# Fatty Acid Composition of Breast and Thigh Muscles of Broilers Fed Diets Supplemented with Candle Nut Kernel Meal Subjected to Different Heat Treatments

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## Abstract

An experiment was conducted to determine the effects of heat treated candlenut kernel meal (CNKM) supplementation in broiler diet on fatty acid profiles in breast and thigh muscle of broiler chickens at grower-finisher stage. A total of 270 male broiler chickens (Cobb500), 21 days of age , were given six dietary treatments (control, control supplemented with unheated, oven-heated, roasted, boiled, and autoclaved ground candlenut kernel meal). Feed intake and body weight were measured over three weeks. There were no significant effects of different types of heat treatment on the chemical composition of CNKM. However, there were significant differences on the saponin content, in which roasted, boiled and autoclaved CNKM had significantly lower ($p<0.05$) saponin than untreated or oven-heated CNKM. Fatty acid composition of CNKM was not significantly affected by the heat treatment for some fatty acids. Birds fed diets supplemented with autoclaved candlenut meal showed the highest growth rate amongst the treatments. In conclusion, supplementing either treated or untreated candlenut meal at 2% level in broiler diet increased the omega-3 fatty acids, and the presence of saponin in the diet due to CNKM had no major influence on meat fatty acid profiles.

**Key words:** *Aleurites moluccana*, heat treatment, broiler chicken, fatty acids, saponin

## Introduction

Candlenut (*Aleurites moluccana*), like other oilseeds, is a potentially valuable source of fatty acid which is rich in alpha-linolenic acid (ALA), linoleic acid (LA) and oleic acid (OC). Unfortunately, the use of candlenut in broiler diet may be toxic to the animal due to the presence of anti-nutritional factors such as saponin when fed in raw form (Duke, 1983; Covacevich *et al*., 1987). The presence of saponin in animal diet at high levels may cause retardation of growth rate and reduction in feed intake (Cheeke and Shull, 1985) and increased mortality (Alexander *et al*., 2009). Many saponins when administered intravenously have a general action on lipid membranes and cause lysis of red blood cells *in vitro* and *in vivo.* Even though the saponins have low bioavailability and toxicity, they may be hydrolysed in the intestinal tract and eventually cause systemic toxicity. However, it depends on the structure and absorption at the aglycone. Alexander *et al*. (2009) reported that some saponins have an adjuvant effect. Inclusion of 12% of 'Mahua' cake from *Madhucalatifolia* sp. (known to contain saponins) in broiler diets killed all chicks within five hours (Jakhmola *et al*., 1987) while Quillaja saponins fed at 0.9% in feed caused low weight gains in chickens (Jenkins and Atwal, 1994). Defatted Quillaja seed meal contained 98 g/ kg saponin which were toxic at that level. Alexander *et al*. (2009) demonstrated that detoxification of saponins

can be achieved by heat treatment. Heating (Nwosu *et al*., 2010), roasting (Yang *et al*., 2004; Nwosu *et al*., 2010), boiling (Aderibighe, 1997; Yang *et al*., 2008; Nwosu *et al*., 2010) and autoclaving (Yang *et al*., 2008; Zilic *et al*., 2010; Sumiati *et al*., 2010) had been shown to detoxify anti-nutritional factors in feed. On the other hand, Alexander (2009) reported that the digestibility of feed decreased significantly through a heat treatment.

Heat treatments are also known to affect fatty acids content of feeds. Loopez-Ferrer *et al*. (1999) reported that the proportion of polyunsaturated fatty acids (PUFA) in poultry meat decreased by 12.4% when subjected to boiling or cooking at higher temperature (200 ˚C). Similarly, Zilic *et al*. (2010) also found a correlation between degree of temperature and time of heating in which a reduction of fatty acid profile can occur at as low as 60 ˚C. In contrast, no difference was observed in the proportion of fatty acid either in raw or cooked broiler thighs exposed to the temperature from 80 to 90 ˚C (Grau *et al*., 2001).

The present experiment conducted was based on the hypothesis that the heat treatment of raw candlenut kernel meal could reduce or inactivate saponin in CNKM, and that supplementation of broiler diets with CNKM would improve the PUFA content of muscles in broiler chickens. CNKM is known to be toxic to some animals and it is possible that while providing sufficient nutrients it may also cause toxicity if given at high levels. Hence, the objective of the study was to examine if different types of heat treatment affect saponin content of CNKM, and improve the fatty acid composition in broiler meat.

