

## Effect of dietary oil seeds on n-3 fatty acid enrichment, performance parameters and humoral immune response of broiler chickens

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(Received 19 Aug 2008; revised version 28 Dec 2008; accepted 9 Feb 2009)

### Summary

A 42-day 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, serum lipid content and antibody response to sheep red blood cells antigen (SRBC). A total of 324 one-day-old chicks were attributed to 6 experimental groups. C: control (soybean-corn); CS1:7.5% CS; CS2:15% CS; CS-FS:10% FS + 10% CS; FS1:7.5% FS; FS2:15% FS. The diets containing FS and CS had a significant negative effect on performance parameters ( $P<0.01$ ), however, feed consumption was not significantly ( $P>0.05$ ) different among treatments. Inclusion of FS and CS significantly increased ( $P<0.01$ ) the concentration of omega-3 FA and decreased the content of the arachidonic acid and n-6:n-3 polyunsaturated FA ratio. The serum lipid content and antibody titre against SRBC were not affected by dietary oil seeds ( $P>0.05$ ).

**Key words:** Broiler, Immune response, Omega-3 fatty acid, Performance, Serum lipid

### Introduction

From the human health aspect, the fatty acid (FA) composition of meat products is an important parameter of the meat quality. In this regard, n-3 polyunsaturated fatty acid (PUFA) is the most important fatty acid. Dietary n-3 fatty acids have benefit in prevention of cardiovascular disorders, improvement of immune response and reduction of the serum cholesterol concentration (Phillipson *et al.*, 1985; Leaf and Weber, 1988; Simopoulous, 1991; Leaf and Kang, 1998). It has been already demonstrated that the fatty acid composition of broiler meat can be altered by changing the FA content of the broiler's diet (Yau *et al.*, 1991). Therefore, studies are directed toward the manipulation of the fatty acid composition of broiler chicks in order to increase n-3 PUFA content and decrease in n-6:n-3 ratio in poultry meat. It is known that n-6 PUFA act as a pro-inflammatory factor and n-3 PUFA acts as an anti-

inflammatory factor on immunity functions and inflammatory processes in animals and humans (Calder, 2001). Immune cells with membranes enriched in n-3 PUFA at the expense of n-6 PUFA release lower amounts and less potent mediators of inflammations, (e.g., eicosanoids) and appear to enhance antibody production (Billiar *et al.*, 1988; James *et al.*, 2000). Flaxseed (FS) and canola seed (CS) are the main sources of  $\alpha$ -linolenic acid (ALA) of terrestrial origin. Several attempts have been successfully made to enrich broiler muscle tissues or products with linolenic acid and its elongated n-3 fatty acids by using vegetable sources in their diet (Mantzioris *et al.*, 2000; Ayerza *et al.*, 2002). In addition, FS and CS might serve as an alternative source of dietary energy for the bird. However, the drawbacks associated with dietary inclusions of FS and CS are the presence of anti-nutritional factors and the low available nutrient content, which may limit their use in poultry diets. The literature shows that

dietary inclusion of FS more than 10-15% may depress broiler growth (Ajuyah *et al.*, 1993; Najib and Al-Khateeb, 2004).

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, immune response and serum lipid profile of the birds.

## Materials and Methods

Three hundred and twenty four straight run 1-day-old broiler chicks (Cobb 500) were divided randomly into six dietary treatments. Each treatment was replicated 3 times with 18 chicks per replicate. Birds were housed in deep litter pens (1 × 2 m). Environmental temperature was set at 32°C on day 1 and lowered stepwise to 23-24°C for the rest of the experiment. Relative humidity and ventilation were under standard conditions. Birds were fed experimental diets from day 1 until 42 days of age with two-time periods: the starting period (day 1 to 21) and the finishing period (day 22 to 42). The 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 calculated to be isocaloric and isonitrogenous. The following six dietary treatments were used, C: control

diet (soybean-corn); CS1: 7.5% ground CS; CS2: 15% ground CS; CS-FS: 10% ground FS + 10% ground CS; FS1: 7.5% ground FS; FS2: 15% ground FS. The composition of the diets is shown in Table 1.

## Performance record

Body weight and feed consumption of all birds in each pen was recorded weekly. Weight gain and feed conversion were calculated at the end of each feeding period.

