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Omega-3 Fatty Acids Enrichment and Organoleptic Characteristics of Broiler Meat

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Abstract: This experiment was conducted to assess the effect of replacing Poultry Fat (PF) with Fish Oil (FO) in broiler diets on Fatty Acid (FA) composition and sensory quality of broiler meat. Three percents oil in 4 diets were altered with replacing PF by FO in completely randomized design at four treatments with 4 replicates (T1 = 3% PF, T2 = 2% PF+1% FO, T3 = 1% PF+2% FO and T4 =3% FO) and were given *ad libitum* to the birds fed throughout the growth period. The fatty acids profile and quality of the breast tissue were determined. Removing PF from diet by replacement of FO decreased total saturated (TSAT) ($p<0.01$) and total monounsaturated FA (TMUFA) ($p<0.001$). The amounts of total polyunsaturated FA (TPUFA) were significantly increased when FO level was increased ($p<0.001$). Altering of substituting PF by FO resulted in higher values of Linolenic Acid (LNA) and long-chain n-3 PUFA (C22:6n-3, C22:5n-3, C20:5n-3) in the breast tissue ($p<0.001$)and therefore decrease in the n-6: n-3 ratio ($p<0.001$). In the sensory quality evaluations (flavor and normal smell), the meat of T3 was acceptable. Therefore, with replacing PF by FO in diets of broiler chicks, meat of T3 could be enriched without significant sensory losses, which may improve nutritional content and promote of human health.

Key words: Poultry fat, fish oil, Omega-3 fatty acids, meat, broiler

INTRODUCTION

The recent of studies indicated that marine oils (fish oil and certain marine alga) contain the long chain (C_{20} and longer) omega-3 fatty acids as being an important factor in the diet is for promote of health in man and animals than other origins (Kinsella *et al*., 1990; Bezard *et al*., 1994; Scaife *et al*., 1994).

Fish oils are contain eicosapentaenoic acid (C20:5 n-3, EPA) and docosahexaenoic acid (C22:6 n-3, DHA), in a high proportion, whereas other origins as animal and vegetable oils contain respectively Linoleic Acid (LA) and Linolenic Acid (LNA), is converted in liver to longer-chain derivatives and deposition in peripheral tissues that are not nutritionally valuable modified products (Cherian and Sim, 1991).

The long chain n-3 PUFA (LC n-3 PUFA) of tissue obtained from direct deposit from dietary fat; therefore, the use from a marine origin is necessary for provides of EPA and DHA animal products such as meat/eggs and beneficial effects on human health (Kinsella *et al*., 1990; Knapp, 1991).

The enrichment of eggs and chicken meat with use of fish oil in order to enhance of LC n-3 PUFA contents is from important aims of nutrition engineers (Chanmugam *et al*., 1992), but entails several Organoleptic problems that adversely affect the meats acceptability (Edwards and May, 1965; Miler and Robish, 1969; Lo´pez-Ferrer *et al*., 1999a, b).

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This experiment was designed on two aims to study the effect of replacing PF by FO in breast tissue. The first aim was to evaluate the progressive deposition of the relative LC n-3 PUFA, with increase of diet fish oil level and the second aim, was to determine the best composition level of fish oil with poultry fat for acceptability of meat.

With a view to increasing the nutritional quality of male broiler chickens with indicating optimal level of fish oil to preventing deterioration of sensory quality, we used various dietary based on alternative substitution 3% fat (replacing poultry fat by fish fat) with 4 levels in diet.

