

**PRODUCTION OF FUNCTIONAL CHICKEN MEAT BY  
DIETARY SUPPLEMENTATION OF OIL RICH IN N-3  
FATTY ACIDS AND TURMERIC OLEORESIN**



**Tran Thi Thuy Hang**

**A Thesis Submitted in Partial Fulfillment of the Requirements for the  
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การผลิตเนื้อไก่สุภาพ โดยการเสริมแหล่งไขมันที่มีกรดไขมันชนิดโอเมก้า-3  
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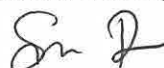


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Suranaree University of Technology has approved this thesis submitted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy.

Thesis Examining Committee



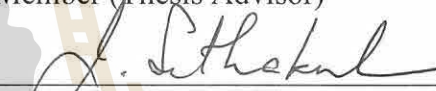
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ตรัง ถิ ทวิ ฮัง : การผลิตเนื้อไก่สุภาพ โดยการเสริมแหล่งไขมันที่มีกรดไขมันชนิด  
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โหมพี, 158 หน้า.

การศึกษาในครั้งนี้ มีวัตถุประสงค์เพื่อศึกษาการใช้อาหารในการผลิตเนื้อไก่สุภาพที่เป็น  
เนื้อไก่ที่มีกรดไขมันชนิด n-3 polyunsaturated fatty acids (PUFA) สูง การทดลองที่ 1 เป็น  
การศึกษาการเพิ่มปริมาณ n-3 PUFA ในเนื้อไก่ โดยใช้ไก่พื้นเมืองลูกผสม คณะแพศ อายุ 21 วัน  
จำนวน 560 ตัว แบ่งออกเป็น 7 กลุ่มการทดลอง คือ กลุ่มควบคุม (เสริมน้ำมันรำข้าว 6%) กลุ่มที่  
เสริมน้ำมันปลาทูน่าหรือน้ำมันลินซีดทดแทนน้ำมันรำข้าวที่ระดับ 2, 4 และ 6% ตามลำดับ ผลการ  
ทดลองพบว่า การเสริมน้ำมันปลาทูน่า 4% มีผลทำให้เปอร์เซ็นต์กรดไขมันชนิด EPA และ DHA  
ในเนื้อไก่สูงขึ้นเมื่อเทียบกับไก่กลุ่มที่เสริมน้ำมันปลาทูน่า 2 และ 6% นอกจากนี้ยังพบว่าเมื่อระดับ  
ของน้ำมันลินซีดในอาหารเพิ่มขึ้น มีผลทำให้เปอร์เซ็นต์ของกรดแอลฟา-ลิโนเลนิกสูงขึ้นทั้งในเนื้อ  
อกและเนื้อสะโพก โดยมีความสัมพันธ์กันในเชิงเส้นตรง (linear,  $P < 0.0001$ ) จากการทดลองนี้  
สรุปได้ว่าอาหารที่เสริมน้ำมันปลาทูน่า 4% มีผลทำให้การสะสมกรดไขมันชนิด n-3 PUFA สูงที่สุด

ในการทดลองที่ 2 เป็นการศึกษาผลของการใช้สารเคอร์คูมินอยด์ (curcuminoids) จาก  
ขมิ้นชันในอาหาร ต่อการเกิดออกซิเดชันและสัดส่วนของกรดไขมันในเนื้อไก่ทั้งในเนื้อสดและเนื้อ  
แช่แข็ง ที่เก็บไว้ 3 เดือน ที่อุณหภูมิ -20 องศาเซลเซียส โดยสุ่มไก่พื้นเมืองลูกผสม คณะแพศ อายุ 21  
วัน ตามแผนการทดลองแบบสุ่มตลอด (CRD) แบ่งออกเป็น 6 กลุ่มการทดลอง ๆ ละ 4 ซ้ำ โดยใช้  
อาหารที่เสริมน้ำมันปลาทูน่า 4% และน้ำมันรำข้าว 2% (อาหารพื้นฐาน) เป็นกลุ่มควบคุมแบบ  
negative control และใช้อาหารพื้นฐานร่วมกับวิตามินอี (dl- $\alpha$ -tocopheryl acetate) 200 ppm เป็น  
กลุ่มควบคุมแบบ positive control กลุ่มทดลองอื่น ๆ ทำการเสริมสารเคอร์คูมินอยด์ที่ได้จากกาก  
ขมิ้นชันที่เหลือจากการสกัดสารเคอร์คูมิน (curcumin removes turmeric oleoresin) ในอาหาร  
พื้นฐาน ที่ระดับ 20, 40, 60 และ 80 ppm ตามลำดับ ผลการทดลองพบว่า การเสริมเคอร์คูมินอยด์ใน  
อาหารมีผลต่อค่า thiobarbituric acid reactive substances (TBARS) ในพลาสมา และค่า drip loss  
ในเนื้ออกและเนื้อสะโพก เป็นไปในทิศทางเดียวกัน โดยมีค่าต่ำในกลุ่มที่เสริมที่ระดับ 40 และ 80  
ppm. เปอร์เซ็นต์ของกรดลิโนเลนิกในเนื้ออกเพิ่มขึ้นในไก่ทุกกลุ่มที่กินอาหารที่เสริมสารเคอร์คูมิน  
อยด์ และกรดไขมันชนิด DHA ในเนื้ออกลดลงในไก่ที่กินอาหารที่เสริมสารเคอร์คูมินอยด์ใน  
ระดับ 20 และ 40 ppm ส่วนในเนื้อไก่แช่แข็ง (-20°C) พบว่า มีแนวโน้มทำให้กรดไขมันชนิด  
monounsaturated fatty acids (MUFA) ลดลง ในขณะที่กรดไขมันชนิด PUFA สูงขึ้น นอกจากนี้ยัง

พบการเกิดออกซิเดชันในเนื้ออกตลอด 3 เดือนที่เก็บรักษาโดยการแช่แข็ง แต่ไม่พบการเกิดออกซิเดชันในเนื้อสะโพก

การทดลองที่ 3 เป็นการศึกษาผลของการเสริมสารต้านอนุมูลอิสระและวิธีการทำให้สุกที่สามารถป้องกันการสูญเสียกรดไขมันชนิด n-3 PUFA ในเนื้อไก่ที่มี n-3 PUFA สูง โดยใช้เนื้อไก่ที่ได้จากการเสริมอาหารด้วยวิตามินอี (dl- $\alpha$ -tocopheryl acetate) ที่ระดับ 200 ppm และเนื้อไก่ที่ได้จากการเสริมสารเคอร์คูมินอยด์ในระดับ 80 ppm ทำให้เนื้อไก่สุกด้วยกรรมวิธีที่ต่างกัน 4 วิธี คือ ต้ม นึ่ง ทอด และอบ โดยใช้แผนการทดลองแบบ Factorial in CRD แบ่งออกเป็น 8 กลุ่มการทดลอง ๓ ละ 4 ซ้ำ ผลการทดลองพบว่า ค่า TBARS สูงสุด ในเนื้ออกและเนื้อสะโพกที่ผ่านการอบ ทั้งอายุการเก็บ 1 วัน และ 4 วัน ( $P < 0.05$ ) โดยในเนื้ออกพบว่าวิธีการทำให้สุกที่เหมาะสมที่สุดในการป้องกันการสูญเสียกรดไขมัน n-3 PUFA คือการอบและการนึ่ง ส่วนวิธีการทำให้สุกที่เหมาะสมสำหรับเนื้อสะโพก คือ การต้ม การทอด การอบ และการนึ่ง



สาขาวิชาเทคโนโลยีการผลิตสัตว์  
ปีการศึกษา 2559

ลายมือชื่อนักศึกษา \_\_\_\_\_

ลายมือชื่ออาจารย์ที่ปรึกษา \_\_\_\_\_

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม \_\_\_\_\_

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม \_\_\_\_\_

TRAN THI THUY HANG : PRODUCTION OF FUNCTIONAL CHICKEN MEAT BY DIETARY SUPPLEMENTATION OF OIL RICH IN N-3 FATTY ACIDS AND TURMERIC OLEORESIN. THESIS ADVISOR : WITTAWAT MOLEE, Ph.D., 158 PP.

CHICKEN/STORAGE/COOKING/CURCUMINOIDS/LINSEED OIL/TUNA OIL/  
N-3 FA/MEAT QUALITY

The purpose of the present study is to produce Thai crossbred chicken meat as functional meat and that can be labeled as “high in n-3 PUFA” by dietary strategies. The first experiment aims to improve the n-3 polyunsaturated fatty acids (PUFA) content of chicken meat. Five hundred and sixty mixed-sex, 21-d-old chicks were randomly allocated to seven dietary treatments: 0, 2, 4, 6% of added tuna oil (TO) or linseed oil (LO) plus rice bran oil (RBO) to make up for a total of 6% added oil, and labeled as Control, TO2, TO4, TO6, LO2, LO4, and LO6. The addition of tuna oil at 4% was more effective than either 2% or 6% for increasing the EPA and DHA content of the chicken meat. As more LO was added, there was a linear increase in the  $\alpha$ -linolenic acid content of the breast and thigh meat ( $P < 0.0001$ ). The best treatment for incorporating long-chain n-3 PUFA into Thai crossbred chicken meat was 4% tuna oil.

In experiment 2, curcuminoids were subsequently examined for its antioxidant effect on chicken oxidation status and meat fatty acid profile, in particular, fatty acid composition of fresh and frozen storage (-20°C) meat for three months. A total of 480 Thai crossbred chickens (21-d-old; mixed-sex) were assigned to a CRD model with six treatment diets and four replicates. The basal diets based on corn-soybean meal with 2% RBO and 4% TO were used as the negative control. The experimental treatment

diets were supplemented with dl- $\alpha$ -tocopheryl acetate at 200 ppm or curcuminoids from curcumin removed turmeric oleoresin at 20, 40, 60 or 80 ppm (E-200, CUR-20, CUR-40, CUR-60, and CUR-80, respectively) into the basal ration. The effects of curcuminoids on plasma thiobarbituric acid reactive substances (TBARS) and drip loss of either the breast or thigh meat shared a similar pattern with lower values in CUR-40 and CUR-80. The significant higher content of linoleic acid in all curcuminoids treatments along with a lower content of DHA in CUR-20 and CUR-40 were observed in the breast meat. The frozen (-20°C) chicken meat decreased the proportion of monounsaturated fatty acids (MUFA) and significantly increased PUFA. During three months of storage, auto-oxidation found in the breast meat but not in the thigh meat.

The experiment 3 was conducted to determine the effects of dietary antioxidants and heating methods which can preserve more n-3 PUFA enriched chicken meat. The breast and thigh meat from the chickens fed either 200 ppm dl- $\alpha$ -tocopheryl acetate or 80 ppm curcuminoids was boiled, steamed, deep-fat fried or roasted following a factorial arrangement in CRD with two factors (Diet and Heat), 8 treatments, and 4 replicates. The highest TBARS value ( $P < 0.05$ ) was found in roasted meat of either breast or thigh meat at first-day and fourth-day storage (4°C) compared to other heating treatments. Roasting and steaming were good choices for cooking n-3 PUFA enriched breast meat. Boiling, frying, roasting, or steaming can be applied without any effect on n-3 PUFA content of the thigh meat.

School of Animal Production Technology      Student's Signature \_\_\_\_\_

Academic Year 2016      Advisor's Signature \_\_\_\_\_

Co-advisor's Signature \_\_\_\_\_

Co-advisor's Signature \_\_\_\_\_

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Tran Thi Thuy Hang



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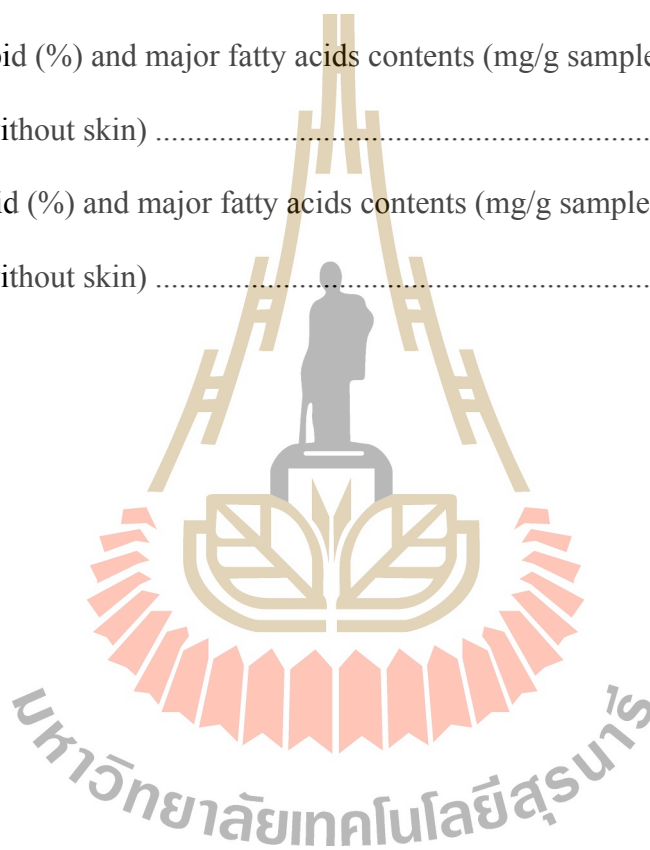
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## LIST OF ABBREVIATIONS

AA	=	Arachidonic acid or C20:4n-6
ALA	=	$\alpha$ -linolenic acid or C18:3n-3
<i>c</i>	=	<i>cis</i>
CRD	=	Completely randomized design
CRTO	=	Curcumin removed turmeric oleoresin
CUR-20	=	The diet contain 20 ppm curcuminoids
CUR-40	=	The diet contain 40 ppm curcuminoids
CUR-60	=	The diet contain 60 ppm curcuminoids
CUR-80	=	The diet contain 80 ppm curcuminoids
DHA	=	Docosahexaenoic acid or C22:6n-3
E-200	=	The positive control diet contain 200 ppm vitamin E
EPA	=	Eicosapentaenoic acid or C20:5n-3
FA	=	Fatty acid or fatty acids
FCR	=	Feed conversion ratio
FI	=	Feed intake
GLM	=	General linear model
HDL-C	=	High-density lipoprotein cholesterol
LA	=	Linoleic acid or C18:2n-6
LDL	=	Low-density lipoprotein
LDL-C	=	Low-density lipoprotein cholesterol

**LIST OF ABBREVIATIONS (Continued)**

LO	=	Linseed oil
LO2	=	The diet contain 2% linseed oil
LO4	=	The diet contain 4% linseed oil
LO6	=	The diet contain 6% linseed oil
MUFA	=	Monounsaturated fatty acids
PUFA	=	Polyunsaturated fatty acids
RBO	=	Rice bran oil
ROS	=	Reactive oxygen species
SEM	=	Standard error of the mean
SFA	=	Saturated fatty acids
<i>t</i>	=	<i>trans</i>
TBARS	=	Thiobarbituric acid reactive substances
TC	=	The diet contain 80 ppm curcuminoids
TE	=	The positive control diet contain 200 ppm vitamin E
TO	=	Tuna oil
TO2	=	The diet contain 2% tuna oil
TO4	=	The diet contain 4% tuna oil
TO6	=	The diet contain 6% tuna oil
TRIG	=	Triglyceride
TRP	=	Turmeric rhizome powder
VLDL	=	Very low-density lipoprotein
VLDL-C	=	Very low-density lipoprotein cholesterol

# CHAPTER I

## INTRODUCTION

### 1.1 Rationale of the study

The important of n-3 polyunsaturated fatty acids (PUFA) in maintaining heart health, protecting against cancer, birth defect, and offsetting symptoms of diabetes has emphasized since the past many decades. The low levels of blood C20:5n-3 (EPA) and very low levels of C22:6n-3 (DHA) have been associated with an increased risk of cardiovascular-related mortality of human being (Stark et al., 2016). Therefore, consuming more n-3 PUFA by supplements or functional food has recommended. De Smet and Vossen (2016) had reported that poultry exhibited the best response in their meat to dietary supplementation of n-3 PUFA compared with pork, lamb, and beef.

It has been well established that FA profile of chicken meat strongly reflects the pattern of FA in the diet. In addition, the conversion of C18:3n-3 (ALA) to long-chain n-3 PUFA is better in slow- and medium-growing birds than in fast-growing birds fed the same diet (Boschetti et al., 2016). The n-3 PUFA composition of crossbred chicken meat was higher than that of slow-growing chickens but lower than fast-growing strain (Chartrin et al., 2005; Dal Bosco et al., 2012). The effectiveness of ALA deposition in breast muscle was higher in standard (high growth rate) than in slow-growing (Label Rouge) chickens fed with linseed oil or extruded linseed diet (Baeza et al., 2013) because the fat content of slow-growing chickens has been found to be lower than that of commercial broilers (Jayasena et al., 2013; Wattanachant et al., 2004). The aforementioned studies have been raised the question whether Thai crossbred chickens can accumulate enough n-3 PUFA for their meat to be claimed as being high in n-3 PUFA.

