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Effect of Vegetable Oil Types on Energy Expenditure, Abdominal Fat Deposition and Fatty Acid Profile of Breast and Thigh Muscles in Broilers

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Abstract

This experiment was conducted to determine the effect of different vegetable oil types on broiler energy expenditure, abdominal fat deposition and fatty acid profile of breast and thigh. A total number of 300 un-sexed day-old cobb 500 broiler chickens were used in a completely randomized design, consisted of five treatments (five different vegetable oil sources including soy, flaxseed, canola, corn, and sunflower oil), with five replicates and 10 chicks in each. Different vegetable oil sources had no significant effect on energy efficiency ratio and abdominal fat deposition. Flaxseed oil increased C18:3 and C20:5 in breast and C18:3, C20:3, C20:4 and C20:5 in thigh muscle ($P < 0.05$). The highest content of n-3 fatty acids was observed in breast of broilers fed diets treated with flaxseed oil ($P < 0.05$). The C18:3 content of thigh of broilers fed flaxseed was significantly higher than those chicken received other oil sources ($P < 0.05$). A significant increase in C20:5 was seen in the thigh of chicken received flaxseed oil, too ($P < 0.05$). The highest content of C18:2 was observed in the breast of the chickens fed corn oil and the lowest was seen in broilers received canola oil ($P < 0.05$). The results showed that dietary oil type could affect fatty acid profile of broiler breast and thigh despite lack of significant difference in broiler energy expenditure or abdominal fat deposition.

Introduction

In the most regions, the dietary ratio of n-6 to n-3 fatty acids has shifted from 8:1 to as high as 20:1 (Simopoulos, 2016). This ratio is too high and very far from acceptable ratio of 4:1 (Barceló-Coblijn and Murphy, 2009). Increasing n-3 fatty acid consumption is not always an easy goal because n-3 fatty acid natural sources are scarce and low popular and their synthetic sources (e.g. n-3 fatty acid capsules) exhibit limited availability. Using functional foods seems to be necessary to provide omega-3 fatty acids to the general population (Ruxton *et al.*, 2004).

Broiler chicken is an important ingredient for our regimen (Tang *et al.*, 2007). Unfortunately, because chicken's diets are composed of grains that are rich in linoleic acid and almost free from alpha linolenic acid, their meat is low in essential n-3 fatty acids, so, it is not such healthy (Pumchasov and Nir, 1992).

Inclusion of vegetable derived n-3 fatty acids to broiler diets is a useful method to increase n-3 fatty acid consumption (Ian Givens and Gibbs, 2008). However, the common crops such as corn, soybean, and the most widely consumed

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vegetable oils consumed are rich in linoleic acid, which competes with alpha linolenic acid for enzymes involved in fatty acid elongation and desaturation (Truswell, 2002). Therefore, high dietary intake of n-6 fatty acids affects the bird's health and nutritional quality of produced meat (Chanmugam *et al.*, 1992). Different vegetable oil sources had been used in broiler diets (Zollitsch *et al*, 1997). However, it is important to evaluate the direct deposition of fatty acids in different tissues. The inclusion of linolenic acid containing sources such as flaxseed oil in broiler diets is a more functional and sustainable mean to enhance dietary intake of linolenic acid (Konieczka *et al*., 2017). In addition, birds genetically have the ability to synthesis very long-chain PUFAs, mainly docosahexaenoic acid if alpha linolenic acid is the main poly unsaturated fatty acid in the diet, thus, it is possible to take advantage of the influence of dietary lipids on the depots of fatty acids in the body and composition of body fatty acids (Sampson, 2015). While, most of the situations that broilers were fed a diet high in alpha linolenic acid, just a relatively small increase in eicosapentaenoic acid and docosahexaenoic acid were observed that may be due to enzymatic activity or direct deposition of alpha linolenic acid (Carragher *et al*., 2016).

N-3-enriched broiler meat have a considerable potential for altering the balance of n-3 to n-6 fatty acids in the human diet, because broiler meat has a high consumption (Kanakri *et al*., 2017). In this experiment five different vegetable oil sources (soybean, canola, sunflower, linseed, and corn oils) in broiler diets were incorporated to determine their effects on fatty acid profile of broiler breast and thigh meat and evaluate their effects on energy expenditure and abdominal fat deposition. The data will increase our understanding on the characteristics of fatty acid metabolism of broiler chicken, and provide direction for the selection of lipid sources in developing formulated diet of broiler chickens towards producing value added products.

