18:2 and C22:6 ($p<0.05$). After withdrawing DHA for 2 weeks, the concentration of ΣSFA and ΣPUFA both had a little increase triggered by the increase of C16:0 and C18:3 following DHA treatment. A slight decrease was noted in ΣMUFA and ΣHUFA which was produced by the decrease of C18:1, C22:5 and C22:6.

Feeding 4 weeks old chickens DHA for 2 weeks caused a significant increase of ΣSFA and ΣHUFA ($p<0.05$). This contributed to the increase of C16:0, C22:4 and C22:6 ($p<0.05$). The decrease of C18:1 and C18:2

Table 5: The composition of fatty acids in leg muscleand total fatty acids percentage

FA	A3	B3	A5	B5	C5	D5	A6	C6	D6
C14:0	1.075±0.03	0.74±0.00	0.32±0.00	0.49±0.00	0.60±0.00	0.61±0.00	0.39±0.00	0.59±0.00	0.58±0.00
C16:0	38.51±0.260	36.21±0.25	38.88±0.42	34.85±0.35	38.44±0.37	34.12±0.38	34.41±0.29	37.43±0.32	37.34±0.52
C18:0	37.79±0.540	36.87±0.40	43.85±0.36	38.88±0.42	42.00±0.40	39.19±0.38	37.47±0.36	41.85±0.50	40.15±0.30
C20:0	0.05±0.000	0.07±0.00	0.03±0.00	0.06±0.00	0.05±0.00	0.06±0.00	0.14±0.00	0.05±0.00	0.04±0.00
ΣSFA	77.43±0.810	73.90±0.64	83.08±0.77	74.28±0.78	81.08±0.76	73.98±0.76	72.41±0.65	79.91±0.81	78.12±0.82
C18:1	19.99±0.180	21.93±0.25	15.01±0.23	23.87±0.24	16.10±0.25	22.68±0.27	24.65±0.27	17.94±0.25	19.70±0.32
C20:1	0.03±0.000	0.04±0.00	0.05±0.00	0.06±0.00	0.03±0.00	0.05±0.00	0.07±0.00	0.04±0.00	0.03±0.00
ΣMUFA	20.02±0.180	21.97±0.25	15.06±0.23	23.93±0.24	16.13±0.25	22.73±0.27	24.72±0.27	17.98±0.25	19.73±0.32
C18:2	0.63±0.000	0.46±0.00	0.52±0.00	0.67±0.00	0.36±0.00	0.45±0.00	0.36±0.00	0.58±0.00	0.42±0.00
C18:3	0.28±0.000	0.30±0.00	0.32±0.00	0.30±0.00	0.31±0.00	0.35±0.00	0.31±0.00	0.30±0.00	0.34±0.00
ΣPUFA	0.91±0.000	0.76±0.00	0.84±0.00	0.97±0.00	0.67±0.00	0.81±0.00	0.67±0.00	0.88±0.00	0.75±0.00
C20:4	0.82±0.010	1.55±0.03	0.51±0.00	0.37±0.00	0.16±0.00	0.97±0.01	1.35±0.02	0.59±0.00	0.61±0.00
C20:5	0.10±0.000	0.20±0.00	0.08±0.00	0.10±0.00	0.71±0.00	0.21±0.00	0.06±0.00	0.11±0.00	0.17±0.00
C22:4	0.17±0.000	0.18±0.00	0.13±0.00	0.10±0.00	0.10±0.00	0.14±0.00	0.40±0.00	0.06±0.00	0.07±0.00
C22:5	0.08±0.000	0.12±0.00	0.06±0.00	0.08±0.00	0.13±0.00	0.07±0.00	0.37±0.00	0.05±0.00	0.03±0.00
C22:6	0.48±0.000	1.33±0.02	0.22±0.00	0.14±0.00	0.99±0.01	1.11±0.02	0.02±0.00	0.44±0.00	0.52±0.00
ΣHUFA	1.64±0.010	3.37±0.04	1.01±0.00	0.79±0.00	2.10±0.01	2.50±0.03	2.20±0.02	1.25±0.00	1.40±0.00

induced the decrease of ΣMUFA and ΣPUFA ($p<0.05$). After the withdrawal of DHA for 1 week, a significant decrease of ΣSFA and ΣPUFA was detected which was brought about by the decrease of C16:0 and C18:2 ($p<0.05$). A significant increase in ΣMUFA and ΣHUFA happened due to the increase of C18:1, C22:4 and C22:6 following DHA treatment ($p<0.05$). After withdrawing DHA for 2 weeks, a decrease in ΣSFA and ΣHUFA was observed. An increase in ΣMUFA was caused by the increase of C18:1.

