Fatty acid profile, cholesterol and oxidative status in broiler chicken breast muscle fed different dietary oil sources and calcium levels

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Abstract

The aim of the present study was to evaluate the effects of three feeds containing 6% oils: palm oil (PO), soybean oil (SO) and linseed oil (LO); and three calcium levels (NRC recommendation, 1.25% and 1.50%) on the fatty acid profile, lipid oxidation and cholesterol concentrations of broiler breast meat in a 3 × 3 factorial experiment. A total of 378 one-day-old chicks were randomly assigned to the diets and fed for six weeks. Birds fed diet supplemented with LO, SO and PO had higher proportions of α-linolenic, linoleic and oleic acids, respectively. The LO diet increased the total n-3 fatty acids and decreased the n-6 : n-3 compared with the PO and SO diets. Birds fed the PO diet had higher oxidative stability and cholesterol compared with those fed the SO and LO diets. However, the level of cholesterol in all treatments was within the normal range. The level of calcium and interaction between source of oil and calcium level did not influence lipid oxidation, fatty acid profile and cholesterol level of broiler breast muscle. It can be concluded that dietary LO and SO enhanced n-3 and n-6 polyunsaturated fatty acids, respectively, while dietary PO enhanced the oleic acid and oxidative stability of broiler breast muscle. Thus, this study showed that PO can be used as an alternative oil source in broiler diets with a positive effect on the oxidative stability of chicken meat refrigerated at seven days when compared with vegetable oils that are rich in linoleic and α-linolenic acid.

Keywords: Chicken meat quality, dietary calcium levels, linseed oil, palm oil, soybean oil
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Introduction

Fat and fatty acids in muscle and adipose tissues are among the major factors that influence meat quality, particularly nutritional value and palatability (Coetzee & Hoffman, 2002). Changes in the dietary fatty acid (FA) composition could be reflected in the blood, which in turn would be transported to target organs such as muscle (Aghwan *et al.*, 2014). Poultry meat is considered healthier owing to its relatively lower fat content compared with other animal meat (Brenes & Roura, 2010). Generally, the lipids of the muscle fibres contain a proportion of phospholipids, triacylglycerol and cholesterol (Pikul *et al.*, 1984). The fatty acids of triacylglycerol are made up mainly of saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA). Conversely, the fraction of phospholipid has a higher proportion of polyunsaturated fatty acids (PUFA), which is strictly controlled as a component of cellular membranes (De Smet *et al*., 2004). Red muscles reportedly contain a higher proportion of phospholipids than white muscles and thus a relatively higher amount of PUFA (Wood *et al*., 2004). However, a higher level of PUFA in muscle membranes is related to increasing susceptibility of meat and meat products to lipid oxidation (Adeyemi & Olorunsanya, 2012b). PUFA undergo

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rapid oxidative changes, which impair organoleptic characteristics, shorten meat shelf-life and produce off-flavours (Adeyemi & Olorunsanya, 2012a).

Manipulation of PUFA composition without affecting product quality has been a challenge for poultry scientists. Although poultry meat contains high levels of UFA, levels of natural antioxidants such as tocopherols are low (Melton, 1983). On the other hand, the current interest in increasing the n-3 PUFA content of meat and eggs increases the need for additional antioxidant protection. Methods that are effective, safe and of low cost for controlling storage stability of the poultry meat are extremely important to the industry. Recently there has been increased interest in the role of antioxidant nutrients owing to their health benefits in disease conditions such as cancer, coronary heart disease and immune functions (Bendich, 1990; Diplock, 1991). Additionally, the incorporation of vegetable oils that are rich in natural antioxidants has been suggested as an effective and economical means of controlling post-mortem lipid peroxidation and an alternative way of increasing these health-enhancing nutrients in human diets.

Several reports have shown that the level of dietary calcium affects the fatty acid profile of broiler chickens. Earlier studies (Atteh & Leeson, 1983; Ajakaiye *et al*., 2003) on chicken showed that dietary calcium can form calcium soaps with fatty acids, resulting in lower digestion and absorption of fat and calcium. Hence, supplementation of dietary oil in poultry diets calls for adequate dietary calcium to promote absorption and utilization of dietary fat and calcium and to forestall problems that may jeopardize the well-being of the birds and product quality. Therefore, the objective of this study was to determine the effect of palm oil (PO), soybean oil (SO) and linseed oil (LO) with three levels of calcium : phosphorus on fatty acid profile, lipid oxidation and cholesterol concentration in broiler breast meat.

Materials and Methods

This study was conducted according to the animal ethics guidelines of the Research Policy of University of Putra Malaysia.

A total of 378 one-day-old chicks (Cobb 500) were used for this study. On arrival, the chicks were individually wing banded, weighed and randomly assigned to nine treatment groups. Each group consisted of six replicates, and each replicate was made up of seven chicks. After seven days of the rearing period, all birds were vaccinated with IB-ND live vaccine against infectious bronchitis (IB) and Newcastle Disease (ND) through the intraocular route. The IBD vaccine against infectious bursal disease (IBD) was applied on day 14 of the rearing period through this route. The birds had free access to water and feed. The climatic conditions and lighting programme followed commercial recommendations. The environmental temperature in the first week of life was 35 ºC and was then decreased to 28 ºC until the end of the experiment. During the first week, 22 h of light were provided with a reduction to 20 h afterwards.

