

# OMEGA-3 ENRICHMENT OF BROILER DARK MEAT: REDUCING UNLIKE FATS AND FISHY TAIN FOR CONSUMER ACCEPTANCE

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✉ Supporting Information

**ABSTRACT:** Fish oil (FO) rich in the long chain n-3 polyunsaturated fatty acid (LC n-3 PUFAs) such as eicosapentaenoic acid (EPA, C22:5 n-3) and docosahexaenoic acid (DHA, C22:6 n-3) play substantial roles to improve FA composition and enhance health-related effects of animal products like meat, dairy and eggs. However, optimization based solely on omega-3 enrichment could lead to undesirable odours in animal-source foods, unless the FO withdrawal period is applied for 1 week before slaughter. The aim of study was to investigate whether the replacement of FO with poultry fat (PF) in the diet for 21 days followed by its withdrawal for 1 week affected fishy taint of thigh meat, cholesterol (CHOL) and triglycerides (TG) concentrations, n-3 enrichment and growth rate of male broiler chickens. Two hundred and forty birds (21-day-old, Ross 308) were fed 1 of 4 dietary groups (T1=3%PF, T2=2%PF+1%FO, T3=1%PF+2%FO, T4=3%FO) during a 21-d growth period. Broilers fed dietary fish oil indicated an improved rate of growth ( $P<0.01$ ) plus a decline of the CHOL and TG levels as well as the n-6: n-3 ratio in thigh meat. Moreover, amount of LC n-3 PUFAs especially EPA and DHA were increased and hence a lower monounsaturated FA: PUFA ratio was detected on day 42 and also after oil/fat withdrawal from the diet. The juiciness and tenderness of dark meat of broilers fed FO dietary groups were raised. But the fishy taint was unfavorably higher ( $P<0.01$ ) especially in T4 meat that affected flavor and acceptability thereof. The dissatisfaction of the panelists toward cooked samples of T4 scored as neither like acceptable and their satisfaction with group 3 meats scored as good. Since the lowest n-6/n-3 FAs and a good growth rate were also observed for 1%PF+2%FO (T3), group 3 meats were selected as good-quality omega-3 enriched broiler meat. It is concluded that pre-slaughter withdrawal of replaced fish oil in broiler diet seems ensure the good performance, n-3 enrichment of thigh without probable off-flavors or unlike fats (CHOL and TG) of dark meat.

**Keywords:** Broiler, Fish oil, Fatty acids, Cholesterol, Thigh meat, Sensory quality

## INTRODUCTION

Chicken as the most common type of poultry is well known as an appropriate model in lipid nutrition studies. Birds fed diets with the same nutritive values and rations containing fat, present better performance and the carcass than birds fed diets without fat. Moreover, the performance of animal is depended on their health and a healthy immune system (Das, 2002). In recent decade, fish oil known as one of the stronger source of long chain n-3 PUFAs such as alpha-linolenic acid (ALA, C18:3n-3), eicosapentaenoic acid (EPA, C22:5 n-3) and docosahexaenoic acid (DHA, C22:6n-3), play substantial role to decrease n-6: n-3 PUFAs ratio and enhance health-related effects of milk, meat and eggs (Connor, 2002; Kris-Etherton et al., 2004; Rymer and Givens, 2005; Schreiner et al., 2005; Yanovych et al., 2013).

Poultry fat is also known as a potential and more economical energy source in feed formulation without detrimental effects and enriched in linoleic acid (LA), omega-6 and omega-9 fatty acid (Baião and Lara, 2005; Panneerselvam et al., 2011). Saleh et al. (2009) stated that a competitive interdependence between n-6 and n-3 fatty acids and keeping the balance between products of their conversion in an organism result from the mutual relation between linoleic and alpha-linolenic acids in dietary fats. Results showed that an unbalanced ratio from the content of n-3 and n-6 PUFAs standpoint might be one of the causes of cardiovascular and ischemic heart diseases with effect on phospholipids of biological membranes (Frenoux et al., 2001). Dietary saturated and trans fats are the primary culprit in today's society leading to worsening of the lipid profile and increasing the risk of heart disease, especially associated with child health development (American Heart Association, 1988).

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In view of the recent findings, the effects of different dietary combinations of PUFAs sources on fatty acids composition in poultry meat (white vs. dark cuts), have been analyzed (Zanini et al., 2004). Among five well-known sources of polyunsaturated oils (soybean, canola, sunflower, linseed, and fish), canola oil and fish oil were more successful in reducing unlike fats and improving fatty acid composition of the thigh meat. Among n-3 fatty acids, EPA and DHA have attracted particular attention. In a number of clinical studies, these compounds were shown to reduce the risk of several chronic diseases and their best-evidenced beneficial role, are in cardiovascular disease (Kris-Etherton et al., 2004; Kidd, 2004; Lopez-Garcia et al., 2005).