## Fatty acid content

Two birds were randomly selected from each pen (6 birds per treatment) for tissue sampling on day 42. After slaughtering the birds, whole carcass samples including skin were collected from each bird and used for FA determination. The FA composition of feed and carcass samples was determined by gas chromatography (Metcalf *et al.*, 1966) using a gas chromatograph (Unicam 4600, USA) equipped with a BPX70 fused silica capillary column and a flame ionization detector. The operating conditions of the gas chromatograph were as follows; the initial temperature was 160°C, increasing by the rate of 40°C/min to 180°C; after 10 min, the temperature was increased at the rate of 20°C/min to 190°C. The temperature of the injector was 240°C and the detector remained stable at 280°C. The column head pressure of the carrier gas (Helium) was 20 psi and sample volume was 0.2 µl.

**Table 1: Ingredients and composition of experimental diets<sup>1</sup>**

Ingredients	Starter						Finisher					
	C	CS1	CS2	CS-FS	FS1	FS2	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	2.00	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 shell	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>2,3</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	0.03	0.08	2.40	3.25	0.03	0.35	0.15	1.90	3.80	4.40	1.70	2.20
Calculated analysis												
ME (kcal/kg)	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	17.44	17.44	17.44	17.44

<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. <sup>2</sup>Vitamin premix provided per kg 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>3</sup>Mineral premix provided per kg of diet: Mn, 60 mg; Fe, 60 mg; Zn, 51.74 mg; Cu, 4.8 mg; I, 0.69 mg; Se, 0.16 mg

Pentadecanoic acid (Sigma, St. Louis, MO) was used as internal standard. Fatty acids were identified by matching their retention times with those of their respective standards.

### Humoral immune response to SRBC

Non-pathogenic antigen of sheep red blood cells (SRBC) was used to monitor immune response of the birds. The anti-SRBC titres for total antibodies were measured by haemagglutination test (Peterson *et al.*, 1999). Three birds from each replicate were randomly selected and injected with 0.1 ml of 5% SRBC via the brachial vein at 21 and 35 days of age and the antiserum to SRBC was prepared 7 days post-injection. Appropriate paints were used for tracing the immunized birds.

### Serum lipid measurement

Three blood samples from each replicate were taken from the wing vein of the birds at 27 and 41 days of age. The serum cholesterol (CHL) and triglyceride (TG) levels were determined spectrophotometrically (UV-visible S2100, Scinco, Korea) by using commercial kits (Pars Azmun, Iran), according to the method described by Richmond (1973).

### Statistical analyses

Data were analyzed in a completely randomized design using the ANOVA procedure of the SAS (SAS Institute, 1990). Differences between the means of all treatment groups were investigated using the Duncan's multiple range test. The following statistical model was used:

$$X_{ij} = \mu + \tau_j + \varepsilon_{ij}, \text{ where}$$

$X_{ij}$  = the observation of  $j^{\text{th}}$  treatment on  $i^{\text{th}}$  pen

$\mu$  = the overall means of the sampled observation

$\tau_j$  = the effect of treatment

$\varepsilon_{ij}$  = residual

## Results

### Performance parameters

Those birds fed on the control diets performed better than those fed on the other diets in terms of weight gain and feed conversion efficiency ( $P < 0.01$ ) (Table 2). Birds of CS2 and CS-FS groups had 11% lower body weight at the end of experiment than the control diet. Feed consumption was not affected by the diets ( $P > 0.05$ ). Lower body weights and a similar feed consumption resulted in a higher ( $P < 0.01$ ) feed:gain ratio for FS and CS groups and the lower feed conversion for CS-FS group.

### Fatty acid composition of meat and diets

The FA composition of the experimental diets and whole carcass muscles are presented in Tables 3 and 4. In relation to feed FA composition, addition of oil seeds, especially FS, increased ALA levels of diet comparing to the control group. Also the ratio of dietary PUFA to saturated fatty acid (PUFA:SFA) increased by increase in oil seeds usage.