Two basal diets were formulated, namely starter and finisher. The starter diet was fed for the first three weeks, and the finisher was fed for the last three weeks of the experimental period. The compositions of the basal starter and finisher diets are shown in Tables 1 and 2, respectively. The diets contained three types of oil, namely LO, SO and PO as control group, and three levels of calcium : phosphorus, namely 1 : 0.5; 1.25 : 0.63; and 1.5 : 0.75 (NRC, 1994). Palm oil was used as control in this experiment because it is commonly used in poultry production in Malaysia. Diets were formulated to meet the requirements of all nutrients for broiler chickens using FeedLIVE software (FeedLIVE 1.52, Thailand).

At the end of the experiment, 12 birds per treatment (two birds from each cage) were randomly selected and weighed. The birds were slaughtered by neck decapitation, bled and processed. The right *pectoralis major* (breast) muscles were removed within 45 min of slaughter from all carcasses and separated into four sections. The first section was snap frozen in liquid nitrogen before being stored at −80 ºC to determine the fatty acid profile and cholesterol. The other three sections were vacuum packed and kept in a chiller at 4 ºC for thiobarbituric acid reactive substance (TBARS) evaluation on the 1st, 3rd and 7th day post mortem. After the ageing period, the muscle samples were snap frozen in liquid nitrogen and stored at −80 ºC until further analyses.

The total fatty acids were extracted from breast muscles based on the method Folch *et al*. (1957), described by Loh *et al*. (2009), and modified by Ebrahimi *et al*. (2014), using chloroform : methanol (2 : 1, v/v) containing butylated hydrotoluene to prevent oxidation during sample preparation. The extracted fatty acids were transmethylated to their fatty acid methyl esters (FAME) using 0.66 N KOH in methanol and 14% methanolic boron trifluoride (BF_3) (Sigma Chemical Co., St. Louis, Mo, USA) according to the methods by AOAC (1990). The FAME were separated by gas liquid chromatography on an Agilent 7890A GC system (Agilent, Palo Alto, Calif, USA) using a 100 m × 0.25 mm ID (0.20 µm film thickness) Supelco SP-2560 capillary column (Supelco, Inc., Bellefonte, Phil, USA). One microlitre FAME was injected by an autosampler into the chromatograph, which was equipped with a flame ionization detector (FID). The carrier gas was He, and the split ratio was 10 : 1 after the FAME was injected. The injector temperature was programmed at 250 ºC, and the detector temperature was 300 ºC. The column temperature programme initially ran at 120 ºC,

held for 5 min, increased by 2 ºC/min to 170 ºC, held at 170 ºC for 15 min, increased again by 5 ºC/min to 200 ºC, held at 200 ºC for 5 min, then increased again by 2 ºC/min to a final temperature 235 ºC, and held for 10 min. The FA concentrations were expressed as percentage total identified FA. A reference standard (mix C4 - C24 methyl esters; Sigma-Aldrich, Inc., St. Louis, Mo, USA) and CLA standard mix (CLA cis-9 trans-11 and CLA trans-10, cis-12, Sigma-Aldrich, Inc., St. Louis, Mo, USA) were used to establish recoveries and correction factors to determine individual FA composition.

Table 1 Composition the starter diet (g/kg) and calculated nutrient content

Ingredients	PO[1]			SO			LO		
	Ca1[2]	Ca2	Ca3	Ca1	Ca2	Ca3	Ca1	Ca2	Ca3
Maize	399.5	412.8	426.5	399.5	412.8	426.5	399.5	412.8	426.5
Soybean meal	400.0	406.0	412.5	400.0	406.0	412.5	400.0	406.0	412.5
Palm oil	60.0	60.0	60.0	0	0	0	0	0	0
Soybean oil	0	0	0	60.0	60.0	60.0	0	0	0
Linseed oil	0	0	0	0	0	0	60.0	60.0	60.0
Wheat pollard	97.7	68.8	38.9	97.7	68.8	38.9	97.7	68.8	38.9
MDCP[3] 21%	15.9	22.8	29.8	15.9	22.8	29.8	15.9	22.8	29.8
Calcium carbonate	14.7	17.5	20.2	14.7	17.5	20.2	14.7	17.5	20.2
Salt	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
L-Lysine	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
DL-Methionine	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Antioxidant[4]	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Vitamin premix[5]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Mineral premix[6]	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Choline chloride	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Toxin binder[7]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Calculated nutrient content (g/kg)[8]									
ME (MJ/kg)	13.0	13.0	13.0	13.0	13.0	13.0	13.0	13.0	13.0
Crude protein	220.2	220.1	220.1	220.2	220.1	220.1	220.2	220.1	220.1
Crude fat	77.8	78.3	78.9	77.8	78.3	78.9	77.8	78.3	78.9
Crude fibre	45.5	44.0	42.5	45.5	44.0	42.5	45.5	44.0	42.5
Calcium	10.0	12.5	15.0	10.0	12.5	15.0	10.0	12.5	15.0
Total phosphorus	8.3	9.6	11.0	8.3	9.6	11.0	8.3	9.6	11.0
Avail. P for poultry	4.5	5.6	6.8	4.5	5.6	6.8	4.5	5.6	6.8

