

## Omega-3 Enrichment of Broiler Meat by Using Two Oil Seeds

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### ABSTRACT

A 42-d study was conducted to evaluate the influence of full-fat flaxseed (FS) and canola seed (CS) on broiler performance, fatty acid (FA) profile of meat, and the oxidative stability of meat during frozen storage. A total of 324 one-day old broiler chicks were randomly attributed to 6 experimental groups and fed iso-energetic and isonitrogenous diets as follows, C: control (soybean-corn); CS1: 7.5% CS; CS2: 15% CS; CS-FS: 10% FS+10% CS; FS1: 7.5% FS; FS2: 15% FS. Negative effect on performance parameters were found by diets containing FS and CS i.e. feeding oil seeds resulted in significantly ( $P < 0.01$ ) lower body weight gain and higher feed conversion ratio, compared to the control. However, no significant differences of feed consumption were shown ( $P > 0.05$ ) among treatments. Inclusion of FS and CS significantly increased ( $P < 0.01$ ) the concentration of omega-3 fatty acid ( $\alpha$ -Linolenic acid= ALA) and decreased the content of the arachidonic acid (AA). Total omega-6 to omega-3 polyunsaturated fatty acid (PUFA) ratio was significantly lower for all FS and CS fed groups compared with the control ( $P < 0.01$ ). Inclusion of FS and CS decreased the oxidative stability of raw meat (breast and thigh) during frozen storage period based on thiobarbituric acid (TBA) values ( $P < 0.01$ ). In conclusion, by adding vegetable sources of omega-3 to the broiler chicken diets, the omega-3 fatty acid content of broiler meat can be increased, which may have beneficial effects on human health.

**Keywords:** Broiler, Fatty acid, Omega-3, Oxidative stability, Performance.

### INTRODUCTION

The fatty acid composition of poultry muscle is an important quality parameter especially with respect to potentially affecting human health from poultry meat consumption. In this regard, n-3 group of poly-unsaturated fatty acids (PUFA) is one of the most important fatty acid (FA) groups. Dietary n-3 FA has been reported to aid in the prevention of certain diseases, especially cardiovascular disorders (De Lorgeril *et al.*, 1994; Hartman, 1995; Leaf and Kang, 1998). It has been demonstrated that the FA composition of broiler meat can be altered by changing the content of the broiler's diet (Yau *et al.*, 1991).

Therefore, many studies are directed towards the manipulation of the FA composition of

broiler chicks in order to increase n-3 PUFA content and decrease n-6/n-3 ratio in poultry meat. This is desirable because of the action of n-6 PUFA as a pro-inflammatory factor and the action of n-3 PUFA as an anti-inflammatory factor on immune functions and inflammatory processes in animals and humans (Calder, 2001). Regarding the health-related effects of n-3 PUFA, it seems appropriate to provide n-3 enriched products for human consumption. The PUFA rich oil seeds can play an important role in enrichment of broiler muscles with n-3 FA. Flaxseed and canola seed are the main sources of  $\alpha$ -linolenic acid (ALA) of terrestrial origin. Several attempts have been successfully made to enrich ALA and its elongated n-3 FA in broiler muscle tissues or products by using vegetable sources in their diet (Mantzioris *et*

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al., 2000; Ayerza et al., 2002). However, due to the high un-saturation levels, chicken muscles are easily oxidized. 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., 1993a). In addition, for poultry production FS and CS might serve as an alternative source of dietary energy. However, the drawbacks with dietary inclusions of FS and CS are the presence of anti-nutritional factors and the low available nutrient content, which may limit its in poultry diets. The literature shows that more than 10-15% dietary inclusion of FS may depress broiler growth (Ajuyah et al., 1993b; Najib and Al-Khateeb, 2004). Since canola seed and flaxseed are widely produced in Iran, it is expected that they have the potential to become a major non-conventional feedstuff for poultry due to their high energy, omega-3 FA content and the lower cost associated with their inclusion compared to the conventional energy sources.

The objective of this study was to compare the effects of diets containing various levels of oil seeds with high proportions of n-3 PUFA on the performance, fatty acid pattern of chicken muscles, and stability of broiler meat as indicated by malondialdehyde (MDA) levels.

## MATERIALS AND METHODS

All animal care was conducted according to the University approved methods. Three hundred and twenty four 1-d old broiler chicks (Cobb 500) were divided randomly into six dietary treatments. Each of the six treatments was replicated 3 times with 18 chicks per each. Birds were housed in deep litter pens (1x2 m). Environmental temperature was set at 32°C on d-1 and lowered stepwise to 23-24°C for the rest of the experiment. Relative humidity and ventilation were arranged under standard conditions. Birds were fed with the experimental diets from day 1 until 42 d with two-time periods included: the starting and finishing periods (1 to 21 days and 22 to 42

days), respectively. Diets were formulated according to the recommendations of the National Research Council (1994). Feed and water were provided *ad libitum* throughout the experiment. Diets were modulation in iso-energetic and iso-nitrogenous. The following six dietary treatments were used: C: control diet (soybean- corn); CS1: 7.5 % ground canola seed; CS2: 15% ground canola seed; CS-FS: 10% ground flaxseed+10% ground canola seed; FS1: 7.5% ground flaxseed; FS2: 15% ground flaxseed. Composition of the diets is shown in Table 1. Two birds were randomly selected from each replicate (6 birds per treatment) for tissue sampling on day 42. After slaughtering, the birds were apportioned into commercial cuts. Breast muscle (white meat) and leg muscle (dark meat) were collected from each bird and two tissue samples were taken from each. The first set of samples was used for oxidation determination of thiobarbituric acid reactive substances (TBARS). The samples were stored at 4°C for 1, 7, 14 or 21 days until TBARS value determination. The second set of samples was stored at -20°C, and used for FA and TBARS determination after storage for 1, 2, and 3 months.