The feeding of 2 weeks old chickens with DHA for 4 weeks gave rise to an increase of C16:0, C22:4 and C22:6 which consequently produced a significant increase of ΣSFA and ΣHUFA ($p<0.05$). A decrease of ΣMUFA and ΣPUFA was as a result of the decrease of C18:1 and C18:2 ($p<0.05$). After withdrawing DHA for 1 week, an increase in ΣMUFA and a decrease in ΣPUFA were noted following DHA intake. No difference was noted in other FAs. After the withdrawal of DHA for 2 weeks, a significant decrease was detected in ΣSFA and ΣHUFA which was caused by the decrease of C26:0, C22:4 and C22:6 ($p<0.05$). Thus, a significant increase in ΣPUFA was brought about by the increase in C18:3 following DHA treatment ($p<0.05$).

Changes of fatty acids composition in leg muscle: From Table 5 researchers can see that 2 weeks old chickens fed DHA for 2 weeks had a decrease in ΣSFA. A significant increase was noted in ΣHUFA as a result of the increase of C20:4 and C22:6 following DHA treatment ($p<0.05$). No difference was noted in ΣMUFA and ΣPUFA between the two groups. After withdrawing DHA for 1 week, no difference was noted in the concentration of FA (Table 5). After withdrawing DHA for 2 weeks, the concentration of ΣSFA had a significant decrease which was caused by the decrease of C18:0 following DHA intake ($p<0.05$). A significant increase of ΣMUFA was brought about by the increase of C18:1 in the experimental group ($p<0.05$).

Feeding 4 weeks old chicken DHA for 2 weeks resulted in a significant increase in ΣHUFA following DHA intake ($p<0.05$). This contributed to the increase of C20:2 and C22:6 ($p<0.05$). After withdrawing DHA for 1 week, a significant decrease in ΣMUFA and ΣHUFA was caused by the decrease of C18:1 and C20:4 ($p<0.05$). However, a significant increase was detected in C22:6 ($p<0.05$). An increase of ΣSFA resulted from the increase of C16:0 and C18:0 in the experimental group ($p<0.05$). After withdrawing DHA for 2 weeks, no difference was detected in the concentration of FA among all groups (Table 5).

About 2 weeks old chickens that were fed DHA for 4 weeks had a significant decrease of ΣSFA as a result of the decrease of C16:0 and C18:0 ($p<0.05$). A significant increase was noted in ΣMUFA and ΣHUFA which was brought about by the increase of C18:1, C20:5 and C22:6 ($p<0.05$). After withdrawing DHA for 1 week, an increase in ΣSFA and a relative decrease in ΣMUFA and ΣHUFA were noted ($p<0.05$). After the withdrawal of DHA for 2 weeks, no difference was noted following DHA intake (Table 5).

Changes of fatty acids composition in brain: From Table 6 we can see, 2 weeks old chickens that were fed DHA for 2 weeks had a significant decrease of ΣSFA, ΣMUFA and ΣPUFA in the brain ($p<0.05$). C20:5 and C22:6 increased significantly following DHA intake ($p<0.05$). Thus, ΣHUFA is higher following DHA intake ($p<0.05$). After withdrawing DHA for 1 week, a significant decrease was still detected in ΣMUFA and ΣPUFA ($p<0.05$). The increase of C22:6 resulted in the increase of

Table 6: The composition of fatty acids in brain and total fatty acids percentage