## Materials and Methods

*Preparation of sample*

Twenty kg of raw candlenut kernel seeds, imported from Indonesia, were purchased from a local market and were divided into five treatments. The treatments were as follows:

(1) *Untreated*; raw candlenut kernel seed,

(2) *Oven-Heated*; the raw seeds were placed onto an aluminum tray and heated in an oven at a temperature of 50±1˚C for 24 h and once completed, the seeds were taken out immediately, cooled at room temperature and ground,

(3) *Roasted*; the raw nuts were placed in an aluminum tray and heated in a standard microwave, set at "grill" indicator and grilled for 25 min,

(4) *Boiled*; the raw seeds were placed in a 600-ml Berzelius beaker (half of the beaker), filled with distilled water and placed in a waterbath set at a temperature of 100 ˚C for 60 min,

(5) *Autoclaved*; the raw seeds were placed in a 1000-ml Berzelius beaker and the beakers were placed in an autoclave set at a temperature of 121±1 ˚C for 15 min. After autoclaving process, the beakers were removed, cooled at room temperature and air-dried.

All the seeds from various heat treatments were ground with a pestle and mortar to obtain meals of about 2 to 3 mm in diameter, and kept in dry and cool place for laboratory analysis and further application. To make sure the meals were in good condition, the candlenut seeds, however, were ground prior to application or when needed.

*Animal and Experimental Design*

Three hundred male broiler chickens, one-day-old, Cobb500 strains, were purchased from a commercial local hatchery, wing-banded, weighed individually and allocated randomly in battery cage with stainless steel wire floor measuring 1.15 m length, 0.86 m width and 0.49 m high in an open-type house. From day 1 to day 21, all chicks were fed a commercial starter diet, containing 21% crude protein and 2900 kcal/ kg metabolizable energy (ME). Feed and drinking water were given *ad libitum* while medication and vaccination were carried out according to the standard practice in poultry management. At 21 days of age, all birds were weighed and selected based on their average body weight and allocated to six dietary treatment groups, with each treatment replicated 5 times, consisting of 9 birds each. The experiment was a Completely Randomized Design (CRD). Feed and water were given *ad libitum*. The feeding trial lasted for three weeks during which feed intake and body weight were determined on a weekly basis.

*Dietary Treatments*

All experimental diets were prepared in mash form using a horizontal mixer. From 22 to 42 days old, the birds were fed *ad libitum* either one of the four dietary treatments (Table 1) namely: (T1) Basal diet containing no supplement (control), (T2) Basal diet supplemented with raw CNKM (2.0%), (T3) Basal diet supplemented with oven-heated CNKM (2.0%), (T4) Basal diet supplemented with roasted CNKM (2.0%), (T5) Basal diet supplemented with boiled CNKM (2.0%) and (T6) Basal diet supplemented with autoclaved CNKM (2.0%). The ration was formulated according to the requirements for Cobb500 broiler strain.

*Laboratory Analysis*

Proximate analyses were conducted on feed ingredients according to the methods of AOAC (1984), saponin content (Nwosu *et al*., 2010) and fatty acid composition based on procedure of Folch *et al*.(1957) and Li *et al*. (2001) with slight modification.

Table 1. Composition (% as fed) of experimental broiler finisher diets

| Ingredients (%) | Dietary treatments | | | | | |
|---|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | T5 | T6 |
| Ground yellow corn | 62.00 | 61.00 | 61.00 | 61.00 | 61.00 | 61.00 |
| Soyabean meal dehulled | 25.00 | 25.00 | 25.00 | 25.00 | 25.00 | 25.00 |
| Fish meal | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 |
| Crude palm oil | 3.40 | 2.40 | 2.40 | 2.40 | 2.40 | 2.40 |
| Limestone | 1.30 | 1.30 | 1.30 | 1.30 | 1.30 | 1.30 |
| Fine salt | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Vitamin premix[1] | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Mineral premix[2] | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Lysine (99%) | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 |
| DL-Methionine (98%) | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 |
| Dicalcium phosphate | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| Choline chloride | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| Candlenut meal | 0.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 |
| Total | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| *Calculated analysis* | | | | | | |
| ME (kcal/kg) | 2848 | 2727 | 2727 | 2727 | 2727 | 2727 |
| CP (%) | 20.51 | 20.81 | 20.81 | 20.81 | 20.81 | 20.81 |

[1]Vitamin premix (per kg): vitamin A 50 IU; vitamin D 10 IU; vitamin E 75 g; vitamin K3 20 g; vitamin B1 10 g; vitamin B2 30 g; vitamin B6 20 g; vitamin B12 0.1 g; D-calcium pantothenate 60 g; nicotinic acid 200 g; folic acid 5 g; biotin 235 g.