Currently the main objective of the broiler industry is the production of saleable chicken meat. The industry tries to produce poultry products with a lower cholesterol and triglyceride levels and rich in LC n-3 FAs being studied (Schreiner et al., 2005, Koreleski and Świątkiewicz, 2006). To this end, it is important to limit a minimum number of the negative parameters of meat product such as unlike fats and also to maximize n-3-enriched meat yield and its quality attributes (Bou et al., 2004; Villaverde et al., 2006).

Poultry meat is a desirable component of the human diet, not only the amount but each types of breast and thighs and especially the sensory quality of meat have a great importance for the producers and the consumers. Because the enrichment of poultry meat with PUFAs results in development of undesirable odours “fishy taints” (Betti et al., 2009, Zuidhof et al., 2009) and a more technical way to improve the sensory characteristics of meat of birds fed fish oil is necessary to remove these components from the feeding mixture. Therefore, an optimization based solely on n-3 PUFAs enrichment in chicken meat could lead to lower meat quality attributes (Bou et al., 2004; Zelenka et al., 2008), unless the plans like the dietary fat substitution (Lopez-Ferrer et al., 1999), antioxidant implementation (Lopez-Ferrer et al., 1999; Zanini et al., 2004), and the dietary fat withdrawal for at least 5 days (Betti et al., 2009; Zuidhof et al., 2009) or one week (Aghaei et al., 2012) are applied.

In the present study we aimed to minimize the effects of off-flavors on the sensory quality of FO enriched broiler dark meat, by two technical methods including 1) dietary supplementation with a mixed fat of fish oil and poultry fat and 2) applying a FO withdrawal period for 1 week before slaughter. Therefore the main aim was to investigate whether the replacement of FO with poultry fat (PF) in the diet for 21 days followed by its withdrawal for 1 week affected fishy taint of thigh meat, cholesterol (CHOL) and triglycerides (TG) concentrations, n-3-enrichment and growth rate of male broiler chickens.

## MATERIAL AND METHODS

### Animal and diets

A total of 600 broiler chickens (Ross 308) obtained from a commercial hatchery (Sefidan Morgh Co., Tabriz, Iran) and fed a same starter diet, up to 3rd wk of age. Two hundred and forty male chickens were separated, individually weighed and randomly assigned on four groups and five replicates (20 floor pens of 1.5 × 1.5 meters, 12 birds per pen). The experimental diets were supplemented with poultry fat (PF) or fish oil (FO) at 3% of feed, as replacement, that formulated in accordance with the NRC (National Research Council, 1994). Both grower and withdrawal plan diets fortified with antioxidants (vitamins E and A). Ingredient composition and nutrient calculation for diets are shown in Table 1. Fish oil (*Clupeonella oil*) and PF were obtained from Iranian sources (Mehregan Khazer Co., Bander Abbass, Iran) and was stored at 4 °C before being mixed with other ingredients. The analytical results of fatty acid composition for supplemented PF and FO are shown in Table 2.

### Housing and measurement

The experimental treatments were consisted of 3% PF (T1), 2% PF + 1% FO (T2), 1% PF + 2% FO (T3), or 3% FO (T4), and fed to birds from 21 to 42 days of age. The chicks maintained on a 24-h constant lighting schedule (at ~20 lux) with average relative humidity of 60–65%. Both diets and fresh water offered *ad libitum* to birds. Male chickens were individually weighed every week and only the body weight (BW) presented as growth performance at the end of each week of a 21-d growth period. The mean BW, using the pen as the experimental unit at the beginning of the experiment (day 21), were not significantly different ( $P>0.05$ ). In day 21, 3% fat was withdrawn from the diets to avoid any organoleptic problems that might adversely affect the meat quality, as all birds fed on a commercial finisher diet (withdrawal plan diet). Fifteen birds per group (3 observations per pen) were slaughtered and eviscerated during 6 h and after a 12 h feed withdrawal period and the carcasses were apportioned by hand into commercial cuts and the breasts and thighs, with skin, were separated, packed in plastics bags and chilled during transportation to the laboratory and the samples were frozen in a deep freezer at  $-20^{\circ}\text{C}$  until analyze proceed, according to work of Lopez-Ferrer et al. (2001). Values in Tables are means of eight observations per treatment and their standard errors. All stored briskets and thighs (remained from fifteen carcasses) used for sensory quality assay.

**Table 1 - Ingredient composition and nutrient content of the experimental diets**

Feedstuffs (%)	Starter diet	Experimental diet <sup>1</sup>	Withdrawal plan diet <sup>2</sup>
Poultry fat/Fish oil <sup>1</sup>	–	3.00	–
Fish meal	4.00	1.00	1.55
Soybean meal	30.50	31.00	20.10
Yellow corn	62.50	61.50	55.50
Wheat	–	–	20.00
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 <sup>2</sup>	0.45	0.45	0.45
Cocciostat	0.05	0.10	0.10
Vitamin E	–	0.10	0.10
Vitamin A	0.10	0.05	0.10
<b>Calculated nutrient content</b>			
ME (kcal/kg)	2,950	3,136	3,020
CP (%)	21.20	19.50	17.11
Calcium (%)	0.32	0.14	0.15
Available P (%)	0.32	0.21	0.23
Lysine (%)	1.22	1.07	0.90
Methionine (%)	0.37	0.31	0.28
Methionine + cysteine (%)	0.65	0.56	0.52

<sup>1</sup>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. <sup>2</sup>Remove oil for one wk before slaughter (to decrease unacceptable odors). <sup>3</sup>Each kg of premix contained: 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. Starter diet fed to birds from 0 to 21 days. ME metabolizable energy. CP= Crude protein.

