

Effect of Dietary Fish Oil on Oxidative Stability and Lipid Composition of Broiler Chickens Breast and Thigh Meat

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Abstract: A study was conducted to evaluate the effect of Fish Oil (FO) on Fatty Acids (FA) profile and oxidative stability of broiler meat during storage. About 215 days old broiler chicks from a commercial hybrid (Cobb 500) were divided into 12 groups of 18 birds each. Total four diets were provided with of 0.0, 1.5, 3.0 and 6% of fish oil. Each diet was randomly assigned to 3 groups of birds for 42 days. Birds had *ad libitum* access to feed and water throughout the experiment. Two birds from each replicate were randomly selected and slaughtered on day 42 for meat FA determination. The omega-3 fatty acid profiles Linolenic Acid (LNA) and long chain unsaturated fatty acid, Eicosapentaenoic Acid (EPA), Docosahexaenoic Acid (DHA)) of skinless breast meat and thigh meat were determined. Oxidation stability of samples was determined after storing in -20°C for 1-3 months or in 4°C for 7, 14, 21 and 28 days. Inclusion of FO in the diets significantly ($p < 0.01$) increased LNA, EPA and DHA value in breast and thigh meat. The birds in diet contained 6% fed group had the highest level of n-3 fatty in breast and thigh. Lipid oxidation (malondialdehyde concentration) in breast and thigh meat after storage was higher in birds fed supplemented of FO diet than those fed control diet ($p < 0.05$). These results demonstrated that the supplementation FO in broiler diet may increase long-chain n-3 PUFA content of chicken meat. Supplementation of 3% fish oil led to enrich the meat with n-3 FA with little deterioration of oxidative stability. Addition of >3% FO to diet increased the level of meat n-3 content that was coincided with increase in oxidative susceptibility.

Key words: Long chain unsaturated fatty acid, fish oil, oxidation stability, broiler meat, oxidative susceptibility, significantly

INTRODUCTION

Evidences from epidemiological studies and clinical trials have emphasized on the reduction risk of heart disease long-chain polyunsaturated fatty acids n-3 (LC PUFA; n-3), Eicosapentenoic Acid (EPA; 20:5) and Docosahexanoic Acid (DHA; 22:6) (Kinsella *et al.*, 1990). The Fatty Acid (FA) composition of broiler meat may be modified considerably by modifying the fatty acid content of diet.

It seems that the enrichment of poultry meat with PUFA, particularly with LC- n-3, from vegetable oil sources may be less effective than marine oils. The content of n-3 fatty acids of marine oils are composed of EPA and DHA in a variable amount but generally in high proportion whereas vegetable oils contain α -linolenic acid (C18:3 LNA) whose conversion to longer-chain derivatives and deposition in peripheral tissues is not

sufficient to give nutritionally valuable modified products (Cherian and Sim, 1991). Many studies have examined the effects of dietary LC-PUFA, supplied as Fish Oil (FO) or fish meal, on the FA composition of the broiler carcass (Bou *et al.*, 2004; Cortinas *et al.*, 2005; Lopez-Ferrer *et al.*, 1999, 2001). However, higher PUFA content of poultry meat increases the degree of unsaturation as a result increasing the susceptibility to oxidation.

In general, oxidation is influenced by dietary factors such as fat composition (Huang *et al.*, 1990), storage times (Grau *et al.*, 2001) and by the type of muscle involved (Ajuyah *et al.*, 1993). With regard to the measurement of Malondialdehyde (MDA) as secondary product of oxidation, the aqueous acid extraction method seems to be the most appropriate for routine assessment of lipid oxidation in meat and meat products (Grau *et al.*, 2001). The objective of this study was to evaluate the effects of different levels of fish oil on type and amount

of FA composition in thigh, breast and whole carcass and the oxidative stability of broiler meat stored in refrigerator and freezer over time.

MATERIALS AND METHODS

Birds and diets: About 216 days old broiler chicks from a commercial hybrid (Cobb 500) were divided into 12 groups of 18 birds each. Four diets were provided with of 0% (control), 1.5, 3 and 6% of fish oil. Each diet was randomly fed to 3 groups for 42 days. Birds had *ad libitum* access to feed and water throughout the experiment. Diets were formulated to be isocaloric and isonitrogenous according to the National Research Council (NRC, 1994) recommendation (Table 1).