[2]Mineral premix (per kg): selenium 0.2 g; iron 80 g; manganese 100 g; zinc 80 g; copper 15 g; potassium chloride 4 g; magnesium oxide 0.6 g; sodium bicarbonate 1.5 g; iodine 1 g; cobalt 0.25 g.

T1: Control; T2: Unheated; T3: Oven Heated; T4: Roasted; T5: Boiled; T6: Autoclaved

*Determination of Saponin Content*

The total saponin content in the feed samples was determined according to Nwosu *et al*. (2010). Approximately 5 g of the ground sample was weighed into an Advantec cellulose thimble and placed into the Soxhlet extractor chamber. A total of 150 ml of petroleum benzine (Merck®, boiling point 40-60 ˚C) was poured into a 250-ml round bottom flask. Extraction was run for 3 h to extract the lipids and interfering pigments. The defatted material in the thimble was used for the second extraction for saponin.

A 250-ml round bottom flask was weighed, added in with 150 ml of methanol, fitted to the Soxhlet apparatus, and extraction was done for 3 h. The flask was removed from the apparatus when the methanol containing saponin was almost dried. Then, the flask was placed in an oven set up at temperature of 70±1˚C for 2 h to evaporate the methanol. The flask bearing the saponin was taken out from the oven, cooled at room temperature, placed in a desiccator, weighed and the calculation for saponin was as follows:

$$\% \text{ Saponin} = \frac{\text{Mass of saponin (g)} \times 100}{\text{Mass of sample (g)} \times 1}$$

*Determination of Fatty Acid Composition*

At the end of the experiment, two birds from each replicate were selected randomly and slaughtered according to the HALAL procedure. The birds were defeathered manually, and an approximately 20 g of *pectoralis* muscle of breast and *iliotibialis* muscle of thigh from each bird were sampled from the left side of carcass, placed in a plastic bag and stored immediately in a -20 ˚C freezer. To determine fatty acid composition, the samples were taken out from the freezer and thawed in chiller overnight. Fatty acid composition of CNKM, diets and meat samples were analysed based on the methods described by Folch *et al*. (1957) and Li *et al*. (2001) with slight modification.

Fatty acid compositional analysis of lipids was carried out by Agilent 7890A gas chromatography (GC) equipped with an injector and a FID detector and using a 30 m x 0.25 mm, 0.2 µm thickness, Supelco SP-2330 capillary column (Supelco, Inc., Bellefonte, PA, USA). Twenty percent boron trifluoride in a complex methanol solution from Merck® was used to convert the fatty acids in a complex lipid to fatty acid methyl esters (FAMEs). An internal standard (C21:0 acid, 100 µL, Sigma Aldrich) was added to each sample. Analysis was performed with a temperature programmed from 168 ˚C to 250 ˚C at a rate of 1 ml/ min constant flow with the linear velocity of 26 CNM/s, and hydrogen as the carrier gas. Fatty acid samples were identified by comparing their retention times with fatty acid standard (Supelco® 37 component FAME Mix) that had been analyzed prior to, during, and at the end of the sample analysis to compensate for shifts in retention times. Results were expressed as percentages of total fatty acids.

*Statistical Analysis*

The data were analyzed based on one way ANOVA using the General Linear Model (GLM) procedure of Statistical Analysis System (SAS 9.2). Significant differences among treatment means were determined by Duncan's Multiple Range Test (DMRT) at $p<0.05$.

**Results and Discussion**

*Chemical Composition of Treated Candlenut Meal*

The amount of saponin (%) in treated CNKM was significantly ($p<0.05$) lower than that in the control (Table 2). The saponin content was significantly reduced by 62% after roasting compared to the control, while boiling reduced the saponin content by about 59%. After heat treatments, the saponin content of heated CNKM were in the increasing order, roasting (3.1%), followed by boiling (3.4%), autoclaving (5.2%), and oven-heated (7.9%). These results were in agreement with those obtained in earlier studies that the nut can be eaten or used as animal feed after cooking or roasting (Duke, 1983; Morton, 1992; Elevith and Manner, 2006).