## Performance Record

Body weight of all birds in each pen was recorded on days 7, 14, 21, 28, 35 and 42, without withdrawing feed before taking the weight. Feed consumption was recorded weekly for birds in each pen. Weight gain and feed conversion ratio were calculated on a pen basis at the end of each feeding period.

## Fatty Acid Content

The FA composition of the feed, thigh, and breast samples was determined by gas chromatography according to the method described by Metcalfe et al. (1996). The FA content was determined using a gas chromatograph (Unicam 4600, USA)

**Table 1.** Ingredients and composition of experimental diets.

Ingredients	Starter						Finisher					
	C <sup>a</sup>	CS1 <sup>b</sup>	CS2 <sup>c</sup>	CS-FS <sup>d</sup>	FS1 <sup>e</sup>	FS2 <sup>f</sup>	C	CS1	CS2	CS-FS	FS1	FS2
Corn	56.30	55.00	47.30	43.00	53.70	50.00	68.50	62.70	56.60	53.20	62.30	58.00
Soybean meal	34.50	31.30	30.00	28.30	31.70	29.10	23.40	20.10	18.00	15.90	19.90	18.00
Corn gluten meal	2.30	2.10	1.45	1.50	1.95	1.70	1.80	2.30	2.00	2.20	2.50	1.80
Flaxseed	0.00	0.00	0.00	10.00	7.50	15.00	0.00	0.00	0.00	10.00	7.50	15.00
Canola seed	0.00	7.50	15.00	10.00	0.00	0.00	0.00	7.50	15.00	10.00	0.00	0.00
Tallow	3.22	0.47	0.30	0.40	1.52	0.30	2.80	1.70	0.80	0.50	2.30	1.20
Dicalcium phosphate	2.00	2.00	2.00	2.00	2.00	2.00	1.80	1.80	1.80	1.80	1.80	1.80
Salt	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32
Oyster	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Vit and Min premix <sup>1,2</sup>	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
DL-Met	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
L-Lys	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
Fine sand	0.08	0.03	2.35	3.20	0.03	0.30	0.10	2.30	3.80	4.40	2.10	2.60
Calculated analysis												
ME (kcal kg <sup>-1</sup> )	3000	3000	3000	3000	3000	3000	3100	3100	3100	3100	3100	3100
CP (%)	21.56	21.56	21.56	21.56	21.56	21.56	17.44	17.44	17.444	17.44	17.44	17.444
Dry matter	86.30	86.32	84.53	83.79	86.35	86.08	86.41	84.62	83.12	82.56	84.73	84.31
Fiber	3.68	3.88	4.06	4.20	3.91	4.13	3.17	3.27	3.43	3.56	3.28	3.53
Ca	0.93	0.95	1.25	1.44	0.94	1.07	0.89	1.10	1.45	1.64	1.50	1.52
Available P	0.53	0.55	0.58	0.54	0.52	0.51	0.47	0.49	0.59	0.52	0.48	0.45
Crude fat	5.69	5.86	8.37	9.70	6.41	7.58	5.64	7.30	9.14	10.10	7.44	8.69

<sup>a</sup> Basal diet; <sup>b</sup> Diet with 7.5 % canola seed; <sup>c</sup> Diet with 15 % canola seed; <sup>d</sup> Diet with 10% canola seed and 10% flaxseed;

<sup>e</sup> Diet with 7.5% flaxseed, <sup>f</sup> Diet with 15 % flaxseed.

<sup>1</sup> Vitamin premix provided per kilogram of diet: vitamin A, 7,040 IU; vitamin D3, 2,000 IU; vitamin E, 8.8 IU; vitamin K3, 1.76 mg; biotin, 0.12 mg; thiamine, 1.2 mg; riboflavin, 3.2 mg; pantothenic acid, 6.4 mg; pyridoxine, 1.97 mg; niacin, 28 mg; vitamin B12, 0.008 mg; choline, 320 mg; folic acid, 0.38 mg.

<sup>2</sup> Mineral premix provided per kilogram of diet: Mn, 60 mg; Fe, 60 mg; Zn, 51.74 mg; Cu, 4.8 mg; I, 0.69 mg; Se, 0.16 mg.

equipped with a BPX70 fused silica capillary column and a flame ionization detector.

The column head pressure of the carrier gas (Helium) was 20 psi and sample volume injected was 0.2 µL. Pentadecanoic acid (Sigma, St. Louis, MO) was used as internal standard. The FA's were identified by matching their retention times with those of their corresponding standards.

### Determination of TBARS

The extent of lipid peroxides in breast and thigh muscle samples was assessed by measuring TBARS according to the method described by Botsoglou *et al.* (1994) using third derivative spectrophotometry. The height of the third-order derivative peak that appeared at 521.5 nm was used for calculation of the

MDA concentration, as secondary oxidation product, in the samples. Tetraethoxypropane (1, 1, 3, 3- Tetraethoxy propane, T9889, 97%, Sigma, USA.) was used as a MDA precursor in the standard curve. The TBARS was expressed as micrograms of MDA per kilogram of sample.

### Statistical Analyses

The experiment was carried out as a completely randomized design with 3 replications. The pen was the experimental unit. Each variable was compared using the ANOVA procedure of SAS (1990) to assess treatment differences on FA composition and MDA levels of thigh and breast muscle. Differences among treatment means were



separated using a Duncan's multiple range test. The following model was used:

$$X_{ij} = \mu + \tau_j + \varepsilon_{ij}$$

Where,  $X_{ij}$  = The observation of the  $j^{\text{th}}$  treatment in the  $i^{\text{th}}$  pen,  $\mu$  = The overall means of the sampled observation,  $\tau_j$  = The effect of treatment,  $\varepsilon_{ij}$  = The experimental error component.