Sample preparation: Two birds from each replicate (6 birds per treatment) were slaughtered and weighed after removal of the head, feet and abdominal fat and then was cut into 2 equal parts (left and right sides) at day 42 of age. The right side (skinless breast and thigh) was hand-deboned and used to determine the FA composition and oxidation by Thiobarbituric Acid Reactive substance (TBAR_s). The skinless breast and thigh samples were mixed and packed in plastic bags (approximately 20 g bag⁻¹) and stored at -20°C until analysis. The samples which used to study the oxidation reaction by TBAR_s were divided in seven groups and were stored at two different temperatures (4 and -20°C). Four samples were stored at 4°C in refrigerator for 4 weeks and tested on days 7, 14, 21 and 28. There samples were stored at -20°C in frozen condition for 3 months and tested for TBA value determination on days: 30, 60 and 90.

Fatty acid and fat content: The total lipid of diets and tissues was extracted according to the method of Folch *et al.* (1957). The fatty acid compositions of diet and tissue samples were determined by gas chromatography according to the method presented by Metcalf *et al.* (1996). The lipid composition of feed and meat samples was determined by gas chromatography in a UNICAM (Ion Path, Road Three, Winford, Cheshire, CW7 3GA, United Kingdom) system equipped with a BPX70 fused silica capillary column (SGE capillary column, length 30 m, i.d. 0.22 mm; 70% cyanopropyl polysilphenylene-siloxane stationary phase SGE analytical Science Pty Ltd. 7 Argent Place, Ringwood Victoria 3134, Australia) film and a flame ionization detector. Each FA was identified in the form of a methyl ester by comparing the retention times with the internal standard (Pentadecanoic acid, Merck-Darrm Germany).

Table 1: Ingredients and compositions of the basal diets

Items	Percentage
Ingredients	
Corn	56.90
Soybean meal	33.50
Corn gluten meal	2.90
Limestone	0.00
Animal fat	2.75
Fish oil	0.00
Oyster	1.10
DCP	2.00
Sodium chloride	0.30
Vitamin premix ¹	0.25
Vitamin mineral ²	0.25
Lysine-L	0.01
Methionine-D-L	0.10
Calculated nutrient content	
Dry matter (%)	89.03
Crude fat (%)	6.10
Crude portion (%)	21.56
ME (kcal kg ⁻¹)	3000.00
Calcium	1.02
Available P	0.45
Methionine+Cystine	0.86
Lysine	1.15

¹Mineral premix provided per kg of ration with 50 mg Fe, 70 mg Mn, 50 mg Zn, 7mg Cu, 0.4 mg Co, 0.17 mg Se and 0.75 mg I. ² Vitamin premix provided per kg of ration with 6,000,000 IU vitamin A, 1,500,000 IU vitamin D3, 15,000 IU vitamin E, 2.5 mg vitamin K3, 0.02 mg vitamin B12, 3,000 mg riboflavin, 7000 mg pantothenic acid, 2500 mg niacin, 500 mg folic acid 8000 mg biotin

Also, fat content of the ground breast and thigh samples were determined by AOAC (2000)'s method.

Determination of TBAR_s value: Breast and thigh samples from right side were used to study the oxidation susceptibility. The extent of meat samples peroxides were assessed by measuring TBAR_s according to the method described by Grau *et al.* (2003). The TBAR_s were expressed as µg Malonaldehyde (MDA) per g of fresh sample.

Statistical analysis: Data were analyzed by analysis of variance in a factorial arrangement with 3 levels of fish oil where meat shelf life was under consideration the third factor in model was the storage time and simple linear regression analysis using the procedures described by the Statistical Analyses System (SAS Institute, 1999). Significant differences among treatments were determined according to GLM procedure. Mean significant differences (<0.05) were compared, using Duncan's method of the same statistical package.