[1] PO: 6% palm oil in diet; SO: 6% soybean oil in diet; LO: 6% linseed oil in diet.
[2] Ca1: 1% calcium and 0. 45% available phosphorus; Ca2: 1.25% calcium and 0. 56% available phosphorus; Ca3: 1.50% calcium and 0.67% available phosphorus.
[3] Mono dicalcium phosphate
[4] Antioxidant contains butylated hydroxyanisole (BHA).
[5] Supplied per kg diet: Vitamin A 11 494 IU; vitamin D 1 725 IU; vitamin E 40 IU; vitamin K_3 2.29 mg; cobalamin 0.05 mg; thiamine 1.43 mg; riboflavin 3.44 mg; folic acid 0.56 mg; biotin 0.05 mg; panthothenic acid 6.46 mg; niacin 40.17 mg; pyridoxine 2.29 mg.
[6] Supplied per kg diet: Fe 120 mg; Mn 150 mg; Cu 15 mg; Zn 120 mg; I 1.5 mg; Se 0.3 mg; Co 0.4 mg.
[7] Toxin binder contains natural hydrated sodium calcium aluminium silicates (HSCAS).
[8] The diets were formulated using feed live International software (Thailand).

The determination of muscle cholesterol was carried out using the method described by Rudel & Morris (1973). A crushed meat sample (1 g) was homogenized with 3 mL 95% ethanol and 2 mL 50% potassium hydroxide. The homogenates were incubated in a water bath at 60 ºC for 10 min following by

cooling to room temperature. A 5 mL hexane was added to homogenates then mixed for 20 s. The final homogenate was raised with additional 3 mL deionised water and vortexed then allowed to settle at room temperature for 15 min to complete phase separation. About 2.5 mL of the upper phase (hexane layer) was transferred into a clean glass tube followed by evaporating the hexane to dryness under nitrogen gas flow at 60 ºC. The residue was re-suspended with 4 mL o-phthalaldehyde reagent and kept at room temperature for 10 min followed by adding 2 mL concentrated sulphuric acid slowly, mixing and standing for an additional 10 min at room temperature. Cholesterol standards (Sigma L-4646) were prepared according to the method of Rudel & Morris (1973), to make final concentrations of 0, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 µg cholesterol/mL. The concentration of cholesterol and standard were tested at absorbance 550 nm using a spectrophotometer (Secomam, Domont, France).

Table 2 Composition of the finisher diet (g/kg) and calculated nutrient content

Ingredients	PO[1]			SO			LO		
	Ca1[2]	Ca2	Ca3	Ca1	Ca2	Ca3	Ca1	Ca2	Ca3
Maize	483.5	495.0	507.0	483.5	495.0	507.0	483.5	495.0	507.0
Soybean meal	328.7	334.4	339.6	328.7	334.4	339.6	328.7	334.4	339.6
Palm oil	60.0	60.0	60.0	0	0	0	0	0	0
Soybean oil	0	0	0	60.0	60.0	60.0	0	0	0
Linseed oil	0	0	0	0	0	0	60.0	60.0	60.0
Wheat pollard	89.0	63.5	38.0	89.0	63.5	38.0	89.0	63.5	38.0
MDCP[3] 21%	10.7	16.1	21.6	10.7	16.1	21.6	10.7	16.1	21.6
Calcium carbonate	15.4	18.3	21.2	15.4	18.3	21.2	15.4	18.3	21.2
Salt	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
L-Lysine	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
DL-Methionine	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Anti-oxidant[4]	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Vitamin premix[5]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Mineral premix[6]	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Choline chloride	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Toxin binder[7]	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Calculated nutrient content (g/kg)[8]									
ME (MJ/kg)	13.3	13.3	13.3	13.3	13.3	13.3	13.3	13.3	13.3
Crude protein	195.1	195.2	195.0	195.1	195.2	195.0	195.1	195.2	195.0
Crude fat	80.1	80.6	81.1	80.1	80.6	81.1	80.1	80.6	81.1
Fibre	42.0	40.7	39.4	42.0	40.7	39.4	42.0	40.7	39.4
Calcium	9.0	11.3	13.5	9.0	11.3	13.5	9.0	11.3	13.5
Total phosphorus	6.9	7.9	9.0	6.9	7.9	9.0	6.9	7.9	9.0
Avail. P for poultry	3.5	4.4	5.3	3.5	4.4	5.3	3.5	4.4	5.3