Table 2. Proximate composition (% dry matter) and saponin content (%) in candle nut kernel meal subjected to different heat treatments

| | Heat treatments | | | | |
|---|---|---|---|---|---|
| Components | Unheated | Oven heated | Roasted | Boiled | Autoclaved |
| Dry matter | 94.4±0.07 | 94.6±0.57 | 96.0±0.04 | 95.8±0.17 | 95.5±0.14 |
| Ash | 1.0±0.01 | 1.0±0.01 | 1.0±0.02 | 1.0±0.02 | 1.0±0.01 |
| Crude fibre | 4.7±0.56 | 5.6±0.19 | 4.6±0.14 | 4.9±0.16 | 5.3±0.15 |
| Crude protein | 23.6±0.27 | 21.8±0.31 | 23.7±0.25 | 23.3±0.16 | 22.3±0.16 |
| Ether extract | 65.9±0.09 | 65.8±0.09 | 67.6±0.22 | 66.4±0.21 | 66.6±0.17 |
| NFE | 4.8±0.89$^{ab}$ | 5.9±0.49$^{a}$ | 3.1±0.29$^{b}$ | 4.4±0.48$^{ab}$ | 4.7±0.44$^{ab}$ |
| GE (MJ/kg) | 32.1±0.21 | 32.3±0.13 | 32.8±0.16 | 32.2±0.07 | 32.5±0.27 |
| Total saponins | 8.2±2.2$^{a}$ | 7.9±2.0$^{a}$ | 3.1±1.9$^{c}$ | 3.4±1.9$^{c}$ | 5.2±2.1$^{b}$ |

$^{ab}$Means within a row with different supercripts differ significantly ($p<0.05$)
All the data were on fresh weight basis

In a similar study, but using linseed meal, the toxicity of the meal was successfully reduced or eliminated by boiling in water (Madhusudhan and Singh, 1983; Madhusudhan and Singh, 1985a; Madhusudhan and Singh, 1985b). Furthermore, MacGregor and McGinnis (1948) reported that simple water extraction of linseed meal apparently destroyed the toxic factors. In the present study, roasting was found to be more effective than other heat treatments in reducing the saponin content. Cooking by microwave treatment has also been introduced to reduce saponin in legumes (Pirrock *et al*., 1984), and suggested that cooking by microwave is the best method of preparing legumes in households and restaurants. Boiling is also one of food processing methods that reduce the percentage of the plant chemicals in plant products (Pirrock *et al*., 1984).

Heat treated CNKM (heated; roasted; boiled; autoclaved) significantly ($p<0.05$) increased the percentage of C16:0 (palmitic acid), C18:0 (stearic acid) and C18:1n-9 (oleic acid) than in control (untreated) group (Table 3). Heat treatment increased the palmitic acid (42 - 63.3%), stearic acid (29.2 - 37.5%), and oleic acid (6 - 17.1%) when compared to the control. Similarly, Zilic *et al*. (2010) reported that heat treatment significantly increased the oleic acid content in soybean products because it was more thermo stable. The changes in polyunsaturated fatty acids, though not statistically different, could be due to thermal oxidation leading to the relative increase of palmitic acid, stearic acid and oleic acid in the heat treated candlenut meal.

Table 3. Fatty acid composition (% of total fatty acids) of treated and untreated CNKM

| | Treatments | | | | |
|---|---|---|---|---|---|
| Components (%) | Unheated | Oven-heated | Roasted | Boiled | Autoclaved |
| Linolenic acid | 29.5±0.07 | 27.0±0.01 | 29.7±2.46 | 27.0±0.25 | 26.4±0.43 |
| Linoleic acid | 41.6±0.10 | 39.6±0.18 | 34.3±5.51 | 39.4±0.15 | 39.4±0.52 |
| Oleic acid | 21.6±0.08[b] | 23.2±0.03[ab] | 25.3±2.21[a] | 23.4±0.29[ab] | 22.9±0.09[ab] |
| Palmitic acid | 4.9±0.03[b] | 7.0±0.10[a] | 7.6±0.69[a] | 7.1±0.05[a] | 8.0±0.79[a] |
| Stearic acid | 2.4±0.08[b] | 3.2±0.12[a] | 3.2±0.21[a] | 3.1±0.07[a] | 3.3±0.19[a] |

[ab]Means within a row with different supercripts differ significantly ($p<0.05$)
All the data were on fresh weight basis