The n-3 PUFA are more susceptible to lipid oxidation; therefore, the antioxidant is used to prevent oxidation in the feed and subsequently poultry products (Leeson, 2012). The natural antioxidant derives from herbs and spices has generally recognized as safe. Curcumin has strong antioxidant properties (Barclay et al., 2000; Fujisawa et al., 2004; Masuda et al., 1999) by stabilizing free radicals (Barzegar and Moosavi-Movahedi, 2011) and increasing the activities of antioxidant enzymes (Aggarwal, 2010). Its derivatives, demethoxycurcumin and bisdemethoxycurcumin also have antioxidant effects (Chattopadhyay et al., 2004). Curcuminoids, in the form of curcumin-removed turmeric oleoresin (CRTO), are bioactive compounds of turmeric rhizome (*Curcuma longa*), comprising mainly demethoxycurcumin, bisdemethoxycurcumin and leftover curcumin. Such substances work as antioxidants, digestive stimulants, hypolipidemic and pose antibacterial, antiviral, anti-inflammatory activities (Viuda-Martos et al., 2010). Hence, there is a hypothesis that curcuminoids could act as antioxidant in the chicken diets rich in n-3 PUFA.

Dietary turmeric powder (contains curcuminoids as the main bioactive compound) in broiler diets decreased the triglycerides and SFAs in thigh meat (Daneshyar et al., 2011). Curcumin (from turmeric) inhibited delta 5-desaturation of n-6 fatty acid, but not n-3 fatty acid in rat hepatocytes (Fujiyama-Fujiwara et al., 1992). The combination of curcumin and long-chain n-3 PUFA might have synergetic effects, then caused a decrease of fat accumulation in tissue (Forman et al., 1997). The aforementioned facts lead to a hypothesis that dietary curcuminoids might inhibit oxidation and favor the accumulation of n-3 PUFA in chicken meat.

Chicken meat is normally consumed after cooking and the effects of cooking methods on the change in nutrient values of chicken meat can vary (Al-Khalifa and Dawood, 1993). Fatty acid composition of raw meat was predominantly stated (Kartikasari et al., 2012; Lopez-Ferrer et al., 2001; Shin et al., 2012) and there were

some reports (Cortinas et al., 2004; Gibbs et al., 2010) about fatty acid profile of ready-to-eat chicken meat. To what the extent does the FA content of enriched meat change after cooking? The cooking study following feeding trial could bring valuable information for marketing the n-3 PUFA enriched chicken meat and revealed the effect of either dl- $\alpha$ -tocopheryl acetate or curcuminoids and heating method on n-3 PUFA content of cooked meat.

## 1.2 Research objectives

- 1.2.1 To find out whether supplementation of tuna oil or linseed oil might be better for enhancing n-3 PUFA in crossbred chicken meat; if possible, which level of oil should be applied?
- 1.2.2 To improve meat quality by dietary supplementing oil rich in n-3 PUFA and curcumin removed turmeric oleoresin.
- 1.2.3 To study the influence of dietary antioxidants and cooking methods on the amount of n-3 PUFA and meat oxidation stability.

## 1.3 Research hypotheses

- 1.3.1 Inclusion of tuna oil in the chicken diet has projected to be more efficacy than that of linseed oil to produce “source of n-3 PUFA” meat.
- 1.3.2 Curcumin removed turmeric oleoresin may ameliorate meat quality and protect raw and frozen meat from oxidation.
- 1.3.3 The loss of n-3 PUFA when cooking can be moderate and the diet with and without antioxidant might cause discrepancy in loss of n-3 PUFA and oxidation stability of cooked meat.

## 1.4 Scope and limitation of the thesis

In the present study, linseed oil and tuna oil are represented for plant and marine source of n-3 PUFA, respectively. Turmeric in general and curcumin in particular is the most intensively studied in pharmaceutical. Curcumin removed turmeric oleoresin is a co-product of purified curcumin production. Therefore, curcumin removed turmeric oleoresin is affordable and commercially available for using in animal feed as natural antioxidant and pigment. Chicken will be raised in open house so that housing climate and other factors cannot be controlled on condition that somehow it may influence on chicken health and experimental results.

## 1.5 Expected results

- 1.5.1 Tuna oil (at the rate of 4%) and linseed oil (6%) supplementation is effectively accumulated n-3 PUFA in meat in order to be labeled as “source of n-3 PUFA” or “high in n-3 PUFA” product. Expected amount is based on Regulation (EC) No 10.1924/2006.
- 1.5.2 It is estimated that combination of curcumin removed turmeric oleoresin and n-3 PUFA rich oil will enhance n-3 LC-PUFA content and oxidation stability of meat.
- 1.5.3 Steaming is projected to be the best method of cooking which preserve the most n-3 PUFA in meat.
- 1.5.4 The outcomes of the present studies provide the strategy to produce n-3 PUFA enriched chicken meat from “Korat” chicken in particular and Thai crossbred chicken in general. By application the knowledge from present studies, the producers can guarantee the quality of value-added chicken meat in raw and frozen meat as well as the ready-to-eat product.



1.5.5 The combination of curcuminoids from curcumin removed turmeric oleoresin and oil rich in n-3 PUFA in the chicken diet could have synergetic effects and bring the new knowledge. Base on the output of the present study, it might inspire further investigations about the usefulness of curcumin removed turmeric oleoresin as a feed additive.

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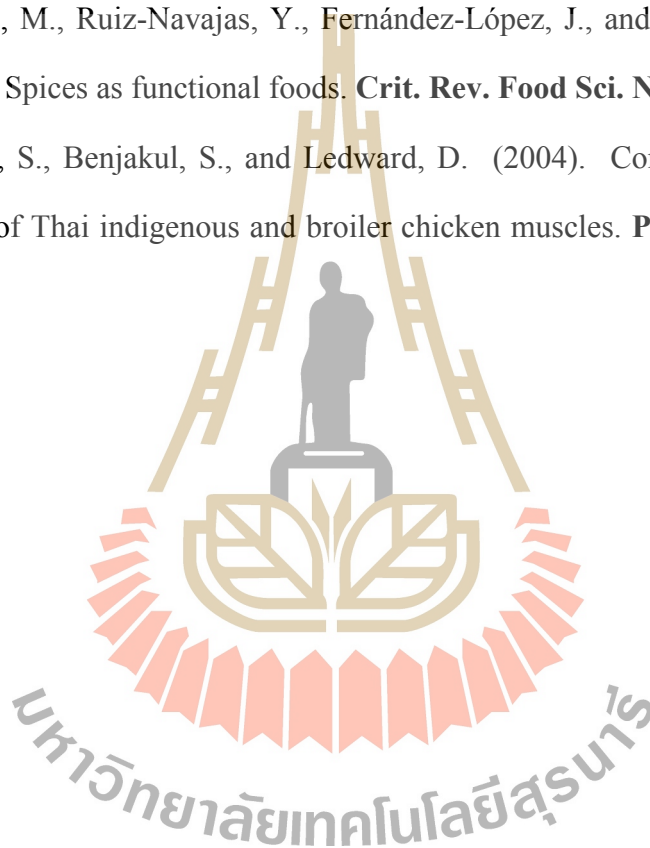
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## CHAPTER II

### REVIEW OF THE LITERATURE

#### 2.1 Polyunsaturated fatty acids

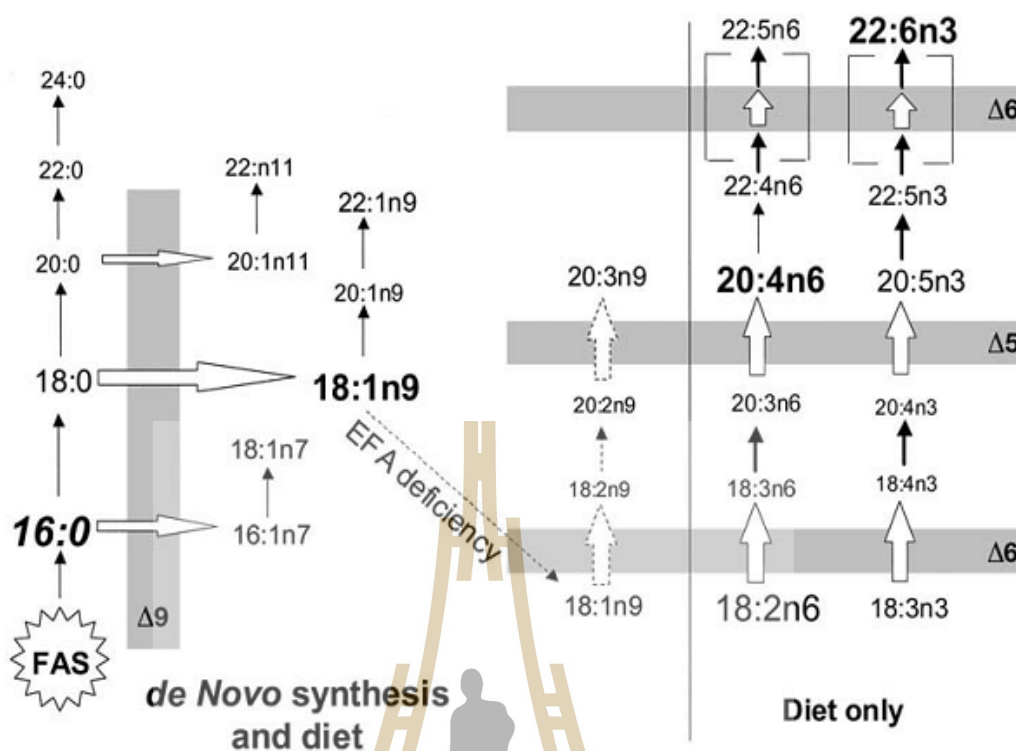
##### 2.1.1 Biosynthesis of polyunsaturated fatty acids in chicken

One of the main functions of the polyunsaturated fatty acids (PUFA) is their conversion to metabolically active prostaglandins and leukotrienes. These eicosanoids are hormone or hormone-like roles in vasoconstriction and blood platelet aggregation. By far information can be found in other reviews (FAO, 2010; Simopoulos, 2000).

Unlike plants, animals possess  $\Delta 5$ ,  $\Delta 6$ , and  $\Delta 9$  desaturase enzymes and are unable to synthesize the n-3 and n-6 PUFAs *de novo* (lack of  $\Delta 12$  and  $\Delta 15$  desaturases). However, extensive elongation and desaturation of essential fatty acids (FA) occur (primarily in the liver). The elongation of eicosapentaenoic acid (EPA, C20:5n-3) to docosahexaenoic acid (DHA, C22:6n-3) was thought for many years to be via  $\Delta 4$  desaturase. The inexplicable difficulty in identifying and isolating the putative  $\Delta 4$  desaturase led to the conclusion that it did not exist, and the pathway from EPA to DHA was elucidated as a double elongation, desaturation, and  $\beta$ -oxidation (Akoh and Min, 2002). The sharing of the elongating and desaturating enzymes by n-6 and n-3 fatty acids (Figure 2.1) causes them to compete for conversion into the various long chain PUFA (Murray et al., 2003). It must, however, be appreciated that the n-3 and n-6 fatty acids also compete in many other pathways, such a  $\beta$ -oxidation, incorporation into lipids, release from lipids, conversion into highly active metabolites, and binding to receptors (Muskiel, 2010).

Notably, mammals absorb fat into lymph, whereas poultry absorbs fat directly into the portal vein. As a result, adipose tissue can access incoming FA directly in mammals, but fat passes by liver first in the avian. Thus, there is considerably more hepatic metabolism of ingested FA in poultry tissue. In fact, the liver is the principal site of lipid biosynthesis; two third of total lipid biosynthesis in the domestic fowl occurs in the liver. The rate of *de novo* synthesis is reduced when the dietary lipid intake is increased (Stevens, 2004). Allometric variation in the acyl composition of bird muscle phospholipids is similar to that observed in mammals (Hulbert et al., 2002). Moreover, the study of Gregory et al. (2013) suggested that chickens may be able to metabolize more C22:5n-3 through to C24:5n-3, the precursor of DHA, compared with other species.

The FA metabolism of poultry is influenced by a number of factors. Sex has a marginal effect on the PUFA metabolism where females showed higher rate of bioconversion of  $\alpha$ -linolenic acid (ALA, C18:3n-3) and lower rate of  $\beta$ -oxidation compared with males; accumulation of LA and ALA increased and  $\beta$ -oxidation decreased with age (Poureslami et al., 2010). Genotype are can affect not only total body fat content in broilers (Rymer and Givens, 2006) but also on FA profile of chicken. In fact, Dal Bosco et al. (2012) found that slow-growing genotypes had higher elongase, thioesterase, and  $\Delta 5/\Delta 6$  desaturase indices accompanied by a lower  $\Delta 9$  index. The conversion of ALA to long-chain n-3 PUFA is better in slow- and medium-growing birds than in fast-growing birds fed the same diet (Boschetti et al., 2016). Dietary FA composition has a large impact on the fatty acids composition of muscle and fat tissue in animals and so saturated FA (SFA), monounsaturated FA (MUFA), and PUFA tends to resemble the profile of the feed. In this context, the adipose tissue is, in general, manipulated by diet more easily than are muscle lipids in the carcass (González-Esquerra and Leeson, 2001).



**Figure 2.1** A generalized scheme of fatty acid metabolism. Gray bars represent desaturase activities, black arrows represent elongase or chain-shortening activity, and the relative abundance of each fatty acid is represented by its size (Akoh and Min, 2002).

### 2.1.2 Sources of n-3 PUFA in chicken diet

Typical sources of PUFA in poultry diet come from certain fish oil, marine algae, and vegetable oil. Although both soybean oil and rapeseed (canola) oil contain significant amounts of ALA, high contents of LA in soybean oil and oleic acid in canola limit their general usefulness. Linseed oil could be better alternative source due to majority ALA content (over 50% of total FA). Some novel oilseeds and biofuel by-product can be used in diets of poultry to modify meat quality such as echium, camelina seed, and chia seed. Since ALA seem poorly converted to EPA and DHA, it is now

possible to engineer common n-6 rich oilseeds such as soybean and canola to produce EPA and DHA (Damude and Kinney, 2007). Oils and fat are also a good source of energy for chicken diet alongside supplying essential fatty acid (Table 2.1).

**Table 2.1** Metabolizable energy of common fat sources used in poultry nutrition (Leeson and Summers, 2005).

Ingredient	Metabolizable energy (kcal/kg)		Fat (%)	M.I.U. <sup>1</sup> (%)
	0-21 d of age	After 21 d of age		
Tallow	7,400	8,000	98	2
Poultry fat	8,200	9,000	98	2
Fish oil	8,600	9,000	99	1
Vegetable oil	8,800	9,200	99	1
Palm oil	7,200	8,000	99	1

<sup>1</sup>Moisture, impurities, unsaponifiable.

There is current interest in the use of fish oil in diets for humans and animals since its distinctive component of long chain fatty acids is thought beneficial for human health. Non-food grade tuna oil (TO) and rice bran oil (RBO) was available with large amounts in tropical Asia which are useful to enhance n-3 PUFA in meat by feeding strategy (Jaturasitha et al., 2016). Tuna oil is mainly produced in Thailand and Australia, is a by-product of processing tuna fish. It is generally high-quality oil and provides a useful source of n-3 fatty acids (Table 2.2 and Table 2.3). Feeding moderate levels of fish oil to broiler chickens has been shown to increase the EPA content of meat. However, with dietary levels in excess of 1%, distinct fish type odor is often



present in both meat and eggs, which is due mainly to the contribution of the n-3 fatty acids (Leeson and Summers, 2005). The oil content in rice bran varied relatively strongly which major FA in bran oil was palmitic, oleic, linoleic acids, and linolenic acid (1.0 - 1.9 % of total fatty acid) (Goffman et al., 2003). The high level of MUFA and n-6 PUFA of RBO was its limitation for utilization in animal diets.

The world marine vertebrate population includes tuna fish remarkably declined (WWF-UK, 2015). Linseed oil (LO) could be better alternative source due to majority  $\alpha$ -linolenic acid (ALA) content (50-60%) (Table 2.4). With a recognition of the importance of n-3 PUFA in the diet, the oils and seed – under the name flaxseed – are using increasingly in food products both for humans (cereals and bread) and for animals (Gunstone, 2004).