[1] PO: 6% palm oil in diet, SO: 6% soybean oil in diet, LO: 6% linseed oil in diet.
[2] Ca1: 0.90% calcium and 0.35% available phosphorus; Ca2: 1.3% calcium and 0. 44% available phosphorus; Ca3: 1.35% calcium and 0.90% available phosphorus.
[3] Mono dicalcium phosphate.
[4] Antioxidant contains butylated hydroxyanisole (BHA).
[5] Supplied per kg diet: vitamin A 11 494 IU; vitamin D 1 725 IU; vitamin E 40 IU; vitamin K_3 2.29 mg; cobalamin 0.05 mg; thiamine 1.43 mg; riboflavin 3.44 mg; folic acid 0.56 mg; biotin 0.05 mg; panthothenic acid 6.46 mg; niacin 40.17 mg; pyridoxine 2.29 mg.
[6] Supplied per kg diet: Fe 120 mg; Mn 150 mg; Cu 15 mg; Zn 120 mg; I 1.5 mg; Se 0.3 mg; Co 0.4 mg.
[7] Toxin binder contains natural hydrated sodium calcium aluminium silicates (HSCAS).
[8] Diets were formulated using FeedLIVE software (Thailand).

Table 3 Fatty acid composition (% of total identified fatty acids) of the starter diet

Fatty acid	PO[1]			SO			LO			SEM[9]
	Ca1[2]	Ca2	Ca3	Ca1	Ca2	Ca3	Ca1	Ca2	Ca3	
C14:0	0.7	0.7	0.8	0.1	0.1	0.1	0.1	0.1	0.1	0.06
C16:0	31.4	31.9	32.6	11.6	11.6	11.8	8.0	8.1	8.3	2.06
C16:1n-7	0.2	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.2	0.01
C18:0	3.8	3.9	4.0	4.4	4.5	4.5	3.7	3.6	3.7	0.07
C18:1n-9	40.0	38.6	38.8	24.3	23.7	24.4	21.3	21.8	22.2	1.48
C18:2n-6	23.5	22.4	22.5	52.9	53.3	52.6	26.9	27.5	27.7	2.58
C18:3n-3	1.4	1.3	1.3	6.6	6.7	6.5	40.0	38.8	37.8	3.25
SFA[3]	32.4	32.8	33.6	12.8	11.7	12.0	8.2	8.3	8.5	2.13
UFA[4]	67.6	66.2	66.4	88.2	88.3	88.0	91.8	91.7	91.5	2.13
MUFA[5]	42.8	42.5	42.7	28.7	28.2	28.9	24.9	25.4	25.9	1.47
PUFAn-3[6]	1.4	1.3	1.3	6.6	6.7	6.5	40.0	38.8	37.8	3.25
PUFAn-6[7]	23.5	23.4	22.5	52.9	53.3	52.6	26.9	27.5	27.7	2.58
n-6 : n-3 ratio[8]	17.0	17.7	17.8	8.0	7.9	8.1	0.7	0.7	0.7	1.35
UFA : SFA	2.1	2.0	2.1	7.5	7.5	7.3	11.2	11.1	10.7	0.72
PUFA : SFA	0.8	0.8	0.7	5.0	5.1	4.9	8.2	8.0	7.7	0.58

[1] PO: 6% palm oil in diet, SO: 6% soybean oil in diet, LO: 6% linseed oil in diet.
[2] Ca1: 1% calcium and 0. 45% available phosphorus; Ca2: 1.25% calcium and 0. 56% available phosphorus; Ca3: 1.50% calcium and 0.67% available phosphorus.
[3] Total saturated fatty acid = sum of C14:0 + C16:0 + C18:0.
[4] Total unsaturated fatty acid = sum of C16:1 + C18:1n-9 + C18:2n-6 + C18:3n-3 + C20:4n-6 + C20:5n-3 + C22:5n-3 + C22:6n-3.
[5] Total mono unsaturated fatty acid = sum of C16:1 + C18:1n-9.
[6] Polyunsaturated fatty acid n-3 = sum of C18:3n-3 + C20:5n-3 + C22:5 n-3 + C22:6n-3.
[7] Polyunsaturated fatty acid n-6 = sum of C18:2n-6 + C18:3n-6 + C20:4n-6.
[8] Polyunsaturated fatty acid n-6: polyunsaturated fatty acid n-3= (C18:2n-6 + C18:3n-6 + C20:4n-6) ÷ (C18:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3).
[9] SEM: standard error of means.

Lipid peroxidation in muscle was determined using a malondialdehyde assay. Malondialdehyde is a secondary product of lipid peroxidation and is the major substrate in the TBARS test (Pryor, 1991). Measurements were taken on the 1st, 3rd and 7th day of ageing refrigerated at 4 ºC. Lipid oxidation was measured using thiobarbituric acid-reactive substances (TBARS) according to the method of Lynch & Frei (1993), modified by Mercier *et al*. (1998). One gram meat sample was homogenized in 4 mL 0.15 M KCl + 0.1 mM BHT with Ultraturrax (1 min, 6000 rpm). After homogenization, 200 µL of the sample were mixed with TBARS solution and then heated in a water bath at 95 ºC for 60 min until a pink colour developed. After cooling, 1 mL distilled water and 3 mL n-butyl alcohol were added to the extracts and homogenized. The mixture was centrifuged at 5000 rpm for 10 min. Absorbance of supernatant was read against an appropriate blank at 532 nm using a spectrophotometer (Secomam, Domont, France). The TBARS value was calculated from a standard curve of 1,1,3,3-tetraethoxypropane and expressed as mg malondialdehyde (MDA)/kg meat.