**Table 2 - Selected major fatty acid composition (%) of supplemented fat type <sup>1</sup>**

Fatty acid (FA) <sup>3</sup>	Dietary fat/oil % wt/wt of total lipids	
	Poultry fat	Fish oil
C14:0	4.43	7.33
C16:0	25.08	19.61
C18:0	8.36	5.36
C24:0	ND	3.46
∑ Saturated fatty acids (SFAs)	37.87	35.76
C16:1 (n-7, cis-9)	5.31	7.76
C18:1 (n-9, cis-9)	26.84	18.95
C18:1 (n-7, trans-9)	8.01	0.17
C20:1 (n-9, cis-13)	0.20	0.45
∑ Monounsaturated fatty acids (MUFAs)	40.36	27.33
C18:2 (n-6, cis-9,12) (LA)	17.70	3.41
C20:4 (n-6, cis- 5,8,11,14) (AA)	0.40	0.79
C18:3 (n-3, cis-6,9,12) (ALA)	1.70	9.93
C20:5 (n-3, cis-5,8,11,14,17) (EPA)	ND	11.50
C22:6 (n-3, cis-4,7,10,13,16,19) (DHA)	ND	8.30
∑ Polyunsaturated fatty acids (PUFAs)	19.80	34.14
Other fatty acids <sup>3</sup>	1.97	2.77
∑ (n-6)	18.10	4.20
∑ (n-3)	1.70	29.94
n-6: n-3	10.64	0.14

<sup>1</sup>Values are means of 3 determinations. <sup>2</sup>PF= poultry fat, FO= fish oil; FAME, fatty acid methyl ester; ALA, α-linolenic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; ND, not detected methyl esters.

### Analytical methods

The consumer panel test carried out by cooking thighs, with the skin, which have frozen in refrigerator at  $-20^{\circ}\text{C}$  up to 1 month of storage following Lopez-Ferrer et al. (2001). Fifteen consumer panelists to testing thigh meats were selected from department and all had experience in poultry meat sensory analysis. Criteria for selection were: 1) age between 20 to 45 years old, 2) not allergic to chicken, 3) consumption of chicken at least once per week, and 4) willingness to evaluate meat from chickens that fed with experimental diets. Vacuum-packed cooked chicken meats served to the panelists in a professional taste panel including normal smell, flavor, juiciness (water-holding capacity) and tenderness of meat using a 5-point scale ranking following Bou et al. (2004). Random 4-digit numbers identified samples, and all dietary treatments presented to the consumer panelists in one session. They were also, asked to rank the total acceptability of the product using four total scale (very good, good, acceptable, bad).

All the samples sliced with a homogenized blade cutter during 4 minutes. Amount of total lipid extracted from all samples by Folch reagent (Folch et al., 1957). After extraction process, 4-milliliters aliquots from ready samples were transported to commercial kits (Kone Specific, Kone Instruments Corp., Kone commercial kit, Japan), and the CHOL and TG were analyzed by means of Autoanalyzer (ALCYON-300, Autoanalyzer, Abbott, American).

The FA composition of the meat samples were determined by a Gas Chromatography, Dany GC-1000 instrument, (Dany GC-1000, Dani Instruments S.P.A., Rome, Italy) equipped with a flame ionization detector, data processor (DS-1000, Dany. Dani Instruments S.P.A. Rome, Italy), hydrogen generator (GLAIND-2200, Via Regina, Lenn Rome, Italy) and a split-splitless injector. Separation of methyl esters performed on an Altech Econo-Cap (Alltech Econo-Cap., Alltech Association Inc., Deerfield, IL), EC-1000 capillary column (30 m  $\times$  0.25 mm i.d., film thickness of 0.25  $\mu\text{m}$ ). Methanol, *n*-heptane, diethyl ether, and other chemicals from E. Merck (E. Merck and Co. Inc., Munich, Germany), FA standards (Supelco Inc., Bellefonte, PA), and high-purity helium (99.999%) (Roham Gas Co., Dubai, UAE) obtained commercially. The total lipid fraction extracted according to the method of Folch et al. (1957). To determine FA, approximately 500-mg of the samples were freeze-dried and extracted with 1 chloroform: 2 methanol (v: v). After vaporization of the solvent, a derivatization reaction was carried out on the remaining residue via the addition of 1 mL of 2 M KOH in pure methanol and then vibrated for 1 h at room temperature ( $25 \pm 1^{\circ}\text{C}$ ). The methyl esters were extracted in 0.5 mol of three  $\times$  *n*-heptanes, and all was injected into the gas chromatograph. The initial column temperature was maintained at  $75^{\circ}\text{C}$  for 1 min and then increased at the rate of  $30^{\circ}\text{C}/\text{min}$  to  $182^{\circ}\text{C}$  and held at this temperature for 8 minutes. The temperature was then increased further at  $7.5^{\circ}\text{C}/\text{min}$  to  $200^{\circ}\text{C}$  and held for 1 min. Helium was used as the carrier and makeup gases at flow rates of 1.2 and 25 mol/min, respectively. The injector and detector temperatures held at 250 and  $260^{\circ}\text{C}$ , respectively. Injection of the samples done in split less mode.