MATERIALS AND METHODS

This experiment was carried out in research farm of Islamic Azad University, Shabestar branch in summer 2006. Six hundred unsexed one-day-old ROSS strain chicks were obtained from a commercial hatchery. The chicks were fed a common basal broiler starter diet from 1 to 20 days (starter period) in completely randomized design at four treatments with 4 replicates. At 21st day, 240 male chickens were sexed and randomly design with (15 bird per pen) and fed experimental diets [diets of containing 3% PF (T1), 2% PF+1% FO (T2), 1% PF+2% FO (T3) and 3% FO (T4)] throughout a 21 days growth period. The experimental diets formulated to be isonitrogenouse (19.5% CP) and isoenergetic (3136 kcal kg^{-1} ME), in accordance with the NRC-1994. The birds were given access to water and diets *ad libitum*. The measured and calculated nutrient composition of the starter and experimental diets were shown in Table 1.

Table 1: Composition and calculated nutrient content of diets fed to chicks

Ingredients and composition (%)	Starter diet[1]	Experimental diet	Withdrawal plan diet[2]
Yellow corn	62.50	61.50	55.50
Wheat	-	-	20.00
Soybean meal	30.50	31.00	20.10
Fish meal	4.00	1.00	1.55
Added fat/oil [3]/PF/FO	-	3.00	-
Monocalcium phosphate	0.80	-	-
Dicalcium phosphate	-	0.90	-
Bone meal	-	-	0.80
Oyster shell	1.20	1.40	1.00
DL-Methionine	0.30	0.20	0.07
Salt	0.20	0.30	0.23
Vitamin/mineral premix[4]	0.45	0.45	0.45
Coccidiostat	0.05	0.10	0.10
Vit E	-	0.10	0.10
Vit A	0.10	0.05	0.10
Total	100.00	100.00	100.00
Calculated nutrient content			
ME (kcal kg^{-1})	2,950.00	3,136.00	3,020.00
Crude protein (%)	21.20	19.50	17.11
Calcium (%)	0.32	0.14	0.15
Available P (%)	0.32	0.21	0.23
Methionine (%)	0.37	0.31	0.28
Methionine+cystine (%)	0.65	0.56	0.52
Lysine (%)	1.22	1.07	0.90

[1]Starter diet fed to birds from 0 to 21 days. [2]Oil remove for one wk before slaughter (to decreased of unacceptable odors) [3]Three percent added fat: T1, control diet = 3% poultry fat (PF); T2 = 1% fish oil (FO) + 2% PF; T3 = 2% FO + 1% PF; T4 = 3% FO. [4]Provides per kilogram of diet: vitamin A, 9,000,000 IU; vitamin D3, 2,000,000 IU; vitamin B1, 1,800 mg; vitamin B2, 6,600 mg; vitamin B3, 10,000 mg; vitamin B6, 3,000 mg; vitamin B12,15 mg; vitamin E, 18,000 mg; vitamin K3, 2,000 mg; vitamin B9, 1,000 mg; vitamin B5, 30,000 mg; vitamin H2, 100 mg; folic acid, 21 mg; nicotinic acid, 65 mg; biotin, 14 mg; choline chloride, 500,000 mg; Mn, 100,000 mg; Zn, 85,000 mg; Fe, 50,000 mg; Cu, 10,000 mg; I, 1,000 mg; Se, 200 mg

Table 2: Fatty acid composition of added fat/oil to experimental diets

	Total fatty acids (%)	
Fatty acid[2]	Poultry fat	Fish oil
C14:0	4.43	7.33
C16:0	25.08	19.61
C16:1n7 trans	5.31	7.76
C18:0	8.36	5.36
C18:1n9	26.84	18.95
C18:1n7	8.01	0.17
C18:2n6cis	17.70	3.41
C18:3n3	1.70	9.93
C20:1n9	0.20	0.45
C20:4n6	0.40	0.79
C20:5n3	0.00	11.50
C24:0	0.00	3.46
C22:5n3	0.00	2.21
C22:6n3	0.00	8.30
Others[3]	1.97	2.77
Total SAT	37.87	35.76
Total MUFA	40.36	27.33
Total PUFA	19.80	34.14
Total n6	18.10	4.20
Total n3	1.70	29.94

[1]Values are means of two determinations, [2]SAT = Saturated Fatty Acid; MUFA: MonoUnsaturated Fatty Acid; PUFA: Poly Unsaturated Fatty Acid; [3]Others fatty acids that no detected

At 42 days, the performance parameters was calculated and the birds were slaughtered at 49 days (two samples per pen) after to exert of withdrawal of FO from diet to determine of parameters. The total breast tissue was also obtained and frozen at -20°C for two month (Lopez-Ferrer *et al*., 2001) until the appropriate sensory evaluation tests were carried out.