The experiment followed a three (sources of oil) by three (levels of calcium and phosphorus) factorial arrangement in a completely randomized design. The data obtained for fatty acids and cholesterol were analysed using the generalized linear model of SAS (SAS, 2007) while the data obtained for lipid oxidation was subjected to a generalized linear model with sampling time as a repeated measure. Significant differences between treatment means were compared using Duncan's multiple range test.

Results and Discussion

The fatty acid profile of experimental diets is shown in Tables 3 and 4. The supplementation of the diets with PO, SO and LO increased the C18:1n-9, C18:2n-6 and C18:3n-3, respectively. The ratio of n-6 : n-3 was in the order of PO > SO > LO.

Table 4 Fatty acid composition (% of total identified fatty acids) of the grower diet

Fatty acid	PO[1]			SO			LO			SEM[9]
	Ca1[2]	Ca2	Ca3	Ca1	Ca2	Ca3	Ca1	Ca2	Ca3	
C14:0	0.8	0.8	0.8	0.1	0.1	0.1	0.1	0.1	0.1	0.07
C16:0	32.5	32.4	33.2	12.2	11.6	11.5	7.8	8.0	8.5	2.13
C16:1n-7	0.2	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.01
C18:0	3.8	3.8	3.9	4.4	4.3	4.3	3.5	3.8	3.7	0.06
C18:1n-9	39.0	39.1	38.9	24.2	24.6	24.7	22.2	22.0	23.8	1.44
C18:2n-6	22.5	22.5	21.9	52.4	53.2	53.1	28.0	26.8	29.0	2.61
C18:3n-3	1.2	1.2	1.2	6.5	6.1	6.2	38.4	39.3	34.8	3.15
SFA[3]	33.4	33.4	34.1	12.4	11.8	11.7	7.9	8.2	8.7	2.20
UFA[4]	66.6	66.6	65.9	87.6	88.2	88.3	92.1	91.8	91.3	2.20
MUFA[5]	42.9	42.9	42.8	28.7	28.9	29.0	25.7	25.8	27.5	1.43
PUFAn-3[6]	1.2	1.2	1.2	6.5	6.1	6.2	38.4	39.3	34.8	3.15
PUFAn-6[7]	22.5	22.5	21.9	52.4	53.2	53.1	28.0	26.8	29.0	2.61
n-6 : n-3 ratio[8]	19.4	18.8	18.2	8.0	8.8	8.5	0.7	0.7	0.8	1.45
UFA : SFA	2.0	2.0	1.9	7.1	7.5	7.5	11.6	11.2	10.4	0.74
PUFA : SFA	0.7	0.7	0.7	4.8	5.0	5.1	8.4	8.0	7.3	0.58

[1] PO: 6% palm oil in diet; SO: 6% soybean oil in diet; LO: 6% linseed oil in diet.
[2] Ca1: 0.90% calcium and 0.35% available phosphorus; Ca2: 1.3% calcium and 0. 44% available phosphorus; Ca3: 1.35% calcium and 0.90% available phosphorus.
[3] Total saturated fatty acid = sum of C14:0 + C16:0 + C18:0.
[4] Total unsaturated fatty acid = sum of C16:1 + C18:1n-9 + C18:2n-6 + C18:3n-3 + C20:4n-6 + C20:5n-3 + C22:5n-3 + C22:6n-3.
[5] Total mono unsaturated fatty acid = sum of C16:1 + C18:1n-9.
[6] Polyunsaturated fatty acid n-3 = sum of C18:3n-3 + C20:5n-3 + C22:5 n-3 + C22:6n-3.
[7] Polyunsaturated fatty acid n-6 = sum of C18:2n-6 + C18:3n-6 + C20:4n-6.
[8] Polyunsaturated fatty acid n-6: polyunsaturated fatty acid n-3= (C18:2n-6 + C18:3n-6 + C20:4n-6) ÷ (C18:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3).
[9] SEM: standard error of means.