Fatty acid composition: The Fatty Acid (FA) composition of the experimental diets is shown in Table 2. The FA composition of the diets, particularly long-chain n-3 PUFA were modified and increased based on the dietary

Table 2: Fatty acid composition of starter, grower and finisher diets (g kg⁻¹)^{1,2}

Diets	Fatty acid															
	Fish oil (%)	C ₁₄	C _{16:0}	C _{16:1}	C _{18:0}	C _{18:1}	C _{18:2}	C _{18:3}	C _{20:3n-5}	C _{22:3n-6}	SFA ³	MUFA ⁴	PUFA ⁵	n-3	n-6	n-6/n-3
Starter	0.0	1.39	32.16	1.13	15.98	25.44	24.99	1.8	0.08	0.00	49.54	26.57	26.73	1.88	24.99	13.29
	1.5	1.79	31.29	2.94	12.90	25.37	24.01	1.94	1.27	2.57	45.98	28.31	29.79	5.78	24.01	4.38
	3.0	2.25	28.78	4.37	13.50	24.58	22.32	2.20	1.64	3.11	44.54	28.95	32.69	7.00	22.32	3.23
	6.0	3.38	27.40	5.84	7.96	24.15	19.58	2.45	2.95	7.85	38.75	30.03	33.10	13.19	19.58	1.61
Grower	0.0	1.39	32.16	1.13	15.98	25.44	25.85	1.94	0.08	0.00	49.54	27.70	26.97	2.04	25.85	12.69
	1.5	1.79	31.29	2.54	12.90	26.17	24.80	2.04	1.27	2.57	45.98	28.75	30.89	5.88	24.80	4.38
	3.0	1.89	27.79	4.37	13.50	24.88	22.32	2.41	2.26	5.30	43.14	29.15	32.29	6.80	22.32	3.28
	6.0	2.00	26.40	5.84	7.96	24.15	19.58	2.60	2.95	7.85	36.36	31.69	32.98	13.39	19.58	1.58
Finisher	0.0	2.00	33.18	1.19	15.24	25.55	26.74	1.74	0.06	0.00	50.42	26.74	28.56	1.80	26.74	14.19
	1.5	2.00	30.88	3.15	11.92	25.10	24.26	2.20	1.18	2.26	44.80	28.26	31.09	5.64	24.26	4.30
	3.0	3.00	29.76	4.72	11.07	24.84	23.96	2.27	1.89	4.42	43.83	29.56	34.52	8.58	23.96	2.73
	6.0	3.00	27.62	5.90	8.87	24.06	21.06	2.47	2.85	6.66	39.49	29.96	36.64	11.99	21.06	1.70

¹The values are means of triplicate determinations. ²Fish oil was provided by Mehregan Khazar, Rasht, Iran. ³SFA = Saturated Fatty Acid, ⁴MUFA = Mono Unsaturated Fatty Acid, ⁵PUFA = Poly Unsaturated Fatty Acid

Table 3: Percent of Ether Extract (EE%) and fatty acid composition of breast and thigh samples of chickens fed diets contained various level of fish oil (g kg⁻¹ meat)