**Table 2.2** Typical fatty acid composition of some commercial fish oils (Gunstone, 2004).

<b>Fatty acids</b>	<b>Anchovy</b>	<b>Capelin</b>	<b>Cod liver</b>	<b>Menhaden</b>	<b>Sardine</b>	<b>Salmon (farmed)</b>	<b>Tuna</b>
C14:0	9	7	4	9	8	5	3
C16:0	17	10	10	19	18	12	22
C16:1	13	10	8	12	10	6	3
C18:1	10	14	25	11	13	20	21
C20:1	1	17	10	1	4	10	1
C22:1	1	15	7	-	3	9	3
C20:5	22	8	10	10	16	7	6
C22:5	2	-	1	1	2	3	2
C22:6	9	6	10	10	9	11	22

**Table 2.3** Fatty acid compositions of tuna fish and tuna oil

Name	Yellowfin Tuna <sup>1</sup>	Bigeye Tuna <sup>1</sup>	Tuna oil <sup>2</sup>	Refined Tuna oil <sup>3</sup>
	(% of total FA)	(% of total FA)	(w/w)	(% oil)
C14:0	1.30	1.66	2.0	2.335
C15:0	0.6	0.73	0.44	ND
C16:0	26.18	24.55	12.93	16.174
C16:1	3.08	3.42	2.55	4.917
C17:0	1.47	1.07	0.54	ND
C17:1	0.83	0.92	ND	ND
C18:0	14.5	8.43	3.07	3.705
C18:1n9c	20.32	24.19	11.18	13.172
C18:2n6c	1.31	0.92	0.74	2.25
C20:0	0.55	0.37	0.17	ND
C20:1	1.3	2.15	1.96	ND
C18:3n3	ND	ND	0.32	ND
C20:2	0.38	0.49	0.24	ND
C22:0	0.41	0.2	0.06	ND
C22:1n9 + C20:3n3	1.65	1.11	0.46	ND
C20:4n6	5.04	4.03	0.92	1.597
C20:5n3	2.39	3.27	7.81	4.109
C24:1	0.58	0.9	0.46	ND
C22:6n3	16.91	20.22	24.56	26.612

ND: not detected; <sup>1</sup> Peng et al. (2013); <sup>2</sup> Suseno et al. (2014); <sup>3</sup> Klinkesorn et al. (2004).

**Table 2.4** Fatty acid compositions of fish oil, tuna oil, linseed oil, and rice bran oil

Fatty acids (wt.%)	Fish oil <sup>1</sup>	Tuna oil <sup>2</sup>	Linseed oil <sup>1</sup>	Rice bran oil <sup>3</sup>
C16:0	15.0	15.78	4.23	15.6
C18:0	2.60	4.52	2.74	2.0
C18:1n-9	15.19	19.35	17.12	42.2
C18:2n-6	1.17	1.84	15.8	36.2
C18:3n-3	0.91	0.39	57.8	
C18:3n-6	0.25	ND	<0.01	1.3
C18:4n-3	2.95	ND	<0.01	ND
C20:4n-6	0.79	ND	<0.01	ND
C20:5n-3	16.5	7.62	<0.01	ND
C22:5n-3	1.76	1.57	<0.01	ND
C22:6n-3	10.5	22.85	<0.01	ND

ND = not detected; <sup>1</sup>Shingfield et al. (2011); <sup>2</sup>Koriyama et al. (2002); <sup>3</sup> adapted from Zambiasi et al. (2007).

### 2.1.3 Effect of dietary n-3 PUFA on production performance

Feeding of diets that difference in composition from a standard diet raises the concern about mortality, growth performance of chicken and subsequent economic losses for the producer. There was low mortality rate during n-3 PUFA feeding period, under 3% (Velasco et al., 2010) or 2.5% (Ponte et al., 2008).

Table 2.5 demonstrated that different level, form (fresh or encapsulated), and duration supplementing fish oil had no influence on broiler performance. Mixing corn oil with linseed oil (Chen et al., 2012) also exhibited no effect on productive performance. This can be explained that dietary treatments of these studies were isoenergetic and isonitrogenous. In contract, Farhoomand and Checaniazer (2009)

found the lowest FCR at the diet of 1% of poultry oil + 2% of fish oil. However, the study of Lopez-Ferrer et al. (2001b) showed no effect on FCR and the proportion of either thigh or breast meat by added linseed oil in broiler diet, similar to 3% of dietary rapeseed or echium (Kitessa and Young, 2009).

**Table 2.5** Effect of fish oil or linseed oil supplementation on broiler performance

Fat source: Level in diet	Duration	Key finding	References
Fish oil or linseed oil: 2 & 4% replaced tallow (8% fat added)	1-38 d	Increase in BW ( $P < 0.05$ ); FI, FCR, breast and thigh proportion were no effect.	(Lopez-Ferrer et al., 2001a; Lopez-Ferrer et al., 2001b)
Corn oil switched Fish oil:5%	1, 2, 3, 4, 5 weeks	No effect on BW, FI, FCR	Ali Asghar Sadeghi et al. (2012)
Blended vegetable oil (5%); Fresh fish oil contain 5g DHA/kg diet; Encapsulated fish oil: 5g DHA/kg diet; Algae biomass: 2.5g DHA/kg diet; 5g DHA/kg diet; 7.5g DHA/kg diet.	21-42d	No effect on BW, FI, FCR	Rymer et al. (2010)
Fish oil: 1 & 2% replaced poultry oil (3% fat added)	21-42d	No effect on FI, increase BW and reduce FCR ( $P < 0.01$ )	Farhoomand and Checaniazer (2009)
Sunflower oil rich oleic_SOO or Sunflower oil rich LA_SOL or Linseed oil or Mixing fat <sup>1</sup> :10%	8-42d	No effect on BW and FI; reduce FCR compared with negative and positive control (10% tallow) ( $P < 0.05$ )	Ferrini et al. (2008)
Sunflower oil or Soybean oil or Linseed oil: 3%	1-35d	No effect on BW and FCR	Fébel et al. (2008)
Mixture of corn oil and linseed oil to made different ratio n-6:n-3 (12, 9, 6, 3:1)	1-42d	No dietary effect on growth performance	Chen et al. (2012)

<sup>1</sup>(55% T+35%LO+10%SOL); <sup>2</sup>150 mg/kg of Cu from Cu sulfate.

#### 2.1.4 Fatty acid profile of chicken meat

There are many fatty acids in the profile of chicken meat. In this content, we are going to discuss some of the nutritionally important n-6 and n-3 PUFA. Fatty acid in muscle food can be found in phospholipids (0.2-1% of muscle weight) and triacylglycerols (0.2-5% of muscle weight). Phospholipid content is independent of total fat content while triacylglycerols are depended on the fat level and other genetic as well as nutritional factors (Nollet and Toldra, 2009). In the domestic fowl, the distribution of triglyceride between these tissues as a percentage of body weight ranges between 0.6 and 2.5% in abdominal tissue, 0.9 and 2.6% in subcutaneous tissue and 0.4 and 0.6% in intramuscular tissues. The precise amount depends on whether the breed has been selected for high or low abdominal adipose tissue. The capacity of birds for storing lipids exceeds that of any other class of vertebrate. (Stevens, 2004). De Smet and Vossen (2016) have reported that poultry exhibited the best response in their meat to dietary supplementation of n-3 PUFA compared with pork, lamb, and beef. In comparison with other meat, poultry meat has the highest concentration of EPA and DHA (~2% of total fatty acids), and DPA (22:5n-3) in poultry meat about 0.5% of total fatty acids (Table 2.6). Adipose tissue of domestic fowl has a very limited capacity for fatty acid synthesis, most of it accumulating fat is of dietary origin (Nollet and Toldra, 2009).

A significant linear relationship between the levels of fish oil and either EPA or DHA of breast and thigh was found (Farhoomand and Checaniazer, 2009; Mirghelenj et al., 2009). The long-chain n-3 FA contents (DHA, DPA, and EPA) of breast meat were increased because of dietary fish oil (Lopez-Ferrer et al., 2001a; Surai and Sparks, 2000).

**Table 2.6** Lipid content and fatty acid composition of poultry fed a standard diet (Rymer and Givens, 2005)

Items	White meat	Dark meat	Skin
Lipid content (wt% fresh tissue)	0.9	2.2	30.3
<b>Lipid composition (wt% total lipid)</b>			
Triacylglycerol	43	83	100
Phospholipid	55	16	Trace
<b>FA composition (% total FA)</b>			
ALA	0.5	0.7	1.0
EPA	0.9	0.5	0.1
DHA	1.8	1.0	0.1
<b>Amount of FA (mg/100 g edible tissue)</b>			
ALA	4.5	15.5	304
EPA	6.3	13.3	122
DHA	16.2	22.0	31

It was believed that supplementing broiler diets with conventional oilseeds has a minor effect on the long-chain n-3 PUFA content of meat. However, the significant increase of n-3 long-chain PUFA was investigated (Betti et al., 2009b). Supplementation linseed oil increased the level of ALA, EPA and DHA in tissue lipids (Fébel et al., 2008; Lopez-Ferrer et al., 2001b; Poureslami et al., 2010).

In fact, The European Food Safety Authority (EFSA), noting that DHA contributes to the maintenance of normal brain function and vision, has recommended that the beneficial effect can be obtained with a daily intake of 250 mg of DHA. The health claim requirements in the European Union for functional meat as “high in n-3

PUFA” are 600 mg/100 g fresh meat or 80 mg/100 g EPA+DHA while enriched meat content half of that thresholds can label as “source of n-3 PUFA) (according to Commission Regulation (EU) 1924/2006 (Zduńczyk and Jankowski, 2013) and 432/2012). The amount of ALA, EPA, and DHA in breast and thigh muscle was presented in Table 2.7. The table showed broiler meat can be claimed as “high in n-3 PUFA” when supplementing 4.4% fresh fish oil, 2.6% Encapsulated fish oil, 2.5% Fish oil + 2.5% Olive oil, 4.5% linseed oil + 1% fish oil, 7% linseed oil + 2% fish oil.

**Table 2.7** Long-chain n-3 PUFA content of enriched chicken meat (mg/100 g fresh tissue)

Ref	Source of PUFA	Dietary level (%)	Days old	Breast			Thigh		
				ALA	EPA	DHA	ALA	EPA	DHA
[1]	Fresh fish oil	4.4	21-42	11	31	129	44	58	132
	Encapsulated fish oil	2.6		20	18	122	53	63	153
[2]	Soybean oil+Olive oil (1:1)	5	1-63	15.8	41.2	38.9	41.2	34.4	21.5
	Flaxseed oil+Olive oil (1:1)	5		52.3	36.1	20.4	105	38.8	15.6
	Fish oil+Olive oil (1:1)	5		31.9	17.2	80.2	32.8	32.9	48.3
[3]	Linseed oil+Fish oil (6:1)	3.5	1-44	189	30	40	1,819	106	73
	Linseed oil+Fish oil (4.5:1)	5.5		249	40	42	2,525	169	92
	Linseed oil+Fish oil (3.5:1)	9		410	57	48	3,143	239	113

Ref.= References: [1]= Rymer et al. (2010); [2]= Shin et al. (2012); [3]= Cortinas et al. (2004).

The study of Poureslami et al. (2010) revealed that the accumulation of LA and ALA in the whole body but neither breast nor thigh meat (Table 2.8).

**Table 2.8** Some fatty acid content of diet, whole body, and accumulation rate

	Source of n-3 PUFA			
	Palm oil	Soybean oil	Linseed oil	Fish oil
<b>Some FA composition of the diet (mg/g feed)</b>				
C18:2n-6	14.7	24.1	14.5	12.3
C18:3n-3	0.86	2.38	14.1	1.27
C20:5n-3				4.11
C22:6n-3				3.03
Total FA	89.5	79.9	76.1	79.4
<b>Some FA content of whole body (mg/g broiler)</b>				
C18:2n-6	11.0 <sup>c</sup>	21.5 <sup>a</sup>	13.7 <sup>b</sup>	9.77 <sup>c</sup>
C18:3n-3	0.51 <sup>c</sup>	1.86 <sup>b</sup>	12.1 <sup>a</sup>	0.84 <sup>c</sup>
C20:5n-3	0.03 <sup>c</sup>	0.06 <sup>c</sup>	0.43 <sup>b</sup>	2.55 <sup>a</sup>
C22:6n-3	0.05 <sup>c</sup>	0.11 <sup>c,b</sup>	0.26 <sup>b</sup>	2.06 <sup>a</sup>
Total FA	89.6	99.1	99.4	87.4
<b>Accumulation (% of net intake)</b>				
C18:2n-6	73.1 <sup>b</sup>	74.7 <sup>b</sup>	85.2 <sup>a</sup>	73.0 <sup>b</sup>
C18:3n-3	63.9	64.3	65.3	58.4

<sup>1</sup> Poureslami et al. (2010).



### 2.1.5 Quality of enriched chicken meat

Enrichment of poultry meat with health-promoting substances resulted in a change of the composition of muscle tissues. In this way, meat quality features may also be modified.

**Table 2.9** Some findings of fresh meat quality of broiler fed n-3 PUFA diets

Ref.	PUFA source	Antioxidant inclusion	Measurement	Key finding
Yang et al. (2010)	Fish oil: 3%	0.02 %	Meat quality	Water holding capacity (WHC) is lower than soybean oil diet
Lopez-Ferrer et al. (2001a)	Fish oil: 2, 4%	BHT:0.02 %; Vit.E: 0.013%; Vit.C: 0.007%	Sensory quality of thigh meat	Insignificant differences among 2% fish oil and 1 or 2 weeks withdraw 4% of fish oil replaced by mixture 1% fish oil + 3% linseed oil
Lopez-Ferrer et al. (2001b)	Linseed oil: 2, 4%	BHT:0.02 %; Vit. E: 0.013%; Vit.C: 0.007%	Quality of breast meat	Juiciness, grill losses, and toughness parameters showed a tendency to decrease in female chicks
Betti et al. (2009a)	Flaxseed meal: 10, 7%	Vit.E: 50 IU/kg diet	Sensory evaluation	Average consumer acceptability ratings for all traits ranged from 5 to 7. After only 4 d of dietary flaxseed enrichment, the meat became more susceptible to oxidation

Dietary high in PUFA caused a decrease in cholesterol content of thigh muscle (Crespo and Esteve-Garcia, 2001) and plasma cholesterol (Newman et al., 2002). A study of Yang et al. (2010) found that the L\* value of meat color of the breast was low in the broilers fed the fish oil diet in comparison with those fed the soybean oil diet. A higher b\* value for meat color and boiling losses of the thigh muscle, drip losses for breast and

thigh muscle, and thawing losses for breast muscle were detected in the broilers fed the fish oil diet than in those fed the soybean oil diet. On the contrary, supplemented fish oil from 2 and 4% (plus tallow made up 8% fat added) did not lead to significantly different in juiciness (WHC), grill losses and tenderness compared with 8% tallow diet. When withdrawing fish oil diet and replaced by mixture with linseed oil (1% fish oil + 3% linseed oil + 4% tallow), Lopez-Ferrer et al. (2001a) also found no significant difference between treatment in WHC, grill loss, and tenderness of breast meat. The similar results were investigated in linseed oil supplementation (Lopez-Ferrer et al., 2001b).

There was a reduction of ultimate pH and increment cooking loss and drip loss of breast when prolonging the duration of feeding ground flaxseed meal. Besides, the tenderness of breast meat was significantly reduced with a longer period of feeding. The color of thigh and breast were influenced similarly. Feeding treatment diets for 24 to 35 days were not considered acceptable for consumer panel testing after prescreening evaluation (Betti et al., 2009a). Other results are summary in Table 2.9.

## **2.2 Turmeric and its bioactive compounds**

### **2.2.1 Bioactive compounds and antioxidant capacity**

Turmeric has variation in bioactive composition due to biological factors (growing location, harvested condition), manufacturing (extraction/distillation, stabilization), and storage condition (light, temperature, oxygen tension, time). More than 100 components have been isolated from turmeric. The main component of the root is a volatile oil, containing turmerone (Jayaprakasha et al., 2002), and there are other coloring agents called curcuminoids in turmeric. (Prasad and Aggarwal, 2011). The yellow pigment in turmeric is known as curcuminoids comprising of curcumin,

demethoxycurcumin, and bisdemethoxycurcumin, which are found to be natural antioxidants. Curcumin contributes 2–5% of turmeric (Charles, 2013). Turmeric also contains vanillic acid, caffeic acid, *p*-coumaric acid,  $\alpha$ ,  $\beta$ -pinene, and cymene (Brewer, 2011) and other compounds (Table 2.10). When making comparison among spices by DPPH method, antioxidant properties of turmeric is lower than that of galangal, lemongrass, chili, ginger but antioxidant protection factor of turmeric is higher than chili, shallot, lemongrass, garlic, galangal (Table 2.11) (Wongsa et al., 2012).