The effects of dietary fat sources and calcium levels on the fatty acid composition of the chicken breast muscle are shown in Table 5. It was observed that the fatty acid composition of breast muscle reflected the fatty acid profile of the experimental diet. The major FA detected in the breast muscle and affected by the dietary oil were C18:1n-9, C18:2n-6 and C18:3n-3. The concentration of oleic acid (C18:1 n-9) in the breast meat of broilers fed PO (T1, T2, T3) diet was significantly higher than those groups fed SO (T4, T5, T6) and LO (T7, T8, T9) diets. This observation is similar to the findings of Htin (2006), which indicated that meat from broilers fed a diet containing PO had higher levels of C18:1n-9 compared with birds fed SO and fish oil. The proportion of palmitic acid (C16:0) increased in meat from broiler-fed PO in comparison with those fed diets supplemented with SO and LO. These results are in agreement with the findings of Htin (2006) and Smink *et al*. (2010), who reported that the levels of palmitic acid increased significantly in broiler breast muscle supplemented with palm oil compared with groups supplemented with SO, coconut oil and sunflower oil. The significant increase in the proportion of oleic and palmitic acids in birds fed the PO diet could be owing to the high oleic and palmitic acid content of PO. Birds fed LO and SO diets had significantly higher α-linolenic and linoleic acids, respectively, compared with those fed PO. The proportion of total saturated and total MUFA of meat samples increased, while total UFA content decreased when PO was incorporated in the diet, resulting in a significantly lower UFA : SFA ratio of the broiler breast muscle compared with those fed SO and LO diets. The highest proportion of total UFA and UFA : SFA ratio was found in birds fed the LO diet. This could be attributed to the high α-linolenic acid content in the fatty acid profile of LO. Regardless of the source of dietary oil, no significant differences were observed in most of the FA in the breast muscle among levels of calcium as well as interaction between source of oil and calcium level.

The n-6 : n-3 ratio differs significantly among sources of oil. Birds fed LO (0.87) had significantly lower n-6 : n-3 compared with PO (9.86) and SO (8.00). These results are in line with those of Crespo & Esteve-Garcia (2002), who found that muscle from broiler diet supplemented with LO had lower n6 : n3 ratio compared to the groups supplemented with tallow, olive and sunflower oil. Similar to present findings, Fébel *et al*. (2008) reported that birds fed LO had lower n6 : n3 ratio than the birds fed SO .

There are no significant differences between the level of calcium and interaction between fat source and calcium level of the ratio of omega 6 to omega 3 fatty acids of broiler breast (Table 5).

Cholesterol is an important molecule that plays a vital role in membrane structure, as well as being a precursor for the synthesis of molecules such as steroid hormones, vitamin D and bile acids (Dessi & Batetta, 2003; Loh *et al*., 2013). On the other hand, coronary heart disease and arteriosclerosis are strongly related to the dietary intake of cholesterol (Sacks, 2002). In addition, a strong relationship has been demonstrated between cellular cholesterol concentration and Alzheimer's disease (Michikawa, 2003). The cholesterol content of breast muscle of chickens fed diets containing different oil sources and calcium levels is shown in Table 6. Birds fed PO had higher (P <0.05) cholesterol concentration than those fed LO and SO. However, the cholesterol concentration was within the normal range (40 - 90 mg/100 g) for poultry (Piironen *et al*., 2002; Valsta *et al*., 2005; Honikel, 2010). This may be attributed to the decrease in the concentrations of UFAs in birds fed PO compared to those fed LO and SO (Table 5). Furthermore, the PUFA/SFA ratio was much higher in meat of broiler chickens fed SO and LO, which could explain the decrease in cholesterol. This observation agrees the reports of Duraisamy *et al*. (2013) and Azman *et al*. (2004), which showed that the level of cholesterol in broiler breast meat decreased when fed a diet containing more UFA rather than SFA. Regardless of the oil source, the concentration of meat cholesterol in the breast muscles of broilers was not significantly different among the calcium levels. Interaction between source of fat and calcium level was not significant.

Lipid oxidation is a major cause of food deterioration and affects the colour, flavour, texture and nutritional value of poultry. Incorporation of natural antioxidants by dietary means may be more effective and practical in controlling lipid-oxidation-related products and providing wholesome nutritious products to health-conscious consumers (Kang *et al*., 2001). The TBARS values expressed as mg MDA/ kg meat for breast muscles during post-mortem ageing periods of broiler chickens fed different dietary treatments are shown in Table 6. At d 1, 3 and 7 post mortem, a significant difference in lipid oxidation was observed among the dietary oils. Breast muscles from broilers fed a diet supplemented with PO had a lower TBARS value (P <0.05) compared with SO and LO throughout the post-mortem storage. This may be attributed to the decrease in the concentrations of PUFA in meat of birds fed PO, as shown in Table 3. The increase in lipid oxidation in muscle of birds fed SO and LO diet could be due to the increase in PUFA, which promotes formation of free radicals. This observation is in tandem with the report of Rey *et al*. (2001), Hoz (2004), Wood *et al*. (2004) and Hugo *et al*. (2009) which showed that oxidative stability of meat and meat products decreased with increasing concentrations of PUFA. On the other hand, the present result disagrees with the findings of Teye *et al*. (2006) and Isabel *et al.* (2003), which showed no significant effect of dietary fat source on TBARS values during storage. Irrespective of the oil source, there was no significant difference among the calcium : phosphorus levels for MDA concentration in the breast muscles of chickens at d 1, 3 and 7 post mortem. There was no significant interaction between oil source and calcium levels for MDA concentration in the breast muscles of chickens.

Generally, lipid oxidation increased with increase in post mortem storage at 4 ºC. At d 3 and d 7 post mortem, the TBARS value increased significantly (P <0.05) in all treatment groups, but the increment was lower in birds fed PO compared with other oils (Table 6). These findings are in agreement with those of Coetzee & Hoffman (2001) and Adeyemi & Olorunsanya (2012b) for chicken, who observed that TBARS values of uncooked chicken meat increased gradually during post-mortem storage. However, the threshold value of TBARS (5 mg MDA/kg) for detecting off-odours and off-taste (Insausti *et al*., 2001) was not reached in the current study.