Material and Methods

Gorgan University of Agricultural Sciences and Natural Resources, Iran, approved the protocol for animal experiment. Three hundred unsexed, day old chicks of Cobb 500 were randomly assigned to 30 replicates of 10 birds each and reared on deep litter floor pens for 42 days. Five practical diets formulated according to NRC (1994) recommendation (Table, 1). The diets prepared as mash. Birds received basal diet differed in the oil source (Soybean, Canola, Sunflower, flaxseed or corn oil). The control group received the diet contained soybean oil. Fatty acid profile of the diets (Table, 1) and the, oil sources (Table, 2) were determined too. Birds had free access to feed and water. Birds weighted and feed intake measured weekly.

At 42 days of age, following 6 h fast, chickens were weighted and then slaughtered by cervical dislocation. Thigh and breast samples were immediately trimmed of excess fat and washed under running tap water. Then, the samples were wrapped in air-permeable plastic bags and stored at -20°C until fatty acid analysis. Fatty acid methyl esters (FAME) of feed, oil sources and thigh and breast samples were prepared using direct FAME synthesis method (O'Fallon *et al*., 2011). The fatty acid composition of the FAME was determined by capillary GC on a SP-2560, 100 m × 0.25 mm × 0.20 µm capillary column installed on a Hewlett Packard 5890 gas chromatograph equipped with a HP 3396 injector and HP 7673 controller, a flame ionization detector, and split injection. The initial oven temperature was 140°C, held for 5 min, then increased to 240°C at a rate of 4°C min^{-1}. Helium was used as the carrier gas at a flow rate of 0.5 ml.min-1. Both the injector and the detector were set at 260°C. Fatty acids were identified by comparing their retention times with the fatty acid methyl standard. A completely randomized design with 5 dietary treatments and 6 replicates each differing in the oil source was used. Data were subjected to one-way ANOVA by using the GLM procedure of SAS (SAS Institute, 2003). The statistical significance of the differences checked using the TUKEY test at the level of 0.05.

Table 1. Composition of the experimental diets (g kg^{-1})

Ingredients	Starter (1-21 day)					Grower (22-42 day)				
	Soybean	Flaxseed	Canola	Corn	Sunflower	Soybean	Flaxseed	Canola	Corn	Sunflower
Corn	560.3	560.3	560.3	560.3	560.3	572.2	572.2	572.2	572.2	572.2
Soybean meal (440 g kg^{-1} CP)	372	372	372	372	372	338.4	338.4	338.4	338.4	338.4
Oil source	25	25	25	25	25	50	50	50	50	50
Limestone	14.3	14.3	14.3	14.3	14.3	13.1	13.1	13.1	13.1	13.1
Dicalcium phosphate	17.6	17.6	17.6	17.6	17.6	16.0	16.0	16.0	16.0	16.0
Salt	3	3	3	3	3	3	3	3	3	3
Vitamin premix*	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Mineral premix*	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
DL-Methionine	2.3	2.3	2.3	2.3	2.3	1.8	1.8	1.8	1.8	1.8
Salinomycine	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Chemical composition										
ME (Kcal Kg-1)	3031	3031	3031	3031	3031	3206	3206	3206	3206	3206
Crude protein (g kg-1)	218.1	218.1	218.1	218.1	218.1	200.4	200.4	200.4	200.4	200.4
Crude fat (g kg-1)	38.7	38.7	38.7	38.7	38.7	61.5	61.5	61.5	61.5	61.5
Calcium (g kg-1)	10	10	10	10	10	9.4	9.4	9.4	9.4	9.4
Available phosphorus (g kg-1)	5	5	5	5	5	4.7	4.7	4.7	4.7	4.7
Sodium (g kg-1)	1.9	1.9	1.9	1.9	1.9	1.3	1.3	1.3	1.3	1.3
Methionine+ cystine (g kg-1)	9.2	9.2	9.2	9.2	9.2	8.1	8.1	8.1	8.1	8.1
Lysine (g kg-1)	12.5	12.5	12.5	12.5	12.5	10.4	10.4	10.4	10.4	10.4
Arginine (g kg-1)	13.5	13.5	13.5	13.5	13.5	13.1	13.1	13.1	13.1	13.1
Fatty acid composition (%)										
C14:0	0.05	0.04	0.04	0.05	0.04	0.05	ND	0.06	0.05	0.04
C14:1n5	ND**	ND	ND	ND	ND	ND	ND	ND	ND	ND
C16:0	23.7	28.1	5.8	18.5	20.1	27.4	23.2	3.7	19	22.2
C16:1n7	2.6	3.5	11.4	7.2	3.3	1.3	4.4	8.6	8.1	2.2
C18:0	13.3	16.3	9.2	5.9	7.6	18	19.5	11.8	6.8	10.2
C18:1n9	34.2	22.3	33.6	38.9	36.3	29.7	17	30.7	36.6	29.5
C18:2n6	15.3	6.6	31.5	21.3	24.2	21.6	8.5	37.6	20.1	28.6
C18:3n3	5.9	19.4	2.7	5.6	1.7	5.2	22.5	4.3	6.3	1.6