Diets	Breast																
	fish oil (%)	Lipid	C ₁₄	C _{16:0}	C _{16:1}	C _{18:0}	C _{18:1}	C _{18:2}	C _{18:3}	C _{20:3n-5}	C _{22:3n-6}	SFA	MUFA	PUFA	n-3	n-6	n-6/n-3
0.0	2.66	0.12	2.61	1.88	0.92	2.84	1.62 ^b	0.03 ^b	0.08 ^b	0.03 ^c	0.03 ^c	3.65	3.72	1.76 ^b	0.15 ^c	1.66 ^b	11.95 ^a
1.5	2.79	0.22	2.40	1.07	0.96	2.32	1.75 ^b	0.03 ^b	0.10 ^b	0.31 ^{ab}	3.59	3.41	2.16 ^b	0.43 ^b	1.75 ^b	4.06 ^b	
3.0	2.71	0.13	2.09	1.63	0.82	1.41	2.36 ^a	0.09 ^a	0.17 ^{ab}	0.34 ^b	3.04	3.04	2.96 ^a	0.60 ^b	2.36 ^a	3.86 ^b	
6.0	2.50	0.33	2.85	1.15	0.36	1.54	2.61 ^a	0.16 ^a	0.28 ^a	0.82 ^a	3.54	2.89	3.89 ^a	1.26 ^a	2.67 ^a	2.34 ^b	
SEM	0.09	0.31	0.37	0.23	0.11	0.88	0.18	0.02	0.23	0.45	0.55	1.11	1.12	0.14	0.18	1.28	
Significant	Ns	Ns	Ns	Ns	Ns	Ns	**	**	**	**	Ns	Ns	**	**	**	*	
Diets	Thigh																
	fish oil (%)	Lipid	C ₁₄	C _{16:0}	C _{16:1}	C _{18:0}	C _{18:1}	C _{18:2}	C _{18:3}	C _{20:3n-5}	C _{22:3n-6}	SFA	MUFA	PUFA	n-3	n-6	n-6/n-3
0.0	8.55 ^a	1.31	14.86	9.38	3.56	25.12	6.36	0.26 ^b	0.07 ^c	0.07 ^c	19.72 ^a	34.06	6.75	0.33 ^c	6.42	19.22 ^a	
1.5	8.60 ^a	1.03	13.63	7.93	3.06	25.00	6.14	0.46 ^b	0.48 ^b	0.98 ^{ab}	17.72 ^a	33.05	6.15	1.93 ^b	6.15	4.17 ^b	
3.0	8.24 ^b	0.90	13.54	7.28	2.42	24.98	7.43	0.50 ^b	1.03 ^a	1.71 ^{ab}	16.86 ^{ab}	32.26	10.75	3.25 ^a	7.50	2.48 ^b	
6.0	8.10 ^b	0.73	12.01	6.65	2.23	22.23	7.27	0.65 ^a	1.01 ^a	2.07	14.97 ^b	28.28	11.06	3.73 ^a	7.33	1.93 ^b	
SEM	0.09	0.31	0.37	0.23	0.11	0.88	0.18	0.02	0.23	0.45	1.2	1.11	0.75	0.14	0.18	1.28	
Significant	**	Ns	Ns	Ns	Ns	Ns	Ns	**	**	**	*	Ns	Ns	**	Ns		

^{a-c}Means in the same column for every trait with no common superscript differ significantly. NS>0.05, *p<0.05, ** p<0.01

fish oil level (0.0<1.5<3<6%). The total n-3, PUFA and Monounsaturated Fatty Acid (MUFA) increased when the level of fish oil in diet increased and the ratio of n-6: n-3 and Saturated Fatty Acid (SFA) decreased. The fatty acid profile of breast and thigh lipid in birds fed graded level of FO were significantly (p<0.01) changed after 3 months of storage in -20°C (Table 3). The EPA and DHA fatty acids were significantly higher (p<0.01) in breast and thigh meat of chicken fed FO, compared to those fed control diet and birds fed 6% FO diet produced the highest values in both meat protein.

Supplementation of basal diet with 6% fish oil caused a 4 and 29 folds increase in EPA and DHA of breast, respectively and 15 and 30 folds increase in thigh meat as compared to control ones. Linolenic acid was significantly higher (p<0.01) in breast and thigh meat of chicken fed fish oil, compared to the control diet. Deposition of total n-3 PUFA in thigh was higher than that in breast samples (Table 3). Thigh deposition of LNA increased from 0.26-0.65 (g kg⁻¹ meat) and breast deposition ranged from 0.03-0.16 (g kg⁻¹ meat) with increase of diet fish oil. The

higher deposition of LNA, EPA and DHA were observed in thigh compared to those in breast (statistical comparison was not performed). The Linoleic Acid (LA) and n-6 PUFA in breast meat were significantly (p<0.01) increased with increase of diet fish oil whereas these fatty acids were not significantly changed in thigh meat. The LA and n-6 PUFA decreased with increasing level of fish oil in diet. Addition of FO to diet significantly (p<0.01) lowered the n-6: n-3 ratio of meat compared to those fed control diet (Table 3).