Table 5 Fatty acid composition (% of total identified fatty acids) of the breast muscle in chicken fed experimental diets[1]

Fatty acid	PO[2]			SO			LO				*P*-Value		
	Ca1[3]	Ca2	Ca3	Ca1	Ca2	Ca3	Ca1	Ca2	Ca3	SEM[10]	Oil	Ca	Oil*Ca
C14:0	0.9^{a}	0.8^{a}	0.8^{a}	0.5^{b}	0.5^{b}	0.5^{b}	0.5^{b}	0.5^{b}	0.5^{b}	0.03	0.01	0.80	0.32
C15:0	1.6^{c}	2.2^{bc}	3.0^{a}	1.9^{bc}	1.9^{bc}	1.9^{bc}	2.3^{b}	2.9^{bc}	2.3^{b}	0.33	0.10	0.03	0.01
C16:0	26.8^{a}	27.0^{a}	27.2^{a}	22.8^{bc}	24.2^{b}	22.8^{bc}	20.4^{d}	20.7^{cd}	21.3^{cd}	0.46	0.01	0.54	0.64
C16:1n-7	4.6^{a}	3.2^{bc}	3.8^{b}	2.3^{d}	2.7^{cd}	2.8^{cd}	2.2^{d}	2.1^{d}	2.5^{cd}	0.13	0.01	0.15	0.02
C18:0	6.6^{c}	7.8^{bc}	8.4^{b}	9.3^{ab}	10.3^{a}	9.1^{b}	8.8^{b}	8.7^{b}	8.5^{b}	0.51	0.01	0.09	0.10
C18:1n-9	39.2^{a}	37.2^{a}	36.4^{a}	25.2^{bcd}	25.8^{bc}	26.9^{b}	22.5^{d}	23.0^{cd}	23.7^{cd}	0.80	0.01	0.90	0.17
C18:2n-6	16.4^{c}	18.4^{c}	16.2^{c}	33.0^{a}	29.8^{b}	31.7^{ab}	17.8^{c}	18.4^{c}	18.4^{c}	0.90	0.01	0.91	0.10
C18:3n-6	0.8^{abc}	0.7^{abcd}	0.9^{abc}	0.2^{d}	0.4^{cd}	0.4^{cd}	1.2^{a}	1.1^{a}	01.0^{ab}	0.70	0.01	0.98	0.77
C18:3n-3	0.9^{d}	0.8^{d}	0.8^{d}	3.3^{c}	2.7^{cd}	3.1^{c}	18.0^{a}	17.5^{ab}	15.7^{b}	0.88	0.01	0.30	0.37
C20:4n-6	0.7^{bc}	0.9^{b}	1.2^{a}	0.7^{bc}	0.8^{bc}	0.8^{bc}	0.6^{c}	0.6^{c}	0.6^{c}	0.04	0.01	0.09	0.10
C20:5n-3	2.0^{b}	3.3^{a}	3.6^{a}	3.8^{a}	3.4^{a}	3.5^{a}	1.5^{c}	1.2^{c}	1.5^{c}	0.16	0.01	0.27	0.02
C22:5n-3	0.5^{bc}	0.8^{b}	0.4^{bc}	0.4^{bc}	0.3^{c}	0.3^{bc}	2.4^{a}	2.1^{a}	2.3^{a}	0.12	0.01	0.71	0.37
C22:6n-3	0.9^{b}	0.4^{b}	0.6^{b}	0.9^{b}	0.8^{b}	0.9^{b}	2.8^{a}	2.70^{a}	2.8^{a}	0.13	0.01	0.26	0.81
SFA[4]	33.9^{a}	33.1^{a}	34.8^{a}	27.4^{bc}	29.3^{b}	28.0^{bc}	25.4^{c}	25.3^{c}	26.6^{c}	0.50	0.01	0.46	0.40
UFA[5]	66.1^{c}	66.9^{c}	65.2^{c}	72.6^{ab}	70.7^{b}	72.0^{ab}	74.6^{a}	74.7^{a}	73.4^{a}	0.50	0.01	0.46	0.40
MUFA[6]	45.8^{a}	45.0^{a}	45.6^{a}	34.5^{bc}	36.1^{b}	35.3^{b}	31.2^{d}	31.7^{d}	32.2^{cd}	0.74	0.01	0.67	0.60
PUFAn-3[7]	2.2^{d}	2.1^{d}	1.8^{d}	4.6^{c}	3.8^{c}	4.3^{c}	23.2^{a}	22.4^{ab}	20.8^{b}	1.09	0.01	0.08	0.26
PUFAn-6[8]	17.2^{b}	19.1^{b}	17.0^{b}	33.3^{a}	30.3^{a}	32.0^{a}	19.0^{b}	19.5^{b}	19.4^{b}	0.82	0.01	0.91	0.17
n-6 : n-3 ratio[9]	9.1^{ab}	10.3^{a}	10.3^{a}	7.2^{b}	7.9^{b}	7.5^{b}	0.8^{c}	0.9^{c}	0.9^{c}	0.50	0.01	0.46	0.89
UFA : SFA	2.0^{c}	2.0^{c}	1.9^{c}	2.7^{ab}	2.5^{b}	2.6^{b}	3.0^{a}	3.0^{a}	2.8^{ab}	0.06	0.01	0.44	0.62
PUFA : SFA	0.6^{d}	0.6^{d}	0.5^{d}	1.4^{bc}	1.2^{c}	1.3^{cb}	1.7^{a}	1.7^{a}	1.5^{ab}	0.06	0.01	0.45	0.57