*Supplied the following per kg of diet: Cholecalciferol , 0.038 mg; retinyl acetate, 3.6 mg; α-tocopherol, 50 mg; menadione,1.7 mg; thiamin, 1.1 mg; riboflavin, 5.5 mg; niacin, 44 mg; D -pantothenate, 11 mg; pyridoxine, 2.2 mg; folic acid, 0.6mg; biotin, 0.03 mg; cyanocobalamin , 0.013 mg; choline (0.05% inclusion), 300 mg; Ca, 75 mg; Na, 0.02 mg; K, 1.1 mg; Mg,21 mg; Mn, 144 mg; Zn, 80 mg; Fe, 32 mg; Cu, 8 mg; I, 1.6 mg; and Se, 0.30 mg.
**ND = Not Detected.

Table 2. Fatty acid profile (g kg^{-1} fatty acid methyl ester) of the oil sources

Fatty acids	Oil sources				
	Soybean	Flaxseed	Canola	Sunflower	Corn
C14:0	6	5	4	3	4
C14:1n5	0.4	0.5	0.5	0.06	0.4
C16:0	81	73	44	78	111
C16:1n7	ND*	ND*	ND*	ND*	ND*
C18:0	55	41	38	27	23
C18:1n9	242	208	614	335	252
C18:2n6	488	191	178	531	572
C18:3n3	90	456	82	5	12

*ND: Not Detected

Results and Discussion

Energy efficiency ratio

The energy efficiency ratio was not affected by dietary oil source (Figure, 1). The least dietary recommended ratio of UFA:SFA for best performance of broiler chicken is 3-4:1 to exhibit the optimum performance (Scott *et al*, 1982). All the experimental diets here provided the ratio of 5-10:1, so, the insignificant energy efficiency ratio difference observed for broilers fed different vegetable oil sources with different fatty acid profiles confirms that the experimental diets effectively supplied fatty acid requirement of the birds. In this study the SFA, MUFA and

PUFA content of diets containing different oil sources ranged between 15-45% of total ingested fatty acids, indicating that SFA, MUFA and PUFA have a same rate of digestion and assimilation (Roche *et al*., 1998; Roche and Gibney, 2000; Berry *et al*., 2007). So, these findings indicating that varying dietary SFA, MUFA and PUFA across a range, has no significant effect on energy expenditure, but it has been reported that consuming triglycerides containing C6 to C10 fatty acids, increase thermogenesis in rat and human (Hill *et al*., 1990; St-Onge *et al*., 2014). So, it seems that energy expenditure is higher when C6 to C10 compared to long chain fatty acid fortified diets were consumed (St-Onge *et al*., 2014). Therefore, fortification of chicken meat with these fatty acids would be useful to control body weight and fat accumulation in consumers. Another reason for insignificant difference between treatments for energy efficiency ratio could be differential composition in dietary fatty acids. Such results observed when diets were highly fortified in different individual fatty acids or when food ingredients naturally fortified with different type of fatty acids (Siro *et al*., 2008).