**Table 2.10** Antioxidant active chemicals isolated from turmeric (USDA, 2003 cited by Suhaj (2006))

Name	Part of plant	Quantity (ppm)
Ascorbic-acid	rhizome	0–293
Beta-carotene	rhizome	
Caffeic-acid	rhizome	0–5
Curcumin	rhizome	9–38,888
Eugenol	essential oil	0–2100
<i>p</i> -coumaric acid	rhizome	0–345
Protocatechuic acid	leaf	
Syringic acid	leaf	
Vanillic acid	leaf	

The antioxidant activity of curcumin was reported as early as 1975. It acts as a scavenger of oxygen free radicals. It protects hemoglobin from oxidation. In vitro, curcumin significantly inhibits the generation of reactive oxygen species (ROS) like superoxide anions, H<sub>2</sub>O<sub>2</sub> and nitrite radical generation by activated macrophages, which

play an important role in inflammation. Curcumin also lowers the production of ROS *in vivo*. Its derivatives, demethoxycurcumin and bisdemethoxycurcumin also have an antioxidant effect. They decrease lipid peroxidation in rat liver microsomes, erythrocyte membrane and brain homogenates. Reddy and Lokesh (1994) found that curcumin (1 wt%) enhanced the activities of antioxidant enzyme and lower lipid peroxide in the liver of the rat. This is brought about by maintaining the activities of antioxidant enzymes like superoxide dismutase, catalase and glutathione peroxidase (Chattopadhyay et al., 2004).

**Table 2.11** Polyphenolic compound, DPPH inhibition activity, and antioxidant protection factor of herbs in Thailand (Wongsa et al., 2012)

<b>Common name, Edible part</b>	<b>Antioxidant protection factor (APF)</b>	<b>Total phenolic content (mg GAE/g)*dw</b>	<b>Antioxidant activity (% DPPH inhibition)</b>
Turmeric, rhizome	1.27	9.32	7.66
Peppermint, leaves	1.14	10.32	74.1
Holy basil, leaves	1.12	70.88	51.11
Sweet basil, leaves	1.07	38.48	80.38
Chili, fruit	1.07	10.63	57.17
Lemongrass, stem	1.06	6.5	51.28
Garlic, clove	1.04	3.31	0
Galangal, rhizome	1.03	7.98	67.81
Ginger, rhizome	1.01	12.81	36.43
Finger root, rhizome	1	4.44	57.93

Decreased expression of superoxide dismutase, glutathione S-transferase, and epoxide hydrolase genes due to aflatoxin B<sub>1</sub> was alleviated by the inclusion of turmeric powder content 74 mg/kg curcumin in the diet (Yarru et al., 2009). In addition, curcumin at 200 mg/kg significantly lowered lipid oxidation in the liver of supplemented birds and improved immune responses (Rajput et al., 2013b).

### **2.2.2 Effects on chicken performance**

Improvement on the growth performance and decrease of feed conversion ratio (Table 2.12) were reported by supplementation of 0.5% turmeric meal (Al-Sultan, 2003; Durrani et al., 2006) and a mixture of turmeric and cumin (Al-Kassie et al., 2011). Curcumin (98% purify) added in a diet at 0.02 % resulted in significant enhancement of weight gain and feed conversion ratio (Rajput et al., 2013a). Moreover, Swathi et al. (2012) reported that supplementing turmeric in broiler diet ameliorated growth rate and feed efficiency under heat stress condition, following by vitamin E (200 mg/kg) and holy basil (tulsi). Unfortunately, this study didn't compare difference between means (contrast or pairwise multiple comparisons); therefore, it is unclear there is any significant difference between turmeric and tulsi or turmeric and vitamin E in the research. However, some authors did not find beneficial effects as supplementing diets with turmeric meal at the rate of 0.5 g/kg (Akbarian et al., 2012; Ali et al., 2014a), 1.0 g/kg (Rahmatnejad et al., 2009), 2.0 g/kg (Mehala and Moorthy, 2008) as well as turmeric extract (2.5%) (Purwanti et al., 2014). Differences in the efficacy of turmeric in those research may be attributed mainly to differences in their chemical composition (Applegate et al., 2010).

Some key findings of research about supplementation of turmeric on broiler production can be seen in Table 2.13. Further information can be found in reviews elsewhere (Dono, 2014; Khan et al., 2012).

**Table 2.12** Difference growth performance as effects of dietary curcumin

Ref.	Treatment	BWG (%)	FI (%)	FCR (%)
Abou-Elkhair et al. (2014)	Control	0 <sup>d</sup>	0	0 <sup>a</sup>
	Black Pepper (BP, 0.5%)	8.30 <sup>abc</sup>	-0.23	-7.88 <sup>d</sup>
	Turmeric (TUR, 0.5%)	4.12 <sup>cd</sup>	-0.89	-4.81 <sup>bc</sup>
	Coriander seed (COR, 2%)	6.60 <sup>abc</sup>	-1.16	-7.27 <sup>cd</sup>
	BP (0.5%) + TUR (0.5%)	4.71 <sup>bcd</sup>	0.62	-3.91 <sup>b</sup>
	BP (0.5%) + COR (2%)	10.1 <sup>a</sup>	-0.69	-9.78 <sup>d</sup>
	BP (0.5%) + TUR (0.5%) + COR (2%)	9.54 <sup>abc</sup>	-0.27	-8.96 <sup>d</sup>
Kumari et al. (2007)	Control	0 <sup>b</sup>	0	0
	Turmeric (0.1%)	7.05 <sup>a</sup>	9.43	-2.15
Durrani et al. (2006)	Control	0 <sup>b</sup>	0 <sup>a</sup>	0 <sup>b</sup>
	Turmeric (0.25%)	-0.26 <sup>b</sup>	-0.72 <sup>ab</sup>	4.55 <sup>ab</sup>
	Turmeric (0.5%)	15.2 <sup>a</sup>	-6.53 <sup>b</sup>	-9.09 <sup>c</sup>
	Turmeric (0.75%)	-5.88 <sup>b</sup>	-0.88 <sup>ab</sup>	13.6 <sup>a</sup>
Al-Kassie et al. (2011)	Control	0 <sup>c</sup>	0 <sup>b</sup>	0 <sup>c</sup>
	Tur+cumin (0.25 %)	3.89 <sup>b</sup>	-2.32 <sup>b</sup>	-5.97 <sup>b</sup>
	Tur+cumin (0.5 %)	10.8 <sup>a</sup>	-1.30 <sup>a</sup>	-10.9 <sup>a</sup>
	Tur+cumin (0.75 %)	13.9 <sup>a</sup>	-1.41 <sup>a</sup>	-13.4 <sup>a</sup>
	Tur+cumin (1 %)	12.1 <sup>a</sup>	-1.69 <sup>a</sup>	-12.4 <sup>a</sup>

**Table 2.12** Difference growth performance as effects of dietary curcumin (cont.)

Ref.	Treatment	BWG, %	FI, %	FCR, %
Swathi et al. (2012)	Control (heat stress-free)	31.0 <sup>a</sup>	-	-32.8 <sup>g</sup>
	Positive control (heat stress)	0 <sup>d</sup>	-	0 <sup>a</sup>
	Vit E 200 mg/kg	27.4 <sup>ab</sup>	-	-25.1 <sup>e</sup>
	Vit E 200 mg/kg + 0.15ppm Se	28.9 <sup>a</sup>	-	-29.2 <sup>fg</sup>
	Tulsi (0.25%)	18.2 <sup>c</sup>	-	-19.9 <sup>d</sup>
	Tulsi (0.5%)	19.7 <sup>b</sup>	-	-23.6 <sup>de</sup>
	Turmeric (0.2%)	26.6 <sup>ab</sup>	-	-25.5 <sup>ef</sup>
	Turmeric (0.4%)	26.8 <sup>ab</sup>	-	-39.9 <sup>h</sup>
	Turmeric +Tulsi	5.30 <sup>d</sup>	-	-15.5 <sup>c</sup>
	Turmeric +Tulsi	1.69 <sup>d</sup>	-	-11.1 <sup>b</sup>
Gowda et al. (2009)	Control	0	0	0
	Curcuminoids (444ppm)	-0.76	-4.69	1.67
Rajput et al. (2013a)	Control	0 <sup>c</sup>	0	0 <sup>a</sup>
	Curcumin (0.01 %)	1.17 <sup>bc</sup>	-0.61	-1.69 <sup>ab</sup>
	Curcumin (0.015 %)	1.85 <sup>ab</sup>	0.95	-1.13 <sup>ab</sup>
	Curcumin (0.02 %)	2.97 <sup>a</sup>	0.70	-2.26 <sup>b</sup>

Superscript letter following value showed significant different among treatment within each reference (P < 0.05).

**Table 2.13** Some findings of supplementing turmeric on broiler chicken diets

References	Treatments	Parameters	Key findings
Abou-Elkhair et al. (2014)	Turmeric powder (0.5%)	Carcass traits and relative weight of lymphoid organs	- Dressing percentage and edible giblets was not influence
Akbarian et al. (2012)	Turmeric powder (0.5 g/kg)	- Some enzymes activity - Electrolytes of male broilers serum	- Significantly decreased alanine aminotransferase (ALT) activity (better function of liver) - Serum chloride and total electrolyte balance significantly decreased
Nayaka et al. (2012)	Turmeric (2g/kg)	- Humoral Immunity - Cell Mediated Immune Response	Improving both humoral immunity against Newcastle virus and also cell mediated immunity
Daneshyar et al. (2011)	Turmeric powder (0.25, 0.5, 0.75 %)	- Plasma lipoproteins and triglyceride concentrations - Fatty acids composition of the thigh meat	- Decrease the concentrations of SFA and triglycerides in thigh meat and improve the meat quality - Higher thigh vaccenic acid concentration (0.75%)
Abbas et al. (2010)	1, 2 and 3 % turmeric powder	- Bloody diarrhea of chickens - Oocyst excretions	- Maximum coccidiostatic effect was observed with turmeric (3%) showing mild bloody diarrhea - Concentration-dependent coccidiostatic effect
El-Hakim et al. (2009)	Turmeric (0.2 %) and 18% CP	- Metabolizable energy and N retention - Some blood plasma parameters	No significant effect



**Table 2.13** Some findings of supplementing turmeric on broiler chicken diets (cont.)

References	Treatments	Parameters	Key findings
Gowda et al. (2009)	222 mg/kg total curcuminoids and 1 mg/kg aflatoxin B1	- Serum biochemical parameters - Antioxidant status in liver	Provided the greatest amelioration and demonstrated highest antioxidant activity
Kumari et al. (2007)	Turmeric powder (0.1%)	Serum parameters	Nephroprotective properties (protect kidney from harm)
Samarasinghe et al. (2003)	Dried turmeric root powder (1 g/kg)	-Energy and protein utilization -Antimicrobial activity	- Energy metabolizability and net protein utilization were increased - Coliform bacteria, yeast, and mold, total viable microbes in broiler gut contents have been markedly reduced

### 2.2.3 Effects on cholesterol, lipid metabolism, and meat quality

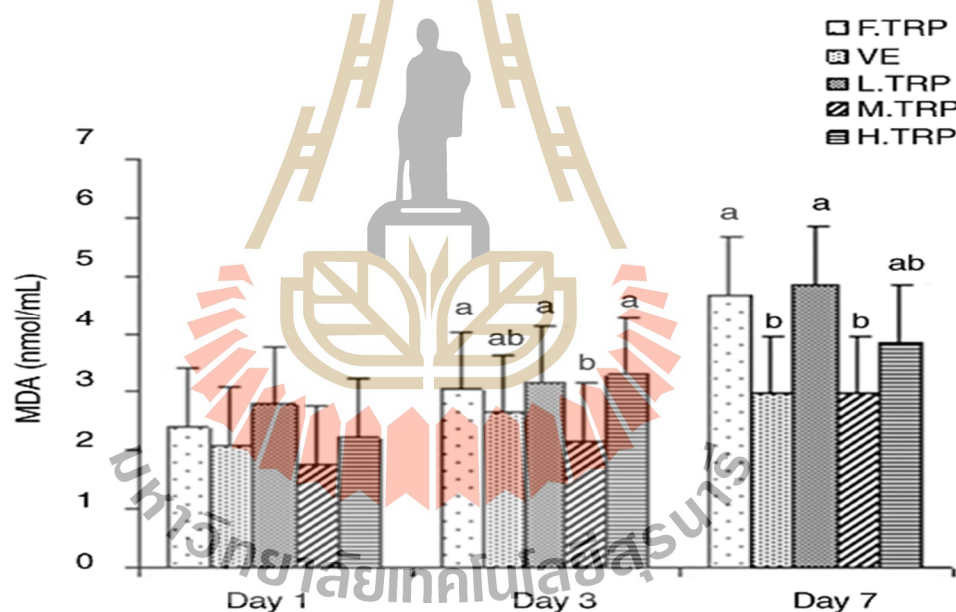
The precursor of cholesterol synthesis is acetyl-CoA with similar to FA synthesis (King, 2017) while transportation of cholesterol and other lipid are carried on plasma lipoprotein. The cholesterol metabolism somehow relates to plasma or serum lipoprotein level. There were studies elsewhere which found no significant change in total serum cholesterol (Mehala and Moorthy, 2008) or serum low-density lipoprotein (LDL), high-density lipoprotein (HDL), and very low-density lipoprotein (VLDL) (Akbarian et al., 2012) of chicken fed turmeric or serum lipid profile of human consumed curcumin (Baum et al., 2007). However, dietary turmeric powder significantly decreased triglycerides, total cholesterol and LDL cholesterol of serum in layers (Kermanshahi and Riasi, 2006). Emadi et al. (2007) reported that 2.5 g/kg

turmeric supplementation in broilers diet significantly increased total cholesterol and HDL-cholesterol and significantly decreased LDL-cholesterol at 42 days of age. The plasma lipid profile of chicken was found significant by dietary turmeric (Daneshyar et al., 2011) and plasma cholesterol by curcumin (Rajput et al., 2013a) or serum cholesterol by turmeric (Ali et al., 2014b) or triglycerides (Nouzarian et al., 2011). Curcumin caused an increase of  $\alpha$ -tocopherol level in rat plasma (Chattopadhyay et al., 2004) but Reddy and Lokesh (1994) reported that curcumin had no appreciable influence on hepatic vitamin E levels of Male Wistar rats fed a diet containing 10 wt% coconut oil, 10 wt% groundnut oil, or 10 wt% cod liver oil.

Supplementation curcumin at 200 ppm decrease abdominal fat (Rajput et al., 2013a; Sugiharto et al., 2011). A study of Daneshyar et al. (2011) determined 0.75% turmeric powder caused a significant decrease in total SFA of thigh meat. Curcumin inhibited microsomal  $\Delta 5$  and  $\Delta 6$  *desaturases* of rat liver (Shimizu et al., 1992); therefore, curcumin is likely to involve in the regulation of biosynthesis of PUFA in chicken. The study in human hepatocyte (Kang et al., 2013) found that curcumin inhibited hepatic lipogenesis and hepatic antioxidative ability by activating adenosine 5'-monophosphate (AMP)-activated protein kinase (AMPK) expression, then reducing sterol regulatory element binding proteins-1 (SREBP-1), down-regulation fatty acid synthase (FAS) expression, and increasing the expression of peroxisome proliferator-activated receptor (PPAR $\alpha$ ). Fan et al. (2016) determined curcumin blocked FAS activity, expression, and mRNA level in a dose-dependent manner in human breast cancer MDA-MB-231 cell. Curcumin (from turmeric) inhibited delta 5-desaturation of n-6 fatty acid, but not n-3 fatty acid in rat hepatocytes (Fujiyama-Fujiwara et al., 1992). Long-chain n-3 PUFA also activated PPAR $\alpha$ , resulting in increased fatty acid  $\beta$  -

oxidation in peroxisomes and decreased lipogenesis (Thota et al., 2016). The combination of curcumin and long-chain n-3 PUFA might have synergetic effects, then caused a decrease of fat accumulation in tissue (Forman et al., 1997).

The study of Daneshyar (2012) found that dietary consumption of 5 mg/kg turmeric rhizome powder (TRP) can increase the thigh meat shelf-life storage and quality in broiler chickens meat, comparable with 50 mg/kg vitamin E (Figure 2.2). Dietary inclusion of TRP in broiler chickens increased the crude protein and moisture contents, and decrease the triglycerides and SFA in thigh meat of broilers and hence improve meat quality (Daneshyar et al., 2011).



**Figure 2.2** Thigh meat malondialdehyde (MDA) content of broiler chickens fed 50 mg/kg vitamin E (VE), and free (F.TRP) or 2.5 TRP (L.TRP), 5 (M.TRP), and 7.5 mg/kg turmeric rhizome powder (H.TRP) diet on days 1, 3 and 7 after refrigerator storage at 4°C. a-b, significant difference ( $P < 0.05$ ) between the treatment within each time (Daneshyar, 2012).

## **2.3 Storage and cooking**

### **2.3.1 Oxidation of enriched meat and effect of dietary antioxidant**

As regard to the oxidative stability of enriched meat, one of the problems is that there are various methods available for measurement of lipid oxidation in foods. However, there is no uniform and standard method for detecting all oxidative changes in all food systems (Akoh and Min, 2002). Therefore, it is difficult to access or make a comparison of different articles. Some key finding was present in Table 2.14 as following.

The most important difference in the susceptibility of meat from different animal species to lipid peroxidation was the balance between anti- and pro-oxidative factors in meat. The thigh meat was more susceptible to lipid peroxidation breast meat, and the high storage stability of chicken breast meat was attributed to a very low concentration of myoglobin and high total antioxidant capacity), which prevented the release of free ionic iron from myoglobin and inhibited free radical chain reactions (Min, 2006).