FA	A3	B3	A4	B4	A5	B5	C5	D5	A6	C6	D6
C14:0	0.19±0.000	0.51±0.00	0.15±0.00	0.20±0.00	0.29±0.00	0.17±0.00	0.16±0.00	0.13±0.00	0.16±0.00	0.21±0.00	0.15±0.00
C16:0	24.33±0.470	22.87±0.59	33.33±0.48	32.78±0.43	33.79±0.50	33.67±0.42	32.59±0.37	30.47±0.38	34.72±0.36	32.35±0.42	32.85±0.47
C18:0	14.39±0.260	10.14±0.14	8.94±0.13	9.64±0.16	8.54±0.13	9.07±0.21	9.39±0.26	8.70±0.14	6.97±0.09	9.51±0.08	5.55±0.08
C20:0	0.43±0.000	0.06±0.00	0.12±0.00	0.20±0.00	0.30±0.00	0.15±0.00	0.15±0.00	0.11±0.00	0.13±0.00	0.27±0.00	0.83±0.01
ΣSFA	39.34±0.700	33.58±0.63	42.53±0.50	42.81±0.58	42.92±0.60	43.06±0.60	42.29±0.61	39.41±0.50	41.97±0.40	42.34±0.50	39.38±0.56
C18:1	13.72±0.210	5.41±0.10	9.66±0.17	8.03±0.09	10.93±0.07	7.02±0.07	8.26±0.10	7.65±0.08	8.43±0.16	8.66±0.12	8.17±0.17
C20:1	0.09±0.000	0.41±0.00	0.35±0.00	0.16±0.00	0.19±0.00	0.20±0.00	0.13±0.00	0.29±0.00	0.17±0.00	0.13±0.00	0.67±0.00
ΣMUFA	13.81±0.210	5.82±0.10	10.01±0.17	8.19±0.09	11.12±0.07	7.22±0.07	8.39±0.10	7.94±0.08	8.60±0.16	8.79±0.12	8.84±0.17
C18:2	5.163±0.04	1.03±0.03	1.10±0.02	0.26±0.00	0.33±0.00	0.39±0.00	0.12±0.00	0.65±0.00	0.09±0.00	0.15±0.00	0.72±0.00
C20:2	0.63±0.000	0.57±0.00	2.78±0.04	1.19±0.01	1.50±0.04	1.21±0.02	1.68±0.03	0.29±0.00	0.76±0.01	1.89±0.04	1.36±0.02
ΣPUFA	5.79±0.040	1.61±0.03	3.89±0.05	1.44±0.01	1.82±0.04	1.60±0.02	1.80±0.03	0.94±0.00	0.85±0.01	2.04±0.04	2.13±0.02
C20:4	14.77±0.140	17.32±0.12	15.91±0.21	15.64±0.17	15.6±0.160	16.86±0.31	17.36±0.17	15.95±0.15	19.01±0.26	16.49±0.24	15.09±0.21
C20:5	0.51±0.000	10.29±0.45	1.21±0.03	0.41±0.00	0.67±0.00	0.37±0.00	0.44±0.00	0.30±0.00	0.43±0.00	0.74±0.00	3.37±0.06
C22:4	3.00±0.070	2.83±0.03	3.14±0.03	3.22±0.02	3.82±0.04	3.15±0.01	3.09±0.03	3.25±0.02	3.44±0.03	3.71±0.01	3.1±0.010
C22:5	4.65±0.020	4.98±0.03	6.82±0.04	4.13±0.04	6.56±0.06	4.71±0.02	7.06±0.05	3.23±0.02	7.95±0.05	6.41±0.05	3.1±0.030
C22:6	18.13±0.530	23.58±0.35	16.49±0.31	24.17±0.32	17.49±0.39	23.03±0.52	19.56±0.49	28.98±0.52	17.73±0.26	19.49±0.37	25.0±0.350
ΣHUFA	41.06±0.750	59.00±0.90	43.58±0.60	47.57±0.53	44.15±0.64	48.12±0.80	47.51±0.65	51.71±0.80	48.56±0.60	46.83±0.65	49.66±0.60

ΣHUFA ($p<0.05$). After the withdrawal of DHA for 2 weeks, ΣMUFA decreased due to the decrease of C18:1 ($p<0.05$). The increase of C22:6 resulted in the increase of ΣHUFA following DHA treatment ($p<0.05$).

Feeding 4 weeks old chickens DHA for 2 weeks brought about no difference in the concentration of ΣSFA, ΣPUFA and ΣHUFA. A significant decrease in ΣMUFA was observed following DHA intake ($p<0.05$). This contributed to the decrease of C18:1 ($p<0.05$). After withdrawing DHA for 1 week, a significant increase of ΣPUFA was triggered by the increase of C20:2 following DHA intake ($p<0.05$). No other difference was observed in ΣSFA, ΣMUFA and ΣHUFA. After withdrawing DHA for 2 weeks, no significant difference was noted among experimental groups. However, a slight increase of C22:6 was noted in the experimental group (Table 6).