### Statistical analysis

All data from the Chol and TG concentrations and FA composition of tissue analyzed by ANOVA using the GLM procedure of SAS software (2001) which is appropriate for completely randomized design. When significances were detected ( $P < 0.05$ ), values were compared post-hoc using the Duncan test. The results expressed as averages and their pooled standard error.

## RESULTS AND DISCUSSION

### Growth performance

Substituting fish oil with dietary poultry fat affected the bird's body weight toward better yield ( $P < 0.01$ , Table 3). T3 group (1% PF + 2% FO) showed the highest BW, followed by T4 group (3% FO). In the present analyses, FO had higher levels of unsaturated FA in compared to the PF. Although, the good performance may be achieved in broiler by FO rich in *n*-3 FAs (Rymer and Givens, 2005; Villaverde et al., 2006) and capable to reduction of catabolic response induced by immune stimulation and promoting growth (Kris-Etherton et al., 2004; Kidd, 2004), but the sensory losses can be occurred in product (Betti et al., 2009) unless FO removed from diet for 1 wk before slaughter to prevent the development of undesirable odors (fishy taints) in the *n*-3-enriched meat (Farrell, 1995).

### The FA profiles of PF and FO sources

The FA profiles of the supplemental PF and FO are shown in Table 2. The major differences between PF with FO were observed in *n*-6 methyl esters and *n*-3 PUFAs especially long chained FAs (C20:5*n*-3, C22:5 and C22:6*n*-3). Poultry fat contained 40.36% MUFA, mostly oleic acid (C18:1*n*9 = 26.84%) and included 18.10% omega-6 PUFA, mainly linoleic acid (C18:2*n*6, LA = 17.70%). Fish oil included 34.14% PUFA, predominantly alpha-linolenic acid (C18:3*n*-3, ALA = 9.93%) and LC *n*-3 PUFA (EPA = 11.50% and DHA = 8.30%). The amount of SFA in PF was slightly

higher than FO (37.87% in PF and 35.76% in FO). Therefore, the main difference in both fat types was related to n-3 and n-6 FAs contents. However, amount of linoleic acid (LA) in PF than FO was above board higher.

**Table 3 - Effect of fish oil and poultry fat on mean body weight in different weeks (g) <sup>1</sup>**

Treatments <sup>2</sup>	Age (days)			
	21	28	32	42
T1	753.25	1044.45	1570.22 <sup>b</sup>	1920.40 <sup>d</sup>
T2	753.42	1054.55	1588.45 <sup>b</sup>	1974.55 <sup>c</sup>
T3	754.28	1105.82	1606.23 <sup>a</sup>	2055.75 <sup>a</sup>
T4	756.40	1098.58	1608.25 <sup>a</sup>	2028.85 <sup>b</sup>
SEM	6.92	9.74	18.92	15.25
Significance <sup>3</sup>	NS	*	**	**

<sup>a-d</sup> Values in the same row and variable with no common superscript differ significantly; <sup>1</sup>Values are means of 5 observations per treatment. <sup>2</sup>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. <sup>3</sup>NS= P>0.05; \*= P<0.05; \*\*= P<0.01.

### The cholesterol and triglyceride concentrations of thigh meat

Replacement of PF with FO in the diet decreased the CHOL and TG values of thighs muscles, significantly (P<0.05, Table 4). The LC n-3 PUFAs series such as EPA and DHA is capable to reduce the very low-density lipoprotein (VLDL) levels in the blood (Lopez-Garcia et al., 2005). This effect is because of acting to lower the circulating free LDL concentration which is normally delivered to tissues for fat storage or deposited directly in the arteries, and thus reduces the rate of TG synthesis in the liver. It is proved that, the fatty acids within the liver can be utilized for a variety of purposes, from oxidation to the synthesis of structural lipids, but a proportion is re-converted into triacylglycerols, and some of this is stored as lipid droplets within the cytoplasm of the cells like adipocytes or many other cell types including leukocytes, epithelial cells and hepatocytes, especially during infectious, neoplastic and other inflammatory conditions. Excessive accumulation of storage triacylglycerols is associated with fatty liver, insulin resistance and type 2 diabetes (Athenstaedt and Daum, 2006). On the other hand, the lipid classes in broilers unevenly distributed in different marketable cuts, i.e. breast or white meat rich in phospholipids, and thigh or dark meat rich in triacylglycerol (Betti et al., 2009).