Freezing is also currently applied to increase the lifetime of meat and meat product. However, both lipolysis and oxidation may occur in the frozen state, depending on the initial freezing conditions, temperature and duration of freezing storage (Michalski et al., 2013). Oxidation of samples (measured as TBARS) was significantly influenced by duration of storage. The TBARS content in chicken leg and breast meats increased substantially during frozen storage, with the most rapid and significant increase occurring during the first 2 months of frozen storage in the chicken leg (Soyer et al., 2010).

**Table 2.14** Some findings of oxidation characteristic of modified chicken meat

Reference	Fat sources	Antioxidant inclusion	Key finding
Bou et al. (2005)	Linseed oil/fish oil: 1.25%	dl- $\alpha$ -TA at 100 mg/kg	TBA values of cooked thigh after 74 days or after 18 months of frozen storage were not affected by any of the dietary factors studied
Narciso-Gaytán et al. (2010a)	Soybean or palm kernel: 5%	33 and 200 to 400mg/kg of dl- $\alpha$ -TA	<ul style="list-style-type: none"> <li>- Oxidation stability of all fresh chicken parts and cooked meat patties showed no interaction between the lipid source and the vitamin E level in the diet.</li> <li>- Starting at day 10 of storage, fresh meat from the low level of dl-<math>\alpha</math>-TA diet showed higher MDA values than the ones from the high level.</li> <li>- In cooked meat, there was a more rapid development of lipid oxidation than in fresh parts.</li> </ul>
Narciso-Gaytán et al. (2010b)	Soybean or palm kernel: 5%	33 and 200 mg/kg of dl- $\alpha$ -TA	<ul style="list-style-type: none"> <li>- Fat source did not affect the MDA values</li> <li>- Supplementation of the high level of vitamin E significantly reduced the MDA values in both breast and thigh meat (<math>P &lt; 0.01</math>)</li> <li>- Refrigerated sous vide chicken meat has a prolonged shelf-life, which is enhanced by high level of vitamin E supplementation</li> </ul>
Cortinas et al. (2005)	PUFA (15, 34, 45, and 61 g/kg)	$\alpha$ -TA: 0, 100, 200, and 400 mg/kg	<ul style="list-style-type: none"> <li>- Dietary PUFA and <math>\alpha</math>-TA supplementation affected lipid oxidation more markedly in cooked and cooked refrigerated meat than in raw meat and raw refrigerated meat.</li> <li>- The oxidative stability of meat was not affected by an increase in the dietary <math>\alpha</math>-TA level from 200 to 400 mg/kg.</li> </ul>

dl- $\alpha$ -TA or  $\alpha$ -TA: dl- $\alpha$ -tocopheryl acetate or vitamin E.

Dietary PUFA and alpha-tocopheryl acetate supplementation affected lipid oxidation more markedly in cooked thigh meat with skin (in a water bath under agitation

for 30 min to an internal temperature of 80°C) and cooked refrigerated meat (2 months, 0-4°C) than in raw meat and raw refrigerated meat (3 days, 0-4°C). Lipid oxidation in cooked meat showed a significant linear increase as the concentration of PUFA in raw meat increased (Cortinas et al., 2005). This was supported by Gaál et al. (2000).

### **2.3.2 Effects of heating processing on lipid and oxidation of meat**

Most of the muscle foods are consumed after cooking step, which is can affect the nutrient properties of the lipids. Indeed, muscle food content both long-chain PUFA and heme iron provided in its majority by the heme protein, myoglobin. The heme molecule breakdown during cooking leads to an increase of the non-heme iron which is one of the most important catalysts of lipid oxidation in meats (Bergamaschi and Pizza, 2011). The thermal treatment, therefore, favors lipid oxidation. The reaction leads to formation products such as volatile compounds involve both in the desirable cook meat flavor and off-flavors and in deleterious oxidation products such as cytotoxic aldehydes that lead to protein carbonylation, which can be take part in colon cancer development and possibly to loss of PUFA and vitamins (Michalski et al., 2013).

Total lipid after cooking generally increased because of the decrease of moisture content (Alfaia et al., 2010). It was observed that poultry meat no matter whether in a pan or by deep-frying, is associated with fat uptake (2 - 14 g per 100 g of raw food) while non-breaded high-fat food of animal origin loses fat during frying (2 - 30%) (Bognar, 1998). Hernández et al. (1999) revealed a slight increase in phospholipid content of meat by roasting and boiling while deep-frying absorbed lipid into non-polar lipid fraction compared to raw meat.

The change of fatty acid composition during meat cooking might result from chemical reactions such as oxidation, hydrolysis, and polymerization (Hernández et

al., 1999). The loss of fat when cooking was 6.2% for SFA, 6.8% for MUFA and 5.7% for PUFA but the ratio SFA:MUFA:PUFA is unchanged (Cortinas et al., 2004). Making a comparison of four methods of cooking, namely boiling, pan frying in olive oil, grilling, and roasting, there was significant differences in fatty acid concentrations of chicken breast cooked by the different methods, and the results suggesting that boiling chicken meat is the most favorable method (Gibbs et al., 2010). Hernández et al. (1999) reported an increase in MUFA after cooking pork by different method, namely deep-frying, swallow-frying, boiling, and roasting.

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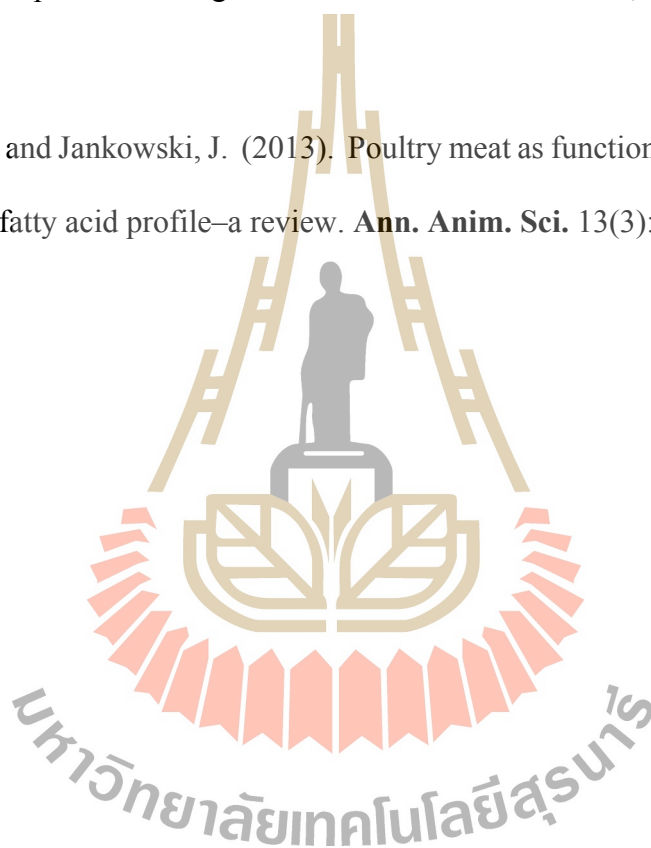
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# CHAPTER III

## EFFECTS OF DIETARY TUNA OIL OR LINSEED OIL ON GROWTH PERFORMANCE, MEAT QUALITY, AND ECONOMIC RETURN OF THAI CROSSBRED CHICKENS

### 3.1 Abstract

The effects of tuna oil (TO) or linseed oil (LO) supplementation in diets on growth performance, carcass composition, and meat quality of Thai crossbred chickens was investigated. Five hundred and sixty unsexed, 21-d-old chicks were randomly allocated to seven dietary treatments: 0, 2, 4, 6% of added TO or LO plus rice bran oil to make up for a total of 6% added oil and labeled as Control, TO2, TO4, TO6, LO2, LO4, and LO6. The TO6 exhibited the lowest ( $P < 0.01$ ) final body weight (day 84) and a similar feed intake, resulting in the highest ( $P < 0.01$ ) feed conversion ratio. The boiling loss of breast meat was highest in LO6 and differed from control and TO2. There were significant differences ( $P < 0.05$ ) between treatments in most of the fatty acids (FA) profile of the breast and thigh meat. Arachidonic acid (AA) content decreased ( $P < 0.001$ ) in the breast of TO or LO group compared with the control group. Including tuna oil at 4% was more effective than TO2 and TO6 for increasing the eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) content of the chicken meat. Overall, as more LO was included, the  $\alpha$ -linolenic acid (ALA) content increased in the breast and thigh

meat ( $P < 0.0001$ ). The n-6/n-3 ratio in the breast and thigh meat decreased more than 4-folds in all groups compared with the control. Despite these differences in the FA profiles of the meat, the contents of cholesterol and thiobarbituric acid reactive substances (TBARS) were similar among the seven diets. From the economic point of view, TO2 was a good option for modifying chicken meat with n-3 PUFA, following by LO2, TO4, and LO4. Tuna oil supplementation at 4% resulted in the highest content of EPA and DHA in the meat; therefore, the best treatment for incorporating long-chain n-3 PUFA into Thai crossbred chicken meat was to supplement the diet with 4% tuna oil.

### 3.2 Introduction

Thai native chickens are thought to have good meat qualities, such as a low level of fat and a chewy texture (Jaturasitha et al., 2008) and adapting well to local climates, thus contributing to food sustainability. While commercial broiler meat dominates the global market, slow-growing chicken is preferred in the premium market (Yang and Jiang, 2005). Consumers not only require food to supply nutrients, they also demand more value-added meat products with beneficial functions for health; for example, meat enriched with n-3 polyunsaturated fatty acid (PUFA). Therefore, producing chicken meat with a lower fat content and a healthier fatty acid profile is currently of great interest.

It has emphasized the significances of n-3 polyunsaturated fatty acids in maintaining heart health, protecting against cancer, birth defect, and offsetting symptoms of diabetes since the past many decades. The low levels of C20:5n-3 (EPA) and very low levels of C22:6n-3 (DHA) recently observed in the blood of people over



most of the world have been associated with an increased risk of cardiovascular-related mortality (Stark et al., 2016). Some sources of n-3 PUFA for human consumption are fish and other marine food, vegetable oil rich in C18:3n-3 (ALA), and functional meat and meat products high in n-3 PUFA. De Smet and Vossen (2016) have reported that poultry exhibited the best response in their meat to dietary supplementation of n-3 PUFA compared with pork, lamb, and beef. The FA profile of chicken meat strongly reflects the pattern of FA in the diet. In addition, the conversion of ALA to long-chain n-3 PUFA is better in slow- and medium-growing birds than in fast-growing birds fed the same diet (Boschetti et al., 2016). However, the fat content of slow-growing chickens has been found to be lower than that of commercial broilers (Jayasena et al., 2013; Wattanachant et al., 2004).

Rice bran oil (RBO) has mainly been used in chicken feed formulation in some countries where this source of oil is available. The RBO contains a mixture of antioxidants such as vitamin E, polyphenols, and  $\gamma$ -oryzanol (Ghosh, 2007; Xu and Godber, 1999). The dietary supplementation using fish oil and linseed oil for broiler was intensively studied, whereas the effect of tuna oil (TO, high in DHA) or linseed oil (LO, high in  $\alpha$ -linolenic acid) combined with RBO in crossbred chickens have yet to be evaluated. The present study will focus on strategy to modify the meat of Thai crossbred chickens by supplementing their diet with either TO or LO replacing part of the usual RBO content. This can be expected to make chicken meat more nutritionally valuable with a higher n-3 PUFA while having only a minor influence on growth performance, sensory evaluation, functional quality, and the contents of cholesterol and thiobarbituric acid reactive substances (TBARS) in the meat.

### **3.3 Materials and methods**

#### **3.3.1 Birds and housing**

All procedures used in the present study were approved by the Ethics Committee on Animal Use of Suranaree University of Technology (SUT), Thailand. A type of Thai indigenous crossbred chicken, the “Korat meat chicken” (male Leung Hang Khao and female SUT line), was used in the study. The chicks were vaccinated against Marek’s disease at the hatchery, against Newcastle disease and Infectious Bronchitis on days of 7 and 21, and against Gumboro disease at 14 d of age. The chickens were kept together after hatching until 21 d of age, with an average BW of  $271.68 \pm 38.07$  g, when they were transferred to the experimental pens. Birds of all treatments were reared on floor pens (8 birds/m<sup>2</sup>) under similar environmental and management conditions until 84 d of age. The floor pens contained a layer of rice hull (depth 5 cm) as bedding. Feed and water were freely available to all chickens.

#### **3.3.2 Experimental design and diets**

The experimental model was a completely randomized design, which included seven treatment diets, four replicates, and twenty 21-d-old mixed-sex chicks per replicate. A basal diet was formulated base on corn-soybean containing 6% of RBO as the control. The other treatment diets were based on substitution part of the RBO oil content with 2, 4 or 6% TO or LO up to a total oil content of 6%. All experimental diets were formulated to have equal nitrogen levels and provided 3,100 kcal/kg ME and 19% CP for the grower (d 22 to 42) and 17% CP for the finisher (d 43 to 84) (Table 3.1). Feed-grade oils were used in all the experimental diets. The fatty acids profiles of oils used for supplementation are shown in Table 3.2.

**Table 3.1** Ingredients and nutrient composition of the basal diets (as-fed basis)

Items	Grower (d 22 to 42)	Finisher (d 43 to 84)
<b>Ingredients</b>		
Soybean meal (44% CP)	33.55	26.55
Corn (8% CP)	50.36	51.50
De-oil rice bran	6.11	12.48
Added oil <sup>1</sup>	6.00	6.00
DL-Methionine	0.25	0.19
L-Lysine	0.13	0.10
L-Threonine	0.10	0.06
Salt	0.35	0.28
Calcium carbonate	1.57	1.42
Monocalcium phosphate (21% P)	1.08	0.92
Premix <sup>2</sup>	0.50	0.50
<b>Calculated nutrients (%)</b>		
ME (kcal/kg)	3,100	3,100
Crude protein	19.00	17.00
Crude fat	8.13	8.24
Crude fiber	4.07	4.45
Digestible lysine	1.00	0.85
Digestible methionine	0.51	0.43
Digestible methionine + cysteine	0.76	0.65
Digestible threonine	0.69	0.58
Calcium	0.90	0.80
Available phosphorus	0.35	0.30
Sodium	0.15	0.12

<sup>1</sup> Different oil and level depend on treatment.

<sup>2</sup> Premix (0.5%) provided the following per kilogram of diet: 15,000 IU of vitamin A; 3,000 IU of vitamin D3; 25 IU of vitamin E; 5 mg of vitamin K3; 2 mg of vitamin B1; 7 mg of vitamin B2; 4 mg of vitamin B6; 25 µg of vitamin B12; 11.04 mg of pantothenic acid; 35 mg of nicotinic acid; 1 mg of folic acid; 15 µg of biotin; 250 mg of choline chloride; 1.6 mg of Cu; 60 mg of Mn; 45 mg of Zn; 80 mg of Fe; 0.4 mg of I; 0.15 mg of Se.

**Table 3.2** Fatty acids profile (%) of oils using in the present study

Items	Crude rice bran oil	Linseed oil	Tuna oil
C8:0	-	0.15	-
C12:0	-	-	0.14
C13:0	-	-	0.12
C14:0	0.77	0.11	8.38
C15:0	-	-	1.29
C16:0	19.15	5.70	24.89
C18:0	1.93	3.43	7.69
C20:0	0.99	0.13	0.84
C21:0	-	0.05	-
C22:0	-	-	0.48
C24:0	-	-	0.77
C14:1	-	-	0.08
C16:1	0.24	0.06	5.42
C18:1n-9c	42.31	16.86	13.59
C20:1	0.39	-	0.73
C24:1	-	-	0.05
C18:2n-6c	32.77	15.40	1.61
C18:3n-6	-	0.23	0.12
C20:2n-6	-	-	0.26
C20:3n-6	-	-	0.14
C20:4n-6	-	-	0.06
C18:3n-3	1.45	57.83	0.54
C20:3n-3	-	0.06	0.07
C20:5n-3	-	-	2.35
C22:6n-3	-	-	30.38
SFA	22.84	9.56	44.60
MUFA	42.94	16.92	19.87
PUFA	34.22	73.52	35.53
n-6	32.77	15.63	2.19
n-3	1.45	57.89	33.34

“-” mean proportion of FA was <0.01% of total FA or undetectable.

### 3.3.3 Sampling and measurements

**Growth performance.** Body weight and feed intake were monitored once a week on a pen basis, whereas weight gain and feed conversion ratio (FCR) values were subsequently calculated. The health status was also recorded daily in each pen. The occurrence of mortality was also recorded.