## RESULTS AND DISCUSSION

### Performance

In terms of weight gain and feed conversion efficiency, birds fed with the control diets performed significantly better ( $P < 0.01$ ) than those fed with other diets (Table 2). Broilers fed with CS2 and CS-FS diets had 11% lower body weight at the end of the test than the birds of the control diet. The final 42d feed consumption of the birds fed with the experimental diets during the test period was not affected by diet. Lower body weights and a similar feed consumption resulted in a significantly higher ( $P < 0.01$ ) feed: gain ratio for birds fed with the diets containing FS and CS. The highest feed conversion was recorded for the birds fed with CS-FS diet.

The inverse effect of high FS and CS inclusion in broiler diets on body weight gain and feed conversion has been reported

by a number of investigators (Roth-Maier *et al.*, 1998; Najib and Al-Khateeb, 2004; Talebali and Farzinpour, 2005). Ajuyah *et al.* (1993b) reported a 17% reduction in body weights when birds were fed diets containing 15% flaxseed (42 days period), and because feed consumption was similar to the control birds, a significantly higher feed conversion was reported.

The negative effects of feeding diets containing FS and CS have been attributed to the lower availability of their fat fraction (Lee *et al.*, 1991), and the presence of anti-nutritional factors (Chadha *et al.*, 1995). The existence of phytic acid in canola seed and flaxseed will cause reduction in calcium absorption in layers (Summers *et al.*, 1988) and inhibit proteolytic enzymes (Caldwell, 1992; Ravidran *et al.*, 1995). In addition, the usefulness of flaxseed and canola seed as energy dense ingredients in monogastric diets has been hampered by the level of soluble fibre which increases intestinal viscosity and leads to reduced nutrient availability by increasing passage rate (Rodriguez *et al.*, 2001). Also, the negative effect of feeding diet containing FS and CS on performance parameters may be due to a higher fat content of the diet (Table 1), which reduces digestion and absorption process and fatty acid synthesis, and increases rate of lipid catabolism (Sanz *et al.*, 2000).

**Table 2.** Broiler chicken performance in response to experimental diets<sup>1</sup>.

performance	Weight gain			Feed consumption			Feed conversion efficiency		
	1-21	22-42	1-42	1-21	22-42	1-42	1-21	22-42	1-42
Age (days)	1-21	22-42	1-42	1-21	22-42	1-42	1-21	22-42	1-42
C	34.43 <sup>a</sup>	86.30 <sup>a</sup>	60.36 <sup>a</sup>	50.81 <sup>a</sup>	173.30	112.05	1.40 <sup>b</sup>	2.01 <sup>b</sup>	1.70 <sup>c</sup>
CS1	33.32 <sup>ab</sup>	80.87 <sup>bc</sup>	57.09 <sup>b</sup>	50.21 <sup>a</sup>	172.82	111.51	1.41 <sup>b</sup>	2.12 <sup>ab</sup>	1.76 <sup>bc</sup>
CS2	30.55 <sup>b</sup>	76.98 <sup>c</sup>	53.76 <sup>c</sup>	47.58 <sup>c</sup>	169.54	108.56	1.46 <sup>ab</sup>	2.18 <sup>a</sup>	1.82 <sup>ab</sup>
CS-FS	30.09 <sup>b</sup>	77.22 <sup>c</sup>	53.65 <sup>c</sup>	49.77 <sup>ab</sup>	174.72	112.25	1.52 <sup>a</sup>	2.25 <sup>a</sup>	1.88 <sup>a</sup>
FS1	32.55 <sup>ab</sup>	82.45 <sup>ab</sup>	57.50 <sup>b</sup>	49.59 <sup>ab</sup>	177.29	113.44	1.43 <sup>ab</sup>	2.12 <sup>ab</sup>	1.78 <sup>bc</sup>
FS2	31.04 <sup>b</sup>	81.47 <sup>abc</sup>	56.25 <sup>bc</sup>	48.50 <sup>bc</sup>	179.35	113.93	1.46 <sup>ab</sup>	2.18 <sup>a</sup>	1.81 <sup>ab</sup>
Significance	**	**	**	**	Ns	Ns	**	**	**
SEM	0.45	0.76	0.59	0.28	1.05	0.57	0.01	0.02	0.02

<sup>a-f</sup> Values in the same column with no common superscript differ significantly.

<sup>1</sup> C: Basal diet; CS1: Diet with 7.5 % canola seed; CS2: Diet with 15 % canola seed; CS-FS: Diet with 10% canola seed and 10% flaxseed; FS1: Diet with 7.5% flaxseed; FS2: Diet with 15 % flaxseed.

Ns:  $P > 0.05$ ; \*  $P \leq 0.05$ , \*\*  $P \leq 0.01$ .

The decreased weight gain with flaxseed and canola seed may be the result of higher dietary fiber and calcium content in these diets relative to the control diet. During digestion of fat, free fatty acids may produce a complex with calcium and formed soap, under which condition fatty acids are unavailable to the birds (Lesson and Summers, 2005). Fiber content of soybean meal is lower than the flaxseed and canola seed, whereas fiber is not very digestible by poultry (Mangold, 1934), and, therefore, its presence may impair the digestibility of other nutrients.