About 2 weeks old chickens fed DHA for 4 weeks had a decrease of ΣSFA, ΣMUFA and ΣPUFA ($p<0.05$). ΣHUFA increased due to the increase of C22:6 ($p<0.05$). After the withdrawal of DHA for 1 week, a decrease was noted in ΣSFA. The increase of C18:2 led to a consequent increase of ΣPUFA ($p<0.05$). After withdrawing DHA for 2 weeks, no difference was noted between the groups. However, an increase of C22:6 was noted (Table 6).

DISCUSSION

In the present study, DHA increased the concentrations of the very long chain fatty acids DHA in broiler tissues, especially in the later phase. Palmitic acid and stearic acid were the main saturated fatty acids in leg and liver, as was palmitic acid in breast, belly and brain. ΣSFA is the main fatty acid in leg muscle. ΣPUFA is the main FA in breast and belly tissues while ΣHUFA is the main FA in liver and brain.

The FA composition of the meat and eggs were affected by the dietary fat source (Bou *et al*., 2005; Gonzalez-Esquerra and Leeson, 2000; Pan *et al*., 2007; Scaife *et al*., 1994; Surai and Sparks, 2000; Yoon *et al*., 2007). It was noted that the FA compositions of broilers' breast, thigh and skin tissues are similar to those of the broilers' diet and it was demonstrated that feeding fish oil to turkeys increases the concentrations of EPA and DHA in their fat deposits and muscle lipids (Neudoerffer and Lea, 1967). This was also demonstrated with rainbow trout that were fed with feed containing menhaden oil, canola oil or fish oils to compare the EPA and DHA level in fillet (Stone *et al*., 2011). The ω-3 fatty acid ALA is the highest in the linseed oil diet and it is significantly higher in the muscle of linseed oil treated birds. The other ω-3 FAs 20:5 (EPA) and 22:5 (DPA) also increased following linseed oil intake (Haug *et al*., 2007) and this may be a result of elongation and desaturation of ALA into EPA and DPA, as has been reported in broilers by others (Bou *et al*., 2005). The same study also examined the birds' ability to convert dietary ALNA to EPA and DHA and then deposit these fatty acids in the edible tissues (Rymer and Givens, 2006). The latter study suggests that although, the birds may be capable of converting ALNA to EPA and DHA to some extent, these acids are not consequently deposited in skeletal muscle but rather sequestered in the liver or transported to other tissues. It therefore seems that ALNA cannot be used to significantly enrich EPA and DHA in edible poultry tissues and therefore fish oil is the most suitable to use since it's rich in EPA and DHA (Lopez-Ferrer *et al*., 2001). The enzyme Δ6-desaturase is the rate-limiting factor in the synthesis of AA and DHA from their 18 carbon precursors (Brenner, 1971). There is a competition between n-6 and n-3 FAs in which n-3 FAs are used as the preferred substrate in the desaturation elongation pathway leading to an increase in the DHA:

AA ratio in tissues. The decrease in the broiler muscle ω-6 fatty acids LA and AA in the linseed oil treatment groups may be as a result of competition between LA and ALA to be incorporated into tissue membranes desaturases and elongases of the 18-carbon FAs to 20-carbon FAs. The ratio between LA and ALA (18:2 n-6 and 18:3 n-3) and between AA and EPA (20:4 n-6 and 20:5 n-3) was significantly lower following the linseed oil treatment, giving a more favorable ω-3 status in the linseed oil-broilers (Haug *et al*., 2007).

However, it is interesting to note that as the hen's age progressed, the pattern of AA and DHA accretion differed in low n-3 and high n-3 eggs (Cherian, 2008). Nielsen (1998) reported that the eggs of young layer hens have 20% more yolk DHA in egg yolks compared to old layer hens. The decrease in DHA observed in Cherian (2008) and Nielsen (1998)'s research suggests that hen's maturation reduces the ability to accrue DHA in yolk lipids or diminishes the desaturation and elongation of n-3 FA as reported in mammals (Ulmann *et al*., 1991). There is little known about the changes of DHA in broilers during the aging progress. In the experiment, different levels of accretion during aging were observed, it was found that age increased the deposit of C22:6 (HUFA) also increased in liver, breast muscle and brain. The efficiency of FA conversion in liver which varies according to the age of the animal (Bourre *et al*., 1990) could modify the differential lipid deposition. Although, LNA is efficiently converted in liver, the n-3 HUFA deposits are not nutritionally valuable in leg muscle. Nevertheless, MUFA decreased during the early phase due to the saturation of FAs in the thigh samples. MUFA decreased when aging increased the HUFA of the liver and brain samples. These may be important for regulation of the brain and liver.