**Slaughter procedure and carcass measurements.** At day 84, a total of 56 birds (four males and four females per treatment) were processed after 12 hours feed withdrawal period. The chosen chickens were transported to the slaughterhouse within one hour and then rested for about 30 minutes. All chickens were hung from the conveyor before stunning by electricity, scalding (60°C), and then de-feathering by machine and eviscerating manually. They were then washed with clean water and put in a chilled room (4°C, 24 h) before cutting into portions. The hot carcass (without viscera, head, neck, feet, and shank), abdominal fat (considered as the fat extending within the ischium, surrounding the cloaca, and adjacent to the abdominal muscle), and edible giblets were weighted. The weights were then calculated as the proportion of the live BW of chicken. The breast fillet, thigh, drumstick, and wing were expressed as the percentage of the chilled carcass weight.

**Meat quality.** The color characteristic [lightness ( $L^*$ ), redness ( $a^*$ ), and yellowness ( $b^*$ )], and meat functional properties (pH, drip loss, cooking loss, and shear force) were measured.

**pH measurement and water-holding capacity.** The pH value was measured at 45 min and 24 h post-mortem in the *pectoralis major* muscle at a depth of 0.5 to 1.0 cm. The breast fillet pH was measured directly using a pH meter with a precision of 0.01

pH units (UltraBasic pH meter, Model UB10A, Denver Instrument, Bohemia, NY, USA) coupled to a probe inserted into the center of the muscle.

After chilling for 24 h, the water-holding capacity was determined as drip loss (hung in an airtight container, chilled 24 h at 4°C) and cooking loss (boiled in a water bath in open plastic bags until an internal temperature of 80°C was reached). The drip loss and cooking loss were expressed as a percentage of the initial weight.

***Skin and meat color measurement.*** The skin color was measured using a Hunter Lab ColorQuest XE spectrophotometer, with QC Software (Reston, VA, USA) calibrated against black and white reference tiles. L\* (lightness), a\* (redness), and b\* (yellowness) values were obtained by an illuminant/observer D65/10°. The measurements were performed through the transparent packaging material. Three random readings were averaged on a sample surface for statistical analysis. The meat samples were ground and mixed before color measurement (Honikel, 1998).

***Shear force measurement.*** Cooked meat samples were used to determine shear force value. At least 3 subsamples were removed (2.0×1.0×0.5 cm) by cutting parallel to the muscle fibers from each cooked sample. A Texture Analyzer (TA-XT2, Texture Technologies Corp., Scarsdale, NY, USA) equipped with a Warner-Bratzler shear force apparatus was used to determine the peak shear force values of subsamples. The crosshead speed was set at 20 cm/min. Peak WBSF values were determined by averaging the values of three subsamples (Froning et al., 1978).

***Plasma lipoprotein.*** All blood samples from 28 chickens (80 days old) were collected between 7:00 AM and 10:00 AM following an overnight fast. Whole chicken blood was collect from wing vein into heparinized tubes. Cells were removed from plasma by centrifuging (2,000 rpm) at 4°C for 20 minutes. The assay was performed

on VITROS 5600 Integrated System (Ortho-Clinical Diagnostics, Raritan, NJ, USA) using the manufacturer's reagents and calibrators. Indirect lipid measures for very-low-density lipoprotein cholesterol (VLDL-C) was calculated based on values obtained for triglyceride (TRIG) ( $VLDL-C = TRIG/5$ ).

**Fatty acid content.** Another group of total 56 birds (four males and four females per treatment) were harvested as the description before. Samples of the breast and thigh meat were collected and stored at  $-20^{\circ}\text{C}$  until analysis when the fatty acid profiles, contents of cholesterol and TBARS were evaluated.

The lipids were extracted from approximately 5 g of each muscle samples using 90 ml of chloroform:methanol (2:1, v/v) (Folch et al., 1957). Then, 20 to 25 mg of extracted fat were methylated (Metcalf et al., 1966). The fatty acid methyl esters (FAME) were analyzed using gas chromatography (Hewlett-Packard 7890A; Agilent Technologies, Santa Clara, CA, USA) with a capillary column (SP 2560, Supelco Inc., Bellefonte, PA, USA,  $100\text{ m} \times 0.25\text{ mm i.d.}$ ,  $0.20\text{-}\mu\text{m}$  film thickness) and a flame ionization detector. The carrier gas was helium at a flow rate of  $0.95\text{ ml/min}$ . The temperatures of the injector and detector were  $260^{\circ}\text{C}$ . The initial column temperature was  $70^{\circ}\text{C}$ , which was then raised to  $175^{\circ}\text{C}$  at a rate of  $13^{\circ}\text{C/min}$ , and finally raised to  $240^{\circ}\text{C}$  at a rate of  $4^{\circ}\text{C/min}$ .

**Meat TBARS.** Duplicate 5-g samples of raw meat were used to analysis TBARS following the protocol of Leick et al. (2010). Samples, blanks, and standards were read at  $530\text{ nm}$  using a Bio-Rad Benchmark Plus<sup>®</sup> Microplate Spectrophotometer (Bio-Rad Laboratories Inc., Hercules, CA, USA).

**Meat cholesterol.** The cholesterol content was measured on the raw breast and thigh meat as describe by Rowe et al. (1999). The cholesterol was analyzed using gas

chromatography (Hewlett-Packard 6890, Agilent Technologies, Santa Clara, CA, USA) with a capillary column (HP 19091A-112, 25 m × 0.32 mm × 0.52 μm film thickness) and a flame ionization detector. The temperatures of injector and detector were 260 and 300°C, respectively. Separation was carried out isocratically at 300°C with a helium gas flow rate of 1 ml/min.

**Descriptive sensory test.** Nine panelists had trained step by step following guideline for Quantitative Descriptive Analysis (QDA) method before taking on the test. During the test, sample containers were coded with 3-digit random numbers and presented to panelists. Panelists evaluated the intensity of the sample attributes and marked their responses on 15-point line scales, low (1) to high (15). Order of sample presentations to panelists was randomized. Warm water and plain crackers were provided to panelists to cleanse their palates between samples.

### 3.3.4 Statistical analysis and calculation

Analysis of variance was performed by GLM procedure for a completely randomized design using SAS<sup>®</sup> University Edition with the following statistical model:  $y_{ij} = \mu + \tau_i + \varepsilon_{ij}$ , where  $y_{ij}$  = the dependent variable,  $\mu$  = the overall mean,  $\tau_i$  = the treatment effect, and  $\varepsilon_{ij}$  = the random residual error. Significant differences between treatment means were assessed by Tukey's multiple comparison tests after a significant F-test. Overall differences between treatment means were considered to be significant at  $P < 0.05$ . Data are expressed as the mean ± standard error of the mean (SEM), which represents the pooled SEM for the model. Differences of different levels of oil and group of treatment were tested using orthogonal contrasts. Data of sensory test was analyzed by Kruskal-Wallis Test of PROC NPAR1WAY of SAS<sup>®</sup> where treatment independence variable and the attributes were dependence variable.



The amount of FA in meat was calculated by the following equation where 0.945 was the conversion factors for poultry meat (Greenfield and Southgate, 2003).

$$\text{FA in meat (mg/100 g)} = [\text{Total lipid (\%)} * 0.945 * \text{FA of total FA of meat (\%)} * 10]$$

### 3.3.5 Site and period of the study

Feeding trial was carried out at University Farm, SUT from 15 May 2015 to 8 August 2015. Chemical analysis was done at Facility Building 3, 9, 10 (F3, F9, F10) of SUT from July to December 2015.

## 3.4 Results and discussion

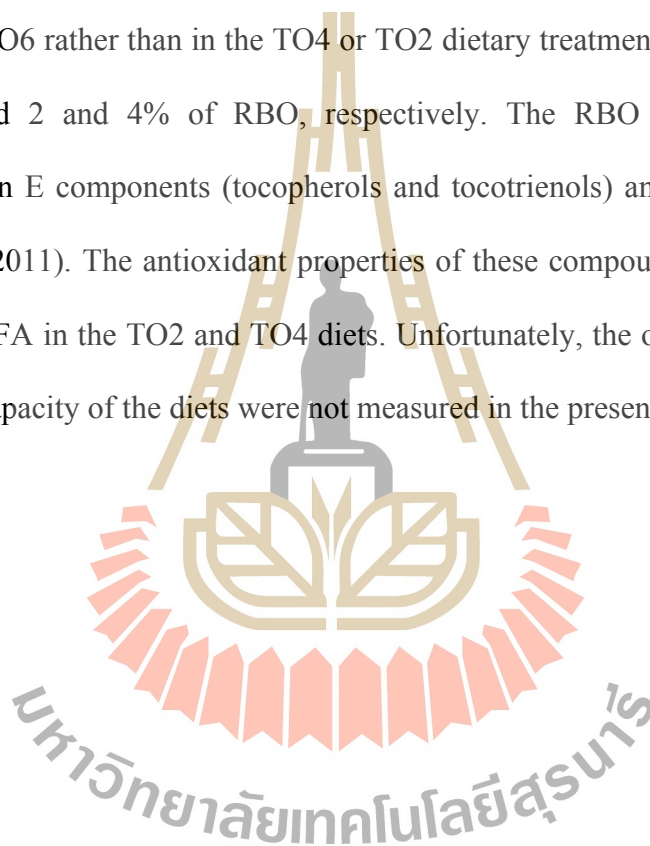
### 3.4.1 Fatty acid composition of oil sources and diets

The FA profiles of TO and LO used in this study revealed that LO was rich in ALA and that TO contained a high concentration of long chain n-3 PUFA, including EPA and DHA. The rice bran oil contained two major fatty acids, namely C18:1n-9c and C18:2n-6c (Table 3.2) Fatty acid profile of RBO, LO or TO were in normal range compared with other studies: ALA content of the LO was nearly the same as that used by Shingfield et al. (2011) and the content of C16:0, C18:1n-9c and EPA in the TO were similar to those of the oil extracted from tuna meat samples by Peng et al. (2013). In particular, DHA content in the TO was slightly higher than that reported elsewhere (Klinkesorn et al., 2004; Peng et al., 2013; Suseno et al., 2014).

The analysis of FA in the experimental diets (Table 3.3 and Table 3.4) showed that the percentage of n-6 PUFA decreased as the level of substitution of TO was increased. The fatty acid profile of the dietary treatments underlined that the TO6 diet had more detectable FA and high level of C16:0 while the percentage of EPA and DHA was lower than that of the TO4 treatment in both the growing and finishing diets. As

the level of LO increased, the percentages of C16:0, C18:1n-9c, and C18:2n-6c FA steadily declined while that of ALA went up dramatically.

The experimental diet was prepared every three weeks and stored in polypropylene woven bags at high ambient temperature, which might contribute to oxidation of oils in the mixture. In particular, long-chain n-3 PUFA in the TO treatments were highly susceptible to oxidation. This detrimental effect was observed more in the TO6 rather than in the TO4 or TO2 dietary treatments. The TO4 and TO2 diets included 2 and 4% of RBO, respectively. The RBO contains about 0.1–0.14% vitamin E components (tocopherols and tocotrienols) and 0.9–2.9% oryzanol (Arab et al., 2011). The antioxidant properties of these compounds may protect long chain n-3 PUFA in the TO2 and TO4 diets. Unfortunately, the oxidative stability and antioxidant capacity of the diets were not measured in the present study.



**Table 3.3** Fatty acids profile (%) of experimental diets in growing phase

Items	Growing diet <sup>1</sup> ( d 22 to 42)						
	Control	TO2	TO4	TO6	LO2	LO4	LO6
C8:0	-	-	-	-	-	-	0.11
C10:0	-	-	-	-	-	-	0.12
C12:0	0.13	0.15	0.15	0.21	0.14	-	0.34
C13:0	-	-	-	0.19	-	-	-
C14:0	1.51	2.83	4.68	7.74	1.04	0.27	1.14
C15:0	0.06	0.31	0.54	1.15	0.05	-	0.06
C16:0	19.67	20.24	22.04	30.57	16.06	12.15	10.06
C18:0	5.37	6.16	9.39	11.94	4.43	3.46	4.60
C20:0	1.62	1.46	1.61	0.74	1.00	0.47	0.50
C21:0	0.03	0.06	0.09	-	-	-	-
C14:1	-	-	-	0.06	-	-	-
C16:1	0.22	1.16	2.03	3.87	0.20	0.13	0.15
C18:1n-9c	35.05	30.00	26.19	22.46	31.02	26.57	21.64
C20:1	0.33	0.38	0.39	0.64	0.28	-	-
C18:2n-6c	34.24	28.35	20.32	15.82	31.82	29.26	24.79
C18:3n-6	-	-	-	-	0.06	0.11	0.14
C20:2n-6	0.10	0.13	0.14	0.24	0.07	0.07	-
C18:3n-3	1.69	1.43	1.13	1.01	13.84	27.51	36.35
C20:3n-3	-	-	-	0.31	-	-	-
C20:5n-3	-	1.07	1.66	0.57	-	-	-
C22:6n-3	-	6.27	9.62	2.49	-	-	-
SFA	28.38	31.21	38.50	52.54	22.72	16.35	16.92
MUFA	35.60	31.53	28.61	27.03	31.50	26.70	21.80
PUFA	36.02	37.25	32.89	20.43	45.79	56.95	61.28
n-6	34.34	28.48	20.47	16.06	31.95	29.44	24.93
n-3	1.69	8.77	12.42	4.37	13.84	27.51	36.35
n-6/n-3	20.33	3.25	1.65	3.67	2.31	1.07	0.69

<sup>1</sup> Treatments consisted of 6% RBO (Control), 2% TO + 4% RBO (TO2), 4% TO + 2% RBO (TO4), 6% TO (TO6), 2% LO + 4% RBO (LO2), 4% LO + 2% RBO (LO4), 6% LO (LO6).

“-“ mean proportion of FA was <0.01% of total FA or undetectable.

**Table 3.4** Fatty acids profile (%) of experimental diets in finishing period

Items	Finishing diet <sup>1</sup> (d 43 to 84)						
	Control	TO2	TO4	TO6	LO2	LO4	LO6
C8:0	-	-	-	-	-	-	0.23
C10:0	-	-	-	-	-	-	0.36
C12:0	-	0.16	-	-	-	0.22	1.01
C14:0	0.74	2.56	3.94	6.85	0.71	1.04	3.16
C15:0	-	0.31	0.57	1.22	-	-	0.21
C16:0	19.34	19.92	20.75	27.35	15.84	12.60	11.92
C18:0	3.54	4.91	5.95	7.78	3.42	4.78	6.03
C20:0	0.80	1.20	1.09	0.86	0.72	0.90	0.89
C22:0	0.37	0.73	0.62	0.47	0.51	0.55	0.50
C16:1	0.22	1.17	2.12	3.73	0.26	0.16	0.31
C18:1n-9c	37.37	31.06	25.53	23.31	32.23	26.00	20.88
C20:1	0.35	0.40	0.45	0.66	0.28	0.21	0.15
C18:2n-6c	35.65	29.46	23.70	18.08	31.77	27.18	21.48
C20:2n-6	-	0.10	0.14	0.19	-	-	-
C18:3n-3	1.62	1.30	1.23	0.96	14.25	26.36	32.88
C20:3n-3	-	-	-	0.92	-	-	-
C20:5n-3	-	0.48	0.99	0.62	-	-	-
C22:6n-3	-	6.26	12.92	7.00	-	-	-
SFA	24.79	29.78	32.92	44.52	21.20	20.09	24.31
MUFA	37.94	32.62	28.11	27.70	32.77	26.37	21.33
PUFA	37.27	37.60	38.97	27.77	46.03	53.54	54.36
n-6	35.65	29.56	23.84	18.27	31.77	27.18	21.48
n-3	1.62	8.04	15.13	9.50	14.25	26.36	32.88
n-6/n-3	21.97	3.68	1.58	1.92	2.23	1.03	0.65

<sup>1</sup> Treatments consisted of 6% RBO (Control), 2% TO + 4% RBO (TO2), 4% TO + 2% RBO (TO4), 6% TO (TO6), 2% LO + 4% RBO (LO2), 4% LO + 2% RBO (LO4), 6% LO (LO6).

“-“ mean proportion of FA was <0.01% of total FA or undetectable.

### 3.4.2 Productive performance

During the experimental period, the mortality was 0.36%. Overall, the TO6 exhibited the lowest BW compared with the control and other oil-supplemented birds (Table 3.5). In particular, the reduction of BW between treatments occurred as a result of the difference between TO and LO groups after feeding for 35 days ( $P < 0.05$  by contrast analysis). The accumulative feed intake was similar between the treatments during the experimental period. The FCR of chickens fed TO6 diet was significantly higher ( $P < 0.01$ ) than all the other treatments except LO2. In other words, the FCR of birds fed the TO4 and LO6 diets was not significantly different from the control group. This finding agreed with many studies indicating no difference in the growth performance parameters of broilers when fed LO and fish oil (Bou et al., 2006; Carragher et al., 2016; Chen et al., 2012; Fébel et al., 2008; Rymer et al., 2010; Sadeghi et al., 2012; Starčević et al., 2014). While LO supplementation did not affect FCR, TO supplementation increased FCR ( $P < 0.05$  by contrast analysis).