**Table 4 - Triglyceride and cholesterol concentrations of thigh tissues of birds fed with experimental diets <sup>1</sup>**

3% fat <sup>2</sup>	Thighs tissues		
	Weight (g)	Cholesterol (mg/100g)	Triglyceride (mg/100g)
T1	360 <sup>b</sup>	12.25 <sup>a</sup>	19.00 <sup>a</sup>
T2	395 <sup>a</sup>	9.25 <sup>ab</sup>	18.75 <sup>a</sup>
T3	390 <sup>ab</sup>	7.00 <sup>b</sup>	16.25 <sup>ab</sup>
T4	384 <sup>b</sup>	6.50 <sup>b</sup>	11.75 <sup>b</sup>
SEM	7.21	1.21	1.80
Significance <sup>3</sup>	*	*	*

<sup>a-d</sup> Values in the same row and variable with no common superscript differ significantly (P<0.05). <sup>1</sup>Values are means of 10 observations per treatment. <sup>2</sup>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. <sup>3</sup>\*= P<0.05.

### Fatty acids composition of thigh meat

Broilers fed by PF presented higher values of predominant SFA and MUFA than birds fed diet contained FO or FO mixed with PF. From among SFAs, the lignoceric acid (C24:0) increased (P<0.001) in thighs tissues while the amount of oleic acid, a predominant MUFA, not significantly decreased (P = 0.07) by substituting FO in the dietary poultry fat. The linoleic acid (LA, C18:2, a predominant n-6 PUFA), significantly increased (P<0.01) and the highest values was related to birds fed 3% FO (T4). Amount of LA in FO source was almost 5 times less than PF source (3.41 vs. 17.70, respectively; Table 2) while, its amount in thigh tissues was substantially lower (Table 5). All LC n-3 PUFA found to be present at higher levels in thigh meat when the dietary FO was at the highest level. The total n-6 and n-3 PUFA in thigh samples were increased. The SAT: PUFA, MUFA: PUFA and n-6: n-3 ratios were decreased (P<0.01) in thighs meat by replacing PF with FO in the diet.

### SFAs and MUFAs

Birds fed PF have higher values of predominant SFA and MUFA than those administered with the FO or both combinatorial PF and FO levels. Indeed, there is a direct deposition of oleic (C18:1, n9) and stearic (C18:0) acids from diet to tissue as well as their endogenous synthesized form in liver to tissue (Lopez-Ferrer et al., 2001). Furthermore, higher palmitic acid (C16:0) content of thigh meat could account for high level of oleic acid in tissue via elongation and desaturation. In the current study, fish oil had lower C16:0 than poultry fat (%19.61 vs. %25.08)



and it could results ( $P < 0.01$ ) a lower C18:1, n9 in thighs of T4 birds (fed 3% FO) with direct deposition or via endogenous elongation and desaturation processes in the liver. In addition, it is proved that deposition of saturated FAs in tissues is depending on their synthesis in the liver and partly their oxidation rate (Cherian et al., 1996) and FA synthesis's inhibition in the liver is more considered to unsaturated fatty acids than saturated fats (Skrivan et al., 2000). Lopez-Ferrer et al. (2001) reported that PUFAs inhibits  $\Delta 9$ -desaturase activity and the formation of MUFA from their precursors.  $\Delta 9$ -desaturase is the key enzyme needed to convert palmitic to palmitoleic acid and stearic to oleic acid (Grønn et al., 1992).

### PUFAs

Amounts of linoleic acid (LA, n-6) and  $\alpha$ -linolenic acid (ALA, n-3) increased by FO. The highest values were related to EPA and DHA contents and other derivatives of n-3 acids. Also, birds fed solely FO did show better results than those fed on PF + FO (T2 and T3). The n-3 PUFAs contents were almost doubled than the n-6 FAs in the thighs (Table 5). Among n-3 fatty acids, ALA (C18:3) which is available in vegetable oils (mainly flaxseed and canola) and EPA and DHA that are present in fish oils are very important. It is reported that, consumption of ALA can be leading to a significant increase of tissue's EPA, but not DHA; while, dietary fish and fish oils are reported to increase both EPA and DHA in animal tissues (Mantzioris et al., 2000; Burdge et al., 2002). It has been demonstrated by Das (2006) that the ALA and LA are transformed into LC PUFAs and their derivatives by enzymes ( $\Delta 6$  and  $\Delta 5$  desaturases and or elongates). These same enzymes metabolize both n-6 and n-3 fatty acids. The fundamental reason for reduction of amount of LA and their derivatives in tissue is because of high levels of LC n-3 PUFAs (mainly, EPA and DHA) in the diet (Hrdinka et al., 1996). On the other hand, the amount of arachidonic acid (C20:4 n6, AA) in tissue of all groups was lower than the predominant n-6 FA (LA); however, a minimum of arachidonic acid might remain constant in tissues to ensure certain metabolic processes (Das, 2006). Researchers reported that, diet supplementation with LC n-3 PUFAs could elevated tissue amount of EPA and DHA with a subsequent reduction in arachidonic acid content within cells of the membrane, especially in the membrane of platelets, erythrocytes, neutrophils, monocytes and liver cells (Boberg et al., 1986; Connor, 2002).