The DHA is the major PUFA of the central nervous system in avians (Ajuyah *et al*., 2003; Noble and Cocchi, 1990). In this research, it was also noted that high concentrations of DHA were stocked in the brain and increased with aging. DHA constitutes the major n-3 fatty acids in the structural lipid of grey matter and is essential for optimum brain development and mental functions (Bourre *et al*., 1989; Hsieh and Brenna, 2009). Some research found dietary ALA didn't affect the concentration of DHA in brain (Bourre *et al*., 1989). While DHA is essential to normal brain function, this need might be covered by dietary alpha-LNA when liver metabolic conversion machinery is intact and the diet has high alpha-LNA concentration (Rapoport and Igarashi, 2009). In this research, researchers also detected that with the increased concentration of alpha-LNA in the brain there was also an increase of concentration of alpha-LNA in the liver. Essentially, all approaches to increase the intake of EPA and DHA rely directly or indirectly on the use of fish oils. There are concerns however, that the continued and possibly increased use of fish oils in the food chain is not sustainable and that alternatives are needed. Although, some data is available, further research on microalgae as dietary sources of EPA and DHA would seem warranted. In the experiment, microalgae oil had been used; it was found that it actually increased the concentration of HUFA in liver, brain and muscle tissues. Recent research findings and recommendations of organizations of public health now a days refine dietary advice and specific requirements are put forward for individual fatty acids within the n-3 class: 1% of daily energy intake for ALA and 0.3% of daily energy intake for DHA and EPA (EPA+DHA).

CONCLUSION

In this study, fortification of poultry products with EPA+DHA can supply these essential nutrients in the diet up to the level recommended by health organizations.

ACKNOWLEDGEMENTS

This research was supported by a grant from The National Natural Science Foundation of China (30871785) and the Program for New Century Excellent Talents in Universities, Chinese Ministry of Education (NCET-06-0865).

REFERENCES

Ajuyah, A.O., Y. Wang, H. Sunwoo, G. Cherian and J.S. Sim, 2003. Maternal diet with diverse ω-6/ω-3 ratio affects the brain docosahexaenoic acid content of growing chickens. Biol. Neonate, 84: 45-52.

Bou, R., F. Guardiola, A.C. Barroeta and R. Codony, 2005. Effect of dietary fat sources and zinc and selenium supplements on the composition and consumer acceptability of chicken meat. Poult. Sci., 84: 1129-1140.

Bourre, J., M. Piciotti and O. Dumont, 1990. Δ6 desaturase in brain and liver during development and aging. Lipids, 25: 354-356.

Bourre, J.M., M. Francois, A. Youyou, M. Dumont, M. Piciotti, G. Pascal and G. Durand, 1989. The effects of dietary α linolenic acid on the composition of nerve membranes enzymatic activity amplitude of electrophysiological parameters resistance to poisons and performance of learning tasks in rats. J. Nutr., 119: 1880-1892.

Brenner, R.R., 1971. The desaturation step in the animal biosynthesis of polyunsaturated fatty acids. Lipids, 6: 567-575.

Cherian, G. and J.S. Sim, 1992. ω-3 fatty acid and cholesterol content of newly hatched chick from α-linolenic acid enriched eggs. Lipids, 27: 706-710.

Cherian, G., 2008. Egg quality and yolk polyunsaturated fatty acid status in relation to broiler breeder hen age and dietary n-3 oils. Poult. Sci., 87: 1131-1137.

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.

Gonzalez-Esquerra, R. and S. Leeson, 2000. Effects of menhaden oil and flaxseed in broiler diets on sensory quality and lipid composition of poultry meat. Br. Poult. Sci., 41: 481-488.

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.

Haug, A., S. Eich-Greatorex, A. Bernhoft, J.P. Wold, H. Hetland, O.A. Christophersen and T. Sogn, 2007. Effect of dietary selenium and ω-3 fatty acids on muscle composition and quality in broilers. Lipids Health Dis., 6: 29-37.