Fatty acid composition of chick tissues generally reflected the fatty acid profile of the diets. Alpha-linolenic acid was higher and arachidonic acid (AA), was significantly lower in the whole carcasses of the broilers fed on diets containing oil seeds ( $P < 0.05$ ) (Table 4). Monounsaturated fatty acids (MUFA) comprised the greatest percentage of FA for carcass meat, with oleic acid, the predominant constituent. The SFA content of carcass remained constant while the

**Table 2: Performance parameters of broilers fed with the experimental diets (1 to 42 d)<sup>1</sup>**

Performance	Weight gain (g/d)			Feed consumption (g/d)			Feed conversion efficiency		
	1-21	22-42	1-42	1-21	22-42	1-42	1-21	22-42	1-42
Age (days)									
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	0.86	0.59	0.29	1.05	0.57	0.01	0.02	0.01

<sup>a-c</sup>Values in the same column with no common superscript differ significantly (\*\* $P < 0.01$ ). <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; CS2: diet with 15% flaxseed. NS: Non significant ( $P > 0.05$ )

concentrations of PUFA increased, when the oil seeds supplemented. Addition of FS and CS to the diet decreased the SFA:PUFA and n-6:n-3 ratios of the carcass meat of the experimental groups compared to the control group ( $P < 0.05$ ).

### Humoral immune response to SRBC

The antibody titres against SRBC were

not affected by dietary treatments ( $P > 0.05$ ) (Table 5). Nonetheless, compared with control, other groups had higher total titres in primary response and higher total titres in secondary response. The lowest antibody titre was 4.17 (1/log2) for the control group in secondary response, and the highest was 5.17 (1/log2) for FS2 group.

**Table 3: Fatty acid composition of diets<sup>1</sup> (%)**

Fatty acid <sup>2</sup>	Treatment <sup>1</sup>						Significance	SEM
	C	CS1	CS2	CS-FS	FS1	FS2		
C14:0	1.23 <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>	3.53 <sup>c</sup>	5.47 <sup>a</sup>	**	0.39
C20:4	ND <sup>3</sup>	ND	ND	ND	ND	ND	-	-
SFA	31.56 <sup>a</sup>	28.54 <sup>bc</sup>	26.63 <sup>cd</sup>	25.46 <sup>d</sup>	29.17 <sup>b</sup>	27.86 <sup>bcd</sup>	**	0.53
MUFA	55.06 <sup>c</sup>	57.24 <sup>ab</sup>	58.07 <sup>a</sup>	57.30 <sup>ab</sup>	55.77 <sup>bc</sup>	55.40 <sup>bc</sup>	*	0.34
PUFA	12.53 <sup>c</sup>	14.27 <sup>bc</sup>	15.33 <sup>b</sup>	17.30 <sup>a</sup>	15.03 <sup>b</sup>	16.84 <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
SFA/UFA	0.47 <sup>a</sup>	0.40 <sup>ab</sup>	0.36 <sup>ab</sup>	0.34 <sup>b</sup>	0.41 <sup>ab</sup>	0.39 <sup>ab</sup>	*	0.01
n6/n3	12.92 <sup>a</sup>	6.13 <sup>b</sup>	3.79 <sup>bc</sup>	2.44 <sup>c</sup>	3.27 <sup>c</sup>	2.08 <sup>c</sup>	*	1.04

<sup>a-f</sup>Values in the same row with no common superscript differ significantly ( $*P \leq 0.05$ ;  $**P \leq 0.01$ ), NS: Non significant,  $P > 0.05$ . <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; CS2: diet with 15% flaxseed. <sup>2</sup>SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid; n6/n3: the ratio of n-6 to n-3 PUFA; UFA: Unsaturated fatty acid. <sup>3</sup>ND: Not detected