The tissue samples eight breast per treatment after freezing for determination of the lipid composition were analyzed by means of gas chromatography (GC-1000).

Fatty Acid Analysis

A GC apparatus consisting of a Dany GC-1000 (Italy), equipped with FID detector, data processor (DS-1000, Dany), hydrogen generator model GLAIND-2200 (Italy) and a split/splitless injector was used. Separation of fatty acids was performed on An Altech Econo-Cap, EC-1000 capillary column (30×0.25 mm i.d., film thickness 0.25 μm) (Table 2).

Methanol, n-heptan, diethyl ether and other chemicals were all from E. Merk (Germany). Fatty acid standards mix was purchased from supelco co. High pure helium (99.999%) was from Roham Gas Co. (Middle East Dubai).

The total lipid fraction was extracted according to Folch *et al*. (1957) method. For determination of fatty acids about 500 mg of the samples were freeze-dried and extracted with chloroform-methanol mixture (2:1). After vaporization of solvent derivatization reaction was carried out on residue by adding 1 mL of potassium hydroxide 2 M in pure methanol and then shacked for 1 h at room temperature (25±1°C). The methyl esters were extracted in 3×0.5 mL n-heptan and 1 μL was injected to GC.

The initial column temperature was maintained at 75°C for 1 min and then raised at 30°C min^{-1} to 182°C and held for 8 min and temperature was then increased at 7.5°C min^{-1} to 200°C and held 1 min. Helium was used as carrier and makeup gas, which their flow rates are 1.2 and 25 mL min^{-1}, respectively. The injector and detector temperature were held at 250 and 260°C, respectively. Injections of samples were made in split less mode.

The objective parameters were conducted 48 h after slaughter of the birds and frozen at -20°C on total breast of eight individuals per treatment following Lopez Ferrer *et al*. (2001). The panel assessed the breast meat in a triangular test (Seemann, 1981) in which flavor of the meat and general impression were evaluated and contrasted twice in accordance with the procedures of Seemann (1981). However, the water-holding capacity (Juiciness), calculated following Grau and Hamm (1953) and using the modified Braunschweiger Model technique (Grashorn, 1995). Tenderness was estimated on cooked samples of breast described by Seemann (1981).

Statistical Analysis

All data were analyzed by ANOVA using the General Linear Model (GLM) procedures of the SAS Institute (SAS, 2000). The differences between means were determined using the Duncan test. The data are expressed as means and their Standard Error (SE).

RESULTS AND DISCUSSION

Fatty Acid Composition

As expected according to the FA profile of the diets, the saturated fatty acid content of the meat was slightly lower when PF was replaced by progressively more FO.

In the present experiments, the saturated FA (SAT) ($p<0.01$) and the monounsaturated FA (MUFA) ($p<0.001$) content decreased when PF was replaced by FO, the predominant SAT being palmitic acid ($C_{16:0}$) and stearic acid ($C_{18:0}$) and the predominant MUFA being oleic acid ($C_{18:1\ n-9}$) (Table 3).