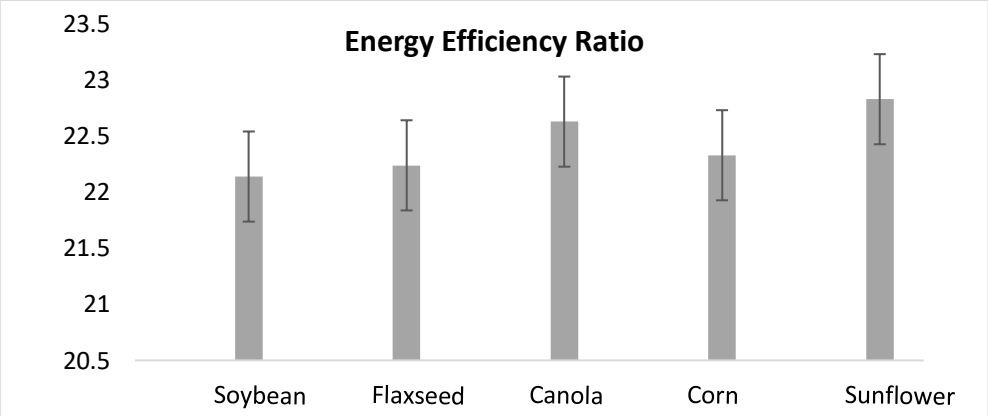

Figure 1. Effect of dietary oil type on energy efficiency ratio from 1-42 days of age

Abdominal fat

The highest adipose tissue was found in chicks received corn oil (contained the highest MUFA) while there was no significant effect between different dietary oil sources (Figure, 2). This small difference may be due to MUFA content of the different treatments. It has been reported that adipose tissue has a special feature in which the proportion of MUFA is greater than that in the diet (Wood *et al*., 2008). In this regards, it has been reported that almost 56% of fatty acids of adipose tissue are in the form of monounsaturated fatty acids while dietary intake was only about one third of total fat intake, so, it is assumed that monounsaturated fatty acids are greater absorbed or lower released by adipose tissue or desaturation of saturated fatty acids in such tissues leading to a higher content of monounsaturated fatty acids (Ailhaud *et al*., 2006). Small differences in abdominal fat accumulation between different treatments in this study could result from differences exist between fatty acid profile of different treatments that may affect the place the fat may accumulate, for example palmitate increases lipid accumulation in visceral but not in subcutaneous adipocytes while oleate increases lipid accumulation in subcutaneous only (Wajchenberg, 2000). During short periods, any extra ingested amino acids or glucose is oxidized while excess in ingested fat are deposited (Baba *et al,* 1982). This shows that energy and fat balance are correlated significantly and chemical structure of fatty acids deeply affects them. The C18:1 and C18:2 are greater and faster oxidized than the C16:0 and C18:0 (Baba *et al,* 1982). Therefore, it seems that diets fortified with specific fatty acids have shown to affect fat balance, body fat depots and energy expenditure.

In a human study the data showed a reduction in lipid oxidation following feeding a highly enriched palmitate diet and no change in postprandial lipid oxidation after the high-oleate diet fed to healthy young adults for 28 days (Bergouignan *et al*., 2009). In another study using virgin olive oil (MUFA source) and cream (SFA source), researchers showed that postprandial fat oxidation rate is higher in men fed MUFA compared to whom fed SFA fortified meal (Chen *et*

al, 2013). Another study showed that replacing SFA by MUFA significantly reduced fat mass without and changes in total energy intake (Heilbronn *et al,* 1999). It was reported that feeding healthy men by a diet rich in C18:1 fatty acid has no effect on daily energy expenditure, while feeding a diet high in C16:0 fatty acid reduced it significantly (Storlien *et al,* 1998). It seems that sympathetic activity in brown adipose tissue in rats fed SFA compared to those fed MUFA falls down, and diet-induced thermogenesis decreases that results in the accumulation of fats in the body (Takeuchi *et al,* 1995).

The present study showed that deposition of broiler abdominal fat was not affected by dietary oil type. This can be explained because all vegetable oil types contain a high proportion of unsaturated fatty acids, which down regulate lipid synthesis and stimulate fatty acid degradation (Schmitz and Ecker, 2008). The evidences show that n-3 fatty acids inhibit body fat accumulation that attributes to the higher lipolysis and lower lipogenesis happens mainly in the liver (Chavez and Summers, 2003). Using isocaloric diets, Hatori *et al,* (2012) showed that feeding a high-fat diet contained n-3 fatty acids reduced body fat deposits compared with a high-fat diet contained n-6 fatty acids or saturated fatty acids or low-fat diets (Hatori *et al.*, 2012). It is believed that effect of n-3 fatty acids on body fat depletion is through activation of PPARα (Haag and Dippenaar, 2005). N-3 fatty acids including alpha linolenic acid and eicosapentaenoic acid have high affinity to PPARα and consequently induce expression of some lipolytic genes which are controlled by PPARα such as Carnitine palmitoyltransferase I (CPT1), uncoupling protein and acyl-coA oxidase (Calder, 2012), On the other hand n-3 fatty acids suppress the expression of ACC, FAD and SCD which involved in fatty acid biosynthesis (Nagao and Yanagita, 2008). Furthermore, n-3 fatty acids inhibits HNF4α activity which inhibit pyruvate kinase that inhibits glucose flux into synthesis of lipids (Nagao and Yanagita, 2008).