Hsieh, A.T. and J.T. Brenna, 2009. Dietary docosahexaenoic acid but not arachidonic acid influences central nervous system fatty acid status in baboon neonates. Prostaglandins Leukotrienes Essent. Fatty Acids, 81: 105-110.

Jiang, Z.R., D.U. Ahn and J.S. Sim, 1991. Effects of feeding flax and two types of sunflower seeds on fatty acid compositions of yolk lipid classes. Poult. Sci., 70: 2467-2475.

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.

Lopez-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.

Mach, N., M. Devant, I. Diaz, M. Font-Furnols, M.A. Oliver, J.A. Garcia and A. Bach, 2006. Increasing the amount of n-3 fatty acid in meat from young Holstein bulls through nutrition. J. Anim. Sci., 84: 3039-3048.

Mendes, A., A. Reis, R. Vasconcelos, P. Guerra and T.L. da Silva, 2009. *Crypthecodinium cohnii* with emphasis on DHA production: A review. J. Applied Phycol., 21: 199-214.

Metcalfe, L.D. and A.A. Schmitz, 1961. The rapid preparation of fatty acid esters for gas chromatographic analysis. Anal. Chem., 33: 363-364.

Millet, S., K. De Ceulaer, M. Van Paemel, K. Raes, S. De Smet and G.P. Janssens, 2006. Lipid profile in eggs of araucana hens compared with lohmann selected leghorn and ISA brown hens given diets with different fat sources. Br. Poult. Sci., 47: 294-300.

Neudoerffer, T.S. and C.H. Lea, 1967. Effects of dietary polyunsaturated fatty acids on the composition of the individual lipids of turkey breast and leg muscle. Br. J. Nutr., 21: 691-714.

Nielsen, H., 1998. Hen age and fatty acid composition of egg yolk lipid. Br. Poult. Sci., 39: 53-56.

Noble, R.C. and M. Cocchi, 1990. Lipid metabolism and the neonatal chicken. Prog. Lipid Res., 29: 107-140.

Pan, C., K. Huang, Y. Zhao, S. Qin, F. Chen and Q. Hu, 2007. Effect of selenium source and level in hen's diet on tissue selenium deposition and egg selenium concentration. J. Agric. Food Chem., 55: 1027-1032.

Rapoport, S.I. and M. Igarashi, 2009. Can the rat liver maintain normal brain DHA metabolism in the absence of dietary DHA? Prostaglandins Leukotrienes Essent. Fatty Acids, 81: 119-123.

Rymer, C. and D.I. Givens, 2006. Effect of species and genotype on the efficiency of enrichment of poultry meat with n-3 polyunsaturated fatty acids. Lipids, 41: 445-451.

Scaife, J.R., J. Moyo, H. Galbraith, W. Michie and V. Campbell, 1994. Effect of different dietary supplemental fats and oils on the tissue fatty acid composition and growth of female broilers. Br. Poult. Sci., 35: 107-118.

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

Schiavone, A., R. Chiarini, M. Marzoni, A. Castillo, S. Tassone and I. Romboli, 2007. Breast meat traits of Muscovy ducks fed on a microalgae (*Crypthecodinium cohnii*) meal supplemented diet. Br. Poult. Sci., 48: 573-579.

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

Stone, D.A.J., A.C.M. Oliveira, S. Plante, S. Smiley, P. Bechtel and R.W. Hardy, 2011. Enhancing highly unsaturated ù-3 fatty acids in phase-fed rainbow trout (*Oncorhynchus mykiss*) using Alaskan fish oils. Aquacult. Nutr., 17: e501-e510.

Surai, P.F. and N.H. Sparks, 2000. Tissue-specific fatty acid and alpha-tocopherol profiles in male chickens depending on dietary tuna oil and vitamin E provision. Poult. Sci., 79: 1132-1142.

Ulmann, L., J. Blond, C. Maniongui, J.P. Poisson, G. Durand, J. Bezard and G. Pascal, 1991. Effects of age and dietary essential fatty acids on desaturase activities and on fatty acid composition of liver microsomal phospholipids of adult rats. Lipids, 26: 127-133.

Yoon, I., T.M. Werner and J.M. Butler, 2007. Effect of source and concentration of selenium on growth performance and selenium retention in broiler chickens. Poult. Sci., 86: 727-730.