The monounsaturated FA (MUFA) content of the breast samples also decreased [mainly in the form of oleic acid (C18:1 n9) ($p<0.001$)] with increasing levels of FO in the diet as reported by Yau *et al*. (1991) and Scaife *et al*. (1994). This effect could be due to the dual origin of oleic acid in meat

Table 3: Fatty acid composition of beast samples of chickens[1]

	Experimental diets[2]					
	T1	T2	T3	T4	SE	p[4]
Fatty acid[3]	(% of total methyl esters of fatty acids)					
C14:0	2.02^{c}	2.72^{bc}	3.32^{ab}	4.05^{a}	0.24	***
C16:0	15.65^{a}	12.80^{b}	12.53^{b}	12.45^{b}	0.37	***
C16:1n7 Trans	0.60^{b}	0.46^{b}	1.91^{a}	0.70^{b}	0.13	***
C18:0	16.39^{a}	14.92^{a}	10.21^{b}	10.12^{b}	1.06	**
C18:1n9	32.67^{a}	29.18^{b}	20.70^{c}	27.81^{b}	0.60	***
C18:1n7	2.22^{a}	0.93^{b}	0.83^{b}	1.17^{b}	0.10	***
C18:2n6cis	2.76^{c}	1.86^{d}	4.92^{b}	12.15^{a}	0.24	***
C18:3n3	1.59^{b}	0.70^{c}	2.17^{a}	2.40^{a}	0.11	***
C20:1n9	0.57^{d}	1.15^{b}	0.75^{c}	6.06^{a}	0.02	***
C20:4n6	1.59^{b}	1.76^{a}	1.80^{a}	1.21^{c}	0.03	***
C20:5n3	1.04^{d}	5.84^{c}	8.53^{b}	10.54^{a}	0.04	***
C24:0	1.09^{d}	3.18^{b}	2.65^{c}	4.13^{a}	0.03	***
C22:5n3	ND	0.10^{b}	0.20^{a}	0.29^{a}	0.02	***
C22:6n3	0.15^{d}	0.66^{c}	2.39^{b}	3.80^{a}	0.15	***
Total SATFA	34.41^{a}	33.63^{ab}	28.63^{c}	30.86^{bc}	1.00	**
Total MUFA	36.07^{a}	31.73^{b}	24.21^{d}	29.69^{c}	0.62	***
Total PUFA	8.14^{d}	19.10^{c}	24.85^{b}	33.16^{a}	0.42	***
Total n6	4.35^{d}	6.68^{c}	8.66^{b}	13.36^{a}	0.26	***
Total n3	3.79^{d}	12.41^{c}	16.27^{b}	20.04^{a}	0.21	***
n6: n3	1.14^{a}	0.53^{c}	0.53^{c}	0.66^{b}	0.02	***

^{a,b,c,d}: Values in the same row and variable with no common superscript differ significantly; [1]Values are means of eight observations per treatment and their standard errors. [2] T1 = Diet with 3% poultry fat (PF); T2 = Diet with 2% PF+ 1% fish oil (FO); T3 = Diet with 1% PF + 2% FO and T4 = Diet with 3% FO. [3] SATFA = saturated fatty acids; MUFA; monounsaturated fatty acid; PUFA; polyunsaturated fatty acid; [4] **: $p<0.01$; ***: $p<0.001$. ND = No detected

(direct depot from diet and de novo synthesis in liver and tissue). The high palmitic acid (C16:0) content in T1 could account for the high level of oleic acid in meat, through elongation and desideration (Lopez Ferrer *et al*., 2001).