In addition, other antinutritional factors like toxic cyanoglycosides (limarin) and vitamin B6 antagonistic factors in flaxseed and lysine-arginine imbalance in canola seed may be related to the reduction of productive performance (Klosterman *et al.*, 1967; Summers and Leeson, 1978; Oomah *et al.*, 1992).

#### Fatty Acid Composition of Meats and Diets

The FA composition of the oil seeds and the experimental diets is shown in Tables 3 and 4. In relation to feed FA composition, when oil seeds, especially flaxseed, were added to the feed, ALA increased compared to the control group. Also, dietary PUFA to SFA ratio increased with the increase of dietary oil seed level. Fatty acid compositions of the white and dark muscle are presented in Table 5, respectively. As shown in the present study, fatty acid composition of the chick's tissues generally reflected the FA profile of the diets. Dietary supplementation with n-3 PUFA increases the content of these FA in poultry meat (Ozpinar *et al.*, 2002; Kahraman *et al.*, 2004; Shen *et al.*, 2005).

Incorporation of flaxseed and canola seed in the diet increased the proportions of the n-3 PUFA in the form of ALA. Alpha linolenic FA, an n-3 PUFA, was significantly higher ( $P < 0.01$ ) in the white and dark muscles of the broilers fed with oil seeds than in the muscles from birds fed

**Table 3.** Fatty acid composition of flaxseed and canola seed (%).

Fatty acid <sup>a</sup>	Flaxseed	Canola seed
C14	0.15	0.27
C16:0	5.70	4.52
C16:1	0.30	0.38
C18:0	4.12	1.93
C18:1	22.5	56.45
C18:2	29.6	26.4
C18:3	37	9.21
C20:4	Nd	Nd
SFA	9.97	6.72
MUFA	22.8	56.83
PUFA	66.6	35.61
n-3	37	9.21
n-6	29.6	26.4
S/U	0.11	0.07
n3/n6	1.25	0.35
Crude fat	34	40

<sup>a</sup> C18:1= Oleic acid; C18:2= Linoleic acid; C18:3= Linolenic acid ; C20:4= Arachidonic acid; n-3= Omega-3 FA; n-6= Omega-6 FA; SFA= Saturated fatty acid; MUFA= Monounsaturated fatty acid; PUFA= Polyunsaturated fatty acid; n3/n6= The ratio of n-3 to n-6 PUFA.

Nd: Not detected.

with the control diet. Also, ALA concentration in the dark and white muscles increased with increasing level of oil seed in the diet. Canola seed has a limited potential for ALA enrichment compared with flaxseed because flaxseed contains more ALA than canola seed. The latter is rich in oleic acid and contains also more linoleic acid (LA) than flaxseed. Similarly, Lopez-Ferrer *et al.* (1999), who used diets with 8.2% flaxseed and canola seed, observed moderate levels of ALA in carcasses using 8.2% canola seed in contrast to flaxseed supplementation.

The AA, a long-chain n-6 PUFA, was significantly lower ( $P < 0.05$ ) in the muscle obtained from the broilers fed with oil seed diets when compared with those fed with the control diet (Table 5). Higher concentration of ALA in muscle tissues may have decreased the formation of AA. Both LA and

**Table 4.** Fatty acid composition of diets (%).

Fatty acid <sup>a</sup>	Treatment						significance	SEM
	C <sup>a</sup>	CS1 <sup>b</sup>	CS2 <sup>c</sup>	CS-FS <sup>d</sup>	FS1 <sup>e</sup>	FS2 <sup>f</sup>		
C14	1.03 <sup>a</sup>	0.97 <sup>bc</sup>	0.90 <sup>bc</sup>	0.83 <sup>c</sup>	1.00 <sup>b</sup>	0.97 <sup>bc</sup>	**	0.03
C16:0	22.96 <sup>a</sup>	21.47 <sup>b</sup>	20.70 <sup>c</sup>	19.30 <sup>d</sup>	21.70 <sup>b</sup>	20.79 <sup>c</sup>	**	0.29
C16:1	8.43	8.77	8.67	8.33	8.60	8.50	ns	0.06
C18:0	7.37	6.10	5.03	5.33	6.47	6.10	ns	0.32
C18:1	46.63 <sup>c</sup>	48.47 <sup>bc</sup>	49.40 <sup>a</sup>	48.97 <sup>ab</sup>	47.17 <sup>bc</sup>	46.90 <sup>c</sup>	*	0.32
C18:2	11.63	12.27	12.13	12.27	11.50	11.37	ns	0.17
C18:3	0.90 <sup>f</sup>	2.00 <sup>e</sup>	3.20 <sup>d</sup>	5.03 <sup>b</sup>	3.52 <sup>c</sup>	5.47 <sup>a</sup>	**	0.39
C20:4	Nd	Nd	Nd	Nd	Nd	Nd	-	-
SFA	31.53 <sup>a</sup>	28.50 <sup>bc</sup>	26.63 <sup>cd</sup>	25.47 <sup>d</sup>	29.20 <sup>b</sup>	27.80 <sup>bcd</sup>	**	0.53
MUFA	55.07 <sup>c</sup>	57.23 <sup>ab</sup>	58.07 <sup>a</sup>	57.30 <sup>ab</sup>	55.77 <sup>bc</sup>	55.40 <sup>b</sup>	*	0.34
PUFA	13.53 <sup>c</sup>	14.27 <sup>bc</sup>	15.33 <sup>b</sup>	17.27 <sup>a</sup>	15.03 <sup>b</sup>	16.83 <sup>a</sup>	**	0.34
n-3	0.90 <sup>f</sup>	2.00 <sup>e</sup>	3.20 <sup>d</sup>	5.03 <sup>b</sup>	3.52 <sup>c</sup>	5.47 <sup>a</sup>	**	0.39
n-6	11.63	12.27	12.13	12.27	11.50	11.37	ns	0.17
S/U	0.46 <sup>a</sup>	0.40 <sup>ab</sup>	0.37 <sup>b</sup>	0.33 <sup>b</sup>	0.40 <sup>ab</sup>	0.40 <sup>ab</sup>	*	0.01
n3/n6	14.20 <sup>a</sup>	6.17 <sup>b</sup>	3.77 <sup>bc</sup>	2.43 <sup>c</sup>	3.26 <sup>c</sup>	2.07 <sup>c</sup>	*	1.04
Crude fat content	6.23 <sup>b</sup>	6.66 <sup>ab</sup>	8.07 <sup>a</sup>	7.59 <sup>ab</sup>	6.15 <sup>b</sup>	7.08 <sup>ab</sup>	**	0.3