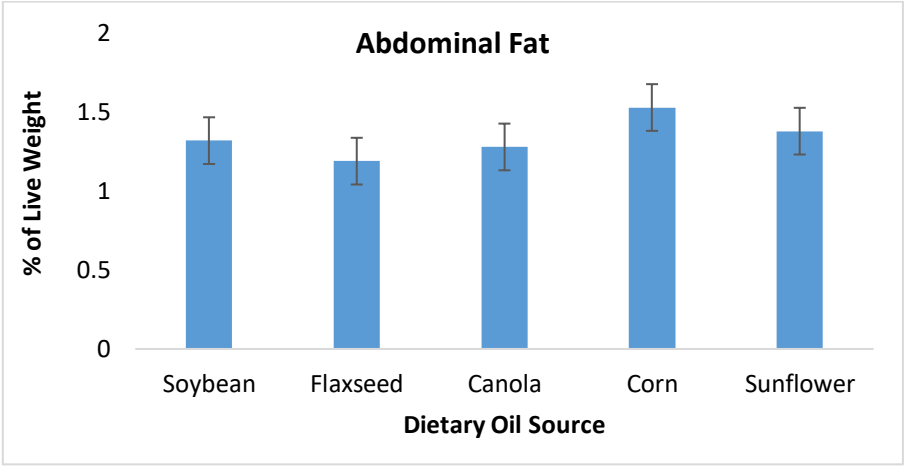

Figure 2. Effect of dietary oil type on abdominal fat deposition (%)

Fatty acid profile of breast and thigh

The fatty acid profiles of the breast and thigh are presented in tables 3 and 4. Dietary oil type greatly affected the fatty acid profile of the tissues ($P < 0.05$) except for the C14:0 in breast ($P > 0.05$). The C14:0 proportion was highest in sunflower fed broilers, compared to the other dietary oil types. In this regards, some researchers believe that through high long chain fatty acid ingestion, saturated fatty acid is deposited preferentially in contrast to monounsaturated fatty acids (Cortinas *et al.*, 2004). A decrease in C14:0 on the flaxseed oil supplemented diet was accompanied with an increase in C14:1 and C16:1 and an increase in C18:1 in both the breast and thigh. This was in parallel with reports showing an inhibition of the delta-9-deasaturase by dietary long chain n-3 fatty acids (Shen *et al.*, 2005).

In both breast and thigh, flaxseed oil fed broilers had the highest C18:3 and C20:5. The n-3 PUFA in breast and thigh meat from flaxseed oil fed birds increased 2.1 to 2.5 fold compared to soybean oil fed birds. The accumulation of C18:3 in these tissues from flaxseed oil is clear evidence that the fatty acid composition of

broiler breast and thigh directly respond to changes in the fatty acid composition of diet. This very large response in alpha linolenic acid following the dietary supplementation of flaxseed oil, may be explained by it's suitable distribution in phospholipids and triglycerides fractions of the both tissues compared to the other oil sources. However, it should be mentioned that reports exist on increased de novo fatty acid synthesis when flaxseed oil was added to the broiler diets (Crespo and Esteve-Garcia, 2002). The C18:1 proportion was the highest in the breast of canola fed broilers, in comparison to the other treatments. The increased tissue content of C18:1 in all diets except flaxseed oil fed birds confirms that deficiency of dietary C18:3 (n-3 fatty acid) results in an increase in the level of C18:1 (n-9 fatty acid). The contents of alpha linolenic acid and eicosapentaenoic acid were higher in the breast and thigh of broilers fed flaxseed oil treated diet (Table, 3). The soybean and sunflower oil fortified diets significantly increased C16:0 in the breast muscle of broilers.