The PUFA content increased ($p<0.001$) with increasing levels of FO in the diet (T2, T3 and T4). The study of important change i.e., increase of the PUFA content, indicated that the precursors of the n-3 and n-6 families (LNA and LA, respectively) increased with addition of FO to the PF diet ($p<0.01$). But, the LNA amounts (especially, LC n-3 PUFA contents) had double increase compared to the LA amounts with replacing FO in dietary fat. All LC n-3 PUFA clearly increased ($p<0.001$) with progressively increasing amounts of FO in the tissue. The prevailing $FA_{,s}$ was EPA and DHA (10.54 and 3.80% for T4 and 8.53 and 2.39% for T3, respectively). However, in comparison with results reported elsewhere (Chanmugam *et al*., 1992; Scaife *et al*., 1994; Lo´ pez-Ferrer *et al*., 1999b, 2001) we observed more of all the LC n-3 PUFA content in breast, particularly EPA. In addition, the FO used was mixed with PF, which diluted the LC-PUFA percentage of the dietary fat. The relative proportion of the n-6 FA, mainly as LA, C18:2 n-6, increased ($p<0.001$) when PF was replaced by FO. Arachidonic acid (C20:4 n-6) content decreased in at all four treatment and T4 has lowest concentration ($p<0.001$). Because high levels of LC n-3 PUFA might have decreased the desaturation and elongation of LA to its derivatives, as reported by Be´zard *et al*. (1994) in mammals. The arachidonic acid content in the tissues was higher than in the diet, although this finding was not closely associated with the LA content in the diet, as suggested by Scaife *et al*. (1994) and Yau *et al*. (1991). A minimum of arachidonic acid might remain constant in tissues to ensure certain metabolic processes. Moreover, most of the n-3 FA are given as LC-PUFA in the diet (EPA, DHA), which inhibits n-3 elongation, desaturation and especially, the metabolism of LA, as shown in rats (Grønn *et al*., 1992). Differences in all LC n-3 PUFA contents in chicken meat between T2 and T3 were minimal which included FO at 1 and 2% of the dietary fat (3%). However, the proportion of the LC n-3 depot was much higher when birds fed diet 4, with 3 % FO, was given throughout experiment (T4). The administration of LNA does not ensure efficient synthesis of its C22 family, which rules out meat enrichment strategies and suggests, as pointed out elsewhere (Chanmugam *et al*., 1992) that in broiler chickens, desaturation and elongation of LNA does not ensure the enrichment of peripheral tissues. Direct supplementation is thus more appropriate than conversion from precursors (Lo´pez-Ferrer *et al*., 2001).

Many studies have examined the effects of dietary LC-PUFA, supplied as FO or fish meal, on the FA composition of the broiler carcass (Miller and Robisch, 1969; Phetteplace and Watkins, 1989; Nash *et al*., 1995; Lo´pez-Ferrer *et al*., 1999b), to encourage the human dietary intake of long chain n-3 PUFA, which have beneficial effects on human health and resistance to various inflammatory diseases. These studies have clearly established that n-3 PUFA-rich diets increase the deposition of these fatty acids in muscle and adipose tissues.

In our experimental conditions, with use of 3% FO for 3 week, in order to enrichment of meat with LC n-3 PUFA, after exert of withdrawal design, provided Higher EPA, DPA and DHA levels. But, consideration to sensory losses, mixture of 2% FO plus 1% PF (T3) is best fat levels than other dietary fats. The produce contains higher EPA, DPA and DHA levels with optimal organoleptic quality are achieved.

Meat Quality Parameters

Table 4 showed the objective quality Meat parameters of the breast samples of chicks at various concentrations of FO after withdrawal plan of substitution. Different amounts of FO in diet indicated significantly differences in meat quality parameter as flavor and normal smell.

The breast meat of T4 birds had least normal smell ($p<0.01$) and flavor of meat increased with adding of FO to dietary fat. The breasts of males that fed from 3% fish oil (T4) had no significantly

Table 4: Meat quality parameters according to different of fish oil in diets[1]

Variable[2]	T1[4]	T2	T3	T4	SE	p[3]
Flavor	4.93	3.93	4.26	4.36	0.146	NS
Normal smell	4.60[a]	4.00[b]	3.66[b]	3.46[b]	0.191	**
Juiciness[3]	3.86	3.70	4.26	4.53	0.237	NS
Tenderness	3.53	3.60	4.06	4.40	0.262	NS