<sup>a-f</sup> Values in the same row with no common superscript differ significantly.

<sup>a</sup> Basal diet; <sup>b</sup> Diet with 7.5 % canola seed; <sup>c</sup> Diet with 15 % canola seed; <sup>d</sup> Diet with 10% canola seed and 10% flaxseed; <sup>e</sup> Diet with 7.5% flaxseed, <sup>f</sup> Diet with 15 % flaxseed.

<sup>a</sup> C18:1= Oleic acid; C18:2= Linoleic acid; C18:3= Linolenic acid; C20:4= Arachidonic acid; n-3= Omega-3 FA; n-6= Omega-6 FA; SFA= Saturated fatty acid; MUFA= Monounsaturated fatty acid; PUFA= Polyunsaturated fatty acid; n3/n6: the ratio of n-3 to n-6 PUFA.

Nd: Not detected, Ns:  $P > 0.05$ ; \*  $P \leq 0.05$ , \*\*  $P \leq 0.01$ .

ALA compete for the same enzyme system responsible for their elongation and desaturation to form the long-chain metabolites. The critical enzyme in these reactions is  $\Delta$ -6 desaturase, for which the greatest affinity appears to be conferred by the greatest number of double bonds in the C18 substrate (Sardesai, 1992).

Because of higher concentrations of ALA and lower concentrations of AA in the lipid of the muscles, addition of oil seeds to the diet significantly ( $P < 0.01$ ) lowered the n -6: n -3 ratios of white and dark muscles. These findings are similar to the results reported by Gonzalez- Esquerria and Leeson (2000) and Shen *et al.* (2005). The ALA deposition values tend to be higher in thigh than in breast. These results could be explained by the higher triglyceride fraction in the thigh in which ALA is normally deposited (Hulan *et al.*, 1988; Ahn *et al.*, 1995). Differences in FA deposition between breast and thigh muscle have been reported. For example, Cortinas *et al.* (2004) reported deposition values for ALA of 26.7% and 22.2% for

thigh and breast muscle, respectively, when 45 g kg<sup>-1</sup> PUFA was added in the diet.

In the present study, no FA with more than 22 carbon atoms was detected. Some researchers have reported that although the chicks are able to desaturate and elongate ALA, their ability appears to be limited, and the deposition rate of these metabolites in muscle tissues is very limited (Lopez-Ferrer *et al.*, 2001; Chanmugam *et al.*, 1992).

The total saturated fatty acid (SFA) and monounsaturated fatty acid (MUFA) content of breast muscle remained constant while the SFA content of thigh muscle was lower when chickens were fed oil seeds diets. In addition, the ability of broiler chickens to alter the SFA content of the breast muscle is limited. The FA in the white muscle tissue serve as structural components that limit its levels of deposition in order to maintain its physical characteristics to ensure fluidity and permeability of the cell to different compounds (Sanz, 1999). Concentrations of PUFA were significantly ( $P < 0.01$ ) increased when the oil seeds were supplemented. On