Gradual increase of short and unsaturated fatty acids, from C14 to C18, in the breast and thigh muscles may be due to oxidation rate, as it was reported dietary SFA and UFA are more oxidized than dietary long and saturated fatty acids (Lands *et al*, 1990), so, it is assumed that UFA are more channeled toward mitochondria for oxidation than saturated fatty acids. Surprisingly, Palmitate content of broiler breast and thigh muscles were lower compared to oleate while dietary intake of palmitate was higher than oleate in all the treatments, this suggest that palmitate oxidation may be favored compared to oleate oxidation. In this regards, it has been reported that carnitine palmitoyl transferase 1, that is an important enzyme for the entrance of LCFA-coA into mitochondria, has low affinity for oleyl-coA compared to palmitoyl-coA (Bouyakdan *et al*., 2015).

Table 3. Effect of different oil sources on fatty acid profile of broiler breast meat

Fatty acid	Oil Source					*SEM*	*P*-value
	Soybean	Flaxseed	Canola	Corn	Sunflower		
C14:0	2.86	2.80	3.00	2.17	3.10	0.372	0.47
C14:1 n-5	1.10^{b}	1.63^{a}	0.90^{bc}	0.50^{d}	0.67^{cd}	0.084	0.05
C16:0	10.03^{ab}	6.33^{d}	7.76^{c}	8.80^{bc}	10.27^{a}	0.406	0.05
C16:1 n-7	1.60^{c}	5.16^{a}	1.56^{c}	2.83^{b}	2.06^{bc}	0.301	0.05
C18:0	20.80^{a}	12.23^{b}	20.70^{a}	19.66^{a}	18.53^{a}	0.801	0.05
C18:1 n-9	32.36^{b}	26.96^{c}	37.36^{a}	29.43^{bc}	29.30^{bc}	0.977	0.05
C18:2 n-6	21.80^{cd}	25.10^{bc}	18.16^{d}	29.46^{a}	26.96^{ab}	1.292	0.05
C18:3 n-3	6.26^{b}	12.96^{a}	6.36^{b}	5.06^{c}	6.23^{b}	0.349	0.05
C20:3 n-6	0.53^{b}	1.36^{a}	1.36^{a}	0.43^{b}	0.43^{b}	0.049	0.05
C20:4 n-6	0.56^{b}	2.36^{a}	0.63^{b}	0.33^{b}	0.56^{b}	0.092	0.05
C20:5 n-3	0.53^{b}	1.50^{a}	0.36^{b}	0.33^{b}	0.53^{b}	0.065	0.05

Data with different superscripts within a row are significantly different ($P < 0.05$).

Table 4. Effect of different oil sources on fatty acid profile of broiler thigh meat

Fatty acid	Oil Source					*SEM*	*P*-value
	Soybean	Flaxseed	Canola	Corn	Sunflower		
C14:0	2.80^{a}	1.90^{b}	3.10^{a}	1.87^{b}	2.30^{b}	0.153	0.05
C14:1 n-5	1.10^{ab}	1.27^{a}	0.93^{ab}	0.93^{ab}	0.90^{b}	0.100	0.05
C16:0	10.67^{b}	5.60^{c}	10.27^{b}	12.03^{a}	10.33^{b}	0.386	0.05
C16:1 n-7	1.77^{bc}	5.73^{a}	1.17^{c}	2.33^{b}	1.87^{bc}	0.223	0.05
C18:0	18.17^{b}	14.90^{c}	22.33^{a}	18.33^{b}	18.40^{b}	0.482	0.05
C18:1 n-9	30.60^{b}	25.33^{c}	34.47^{a}	26.40^{c}	30.17^{b}	0.538	0.05
C18:2 n-6	26.30^{b}	25.83^{b}	17.77^{c}	30.10^{a}	25.57^{b}	0.425	0.05
C18:3 n-3	4.96^{c}	12.30^{a}	5.60^{b}	4.93^{c}	4.83^{c}	0.193	0.05
C20:3 n-6	0.50^{b}	2.53^{a}	0.53^{b}	0.47^{b}	0.70^{b}	0.082	0.05
C20:4 n-6	0.93^{b}	1.87^{a}	0.90^{b}	0.87^{b}	0.83^{b}	0.098	0.05
C20:5 n-3	0.53^{b}	1.60^{a}	0.60^{b}	0.53^{b}	0.43^{b}	0.082	0.05

Data with different superscripts within a row are significantly different ($P < 0.05$).

Conclusion

Using different vegetable oil types in broiler diets is an acceptable method to change fatty acid profile of the breast and thigh muscle in broiler chickens with no any adverse effects on energy metabolism or body fat accumulation.

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