The reduction in performance of chickens fed TO6 diet could be explained by the oxidation of the n-3 long-chain PUFA. Consequently, the presence of free radicals may have caused an alteration in protein quality, and possibly a loss of amino acids. Engberg et al. (1996) also reported that the oxidized oil can decrease the nutrient content of the feed and suppress growth performance by reacting with proteins, lipids, and fat-soluble vitamins, even forming toxic products. With the greater quantity of natural antioxidants present in vegetable oils, these effects can often be less pronounced (Karrick, 1967). Therefore, supplementation with 6% TO alone had a detrimental impact on the growth performance. In fact, supplementation 5% linseed oil (without additional antioxidant) in broiler diet showed similar body weight gain, FI, and FCR compared to lard (Zhong et al.,

2014). In contrast, previous study have shown that adding 8.2% LO or fish oil to the broiler chicken diet led to a similar daily weight gain and FCR (Lopez-Ferrer et al., 1999) possibly as a result of including the antioxidant in the experimental diet (0.02% butylhydroxytoluol). Thus, including TO (6%) in the diet without appropriate amount and type of antioxidant can have a negative influence on growth performance.

**Table 3.5** Growth performance of Thai crossbred chickens fed experimental diets

Treatment <sup>1</sup>	BW d 21 (g/b)	BW d 84 (g/b)	FI (g/b)	FCR
Control	266.19	1,582.51 <sup>a</sup>	3,741.77	2.85 <sup>b</sup>
TO2	264.75	1,570.75 <sup>a</sup>	3,839.09	2.95 <sup>b</sup>
TO4	270.50	1,562.5 <sup>a</sup>	3,829.57	2.96 <sup>b</sup>
TO6	264.00	1,378.95 <sup>b</sup>	3,764.90	3.39 <sup>a</sup>
LO2	265.38	1,577.25 <sup>a</sup>	3,964.36	3.02 <sup>ab</sup>
LO4	264.75	1,525.88 <sup>a</sup>	3,779.76	3.01 <sup>b</sup>
LO6	268.13	1,607.50 <sup>a</sup>	3,867.27	2.89 <sup>b</sup>
SEM	6.81	62.66	155.93	0.16
<i>P</i> -value	0.831	0.001	0.493	0.002
<b><i>P</i>-value of contrast</b>				
Control vs TO <sup>2</sup>	0.955	0.042	0.449	0.012
Control vs LO <sup>3</sup>	0.979	0.737	0.168	0.187
TO vs LO	0.906	0.017	0.362	0.062

<sup>a,b</sup> Means within column carrying no common superscripts are significantly different at  $P < 0.05$ .

<sup>1</sup> Treatments consisted of 6% RBO (Control), 2% TO + 4% RBO (TO2), 4% TO + 2% RBO (TO4), 6% TO (TO6), 2% LO + 4% RBO (LO2), 4% LO + 2% RBO (LO4), 6% LO (LO6).

<sup>2</sup> Effect of group of tuna oil treatment (2, 4, 6%) compared to control.

<sup>3</sup> Effect of group of linseed oil treatment (2, 4, 6%) compared to control.

In addition, feeding 60 g/kg of fish oil decreased the percentage of monocytes and lowered weights of bursa of Fabricius, implying fish oil suppresses some aspects of the

immune response; however, the risk of infection by feeding fish oil was not evaluated (Al-Khalifa et al., 2012). Although the incident of disease was not observed in the present study, tuna oil at 6% might have harmful effect on birds' defense system. Furthermore, an effective immune system is costly (Van Der Most et al., 2011). In other words, the more resource of the body must use for immunity so that chicken performance of TO6 lowered.

### 3.4.3 Carcass composition

The carcass yield, expressed as the percentage of the chilled carcass weight excluding the gizzard, was similar between the treatments ( $P > 0.05$ ) (Table 3.6). The carcass yield results agreed with earlier studies (Lopez-Ferrer et al., 2001a; Lopez-Ferrer et al., 2001b).

**Table 3.6** Carcass yield of Thai crossbred chickens fed experimental diets

Items	Treatment <sup>1</sup>							SEM	P-value
	Control	TO2	TO4	TO6	LO2	LO4	LO6		
Dressing <sup>2</sup> (%)	68.49	68.72	68.55	67.83	69.18	69.02	67.88	1.14	0.561
<b>% of hot carcass</b>									
Abdominal fat <sup>3</sup>	0.20	0.14	0.08	0.16	0.10	0.10	0.14	0.14	0.896
Gizzard	2.54 <sup>ab</sup>	2.26 <sup>c</sup>	2.24 <sup>c</sup>	2.69 <sup>a</sup>	2.27 <sup>bc</sup>	2.39 <sup>bc</sup>	2.40 <sup>bc</sup>	0.21	0.049
Liver	1.70	1.69	1.57	1.87	1.70	1.77	1.84	0.20	0.461
Heart	0.43	0.41	0.46	0.46	0.46	0.42	0.43	0.04	0.238
<b>% of chilled carcass weight</b>									
Breast fillet	16.61	16.16	16.43	16.21	16.18	16.68	16.37	1.06	0.988
Inner fillet	5.07	5.41	5.54	5.14	5.85	5.30	5.40	0.42	0.217
Thigh	17.05	16.54	17.12	17.99	16.96	17.38	16.98	0.95	0.513
Drumstick	15.06	16.17	15.89	16.17	16.77	15.90	15.97	1.03	0.465
Wing	13.04	14.02	13.74	13.95	13.70	13.33	13.65	0.75	0.547

<sup>a-c</sup> Means within row carrying no common superscripts are significantly different at  $P < 0.05$ .

<sup>1</sup> Treatments consisted of 6% RBO (Control), 2% TO + 4% RBO (TO2), 4% TO + 2% RBO (TO4), 6% TO (TO6), 2% LO + 4% RBO (LO2), 4% LO + 2% RBO (LO4), 6% LO (LO6).

<sup>2</sup> without viscera, head, neck, feet, and shank.

<sup>3</sup> considered the fat extending within the ischium, surrounding the cloaca, and adjacent to the abdominal muscle.

Although diet is one of the factors affecting meat yield (Irshad et al., 2013), the dietary treatments in the present study used a similar basal diet with an equal amount of added oil. Thus, substitution of RBO by TO or LO exhibited no impact on carcass composition related to the chilled carcass weight of the chickens.

#### 3.4.4 Meat quality

Color strongly influences the decision to purchase meat and its acceptance by consumers (Garcia et al., 2010) with a more yellow chicken skin being preferred. Skin pigmentation is affected by genetics, dietary pigments, the health status of the birds, and the conditions of slaughter (Sirri et al., 2010). None of the treatments influenced the skin color ( $L^*$ ,  $a^*$ , and  $b^*$ ) (Table 3.7). However, the yellow color ( $b^*$ ) of breast meat in TO4 and TO6 diets exhibited lower ( $P < 0.05$ ) values compared to LO2 diet. The result obtained from the present study showed that the meat of chickens fed LO were more yellow than chickens fed the TO diets ( $P < 0.05$  by contrast analysis). It might be due to more yellow pigment, for example carotenoids, in linseed oil compared with tuna oil. This result differed from the study of Yang et al. (2010) which showed a higher  $b^*$  value for the meat color of thigh meat in broilers fed a fish oil diet than in those fed a soybean oil diet. In the present study, the red value ( $a^*$ ) of thigh meat was highest in the LO2 group and significantly different from the control group ( $P < 0.05$ ).

Lipid oxidation discolors the surface of the meat (Sohaib et al., 2015) by affecting myoglobin, especially the redox state of the ferrous component in the structure of heme protein. Supplementation with tuna or linseed oil with their different FA profiles could induce lipid oxidation in meat to a different extent, which could explain the difference in meat color found in the present study.



**Table 3.7** Skin and meat color value of Thai crossbred chickens

Items	Treatment <sup>1</sup>							SEM	P-value
	Control	TO2	TO4	TO6	LO2	LO4	LO6		
<b>Skin color</b>									
Breast									
L*	64.54	63.41	64.50	66.24	63.88	65.19	65.32	1.36	0.122
a*	2.47	3.24	2.85	2.14	2.51	2.55	2.40	0.66	0.370
b*	5.59	6.45	4.70	4.95	5.75	5.52	5.70	1.38	0.672
Thigh									
L*	65.58	64.21	66.92	66.84	65.21	66.33	65.50	1.56	0.212
a*	2.49	3.45	2.64	1.96	3.00	2.48	2.72	0.64	0.095
b*	3.51	4.55	2.22	2.74	5.23	2.35	2.90	1.80	0.192
<b>Meat color</b>									
Breast									
L*	62.12	61.43	61.59	61.62	62.36	62.82	61.92	2.02	0.959
a*	7.06	7.53	7.49	7.48	7.60	7.38	7.92	0.62	0.639
b*	16.88 <sup>ab</sup>	16.57 <sup>ab</sup>	16.04 <sup>b</sup>	15.53 <sup>b</sup>	17.55 <sup>a</sup>	16.60 <sup>ab</sup>	16.25 <sup>ab</sup>	0.64	0.008
Thigh									
L*	59.14	59.15	59.80	58.24	59.08	59.84	59.61	1.70	0.854
a*	9.03 <sup>b</sup>	9.65 <sup>ab</sup>	10.09 <sup>ab</sup>	9.73 <sup>ab</sup>	10.73 <sup>a</sup>	10.60 <sup>ab</sup>	10.53 <sup>ab</sup>	0.65	0.022
b*	16.37 <sup>ab</sup>	16.59 <sup>ab</sup>	16.63 <sup>ab</sup>	15.23 <sup>b</sup>	17.52 <sup>a</sup>	17.34 <sup>a</sup>	16.94 <sup>a</sup>	0.67	0.002

<sup>a-b</sup> Means within row carrying no common superscripts are significantly different at  $P < 0.05$ .

<sup>1</sup> Treatments consisted of 6% RBO (Control), 2% TO + 4% RBO (TO2), 4% TO + 2% RBO (TO4), 6% TO (TO6), 2% LO + 4% RBO (LO2), 4% LO + 2% RBO (LO4), 6% LO (LO6).

The pH of the breast meat was similar for all the diets at 45 min and 24 h after slaughter (Table 3.8). The L\* value is also the main parameter determining poultry meat color. Barbut (1997) found that the optimal lightness range for chicken fillets was around 49-50, and the lighter the color (>50) the lower the pH value (<5.6). The meat of the breast and thigh in the present study was lighter than normal, although exhibiting a high pH value. It was not expected that the pH value would remain unchanged in the aging process (measured at 45 min and 24 h). This plateau in the pH value may be

explained by the low level of glycogen, as well as an increase in radical oxygen species, oxidation stress, and muscle catabolism (Beauclercq et al., 2016).

The oil supplementation had no effect on shear force values of the meat (Table 3.8). The water-holding capacity is important in whole meat, where it is determined by the drip and boiling losses in this study. Drip loss of the breast and thigh was unaffected by oil inclusion possibly because of the relatively high concentrations of unsaturated FA of all treatments in this study. The cooking loss of breast meat was highest ( $P < 0.05$ ) in the LO6 treatment in contrast to the lower values in the control and TO2 treatments. This seems to be related to the level of PUFA in the diet. This agreed with previous studies (Lopez-Ferrer et al., 2001b; Yang et al., 2010) reporting that supplementation with fish and linseed oil reduced the water-holding capacity of broiler meat.

**Table 3.8** Meat quality of Thai crossbred chickens fed experimental diets

Items	Treatment <sup>1</sup>							SEM	P-value
	Control	TO2	TO4	TO6	LO2	LO4	LO6		
pH 45 min	6.57	6.69	6.72	6.69	6.60	6.73	6.74	0.13	0.457
pH 24 h	6.71	6.50	6.77	6.62	6.52	6.45	6.47	0.16	0.074
<b>Breast meat (% of total)</b>									
Drip loss	10.51	9.30	8.48	10.04	8.73	10.19	8.81	1.25	0.210
Boiling loss	23.07 <sup>b</sup>	22.88 <sup>b</sup>	24.19 <sup>ab</sup>	23.61 <sup>ab</sup>	24.17 <sup>ab</sup>	25.02 <sup>ab</sup>	25.87 <sup>a</sup>	1.09	0.010
<b>Thigh meat (% of total)</b>									
Drip loss	7.76	7.57	7.17	6.16	6.47	6.43	6.19	0.98	0.159
Boiling loss	28.60	26.48	25.25	25.07	26.82	29.15	30.69	2.99	0.157
Breast WBS <sup>2</sup>	3.10	2.61	2.04	3.20	3.18	3.50	2.91	0.79	0.245
Thigh WBS	1.42	1.51	1.22	1.45	1.21	1.41	1.51	0.36	0.821

<sup>a-b</sup> Means within row carrying no common superscripts are significantly different at  $P < 0.05$ .

<sup>1</sup> Treatments consisted of 6% RBO (Control), 2% TO + 4% RBO (TO2), 4% TO + 2% RBO (TO4), 6% TO (TO6), 2% LO + 4% RBO (LO2), 4% LO + 2% RBO (LO4), 6% LO (LO6).

<sup>2</sup> WBS: Warner-Bratzler shear force expressed as kgf/0.5 cm<sup>2</sup>.

### 3.4.5 Fatty acid of chicken meat

The total lipid in the breast meat samples ranged from 1.68 to 2.94 g/100 g of fresh meat. The thigh meat contained approximately twice as much total lipid as the breast meat. In general, the content of most of the fatty acids in the breast and thigh meat samples were significantly different ( $P < 0.05$ ) between treatments, except C14:0 and C18:0 in the breast meat (Tables 3.9 and 3.10). For both types of meat, the highest levels of n-6 PUFA were found in the control. The deposition of arachidonic acid (C20:4n-6; AA) decreased ( $P < 0.001$ ) in the breast meat of chickens fed n-3 fatty acids compared with chickens fed the control diet. The control diets based on the corn-soybean meal with added RBO contained around 70% of C18:1n-9c and C18:2n-6c FA, which subsequently modified the meat to provide the highest level of these FA. The proportion of AA in the meat of chickens fed TO or LO was reduced by almost a half in the present study. This is desirable because this FA is a precursor of prostaglandin E2, a very active pro-inflammatory agent. Shin et al. (2012) also found similar responses in a comparison of feeding Cobb  $\times$  Ross male broilers with n-3 PUFA for 9 weeks or with animal and vegetable oils. This also agreed with Kartikasari et al. (2012) who reported that the AA content of chicken meat was reduced by increasing the dietary ALA content.

The level of dietary TO also had a positive correlation to total n-3 PUFA content ( $P < 0.05$ ) and SFA in the meat. However, the TO6 treatment produced the highest content of SFA in thigh meat, but the n-3 PUFA content was similar to the TO2 treatment. Notably, supplementation of 4 or 6% TO resulted in a similar percentage of EPA in the breast meat but not in the thigh meat. The TO4 treatment was also more effective than the TO2 and TO6 treatment for increasing the EPA and DHA content in chicken meat (Figure 3.1) in the present study. However, if the TO6 was supplemented

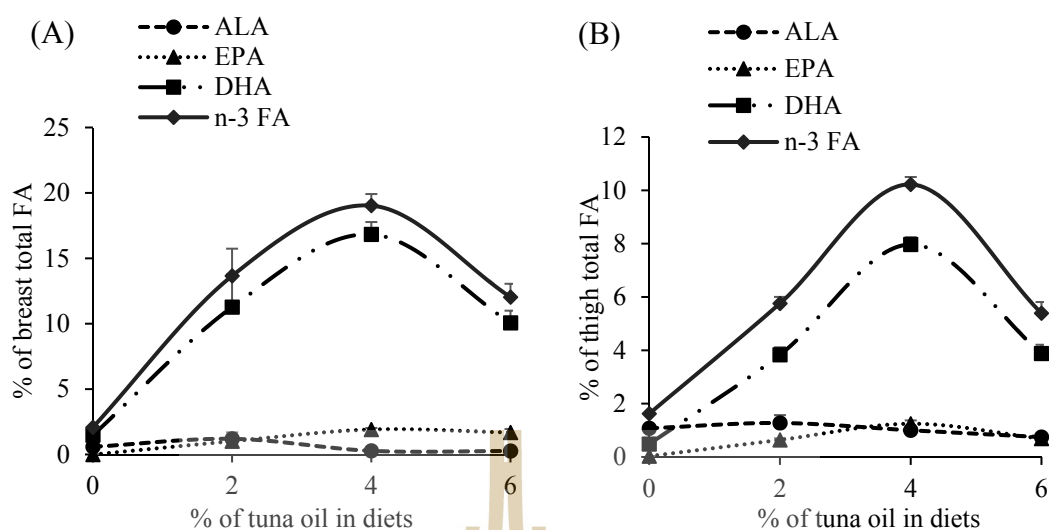
with vitamin E, it could protect the feed from lipid oxidation; and subsequently, the increasing level of tuna oil could linearly increase DHA content of chicken meat.