**Table 5.** Fatty acid composition of broiler breast and thigh muscle lipids<sup>a</sup> (mg g<sup>-1</sup> of meat).

Fatty acid <sup>c</sup>	Breast muscle lipids						Thigh muscle lipids									
	Treatment <sup>b</sup>						Treatment <sup>b</sup>									
	C	CSI	CS2	CS-FS	FS1	FS2	significance	SEM	C	CSI	CS2	CS-FS	FS1	FS2	significance	SEM
C14	0.14	0.11	0.13	0.12	0.11	0.12	ns	0.005	0.56	0.49	0.48	0.49	0.44	0.47	ns	0.02
C16:0	2.99	2.78	2.52	2.58	2.83	2.77	ns	0.07	13.45 <sup>a</sup>	11.68 <sup>ab</sup>	10.52 <sup>b</sup>	11.14 <sup>b</sup>	11.21 <sup>b</sup>	10.73 <sup>b</sup>	*	0.30
C16:1	1.02	0.98	0.99	0.96	1.02	1.00	ns	0.03	7.14	5.04	5.00	5.72	6.31	6.11	ns	0.29
C18:0	1.14 <sup>a</sup>	0.87 <sup>b</sup>	0.87 <sup>b</sup>	0.90 <sup>b</sup>	1.03 <sup>ab</sup>	1.03 <sup>ab</sup>	**	0.03	3.06	2.58	2.35	2.80	2.58	2.25	ns	0.09
C18:1	5.9 <sup>a</sup>	5.84 <sup>a</sup>	5.99 <sup>a</sup>	5.87 <sup>a</sup>	5.53 <sup>ab</sup>	5.32 <sup>b</sup>	*	0.07	23.45 <sup>bc</sup>	24.48 <sup>bc</sup>	26.51 <sup>ab</sup>	28.08 <sup>a</sup>	23.80 <sup>c</sup>	23.5 <sup>c</sup>	**	0.33
C18:2	1.91	1.98	2.00	1.99	1.93	1.98	ns	0.02	6.11 <sup>ab</sup>	6.14 <sup>ab</sup>	6.28 <sup>ab</sup>	6.45 <sup>a</sup>	5.85 <sup>b</sup>	5.92 <sup>ab</sup>	*	0.06
C18:3	0.04 <sup>e</sup>	0.15 <sup>d</sup>	0.19 <sup>c</sup>	0.25 <sup>b</sup>	0.19 <sup>c</sup>	0.29 <sup>a</sup>	**	0.01	0.26 <sup>e</sup>	0.72 <sup>d</sup>	0.85 <sup>c</sup>	0.85 <sup>c</sup>	0.88 <sup>c</sup>	1.15 <sup>a</sup>	**	0.05
C20:4	0.08 <sup>a</sup>	0.05 <sup>bc</sup>	0.05 <sup>b</sup>	0.03 <sup>d</sup>	0.03 <sup>d</sup>	0.40 <sup>cd</sup>	**	0.003	0.26 <sup>a</sup>	0.23 <sup>abc</sup>	0.24 <sup>ab</sup>	0.25 <sup>ab</sup>	0.21 <sup>bc</sup>	0.20 <sup>c</sup>	**	0.006
SFA	4.27	3.76	3.52	3.60	3.97	3.92	ns	0.09	17.16 <sup>a</sup>	14.75 <sup>b</sup>	13.26 <sup>b</sup>	14.42 <sup>b</sup>	14.24 <sup>b</sup>	13.45 <sup>b</sup>	*	0.35
MUFA	6.92	6.81	6.98	6.86	6.55	6.32	ns	0.08	32.30 <sup>ab</sup>	29.53 <sup>b</sup>	31.50 <sup>ab</sup>	33.80 <sup>a</sup>	30.11 <sup>b</sup>	29.56 <sup>b</sup>	*	0.44
PUFA	2.03 <sup>b</sup>	2.18 <sup>ab</sup>	2.24 <sup>a</sup>	2.27 <sup>a</sup>	2.15 <sup>ab</sup>	2.3 <sup>a</sup>	**	0.02	6.63 <sup>c</sup>	7.09 <sup>bc</sup>	7.38 <sup>ab</sup>	7.74 <sup>a</sup>	6.94 <sup>bc</sup>	7.27 <sup>ab</sup>	**	0.07
n-3	0.04 <sup>e</sup>	0.15 <sup>d</sup>	0.19 <sup>c</sup>	0.25 <sup>b</sup>	0.19 <sup>c</sup>	0.29 <sup>a</sup>	**	0.01	0.26 <sup>e</sup>	0.72 <sup>d</sup>	0.85 <sup>c</sup>	0.85 <sup>c</sup>	0.88 <sup>c</sup>	1.15 <sup>a</sup>	**	0.05
n-6	1.99	2.02	2.06	2.02	1.96	2.02	ns	0.02	6.37 <sup>abc</sup>	6.37 <sup>abc</sup>	6.53 <sup>ab</sup>	6.70 <sup>a</sup>	6.06 <sup>c</sup>	6.12 <sup>bc</sup>	*	0.06
S/U	0.48 <sup>a</sup>	0.42 <sup>ab</sup>	0.38 <sup>b</sup>	0.40 <sup>b</sup>	0.45 <sup>ab</sup>	0.46 <sup>ab</sup>	**	0.01	0.44 <sup>a</sup>	0.40 <sup>ab</sup>	0.34 <sup>b</sup>	0.35 <sup>b</sup>	0.38 <sup>ab</sup>	0.36 <sup>b</sup>	**	0.009
n3/n6	47.78 <sup>a</sup>	13.20 <sup>b</sup>	11.16 <sup>bc</sup>	8.14 <sup>cd</sup>	10.14 <sup>cd</sup>	7.12 <sup>d</sup>	**	2.42	26.06 <sup>a</sup>	8.92 <sup>b</sup>	7.70 <sup>bc</sup>	6.45 <sup>bc</sup>	6.92 <sup>bc</sup>	5.35 <sup>c</sup>	**	1.18

<sup>a-e</sup> Values in the same row with no common superscript differ significantly.

<sup>a</sup> Values are the means of six observations per treatment.

<sup>b</sup> C: Basal diet; CSI: Diet with 7.5 % canola seed; CS2: Diet with 15 % canola seed; CS-FS: Diet with 10% canola seed and 10% flaxseed; FS1: Diet with 7.5% flaxseed; FS2: Diet with 15 % flaxseed.

<sup>c</sup> C18:1= Oleic acid; C18:2= Linoleic acid; C18:3= Linolenic acid; C20:4= Arachidonic acid; n-3= Omega-3 FA; n-6= Omega-6 FA; SFA= Saturated fatty acid; MUFA= Monounsaturated fatty acid; PUFA= polyunsaturated fatty acid; n3/n6: the ratio of n-3 to n-6 PUFA.

Ns:  $P > 0.05$ ; \*  $P \leq 0.05$ , \*\*  $P \leq 0.01$ .



the other hand, breast muscle exhibited a higher deposition of PUFA than thigh muscle due to the higher proportion of phospholipid fraction in breast muscle (Ratnayake *et al.*, 1989). Based on the results reported in different studies, the MUFA are deposited in the greatest amount in the triglyceride fraction (Ajuyah *et al.*, 1993a), therefore, as expected, MUFA concentrations were higher in the thigh muscle than in the breast muscle. Thus, differences in tissue FA profile could be attributed to different roles of FA in these tissues or to their different phospholipid contents.