In a similar study, Zilic *et al*. (2010) showed that heat treatment (temperatures ranging from 60 to 150 ˚C for 25 sec to 30 min) decreased PUFA especially LNA. However, no significant difference was observed on C18:2*n*-6 (LA) and C18:3*n*-3 (LNA) by various heat treatments. Similar effects were observed in Maillard reaction in which the ALA increased its stability during prolonged heating which could be due to either the increased content of the products reaction at high temperature that are known to have antioxidant properties or the position of LNA in the triglyceride molecule. Bolton and Sanders (2002) in their study connected the oxidative stability of prolonged roasted peanut oil with the antioxidative activity of products that were made in the process of the Millard reaction. Heat treatments by heating, roasting, boiling, and autoclaving caused no changes in percentage of LA and LNA content in heat treated CNKM. Based on the results, the temperatures as low as 50±1 ˚C and as high as 200 ˚C (true roasting temperature) (http://en.wikipedia.org/wiki/Roasting) with the time of heating from 15 min to 24 h (time decreased as temperature increased) did not affect the percentage value of LA and especially LNA. In the present experiment, saponin contents could be reduced by the heat treated without any effect on the LNA value.

The growth performance of broiler chickens fed diets supplemented with heat-treated and untreated candlenut kernel meals from 22 d of age to 42 d of age is presented in Table 4. Supplementation of either heat-treated or untreated candlenut kernel meal did not affect feed intake and feed conversion ratio of broiler chickens. However, birds fed with Diet T6 (autoclaved candlenut kernel meal) had highest final body weight, 2235±46 g, although they were not significantly different from the other diets, including the control, but significantly ($p<0.05$) higher than those fed T5 (2065±57 g).

Table 4. Growth performance of broiler chickens fed heat-treated and untreated candlenut kernel meal at day 42 of age (Mean±SE)

| | Dietary treatments | | | | | |
|---|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | T5 | T6 |
| IBW (g) | 805±14.8 | 791±10.5 | 815±8.7 | 802±5.61 | 793±6.24 | 810±10.8 |
| BW (g) | 2109±45$^{ab}$ | 2096±50$^{ab}$ | 2174±31$^{ab}$ | 2170±36$^{ab}$ | 2065±57$^{b}$ | 2235±46$^{a}$ |
| BWG (g/d) | 62.1±2.3$^{ab}$ | 62.2±2.0$^{ab}$ | 64.7±1.4$^{b}$ | 65.1±1.7$^{ab}$ | 60.6±2.7$^{ab}$ | 67.9±2.3$^{a}$ |
| FI (g/d) | 105±11.2 | 100±10.5 | 112±7.2 | 107±8.4 | 104±12.9 | 116±10.3 |
| FCR | 1.76±0.2 | 1.62±0.2 | 1.75±0.1 | 1.63±0.1 | 1.76±0.3 | 1.70±0.2 |

$^{ab}$Means within a row with different supercripts differ significantly ($p<0.05$)

T1: Basal diet; T2: Basal diet + 2% untreated candlenut; T3: Basal diet + 2% heated candlenut; T4: Basal diet + 2% roasted candlenut; T5: Basal diet + 2% boiled candlenut; T6: Basal diet + 2% autoclaved candlenut

IBW: Initial body weight; BW: Body weight; BWG: Body weight gain; FI: Feed intake; FCR: Feed conversion ratio

*Fatty Acid Composition in Breast and Thigh Muscles*

There were significant differences in individual fatty acid composition, C16:1 (palmitoleic acid-PA), C18:3 (LNA), C20:5 (eicosapentaenoic acid-EPA), and C22:5 (decosapentaenoic acid-DPA) (Table 5). Supplementation of 2% treated and untreated CNKM in broiler diets had influenced individual meat composition which doubled the composition in LNA, EPA and DPA than in control. However, there was similar impact on the individual fatty acid composition between treated groups and also when compared to the untreated group. The highest increase of *n*-3 content in breast muscle tissue was observed in T3 (heated), 6.22% whereas the lowest was in the T1 (control group), 2.68%.

Supplementation of candlenut kernel meal at a level of 2% in broiler diet, treated or untreated, had significantly increased the proportion of LNA fatty acid and decreased the LA fatty acid in breast muscle, and it changed the ratio of LNA and LA. Compared to the control, the ratio of *n*-6:*n*-3 fatty acids in the muscle tissue also decreased 2.7 times which from 10.3 to 3.9. The presence of saponin with different levels in the broiler diet did not affect the fatty acid composition in breast muscle tissue.