**Table 5 - Fatty acid composition of thighs tissue of broilers as influenced by experimental diets<sup>1</sup>**

Fatty acid <sup>3</sup>	Experimental diet, % wt/wt of total fatty acid methyl esters <sup>2</sup>					
	Before withdrawal plan (day 42)					
	T1	T2	T3	T4	SE	Sign. <sup>4</sup>
C14:0	1.88	1.78	2.52	2.85	0.378	NS
C16:0	11.06	11.05	11.35	10.92	1.074	NS
C18:0	12.14	11.44	10.56	10.37	0.784	NS
C24:0	0.64 <sup>c</sup>	1.07 <sup>cb</sup>	2.06 <sup>a</sup>	1.65 <sup>ab</sup>	0.204	**
∑ Saturated FAs	25.73	25.11	23.95	25.79	1.252	NS
C16:1 (n-7, cis-9)	2.04	1.59	1.06	1.16	0.371	NS
C18:1 (n-9, cis-9)	27.02	25.98	22.99	21.70	2.117	**
C18:1 (n-7, trans-9)	2.05 <sup>a</sup>	1.37 <sup>ab</sup>	1.27 <sup>cb</sup>	0.58 <sup>c</sup>	0.240	*
C20:1 (n-9, cis-11)	0.37 <sup>b</sup>	0.97 <sup>a</sup>	0.94 <sup>a</sup>	1.29 <sup>a</sup>	0.150	**
∑ Monounsaturated FAs	31.00	28.95	26.82	23.45	0.478	NS
C18:2 (n-6 cis-9,12) (LA)	0.92 <sup>b</sup>	0.94 <sup>b</sup>	1.94 <sup>a</sup>	2.68 <sup>a</sup>	0.312	**
C20:4 (n-6, cis- 5,8,11,14) (AA)	0.77	0.57	0.44	0.80	0.176	NS
∑ (n-6)	2.02 <sup>b</sup>	2.50 <sup>b</sup>	3.33 <sup>b</sup>	4.85 <sup>a</sup>	0.466	**
C18:3 (n-3, cis-6,9,12) (ALA)	0.85 <sup>b</sup>	0.77 <sup>b</sup>	1.14 <sup>b</sup>	2.16 <sup>a</sup>	0.263	*
C20:5 (n-3, cis-5,8,11,14,17) (EPA)	0.10 <sup>c</sup>	1.36 <sup>cb</sup>	2.68 <sup>ab</sup>	3.88 <sup>a</sup>	0.478	***
C22:5 (n-3, cis-7, 10, 13, 16, 19)	0.00 <sup>b</sup>	0.02 <sup>b</sup>	0.06 <sup>ab</sup>	0.29 <sup>a</sup>	0.082	NS
C22:6 (n-3, cis-4,7,10,13,16,19) (DHA)	0.00 <sup>a</sup>	0.15 <sup>b</sup>	0.66 <sup>c</sup>	1.51 <sup>a</sup>	0.127	***
∑ (n-3)	0.95 <sup>c</sup>	2.31 <sup>c</sup>	4.55 <sup>b</sup>	7.64 <sup>a</sup>	0.643	***
∑ Polyunsaturated FAs	2.97 <sup>c</sup>	4.81 <sup>c</sup>	7.88 <sup>b</sup>	12.50 <sup>a</sup>	0.932	***
Sum fatty acids	59.92	58.87	58.65	61.74	2.542	NS
SAT: PUFA	9.63 <sup>a</sup>	6.02 <sup>ab</sup>	3.08 <sup>b</sup>	2.17 <sup>b</sup>	1.300	**
MUFA: PUFA	10.97 <sup>a</sup>	6.06 <sup>b</sup>	3.43 <sup>c</sup>	1.99 <sup>c</sup>	0.787	***
n-6: n-3	3.40 <sup>a</sup>	1.18 <sup>b</sup>	0.77 <sup>b</sup>	0.64 <sup>b</sup>	0.673	*

<sup>a-d</sup> Values in the same row with no common superscript differ significantly. <sup>1</sup>Values represent the means of ten observations per treatment and their standard errors. <sup>2</sup>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. AA=arachidonic acid, ALA= $\alpha$ -linolenic acid, EPA=eicosapentaenoic acid, DPA = docosapentaenoic acid, DHA=docosahexanoic acid. Others fatty acids that were not detected. Sign.= \*  $P < 0.05$ ; \*\*  $P < 0.001$ ; \*\*\*  $P < 0.001$ .