[a,b]: Values in the same row and variable with no common superscript differ significantly. [1]Values are means of fifteen observations per treatment and their standard errors. [2]All above score 0 to 5. [3]Juiciness = Water-holding capacity. Proportion of area of liquid in relation to the area of meat. [4]T1 = Diet with 3% poultry fat (PF); T2 = Diet with 2% PF+ 1% Fish Oil (FO); T3 = Diet with 1% PF + 2% FO and T4 = Diet with 3% FO [5]NS = p>0.05; **: p<0.01

Table 5: Eating quality traits: contrasts

Contrasts[2]	Breast
T1×T2	NS
T1×T3	NS
T1×T4	**
T2×T3	NS
T2×T4	**
T3×T4	**

[1]T1: Diet with 3% poultry fat (PF); T2: Diet with 2% PF+ 1% fish oil (FO); T3: Diet with 1% PF + 2% FO and T4: Diet; With 3% FO [2]Seemann, (1981) [3]NS = p>0.05; **: p<0.01

more juiciness. Juiciness is associated with the retention of water within the muscular fibers of raw meat (Grashorn, 1995). On the other hand, with replacing PF by FO, were not significantly increased the tenderness of the breast meat samples. One consideration in the use of fish oil as the n-3 fatty acid source is it's off flavor in bird diets and the reduced shelf life of the chicken meat. A combination of preserving agents and antioxidants may be used to increase shelf life and conceal the distasteful flavors (Farrell, 1995).

Sensory Quality of Meat

Results of the chicken meat sensory tests are shown in Table 5. We did compared all of treatments contain fish oil with T1 (without FO). Instead, T4 (only 3% FO) compared with T3 (composition of 2% FO+1% PF). Researchers reported unacceptable odors were detected in carcasses of chickens fed FO up to 4% (Dansky, 1962) and 2% (Edwards and May, 1965).

Sensory quality of the meat from T2 and T3 did not have a fishy taint when compared to the control diet (T1), which did not include FO, corresponding with the results of Lopez Ferrer *et al.* (2001). The panel did not find differences in flavors between T2 and T3 samples or when these samples were compared to those from T1.

The EPA content increased slightly when FO was added to the dietary fat. However, EPA is among the most biologically important FA included in the human diet. A high EPA content would improve not only the meat but also the regulation of human lipid metabolism (Kinsella *et al*., 1990; Knapp, 1991). This improvement requires assessment of the oxidative control of the LC n-3 PUFA enriched meat; highly polyunsaturated meat is highly susceptible to oxidative processes, which may harm human health (Hamilton, 1989).

Low amounts of DHA in the diet resulted in decreased content in the meat. Meat enrichment in LC n-3 PUFA can only be achieved by adding high proportions of marine products to chicken diets. The conversion of LNA to LC n-3 PUFA and later deposition in peripheral tissues is rather limited in chicken, although results from viscera such as liver are far more controversial and need further research (Lo´pez-Ferrer *et al*., 2001). Enrichment of chicken meat with LNA could be achieved without significant sensory losses, which may improve nutritional content because of its influence on the human lipidic metabolism, if the oxidative control of LC n-3 PUFA enriched meat is assessed (Farrell, 1995). Meat from T4-fed chickens would contain more n-3 (methyl esters of LC-PUFA and LNA) than control (T1).

With consider to recommendations of International Life Sciences Institute (1995) in the human daily requirements to LC n-3 PUFA, were ensured the EPA, DHA content and total n-3 in 300 g of breast meat of T3-fed chickens (1305 mg of EPA, 366 mg of DHA and 330 mg of LNA) without sensory losses.

Nowadays, carried out far most researches and efforts by scientists and researchers and poultry producers in order to improve of nutritionally products, which have health benefits for human. Enrichment of meat especially by fish oil because of including large mount of LC n-3 PUFA contents (mainly EPA and DHA) has a lot of importance. But, further research should be carried out to ensure that is previously mentioned contents in meat or eggs without sensory losses.

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