Table 5. Fatty acid composition (% of total fatty acids) of breast muscle (Mean±SE)

| Numeric name | Dietary treatments | | | | | |
|---|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | T5 | T6 |
| C15:1 | 2.59±0.5 | 2.47±0.30 | 2.86±0.47 | 2.58±0.14 | 3.18±0.30 | 3.12±0.50 |
| C16:0 | 22.0±0.6 | 22.1±0.3 | 22.3±1.0 | 22.7±0.4 | 22.2±0.2 | 21.8±0.4 |
| C16:1 | 4.06±0.3$^{ab}$ | 4.33±0.3$^{ab}$ | 3.00±0.5$^{b}$ | 4.17±0.1$^{a}$ | 3.65±0.3$^{ab}$ | 4.06±0.4$^{ab}$ |
| C17:1 | 1.08±0.47 | 0.97±0.39 | 1.03±0.34 | 1.11±0.23 | 1.24±0.45 | 1.32±0.37 |
| C18:0 | 7.97±0.22 | 7.42±0.32 | 8.65±0.31 | 8.01±0.15 | 8.40±0.49 | 8.52±0.75 |
| C18:1 | 31.3±2.17 | 33.4±0.86 | 31.1±0.89 | 31.9±0.58 | 31.8±1.02 | 30.6±1.36 |
| C18:2*n*-6 | 6.02±0.39 | 5.56±0.87 | 4.71±0.84 | 5.44±0.66 | 5.60±0.80 | 5.54±1.82 |
| C18:3*n*-3 | 1.23±0.18$^{b}$ | 2.35±0.16$^{a}$ | 2.26±0.12$^{a}$ | 2.34±0.17$^{a}$ | 2.13±0.18$^{a}$ | 2.25±0.22$^{a}$ |
| C20:4*n*-6 | 4.19±0.87 | 4.04±0.39 | 5.08±0.93 | 4.58±0.36 | 4.98±0.59 | 5.23±0.83 |
| C20:5*n*-3 | 0.35±0.03$^{b}$ | 0.60±0.10$^{ab}$ | 0.71±0.14$^{a}$ | 0.69±0.15$^{ab}$ | 0.73±0.04$^{a}$ | 0.53±0.11$^{ab}$ |
| C22:5*n*-3 | 0.53±0.12$^{b}$ | 1.23±0.30$^{ab}$ | 1.74±0.47$^{a}$ | 1.23±0.14$^{ab}$ | 1.19±0.30$^{ab}$ | 1.61±0.50$^{a}$ |
| C22:6*n*-3 | 22.0±0.63 | 22.1±0.26 | 22.3±0.97 | 22.7±0.38 | 22.2±0.15 | 21.8±0.43 |
| ΣSFA | 30.7±0.60 | 30.4±0.37 | 31.6±0.10 | 31.5±0.40 | 31.3±0.38 | 31.3±0.54 |
| ΣUSFA | 69.3±0.60 | 69.6±0.37 | 68.4±1.10 | 68.5±0.40 | 68.7±0.38 | 68.7±0.54 |
| *n*-3 | 2.68±0.35$^{b}$ | 5.18±0.36$^{a}$ | 6.22±1.82$^{a}$ | 5.41±0.62$^{a}$ | 4.99±0.71$^{a}$ | 5.70±1.32$^{a}$ |
| *n*-6 | 27.6±3.64$^{a}$ | 23.3±0.63$^{b}$ | 24.2±0.32$^{ab}$ | 23.3±0.43$^{b}$ | 23.7±0.58$^{ab}$ | 23.9±0.28$^{b}$ |
| *n*-6/*n*-3 | 11.5±3.21$^{a}$ | 4.58±0.31$^{b}$ | 4.14±0.48$^{b}$ | 4.36±0.22$^{b}$ | 4.85±0.30$^{b}$ | 4.33±0.33$^{b}$ |

$^{ab}$Means within a row with different superscripts differ significantly ($p<0.05$)
All the data were based on fresh weight, SE: Standard error of mean
T1: Basal diet; T2: Basal diet+2% untreated candlenut meal; T3: Basal diet+2% heated candlenut meal; T4: Basal diet+2% roasted candlenut meal; T5: Basal diet+2% boiled candlenut meal; and T6: Basal diet+2% autoclaved candlenut meal.