Breast total Saturated Fatty Acid (SFA) content remained constant whereas it was significantly lowered in thigh muscle (p<0.01) when birds fed the fish oil diets (Table 3). The results indicated that the broiler chickens have limited ability to alter the SFA content of breast muscle. The decreased in breast and thigh total MUFA content with an increase in dietary fish oil were only numerical. The MUFA content of breast and thigh meat was decreased when n-3 PUFA increased (Table 3). The regression analysis of FA profile of thigh and breast samples as influenced by to the percent of diet fish

Table 4: The relationship between diet fish oil level and linolenic acid, EPA, DHA and n-3 in breast and meat of broiler chickens (x = percent fish oil, y = fatty acid (g kg⁻¹ meat))

Independent variables (mg kg ⁻¹)	Breast			Thigh		
	Equation	Significant	R ²	Equation	Significant	R ²
C 18:3	Y = 0.03x+0.03	**	0.48	Y = 0.31x+0.06	*	0.43
EPA	Y = 0.08x+0.02	*	0.58	Y = 0.24x+0.16	**	0.51
DHA	Y = 0.05x+0.12	**	0.71	Y = 0.32x+0.33	**	0.62
n-3	Y = 0.16x+0.17	**	0.71	Y = 0.88x+0.57	**	0.61

*p<0.05, **p<0.01

Table 5: Effect of diet fish oil and storage refrigerated time (+4°C) on thiobarbituric acid reactive substance values of thigh and breast of chickens (µg of malondialdehyde/g meat)

Diet fish oil (%)	Thigh	Breast
0.0	0.41 ^d	0.35 ^d
1.5	0.51 ^c	0.41 ^c
3.0	0.85 ^b	0.68 ^b
6.0	1.71 ^a	1.24 ^a
p-value	**	**
Refrigerated time (+4°C)		
1 week	0.39 ^d	0.30 ^d
2 week	0.64 ^c	0.50 ^c
3 week	1.00 ^b	0.72 ^b
4 week	1.45 ^a	1.18 ^a
p-value	**	***
Diet fish oil *	NS	NS
Refrigerated time		
SEM	0.04	0.03

p<0.01, *p<0.001

oil is shown in Table 4. The higher correlation between FA profile of thigh and breast samples were observed in DHA and n-3 with 0.61 and 0.71%, respectively.

Lipid oxidation in thigh meat and fat content: The effect of dietary fish oil and storage time on MDA values (as a secondary product of oxidation) in thigh and breast meat were shown in Table 4 and 5. A higher TBAR_s content was found in chickens receiving higher levels of fish oil (6%) as compared to control group. Supplementation of 6% FO in diet caused higher lipid oxidation in breast and thigh (3 and 4 folds, respectively) compared to these fed control diet. The higher TBAR_s value was found in thigh as compared to breast meat.

The fat content of breast and thigh meat is shown as percentage of fresh tissue in Table 3. The thigh meat of control groups and those fed 1.5% FO diet had significantly higher fat content than birds received 3 and 6% FO diet (p<0.01). The decreased in breast fat content of birds fed diet contained 6% FO diet was only numerically lower than fed 3% or lower FO diets.

RESULTS AND DISCUSSION

Fatty acid composition: The FO used in this study was a rich source of LC n-3, EPA (5.74 mg g⁻¹) and DHA (14.55 mg g⁻¹). Fatty acid composition of chick's tissue

generally, reflected the fatty acid profile of the diet. Similar to the study, the increased deposition of n-3 PUFA in birds fed Menhaden oil have been reported by other researches (Schreiner *et al.*, 2005; Cortinas *et al.*, 2004; Lopez-Ferrer *et al.*, 1999, 2001).

Likewise, the higher differences in fatty acid deposition of thigh as compared to breast meat had been reported by Cortinas *et al.* (2004). However in contrast to the finding, other investigator reported higher deposition of LC PUFA in breast muscle compared with thigh muscle (Crespo and Esteve-Garcua, 2001) and higher deposition of LNA in thigh muscle compared to breast muscle (Lopez-Ferrer *et al.*, 1999; Gonzalez-Esquerria and Leeson, 2000). The difference in FA profile could be attributed to the type of calculation basis of FA in these tissues (percent or absolute value). The Linolenic acid and n-6 PUFA in meat were decreased with increasing level of FO in diet.

This revers relationship between fatty acid of tissue and diet could be explained by the competition of fatty acid (LNA and LA) for both enzyme system responsible for the elongation and desaturation to from the long-chain metabolite (Cortinas *et al.*, 2004). The reduced ratio of n-6: n-3 in broilers meat with higher diet FO in this experiment was in agreement with the finding of Gonzalez-Esquerria and Leeson (2000) and Schreiner *et al.* (2005).