### TBARS Concentration

The MDA levels of the dark and white muscles are summarized in Table 6, respectively. Oil seed meal inclusion in broiler diets decreased the oxidative stability of raw meat with TBARS values in breast and thigh muscle from these groups being significantly ( $P < 0.01$ ) higher than in muscle tissues of the birds fed with the control diet.

At any storage time, thigh and breast muscle samples from the chickens fed with oil seed diets gave the highest TBA values except for breast muscle sampled 1 day after slaughtering ( $P < 0.01$ ). The greatest rate of oxidation during frozen storage was produced by feeding the CS-FS diet. There have been other reports of higher TBA values for chicken muscle from unsaturated fat sources (Du *et al.*, 2000; Grau *et al.*, 2001; Cortinas *et al.*, 2005). This is a consequence of the higher PUFA content in meat from unsaturated diets. As mentioned earlier, TBARS formation is mainly increased with the peroxidation of FA with more than two double bonds.

Hence, the TBARS values were higher in the thigh meat than in the breast meat of broilers probably due to its higher unsaturated FA concentration and higher lipid content. The increased MDA levels of birds fed n-3 PUFA in thigh muscle compared with breast muscle has been observed by Ruiz *et al.* (1999) and Zanini *et al.* (2006). In addition, the lower concentrations of the heme pigment

in breast muscle may have been the reason for lower TBARS values in breast in comparison to thigh muscle, since the oxidation of the heme pigment probably has catalysed the lipid oxidation (Akamiitath *et al.*, 1990). The results of oxidative stability of breast and thigh muscle show that oxidation of the breast and the thigh muscle increased with length of storage, which is in agreement with other reports (Grau *et al.*, 2001; Jeun-Horng *et al.*, 2002; Zanini *et al.*, 2006). Furthermore, the susceptibility toward oxidation of the muscle was detected when the levels of oil seeds were increased. This finding indicates that oil seeds, particularly flaxseed, are probably a major contributor to oxidation, because they contain high levels of PUFA that is prone to oxidation.

The pattern of FA deposition in muscle tissue of poultry can be manipulated by changes in the FA composition of dietary fats. Additionally, the origin of dietary fats significantly influences the FA composition and concentration of broiler chicken carcasses reflecting the predominant FA of the diet. Modifying the FA composition in muscle tissues toward a higher degree of unsaturation decreases the oxidative stability of muscles. The susceptibility of stored muscle lipids to oxidation depends on the concentration of unsaturated FA in the muscles. In general, the rate and extent of lipid oxidation in muscle could be influenced by the fat level, profile of fatty acids, and the storage conditions.

### Total Fat Content

The crude fat content (Table 7) of breast and thigh meat was not affected by treatments ( $P > 0.05$ ). However, birds fed with the control diet deposited more lipids in breast muscle. Some authors have shown that total fat content in tissues reduced as the dietary PUFA level increased (Cortinas *et al.*, 2004; Shen *et al.*, 2005). It seems that the lower fat deposition was due to an increased rate of lipid catabolism and a decreased fatty acid synthesis (Sanz *et al.*, 2000). These observations are consistent



**Table 6.** Malondialdehyde (MDA) concentrations in breast and thigh muscle of broiler <sup>a</sup> ( $\mu\text{g kg}^{-1}$  of meat).

Treatment <sup>b</sup>	MDA concentrations in breast muscle						MDA concentrations in thigh muscle							
	Stored at 4°C			Stored at -20°C			Stored at 4°C			Stored at -20°C				
	1 day	7 day	14 day	21 day	30 day	60 day	90 day	1 day	7 day	14 day	21 day	30 day	60 day	90 day
C	52.20	59.98 <sup>d</sup>	86.02 <sup>c</sup>	140.03 <sup>c</sup>	58.73 <sup>d</sup>	68.63 <sup>a</sup>	99.30 <sup>d</sup>	65.25 <sup>d</sup>	74.51 <sup>a</sup>	92.35 <sup>d</sup>	150.07 <sup>a</sup>	74.52 <sup>c</sup>	98.61 <sup>d</sup>	130.74 <sup>d</sup>
CS1	53.50	71.47 <sup>c</sup>	126.82 <sup>b</sup>	179.60 <sup>b</sup>	68.17 <sup>c</sup>	86.80 <sup>c</sup>	159.37 <sup>c</sup>	68.70 <sup>c</sup>	85.81 <sup>c</sup>	170.63 <sup>c</sup>	284.67 <sup>c</sup>	81.10 <sup>b</sup>	125.92 <sup>c</sup>	261.85 <sup>c</sup>
CS2	54.53	78.03 <sup>b</sup>	164.67 <sup>a</sup>	214.47 <sup>a</sup>	74.30 <sup>b</sup>	104.48 <sup>ab</sup>	196.48 <sup>b</sup>	71.53 <sup>bc</sup>	93.60 <sup>b</sup>	210.72 <sup>b</sup>	386.04 <sup>b</sup>	98.37 <sup>a</sup>	170.61 <sup>b</sup>	320.10 <sup>b</sup>
CS-FS	55.25	81.83 <sup>a</sup>	165.62 <sup>a</sup>	220.85 <sup>a</sup>	77.50 <sup>a</sup>	108.48 <sup>a</sup>	206.45 <sup>a</sup>	75.44 <sup>a</sup>	99.25 <sup>a</sup>	217.50 <sup>a</sup>	392.44 <sup>ab</sup>	101.96 <sup>a</sup>	178.59 <sup>a</sup>	333.67 <sup>a</sup>
FS1	53.17	70.18 <sup>c</sup>	123.67 <sup>b</sup>	185.67 <sup>b</sup>	66.03 <sup>c</sup>	84.73 <sup>c</sup>	165.05 <sup>c</sup>	70.18 <sup>c</sup>	87.77 <sup>c</sup>	172.03 <sup>c</sup>	277.33 <sup>c</sup>	81.87 <sup>b</sup>	125.70 <sup>c</sup>	260.62 <sup>c</sup>
FS2	53.77	77.10 <sup>b</sup>	160.15 <sup>a</sup>	213.67 <sup>a</sup>	72.23 <sup>b</sup>	103.00 <sup>b</sup>	204.65 <sup>a</sup>	73.16 <sup>ab</sup>	96.85 <sup>ab</sup>	212.35 <sup>ab</sup>	399.14 <sup>a</sup>	99.26 <sup>a</sup>	164.36 <sup>b</sup>	322.99 <sup>b</sup>
significance	Ns	**	**	**	**	**	**	**	**	**	**	**	**	**
SEM	0.31	1.29	4.91	4.79	1.07	2.40	6.33	0.61	1.46	7.34	15.10	1.88	4.95	11.78