[1] Data are expressed as the percentage of identified fatty acids.
[2] PO: 6% of palm oil in diet; SO: 6% of soybean oil in diet; LO: 6% of linseed oil in diet.
[3] Ca1: 1% calcium and 0. 45% available phosphorus; Ca2: 1.25% calcium and 0. 56% available phosphorus; Ca3: 1.50% calcium and 0.67% available phosphorus
[4] Total saturated fatty acid = sum of C14:0 + C16:0 + C18:0;
[5] Total unsaturated fatty acid = sum of C16:1+ C18:1n-9 + C18:2n-6 + C18:3n-3 + C20:4n-6 + C20:5n-3 + C22:5n-3 + C22:6n-3;
[6] Total monounsaturated fatty acid = sum of C16:1 + C18:1n-9;
[7] Polyunsaturated fatty acid n-3 = sum of C18:3n-3 + C20:5n-3 + C22:5 n-3 + C22:6n-3;
[8] Polyunsaturated fatty acid n-6 = sum of C18:2n-6 + C18:3n-6 + C20:4n-6;.
[9] Polyunsaturated fatty acid n-6 : polyunsaturated fatty acid n-3 = (C18:2n-6 + C18:3n-6 + C20:4n-6) ÷ (C18:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3);
[a-d] Means within the same row for each parameter with different superscripts are significantly different (P <0.05). [10]SEM: standard error of means.

Table 6 Meat cholesterol (mg/100 g of meat) and thiobarbituric acid reactive substance (mg MDA/kg meat) in the breast muscle of broiler chicken at different post-mortem ageing periods

Treat	Lipid oxidation			Cholesterol
	1 day	3 day	7 day	
[1]PO*Ca1[2]	0.3^{bx}	0.6^{by}	0.7^{cy}	46.1^{ab}
PO*Ca2	0.3^{bx}	0.9^{by}	1.0^{bcy}	49.6^{a}
PO*Ca3	0.2^{bx}	0.8^{by}	1.0^{bcz}	46.2^{ab}
SO*Ca1	0.4^{bx}	1.0^{by}	1.3^{by}	41.8^{bc}
SO*Ca2	0.5^{abx}	1.0^{by}	1.2^{by}	42.4^{bc}
SO*Ca3	0.5^{abx}	1.0^{by}	1.3^{by}	44.0^{b}
LO*Ca1	0.7^{ax}	2.5^{ay}	2.9^{ay}	39.1^{cd}
LO*Ca2	0.7^{ax}	2.4^{ay}	2.8^{ay}	38.9^{cd}
LO*Ca3	0.7^{ax}	2.2^{ay}	2.6^{ay}	37.1^{d}
SEM	0.04	0.10	0.11	0.66
Fat/P-value	0.01	0.01	0.01	0.01
Ca/P-value	0.96	0.80	0.88	0.53
Fat*Ca/P-value	0.98	0.48	0.37	0.38

[1] PO: 6% of palm oil in diet; SO: 6% of soybean oil in diet; LO: 6% of linseed oil in diet.
[2] Ca1: 1% calcium and 0. 45% available phosphorus; Ca2: 1.25% calcium and 0. 56% available phosphorus; Ca3: 1.50% calcium and 0.67% available phosphorus.
[a-d] Means within the same column with different superscripts are significantly different (P <0.05).
[x-z] Means within the same row with different superscripts in the different ageing period are significantly different (P <0.05).
SEM: standard error of means.

Conclusion

The results of the current study demonstrated that supplementation of PO, SO and LO increased the proportion of oleic, linoleic and α-linolenic acids, respectively, in broiler breast muscle. Birds fed LO had higher total n-3 and lower n-6: n-3 compared with birds fed PO and SO. Birds fed the PO diet had higher oxidative stability and cholesterol compared with those fed LO and SO. However, the values of cholesterol were within an acceptable range. Calcium level and interaction between calcium level and dietary oil were not significant for fatty acid profile, cholesterol and lipid oxidation. Thus, this study confirms that PO can be used as a vegetable oil in broiler chicken nutrition with positive effects on firmness of meat quality compared with vegetable oils that are rich in linoleic or α-linolenic acid. However, the use of PO in animal feeding may be restricted by its availability in other countries. Further studies on the effects of dietary oils on antioxidant enzyme activities in broiler chicken are suggested.

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