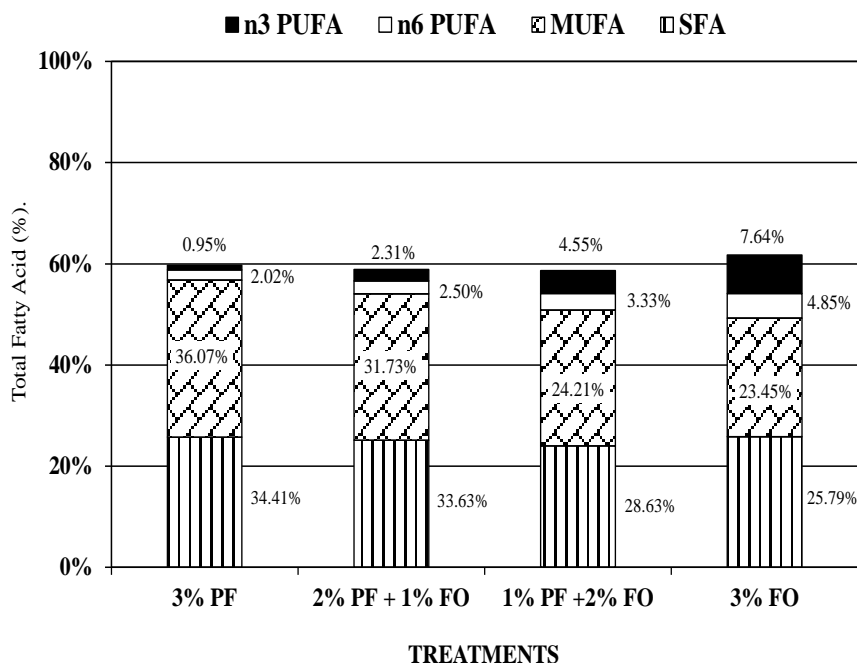
### Total FA Ratio

The SAT: PUFA, MUFA: PUFA and n-6: n-3 ratios were reduced ( $P < 0.01$ ) in tissue by replacement of PF with FO in the diet. These findings are in agreement with the results of those that used menhaden-oil-enriched diets

(Cherian et al., 1996) and replaced or combined FO in diets (Lopez-Ferrer et al., 1999). It is clearly shown in table 5 that FO replacement in dietary fat declined n-6: n-3 ratio and caused omega-3-enriched chicken thigh. A FO-enriched diet could make poultry meat an attractive alternative for those consumers that are not willing to include fish products in their daily diets. Therefore, poultry meat in comparison with an equal sized portion of canned tuna fish could provide between 2.7 and 3.5 times more n-3 PUFA per edible portion of breast and thigh meats, respectively (Sinclair et al., 1998). In addition, an omega-3-enriched meat known to healthy product might guarantee a notable decline of the cholesterol and triglyceride levels that are linked to the total fatty acid ratios or their saturation and unsaturation types.

### Omega-3 enrichment of broiler dark meat

Regarding International Life Sciences Institute (1995) recommendations in the human daily requirements to LC n-3 PUFA, serving of 200 g of chicken thigh meat fed on 3% FO diet, would provide approximately 160 mg of EPA and 1.100 mg of total n-3 FA. In the current experiment, 117 mg of ALA, 271.87 mg of EPA and 69.75 mg of DHA would provide from total n-3 in 200 g of thigh meat of T3-fed chicks (Figure 1). The real n-3 enrichment of meat could therefore accomplish by marine origins like FO rich in LC n-3 PUFA; however, it is highly susceptible to oxidative process and may harm human health. Vitamin E is well-known as a good defender against lipid oxidation (Morrissey et al., 1998). Hence, for adjustment of human lipid metabolism with a high EPA content of n-3-enriched broiler meats, fortifying the diet with antioxidants like vitamin E levels, e.g. 100 IU in broiler diets - relatively high levels can be fed- is necessary (Surai and Spark, 2000). Otherwise, EPA and DHA contents in meat could be limited and off-flavors could occur. Consequently, diets enriched with n-3 PUFA and low or non-vitamin E may exacerbate the formation of reactive oxygen species in chicken breast muscle that could create from the reaction of unsaturated fatty acids with transition metals such as iron oxidation (Morrissey et al., 1998).



**Figure 1 - Fatty acid composition of thighs meat of male broiler fed from 3% fat. Values of figures are correspondent to those shown in Table 4. PF= poultry fat, FO= fish oil; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids. T1=3% PF; T2= 2% PF + 1% FO; T3= 1% PF + 2% FO; T4= 3% FO.**