**Table 4: Fatty acid composition of broiler whole carcass lipids<sup>1</sup> (mg/g of meat)**

Fatty acid <sup>3</sup>	Treatment <sup>2</sup>						Significance	SEM
	C	CS1	CS2	CS-FS	FS1	FS2		
C14:0	0.84	0.66	0.65	0.68	0.63	0.66	NS	0.03
C16:0	20.68	20.17	20.16	18.97	19.49	19.75	NS	0.27
C16:1	8.57	7.82	7.50	8.57	7.57	8.28	NS	0.29
C18:0	4.98	4.69	4.50	4.47	4.46	4.88	NS	0.10
C18:1	41.26 <sup>bc</sup>	41.54 <sup>bc</sup>	45.54 <sup>a</sup>	43.08 <sup>b</sup>	37.9 <sup>d</sup>	39.91 <sup>cd</sup>	*	0.46
C18:2	11.62 <sup>b</sup>	11.7 <sup>b</sup>	12.89 <sup>a</sup>	12.64 <sup>ab</sup>	10.37 <sup>c</sup>	11.46 <sup>bc</sup>	*	0.17
C18:3	0.44 <sup>d</sup>	1.61 <sup>c</sup>	2.39 <sup>b</sup>	3.18 <sup>a</sup>	2.31 <sup>b</sup>	3.01 <sup>a</sup>	**	0.16
C20:4	0.27	0.25	0.24	0.25	0.19	0.16	NS	0.009
SFA	26.50	25.53	25.31	24.12	24.58	25.29	NS	0.28
MUFA	49.83 <sup>ab</sup>	49.36 <sup>ab</sup>	53.03 <sup>a</sup>	51.66 <sup>ab</sup>	45.46 <sup>c</sup>	48.20 <sup>bc</sup>	*	0.53
PUFA	12.33 <sup>d</sup>	13.56 <sup>cd</sup>	15.52 <sup>ab</sup>	16.08 <sup>a</sup>	12.88 <sup>d</sup>	14.64 <sup>bc</sup>	*	0.26
n-3	0.44 <sup>d</sup>	1.61 <sup>c</sup>	2.39 <sup>b</sup>	3.18 <sup>a</sup>	2.31 <sup>b</sup>	3.01 <sup>a</sup>	**	0.16
n-6	11.90 <sup>bc</sup>	11.95 <sup>bc</sup>	13.13 <sup>a</sup>	12.90 <sup>ab</sup>	10.56 <sup>d</sup>	11.63 <sup>cd</sup>	*	0.18
SFA/UFA	0.43 <sup>a</sup>	0.40 <sup>ab</sup>	0.37 <sup>c</sup>	0.36 <sup>c</sup>	0.42 <sup>a</sup>	0.40 <sup>bc</sup>	*	0.006
n6/n3	27.04 <sup>a</sup>	7.43 <sup>b</sup>	5.49 <sup>bc</sup>	4.06 <sup>c</sup>	4.57 <sup>c</sup>	3.86 <sup>c</sup>	**	1.47

<sup>a-d</sup>Values in the same column with no common superscript differ significantly ( $*P \leq 0.05$ ;  $**P \leq 0.01$ ). NS: Non significant,  $P > 0.05$ . <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; CS2: diet with 15% flaxseed. <sup>3</sup>SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid; n6/n3: the ratio of n-6 to n-3 PUFA. UFA: Unsaturated fatty acid

**Table 5: Total anti-SRBC (titre)**

Treatment	27 days of age	35 days of age
C	1.67	4.17
CS1	2.30	5.00
CS2	2.16	4.77
CS-FS	2.27	5.00
FS1	3.23	4.83
FS2	2.00	5.17
Significance	NS	NS
SEM	0.24	0.15

NS: Non significant ( $P>0.05$ )

### Serum lipoprotein parameters

The effects of addition of FS and CS to diets of broiler chickens on levels of serum CHL and TG are shown in Table 6. There was no significant difference between serum CHL and TG in groups ( $P>0.05$ ), however, broilers fed on oil seeds had numerically lower levels of CHL and TG concentrations compared to the control group, and total CHL and TG of serum in broilers fed on oil seed diets were also lower than those of the control.

**Table 6: Lipid content of serum (mg/dl)**

Treatment	CHL		TG	
	TCHL <sup>1,2</sup>	TCHL <sup>2</sup>	TG1	TG2
C	112.6	108.87	120.77	128.53
CS1	106.53	93.30	95.70	108.20
CS2	100.43	87.9	108.00	123.90
CS-FS	101.30	95.13	116.00	108.60
FS1	101.57	87.06	97.67	105.73
FS2	102.93	100.80	109.3	117.87
Significance	NS	NS	NS	NS
SEM	2.52	3.13	3.74	3.45

<sup>1,2</sup> Determined on 7 and 41 day of age. NS: Non significant ( $P>0.05$ ). \*TCHL: Total cholesterol

### Discussion

The negative effects of feeding diets containing FS and CS on bird performance 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; Roth-Maier *et al.*, 1998). The existence of phytic acid in CS and FS will decrease calcium absorption (Summers *et al.*, 1988) and inhibit proteolytic enzymes (Caldwell, 1992; Ravidran *et al.*, 1995). In addition, the usefulness of FS and CS 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

the passage rate (Rodriguez *et al.*, 2001).