Fatty acid composition was different compared to fatty acid composition in thigh muscle tissue (Table 6). Supplementation of 2% candlenut either treated or untreated meal in the broiler diet significantly increased the individual fatty acid proportion, C18:3 (LNA) which was two times higher than in control. The highest content of LNA was in heated (2.86%), followed by untreated (2.77%), roasted (2.67%), autoclaved (2.58%), and boiled (2.32%).

However, the LNA proportion in thigh muscle between the treated and untreated group were slightly different with untreated CNKM better than treated group. Inclusion of 2% heated CNKM in the diet significantly increased the percentage of ΣUSFA in thigh muscle compared to the other treatments with *n*-3 fatty acids 1.8 times more than the control. However, total *n*-3 fatty acids in thigh muscle between the treated and untreated groups were similar.

Table 6. Fatty acid composition (% of total fatty acids) of thigh muscle (Mean±SE)

| Numeric name | Dietary treatments | | | | | |
|---|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | T5 | T6 |
| C16:0 | 23.9±1.00 | 24.1±1.74 | 22.0±0.14 | 22.5±1.06 | 21.8±0.24 | 22.8±0.60 |
| C16:1 | 5.47±0.05 | 5.45±0.45 | 4.76±0.60 | 5.03±0.46 | 5.11±0.45 | 5.51±0.33 |
| C18:0 | 6.66±0.24 | 6.35±0.85 | 5.69±0.13 | 7.72±0.36 | 7.69±0.88 | 5.73±0.66 |
| C18:1 | 36.4±0.85 | 34.0±2.28 | 37.2±1.23 | 34.2±1.06 | 33.6±1.67 | 37.7±1.13 |
| C18:2*n*-6 | 20.3±0.15 | 21.4±0.75 | 20.2±0.35 | 20.2±0.66 | 20.7±0.41 | 20.3±0.63 |
| C18:3*n*-3 | 1.49±0.15$^{c}$ | 2.77±0.27$^{ab}$ | 2.86±0.11$^{a}$ | 2.67±0.05$^{ab}$ | 2.32±0.11$^{b}$ | 2.58±0.07$^{ab}$ |
| C20:4*n*-6 | 2.29±0.56 | 2.32±0.65 | 3.09±1.01 | 3.30±0.79 | 3.78±0.72 | 1.96±0.33 |
| ΣSFA | 31.6±0.94$^{a}$ | 31.2±1.19$^{a}$ | 28.3±0.22$^{b}$ | 30.9±0.74$^{a}$ | 30.3±0.82$^{ab}$ | 29.4±0.16$^{ab}$ |
| ΣUSFA | 68.4±0.94$^{b}$ | 68.8±1.19$^{b}$ | 71.7±0.22$^{a}$ | 69.1±0.74$^{b}$ | 69.7±0.82$^{b}$ | 70.7±0.16$^{b}$ |
| *n*-3 | 2.64±0.42$^{b}$ | 3.99±0.19$^{a}$ | 4.78±0.80$^{a}$ | 4.48±0.39$^{a}$ | 3.90±0.13$^{a}$ | 4.08±0.19$^{a}$ |
| *n*-6 | 22.5±0.68 | 23.8±1.49 | 23.3±0.65 | 23.5±1.40 | 24.5±0.77 | 22.3±0.85 |
| *n*-6/*n*-3 | 9.35±1.76$^{a}$ | 6.04±0.56$^{b}$ | 5.21±0.68$^{b}$ | 5.29±0.29$^{b}$ | 6.28±0.05$^{b}$ | 5.47±0.21$^{b}$ |

$^{abc}$Means within a row with different superscripts differ significantly ($p<0.05$)

All the data were based on fresh weight. SE: Standard error of mean

T1: Basal diet; T2: Basal diet+2% untreated candlenut meal; T3: Basal diet+2% oven-heated candlenut meal; T4: Basal diet+2% roasted candlenut meal; T5: Basal diet+2% boiled candlenut meal; and T6: Basal diet+2% autoclaved candlenut meal.

The decrease in saponin content in the diet did not influence the *n*-3 fatty acid composition in breast and thigh muscles when compared to untreated CNKM. In another study in lambs, Brogna *et al*. (2011) found that supplementing up to 90 ppm of Quillaja saponin did not produce detrimental effects on the overall meat fatty acid profile. Total *n*-3 fatty acids was higher in breast muscle than those in thigh muscle. This could be due to higher deposition of long chain polyunsaturated fatty acid (LCPUFA) in breast muscle compared in thigh muscle. However, the ratio of *n*-6:*n*-3 in thigh muscle was higher (1.8) than in breast muscle (2.7). These results were in agreement with the previous findings (Hulan *et al*., 1988; Lopez-Ferrer *et al*., 1999; Gonzalez-Esquerra and Leeson, 2000; Crespo and Esteve-Garcia, 2001; Cortinas *et al*., 2004).