<sup>a-d</sup> Values in the same column with no common superscript differ significantly.

<sup>a</sup> Values are the means of six observations per treatment.

<sup>b</sup> C: Basal diet; CS1: Diet with 7.5 % canola seed; CS2: Diet with 15 % canola seed; CS-FS: Diet with 10% canola seed and 10% flaxseed; FS1: Diet with 7.5% flaxseed; FS2: Diet with 15 % flaxseed.

Ns:  $P > 0.05$ , \*\*  $P \leq 0.01$ .

**Table 7.** Total fat content of diets, breast and thigh (%)<sup>1</sup>.

Treatments <sup>2</sup>	Feed	Breast	Thigh
C	6.23 <sup>b</sup>	7.45	9.39
CS1	6.66 <sup>ab</sup>	6.80	9.65
CS2	8.07 <sup>a</sup>	7.04	9.45
CS-FS	7.59 <sup>ab</sup>	7.06	9.84
FS1	6.15 <sup>b</sup>	6.79	8.97
FS2	7.08 <sup>ab</sup>	6.98	9.10
significance	**	Ns	Ns
SEM	0.30	0.08	0.10

<sup>a-c</sup> Values in the same column with no common superscript differ significantly.

<sup>1</sup>Values are the means of six observations per treatment.

<sup>2</sup> C: Basal diet; CS1: Diet with 7.5 % canola seed; CS2: Diet with 15 % canola seed; CS-FS: Diet with 10% canola seed and 10% flaxseed; FS1: Diet with 7.5% flaxseed; FS2: Diet with 15 % flaxseed.

Ns:  $P > 0.05$ , \*\*  $P \leq 0.01$ .

with our results. The lack of effect of fat source on crude fat can be related to the moderate enrichment of muscles. Some authors have shown that the dietary polyunsaturation level of fat does not influence intramuscular lipid content of breast (Scaife *et al.*, 1994; Crespo and Esteve-Garcia, 2002).

Furthermore, higher dietary fibers content reduces hepatic lipogenesis and triglyceride synthesis and accelerates lipoprotein lipase activity in the adipose tissue (Akiba and Matsumoto, 1982).

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غنی سازی گوشت طیور از نظر اسیدهای چرب امگا-۳ با استفاده از دو بذر روغنی

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#### چکیده

به منظور ارزیابی تاثیر بذر کتان (FS: Flaxseed) و کانولا یا کلزا (CS: Canola seed) بر عملکرد، پروفیل اسید چرب گوشت و پایداری اکسیداتیو گوشت طی نگهداری آزمایشی به مدت ۴۲ روز انجام

گردید. تعداد ۳۲۴ جوجه یک‌روزه به صورت تصادفی با شش جیره غذایی مختلف شامل C (گروه شاهد)، CS1 (۷/۵ درصد CS)، CS2 (۱۵ درصد CS)، CF-FS (مخلوط ۱۰ درصد CS و FS)، FS1 (۷/۵ درصد FS) و FS2 (۱۵ درصد FS) تغذیه شدند. اثرات منفی بر پارامترهای عملکردی توسط جیره‌های حاوی بذر کتان و کانولا مشاهده گردید، به طوری‌که تغذیه با بذور روغنی منجر به کاهش معنی‌دار ( $P < 0/01$ ) افزایش وزن بدن و افزایش ضریب تبدیل غذایی نسبت به گروه شاهد گردید. با این حال تفاوتی در میزان غذای مصرفی بین گروه‌های آزمایشی مشاهده نگردید ( $P > 0/05$ ). افزودن بذر کتان و بذر کانولا منجر به افزایش معنی‌داری در محتوی اسید چرب امگا-۳ (به فرم اسید لینولنیک) و کاهش در محتوی اسید آراشیدونیک گردید ( $P < 0/01$ ). نسبت اسید چرب امگا-۶ به امگا-۳ به طور معنی‌داری برای طیور تغذیه شده با بذور کتان و کانولا در مقایسه با گروه شاهد کمتر بود ( $P < 0/01$ ). افزودن بذور کتان و کانولا پ منجر به کاهش پایداری اکسیداتیو گوشت خام (سینه و ران) بر اساس شاخص تیوباربیتوریک اسید (TBA) طی زمان نگهداری شد ( $P < 0/01$ ). با افزودن منابع گیاهی امگا-۳ به جیره طیور، محتوی اسید چرب امگا-۳ گوشت طیور افزایش یافته که ممکن است اثرات مفیدی بر سلامتی انسان داشته باشد.