### Sensory evaluation of omega-3 enriched dark meats

Table 6 is shown panel test results. The sensory quality parameters of thigh meat such as flavor and normal smell of chickens fed on solely 3% FO (T4) did significantly ( $P < 0.01$ ) show lower scores (2.73 and 2.35, respectively) after 1 month of storage, but the juiciness and tenderness were acceptable (4.20 and 3.86, respectively). The consumer's acceptance of T2 and T3-cooked thighs were better than T4. Average consumer's acceptance ratings for treatment 3 (1% PF + 2% FO) ranged from 3 to 7, which correspond to a score of "neither like acceptable" to "like good." Consumers commented that thigh samples had more aftertastes which are probably attributable to the greater occurrence of fat-associated flavor volatiles in dark meat (Betti et al., 2009). The highest n-3 FAs content, lowest CHOL and TG levels and a good growth rate were achieved in this group 4 broilers who received omega-3

enriched and Vit-E fortified diet after 1 week withdrawal plan, but panelists were reported the serious sensory losses for T4 cooked meats that couldn't satisfied panelists while, T3 samples presented most advantages with good flavor. In n-3-meat enrichment method of Lopez-Ferrer et al. (2001), withdrawal design (use of 4% FO for 3 or 4 week, followed by a mixture of 3% linseed oil and 1% FO) was more efficient than n-3-enrichment with 2% FO throughout the experimental period. By their method, higher EPA, DPA, and DHA levels in tissues as well as improved organoleptic quality of enriched meat were detected.

In present study, all factors including loss of water holding capacity, tenderness, and flavor need to take into consideration to evaluate the overall quality of n-3 PUFA enriched products for commercialization. Hence, optimization based solely on origins rich in n-3 PUFA enrichment in chicken meat could lead to lower meat quality attributes. Regarding to our findings and current information, such strategies like the incorporation of FO in the diet for 21 d followed by its withdrawal for 1 week before slaughter, may be a preferable option to exploit enriched meats especially thighs that need a shorter time to enrichment with the greater fat content. The study of n-3-enriched thighs meat underlying the effects of vitamin E inclusion and fish oil withdrawal can facilitate a better understanding of the changes that occur in dark tissues following reuse or *de novo* synthesis in the liver or tissue.

**Table 6 - Consumer panelists and acceptability scores of cooked thigh meats after 1 month of storage at - 4 °C according to difference of fish oil in diets<sup>1</sup>**

Thigh meat	Experimental diets <sup>2</sup>	T1	T2	T3	T4	SEM	Sig. <sup>3</sup>
Flavor		4.13 <sup>b</sup>	4.25 <sup>a</sup>	3.93 <sup>c</sup>	2.73 <sup>d</sup>	0.152	***
Normal smell		3.40 <sup>b</sup>	3.86 <sup>a</sup>	3.24 <sup>b</sup>	2.35 <sup>c</sup>	0.146	***
Juiciness		3.53 <sup>c</sup>	3.73 <sup>ba</sup>	3.93 <sup>b</sup>	4.20 <sup>a</sup>	0.161	*
Tenderness		3.60	3.70	3.73	3.86	0.174	NS
Acceptability		7.80 <sup>a</sup>	7.10 <sup>b</sup>	6.20 <sup>c</sup>	4.40 <sup>d</sup>	0.447	***

<sup>a-d</sup> Values in the same row and variable with no common superscript differ significantly. <sup>1</sup>Values are means of 15 observations (for professional taste panel and acceptability) per treatment. At 1 month of storage a freshly cooked commercial chicken meat sample stored for 1 d at -20 °C (vacuum packed) was added to the consumer test as a blind control. The consumers ranked flavor, normal smell, juiciness and tenderness of meat using a 5-point scale and the acceptability of the meats using a 9-point scale (1 = bad; 3= acceptable; 6= good; 9 = very good). Juiciness = Water-holding capacity. <sup>2</sup>T1=3% PF; T2= 2% PF + 1% FO; T3= 1% PF + 2% FO; T4= 3% FO. <sup>3</sup>Sign.= \* = P<0.05; \*\* = P<0.01; NS= P>0.05.

## CONCLUSIONS AND RECOMMENDATIONS

Diet supplementation with fish oil resulted in better n-3 enriched dark meat with lower unlike fats especially in groups 4 and 3 birds, respectively. But based on the dissatisfaction of the panelists toward T4 meats and satisfaction with cooked T3 samples (scored as good), the meat of group 3 animals fed 1%PF + 2%FO was selected as good quality n-3-enriched thigh meats. This study showed that meat enrichment based solely on FO rich in n-3 PUFA couldn't lead to reduction of meat quality detriments, while diet supplementation with combinatorial fish oil and poultry fat with adequate antioxidant for grower period by applying 1 week withdrawal plan could appropriately reduce sensory quality losses. Also, the meat of group 3 (1% PF + 2% FO) seems to be a good combinative fat for supplementation in broiler diets to reach a good growth rate for n-3 enriched dark meat product. Regarding to meat enrichment strategies of broiler chickens, the roles of the physiological mechanism of the FA deposition and vitamin E in the oxidative stability of LC n-3 PUFA sources to remove sensory losses, are substantial for produce health promoting nutritionally products, which are preferable scopes.

## DECLARATIONS

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### Ethical approval

The review board and ethics committee of *Department of Animal Science, Shabestar Islamic Azad University* approved the study protocol.

### Authors' contributions

SCh and HA participated in the design of study and performed the experiments. SCh analyzed the data, wrote and revised the manuscript for important intellectual contents. Both authors read and approved the final manuscript.



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## Competing interests

The authors declare that they have no competing interests.

## Consent to publish

Not applicable

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