Also, other antinutritional factors like toxic cyanoglycosides (limarin) and vitamin B6 antagonists in FS and lysine-arginine imbalance in CS may be the causes of the impaired performances (Klosterman *et al.*, 1967; Summers and Leeson, 1978; Oomah *et al.*, 1992; Talebali and Farzinpour, 2005).

Ajuyah *et al.* (1993) reported a 17% reduction in body weights when birds were fed diets containing 15% FS (42-day period), and because feed consumption was similar to control birds, a significantly poorer feed conversion was reported.

Incorporation of FS and CS in the diet increased the proportions of the n-3 PUFA in the form of ALA. These results are consistent with the observation that 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). Depending on dietary oil seed level, ALA proportions ranged from 1.61 to 3.18 mg/g of meat, while this level in control group was 0.44 mg/g of carcass meat. Canola seed has a limited potential for ALA enrichment of the meat compared with FS, because FS contains more ALA than CS. The latter is rich in oleic acid and contains also more linoleic acid (LA) than FS. Similar to our findings, Lopez-Ferrer *et al.* (1999), who used diets with 8.2% FS and CS, observed moderate levels of ALA in carcass using 8.2% CS in contrast to FS supplementation.

A decrease in AA content indicates a decrease in conversion of LA to long chain n-6 PUFA. Higher concentration of ALA in muscle tissues decreased the formation of AA, since both LA and ALA compete for the same enzyme system responsible for their elongation and desaturation to form 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). Therefore, the relative excess of ALA in muscle inhibits the formation of AA. Because of the higher concentrations of ALA and lower concentrations of AA in the lipid of the muscles, addition of oil seeds to the diet lowered the n-6:n-3 ratios of the carcass muscle ( $P<0.01$ ). These findings are

similar to the results reported by Gonzalez-Esquerria and Leeson (2000) and Shen *et al.* (2005). In our research FA with more than 20 carbon atoms was not detected. Reports indicate that although chicks are able to desaturate and elongate ALA, their ability appears to be limited, and deposition rate of these metabolites in muscle tissues is very low (Chanmugam *et al.*, 1992; Lopez-Ferrer *et al.*, 2001). These results can be explained in part by the low content of ALA in muscles of experimental birds of this study.

Inclusion of oil seeds in the diets increased the PUFA of meat. The presence of PUFA in bird tissues depends on their presence in the diet; therefore, the increase in meat PUFA was due to the higher proportion of PUFA in these diets.

The antibody titers from birds fed with the ALA enriched-diet were higher than the titers from the control birds; however, the differences were not significant. Contrary to our findings, Friedman and Sklan (1995) reported a more rapid, higher, and persistent antibody production in broiler fed high levels of ALA. The conflicting results of the effects of dietary n-3 PUFA on humoral immunity could be due to different levels of incorporation of dietary fatty acid in the diets and the sources of n-3 PUFA. It seems that the level and source of n-3 PUFA affect the antibody production in chicks, as long chain n-3 PUFA (e.g. eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)) have higher ability to increase immune response compared with ALA. However, there are also some reports of non-significant effect of ALA-enriched diets on antibody production (Fritsche *et al.*, 1992; Phetteplace and Watkins, 1992; Wang *et al.*, 2000).

The lack of difference in serum lipoprotein disagreed with other studies showing that lipid content of serum was reduced as the dietary PUFA levels increased (Newman *et al.*, 2002; Celebi and Utlu, 2006). However, Washburn and Nix (1974) showed that serum CHL level was not affected noticeably by dietary PUFA. The discrepancies between studies on lipid content of serum might be attributed to the genetic and dietary variations. It seems that the lack of effect of n-3 FA supplementation on CHL and TG of serum might be related

to the modest omega-3 FA concentration in muscles and particularly the absence of long chain omega-3 PUFA metabolites.

In conclusion, broilers fed diets containing FS, CS or a blend of these oil seeds produced omega-3 FA enriched meat, while their performance was negatively affected by oil seeds supplementation. We suggest the simultaneous use of suitable antioxidants and extracted oils of CS and FS rather than ground seeds, to enrich broiler meat by n-3 FA, avoiding reduction of broiler performance and meat shelf life.

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