Panja *et al*. (1995) reported that including 6% of palm oil and soybean oil either single or mixed in broiler diets significantly increased proportion of LA and LNA in both breast and thigh muscles compared to those in the control group. Feeding linseed oil or full-fat flaxseed increased the tissue content of ALA in pigs (Van Oeckel *et al*., 1996; Romans *et al*., 1995a,b) and cattle (Scollan *et al*., 2001a,b). Feeding diets supplemented with canola or rapeseed oil containing approximately 9% ALA to pigs increased the ALA content of the pork and the *n*-6:*n*-3 ratio to 2.5 (Leskanich *et al*., 1997). Feeding 10% flaxseed containing 37% crude fat in the seeds for 25 d also increased the ALA, but no change in DHA in muscle (Romans *et al*., 1995a).

Bou *et al*. (2004) reported that supplementing broiler diets with 2.5% of fish oil (FO) doubled the amount of EPA and DHA acids in their meat compared to the chickens fed diets supplied with 1.25% of flaxseed oil (FO) (0.06 vs. 1.00 and 0.09 vs. 1.38 for EPA and DHA, respectively). Supplementing broiler chickens with 4% FO decreased the proportion of saturated fatty

acid (from 43.77 to 39.84% of total fatty acids) and monounsaturated fatty acid (from 41.26 to 37.60% of total fatty acids) and increased the proportion of *n*-3 PUFA (from 2.09 to 8.14% of total fatty acids) in thigh samples (Lopez-Ferrer *et al*., 2001). Lopez-Ferrer *et al*. (1999) concluded that the total amount of *n*-3 PUFA in the chicken meat decreased when FO was removed from the diet, while the proportions of *n*-6 PUFA and MUFA were increased by substituting 8.2% amount of linseed and rapeseed. Feeding chickens a diet containing 3-6% FO in the diets significantly decreased the percentage of total *n*-6 PUFA, increased the percentage of total *n*-3 PUFA, and decreased the ratio of *n*-6:*n*-3 fatty acids (Al-Khalifa *et al*., 2012). Feeding 10% and 17% flaxseed in broiler diet for 35 d enriched breast meat to 300 mg/100 g meat or 0.3% (Zaiddhof, 2008). LNA is mainly concentrated in dark meat (thigh) and LA (*n*-6 fatty acid) in light meat (breast) because of the difference in lipid part in dark and light meat while triglyceride is the dominant fat in dark meat and subcutaneous tissue (Miller and Robisch, 1968). Addition to broiler diets oil containing high levels of 18:2*n*-6, for example corn oil, increased the formation of 20:4*n*-6 (Sadeghi *et al*., 2012). Feeding diets containing 200 g purified fish oil/kg between birth and slaughter (52 d) also increased the proportion of saturated and *n*-3 fatty acids and decreased *n*-6 fatty acid of breast and thigh meats (Huang and Miller, 1993). Increasing the levels of dietary PUFA increased the amount and type of FA deposited in chicken tissues, especially in the edible portions (Cortinas *et al*., 2004). The effect of supplementation of α-tocopherol on fatty acids in poultry has rarely been studied and is rather controversial (Bou *et al*., 2004). PUFA content in the tissues increased when the dietary polyunsaturation level increased, ca 3.1 and 2.4 times higher in thigh and breast meats, respectively, than those on the most saturated fatty acid diet. A similar response was also observed in thigh meat that contain PUFA, particularly in LNA, LA, EPA and DHA. It is known that as the SFA and MUFA contents of diet decreased, the process of polyunsaturation increased (Cortinas *et al*., 2004).

In conclusion, supplementing either treated or untreated CNKM in broiler diet increased the *n*-3 fatty acid composition and decreased the ratio of *n*-6:*n*-3 in breast and thigh muscle tissues with no major influence on meat fatty acid profiles. Heat treatment, with the exception of dry oven heating, reduced significantly the amount of total saponins which may have an effect on dry matter intake of broilers. It may be applied in laying diet to produce eggs enriched with omega-3 fatty acids.

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