It is observed that the increased in n-3 content of meat rather than decrease in the level of n-6 FA was responsible for the decrease in n6:n3 ratio. The decrease in oleic (C18:1) and palmitoleic (C16:1) acids is probably explained by the inhibitory effect of PUFA against Δ9-desaturase activity, preventing the formation of MUFA from the precursors.

The Δ9-desaturase is the key enzyme required to convert palmitic (C16:0) to palmitoleic acid and stearic (C18:0) to oleic acid. This interaction between MUFA and PUFA has been reported in other animals as well (Ayerza *et al.*, 2002). The relationships of n-3 LC-PUFA fatty acids in diet with meat were higher rather than their precursor, LNA. The higher R² value for LC-PUFA was observed in study by Lopez-Ferrer *et al.* (1999) compared

to the study. They were used level 0.0-8.2% FO in diet and consequently, the dietary levels of EPA from 0.0-15.8% and DHA from 0.0-9.1%. These dietary levels were highly correlated with the n-3 LC-PUFA content in thigh meat ($R^2 = 0.99$, $y = 0.51x - 0.06$, $p < 0.001$ for EPA; $R^2 = 0.91$, $y = 0.71x - 0.57$, $p < 0.001$ for DHA) without reaching a plateau of the deposited value of the n-3 LC-PUFA in this specific peripheral tissue. The higher correlation between FA profile of thigh and breast samples were observed in DHA and n-3 with 0.61 and 0.71%, respectively.

Fat content and lipid oxidation in thigh and breast meat:

Several studies reported that the feeding of polyunsaturated fatty acids to chickens resulted in reduced total carcass fat as compared to birds fed saturated fatty acid sources (Sanz *et al.*, 2000; Crespo and Esteve-Garcua, 2001; Newman *et al.*, 2002).

Some researchers have suggested that the lower fat deposition in broilers fed polyunsaturated fats was in part due to the increased rate of lipid catabolism and by a decreased rate of FA synthesis (Sanz *et al.*, 2000). Similarly, other researchers reported a significantly lower metabolic lipid oxidation and consequently a lower thermogenesis in tissues of rats fed saturated fats than in rats fed unsaturated fats (Shimomura *et al.*, 1990).

Nevertheless, further studies are needed on why body fat deposition is reduced as dietary polyunsaturated fatty acids increased. The higher TBA of chicken meat from unsaturated fat sources has been reported by some investigators (Grau *et al.*, 2001; Bou *et al.*, 2004; Cortinas *et al.*, 2005). This finding may be related to the fatty acid composition of meats (Table 3) because the short-chain aldehydes that mainly contribute to the oxidative rancidity development in chicken meat are mainly formed from highly unsaturated fatty acids (Ajuyah *et al.*, 1993).

In contrast to the experiment Koreleski and Swiatkiewicz (2006), reported a higher TBAR_s value in breast meat of chickens fed the control diet than for those fed diets contained fish oil. The total PUFA level in stored meat of chickens fed diets contained fish oil were similar or even lower than these fed control diet.

The lower TBAR_s may be due to the use of antioxidant ethoxyquin and low level of FO (5-8 g kg⁻¹ diet) in their experiment. It is likely that ethoxyquin may also have an antioxidative effect in meat of chickens fed antioxidant containing diets.

However, few studies have been devoted to identify the optimum dose of fish oil addition which would not affect the meat quality. The slight relationship between consumer acceptability and TBA values followed sensory test was previously reported (Bou *et al.*, 2004).

CONCLUSION

It is observed that the supplementation of fish oil in diet enhanced long-chain n-3 PUFA content of chicken meat. The meat of chicken fed diet with 3% fish oil was enriched with n-3 FA with little deterioration of meat oxidative stability. However, the higher level of FO in diet increased the level of meat n-3 content coincided with increased oxidative susceptibility of enriched meat. The TBA value obtained was lower in frozen compared to refrigerated meat.

RECOMMENDATION

It is recommended determining ketone volatile by use HPLC compared to with TBA that determination aldehyde more useful for score test in the refrigerated and frozen breast and thigh samples.

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