Increasing the levels of LO in the diet resulted in a linear increase ( $P < 0.05$ ) in the ALA content of the breast and thigh meat (Figure 3.2), with the greatest content provided by the LO6 treatment. The proportion of ALA in the thigh meat was greater than in the breast meat. The EPA and DHA contents of the meat of chickens fed LO were higher than those of the control. Overwhelmingly, the amount of FA in the meat has been found to depend on the amount of these FA in the diet, antioxidative status, and their synthesis in the liver (Farhoomand and Checaniazar, 2009). The conversion efficiency from ALA to EPA and DHA has also been shown to depend on the ratio of ingested C18:2n-6c (LA) and ALA (Harnack et al., 2009). In the present study, the conversion of ALA to its derivatives had nutritionally valuable; although Lopez-Ferrer et al. (2001b) have reported that this was nutritionally meaningless, they used broiler (Cobb) in their research. In fact, medium-growing chickens exhibited a higher  $\Delta 6$  and  $\Delta 5$  desaturase activities and consequently a higher long chain n-3 PUFA content in the breast meat than in fast-growing birds fed the same diet (Boschetti et al., 2016). In the present study, the EPA and DHA content of the intramuscular fat of the breast were higher than those of the thigh muscle, agreeing with the report of Zuidhof et al. (2009). Meanwhile, the ALA content of the thigh meat was considerably higher than in the breast meat. The ALA is mainly deposited in the triacylglycerol fraction of meat (Betti et al., 2009) while breast contains mainly phospholipid and thigh meat has a large amount of triacylglycerol.

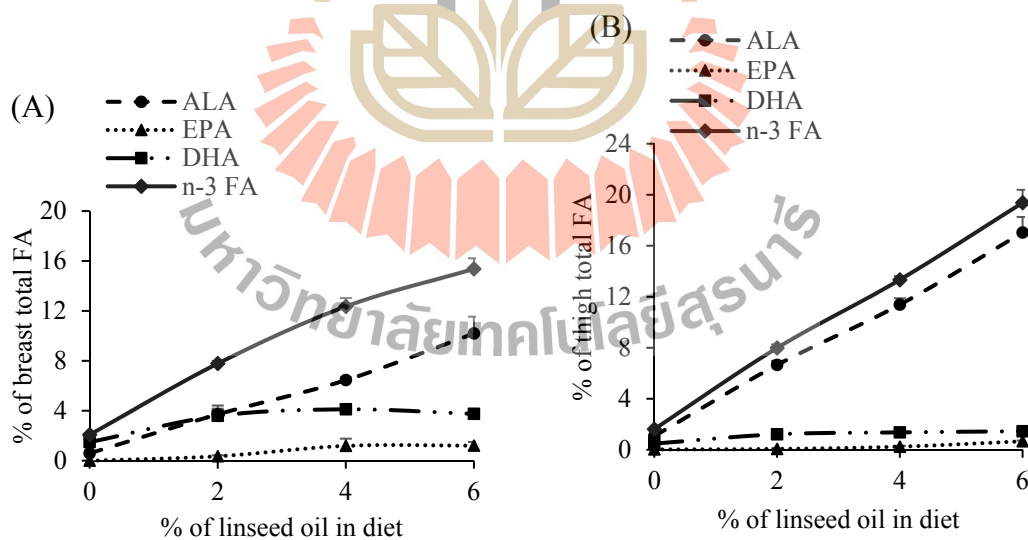
The n-6/n-3 ratios of both the breast and thigh meat were significantly different ( $P < 0.0001$ ) with the highest values in the control. In the breast meat, the n-6/n-3 ratio was less than 4 in the group supplemented with TO or LO, with TO4 exhibited the

lowest ratio. In the thigh meat, the LO6 treatment resulted in the lowest ratio ( $P < 0.05$ ) of n-6/n-3 with similar ratios in thigh meat from the LO4 and TO4 treatments.

Because the total lipid content in the meat was not significantly different between treatments, the mean total lipid of all treatments was used for calculations. The TO2 and TO4 diets for Thai crossbred chickens provided 252.88 mg and 377.53 mg DHA per 100 g raw breast, respectively. Compared with the breast meat, the content of DHA in the thigh meat was lower at 293.92 mg/100 g raw meat for the TO4 diet and approximately 140 mg/100 g meat for the TO2 and TO6 diets (Table 3.11). This implied that Thai crossbred chicken can accumulate a nutritionally valuable amount of n-3 PUFA for human consumption. In fact, The European Food Safety Authority (EFSA), noting that DHA contributes to the maintenance of normal brain function and vision, has recommended that the beneficial effect can be obtained with a daily intake of 250 mg of DHA. The LO treatment also increased the amount of ALA in the meat. However, only the ALA content in the thigh meat of chickens fed the LO6 diet reached the threshold for designating it as “high in n-3 PUFA” meat (according to Commission Regulation (EU) 1924/2006 (Zduńczyk and Jankowski, 2013) and 432/2012). Although the amount of EPA and DHA in the groups of chickens fed LO was significant, especially in the breast meat, the dominant oil for supplying these FA was TO.



**Figure 3.1** Effects of dietary tuna oil on n-3 PUFA profile of the breast (A) and thigh (B) meat. The value presented are mean $\pm$ SEM (n=4/treatment). There were significant differences ( $P < 0.001$ ) in the level of EPA, DHA, and total n-3 of the breast (Quadratic) and thigh meat (Cubic).



**Figure 3.2** Effects of dietary linseed oil on n-3 PUFA profile of the breast (A) and thigh (B) meat. The value presented are mean $\pm$ SEM (n=4/treatment). There were significant differences ( $P < 0.05$ ) in the level of ALA, EPA, DHA, and total n-3 of either breast or thigh meat (Linear), except EPA of the thigh meat (Quadratic).

**Table 3.9** Fatty acid profile (g/100 g total FA) of breast meat of the chickens

Fatty acid <sup>1</sup>	Treatment <sup>2</sup>											SEM	P-value	P-value of contrast		
	Control	TO2	TO4	TO6	LO2	LO4	LO6	C <sup>3</sup> vs TO	C vs LO	TO vs LO						
C14:0	0.53	0.82	0.76	1.47	1.28	0.35	0.72	0.50	0.099	0.152	0.461	0.290				
C15:0	0.07 <sup>b</sup>	0.21 <sup>ab</sup>	0.37 <sup>ab</sup>	0.43 <sup>a</sup>	0.14 <sup>ab</sup>	0.15 <sup>ab</sup>	0.08 <sup>b</sup>	0.14	0.016	0.011	0.594	0.003				
C16:0	22.62 <sup>bc</sup>	22.70 <sup>b</sup>	24.15 <sup>ab</sup>	25.34 <sup>a</sup>	23.28 <sup>ab</sup>	22.20 <sup>bc</sup>	20.14 <sup>c</sup>	0.99	<0.0001	0.037	0.267	<0.0001				
C20:0	0.17	0.49	0.05	0.59	0.08	0.03	0.47	0.43	0.393	0.463	0.932	0.335				
C18:0	8.56	9.83	9.51	11.90	11.72	10.70	10.80	1.47	0.081	0.067	0.019	0.310				
C22:0	-	0.21	-	-	-	-	-	0.17	0.555	0.535	1.000	0.355				
C16:1	0.21 <sup>b</sup>	0.58 <sup>b</sup>	1.02 <sup>b</sup>	2.13 <sup>a</sup>	0.76 <sup>b</sup>	0.11 <sup>b</sup>	0.30 <sup>b</sup>	0.40	<0.0001	0.001	0.525	0.000				
C18:1n-9 <sup>c</sup>	30.12 <sup>a</sup>	24.63 <sup>b</sup>	22.39 <sup>b</sup>	23.90 <sup>b</sup>	26.59 <sup>ab</sup>	23.93 <sup>b</sup>	23.50 <sup>b</sup>	1.68	0.000	<0.0001	0.000	0.168				
C20:1	0.22	0.19	0.11	0.19	0.16	0.11	0.10	0.11	0.636	0.403	0.176	0.393				
C18:2n-6 <sup>c</sup>	24.31 <sup>a</sup>	20.50 <sup>ab</sup>	17.53 <sup>bc</sup>	16.02 <sup>c</sup>	21.19 <sup>ab</sup>	23.97 <sup>a</sup>	23.50 <sup>a</sup>	1.73	<0.0001	<0.0001	0.228	<0.0001				
C18:3n-6	-	-	-	0.02	0.02	0.01	-	0.02	0.582	0.677	0.398	0.498				
C20:2n-6	0.55	1.05	0.15	0.12	0.29	0.19	0.27	0.60	0.359	0.770	0.455	0.478				
C20:3n-6	0.20	0.37	0.31	0.44	0.30	0.53	0.54	0.20	0.318	0.193	0.068	0.357				
C20:4n-6	10.35 <sup>a</sup>	4.76 <sup>b</sup>	4.61 <sup>b</sup>	5.43 <sup>b</sup>	6.39 <sup>b</sup>	5.36 <sup>b</sup>	4.22 <sup>b</sup>	1.24	0.000	<0.0001	<0.0001	0.474				
C18:3n-3	0.59 <sup>cd</sup>	1.20 <sup>cd</sup>	0.29 <sup>d</sup>	0.28 <sup>d</sup>	3.72 <sup>bc</sup>	6.46 <sup>b</sup>	10.19 <sup>a</sup>	1.21	<0.0001	0.995	<0.0001	<0.0001				
C20:3n-3	-	0.16	-	-	0.12	0.59	0.21	0.35	0.355	0.814	0.193	0.104				
C20:5n-3	0.00 <sup>e</sup>	1.02 <sup>abc</sup>	1.92 <sup>a</sup>	1.69 <sup>a</sup>	0.36 <sup>bc</sup>	1.19 <sup>abc</sup>	1.21 <sup>ab</sup>	0.47	0.001	<0.0001	0.009	0.007				
C22:6n-3	1.49 <sup>d</sup>	11.27 <sup>ab</sup>	16.83 <sup>a</sup>	10.07 <sup>bc</sup>	3.59 <sup>d</sup>	4.12 <sup>cd</sup>	3.76 <sup>d</sup>	2.39	<0.0001	<0.0001	0.155	<0.0001				

**Table 3.9** Fatty acid profile (g/100 g total FA) of breast meat of the chickens (cont.)

Fatty acid <sup>1</sup>	Treatment <sup>2</sup>						SEM	P-value	P-value of contrast			
	Control	TO2	TO4	TO6	LO2	LO4			LO6	C <sup>3</sup> vs TO	C vs LO	TO vs LO
SFA	31.96 <sup>b</sup>	34.26 <sup>ab</sup>	34.84 <sup>ab</sup>	39.73 <sup>a</sup>	36.50 <sup>ab</sup>	33.43 <sup>ab</sup>	32.21 <sup>b</sup>	2.74	0.015	0.025	0.263	0.074
MUFA	30.55 <sup>a</sup>	25.40 <sup>b</sup>	23.52 <sup>b</sup>	26.21 <sup>ab</sup>	27.51 <sup>ab</sup>	24.15 <sup>b</sup>	23.90 <sup>b</sup>	2.01	0.004	0.001	0.001	0.872
PUFA	37.49 <sup>abc</sup>	40.33 <sup>abc</sup>	41.63 <sup>ab</sup>	34.06 <sup>c</sup>	35.99 <sup>bc</sup>	42.41 <sup>ab</sup>	43.89 <sup>a</sup>	2.75	0.001	0.512	0.087	0.094
n-6	35.41 <sup>a</sup>	26.67 <sup>bc</sup>	22.60 <sup>cd</sup>	22.02 <sup>d</sup>	28.21 <sup>b</sup>	30.06 <sup>b</sup>	28.53 <sup>b</sup>	1.77	<0.0001	<0.0001	<0.0001	<0.0001
n-3	2.08 <sup>d</sup>	13.66 <sup>b</sup>	19.03 <sup>a</sup>	12.03 <sup>bc</sup>	7.78 <sup>cd</sup>	12.36 <sup>bc</sup>	15.37 <sup>ab</sup>	2.17	<0.0001	<0.0001	<0.0001	0.004
n-6/n-3	17.24 <sup>a</sup>	2.14 <sup>cd</sup>	1.20 <sup>d</sup>	1.91 <sup>cd</sup>	3.64 <sup>b</sup>	2.46 <sup>bc</sup>	1.88 <sup>cd</sup>	0.47	<0.0001	<0.0001	<0.0001	0.000

<sup>a-d</sup> Means within row carrying no common superscripts are significantly different at  $P < 0.05$ .

<sup>1</sup> “-“ mean proportion of FA was <0.01% of total FA or undetectable.

<sup>2</sup> Treatments consisted of 6% RBO (Control), 2% TO + 4% RBO (TO2), 4% TO + 2% RBO (TO4), 6% TO (TO6), 2% LO + 4% RBO (LO2), 4% LO + 2% RBO (LO4), 6% LO (LO6).

<sup>3</sup> C: Control.



**Table 3.10** Fatty acid profile (g/100 g total FA) of thigh meat of the chickens

Fatty acid <sup>1</sup>	Treatment <sup>2</sup>										SEM	P-value	P-value of contrast			
	Control	TO2	TO4	TO6	LO2	LO4	LO6	C <sup>3</sup> vs TO	C vs LO	TO vs LO						
C10:0	-	-	-	0.002	0.01	-	-	-	0.01	0.623	0.932	0.564	0.466			
C12:0	0.01	-	-	0.02	0.06	0.02	0.01	0.05	0.05	0.711	0.839	0.656	0.347			
C13:0	-	-	-	0.01	-	-	-	-	0.003	0.084	0.314	1.000	0.152			
C14:0	0.50 <sup>c</sup>	1.03 <sup>b</sup>	1.38 <sup>b</sup>	1.96 <sup>a</sup>	0.53 <sup>c</sup>	0.35 <sup>e</sup>	0.46 <sup>e</sup>	0.19	<0.0001	<0.0001	<0.0001	0.678	<0.0001			
C15:0	0.09 <sup>d</sup>	0.29 <sup>c</sup>	0.47 <sup>b</sup>	0.63 <sup>a</sup>	0.08 <sup>d</sup>	0.10 <sup>d</sup>	0.08 <sup>d</sup>	0.04	<0.0001	<0.0001	<0.0001	0.663	<0.0001			
C16:0	18.46 <sup>c</sup>	20.09 <sup>b</sup>	20.54 <sup>b</sup>	23.62 <sup>a</sup>	17.24 <sup>cd</sup>	16.03 <sup>d</sup>	14.01 <sup>e</sup>	0.49	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001			
C18:0	7.59 <sup>b</sup>	7.25 <sup>b</sup>	8.79 <sup>ab</sup>	10.39 <sup>a</sup>	7.85 <sup>b</sup>	8.76 <sup>ab</sup>	9.46 <sup>ab</sup>	1.01	0.008	0.081	0.081	0.121	0.792			
C20:0	0.27	0.40	0.26	0.36	0.14	0.20	0.19	0.28	0.872	0.716	0.611	0.211				
C21:0	-	-	-	0.01	-	-	-	0.003	0.082	0.313	1.000	0.151				
C14:1	0.00 <sup>b</sup>	0.03 <sup>b</sup>	0.03 <sup>b</sup>	0.15 <sup>a</sup>	0.01 <sup>b</sup>	0.01 <sup>b</sup>	0.00 <sup>b</sup>	0.03	<0.0001	0.004	0.790	0.000				
C16:1	0.67 <sup>d</sup>	1.93 <sup>bc</sup>	2.29 <sup>b</sup>	4.31 <sup>a</sup>	1.12 <sup>cd</sup>	1.00 <sup>cd</sup>	0.74 <sup>d</sup>	0.34	<0.0001	<0.0001	0.228	<0.0001				
C17:1	0.11	-	-	-	-	-	-	0.07	0.335	0.020	0.022	1.000				
C18:1n-9 <sub>7</sub>	-	-	-	0.06	-	0.13	-	0.05	0.092	0.574	0.238	0.343				
C18:1n-9 <sub>c</sub>	34.37 <sup>a</sup>	32.08 <sup>ab</sup>	27.58 <sup>c</sup>	30.47 <sup>bc</sup>	31.92 <sup>ab</sup>	28.02 <sup>c</sup>	25.05 <sup>d</sup>	1.03	<0.0001	<0.0001	<0.0001	0.002				
C20:1	0.42 <sup>ab</sup>	0.48 <sup>ab</sup>	0.47 <sup>ab</sup>	0.57 <sup>a</sup>	0.35 <sup>bc</sup>	0.15 <sup>cd</sup>	0.14 <sup>d</sup>	0.09	<0.0001	0.154	0.003	<0.0001				
C24:1	-	-	-	-	0.01	0.02	-	0.01	0.414	1.000	0.229	0.083				
C18:2n-6 <sub>7</sub>	-	-	-	0.005	-	-	-	0.004	0.592	0.551	1.000	0.392				
C18:2n-6 <sub>c</sub>	31.02 <sup>a</sup>	28.27 <sup>bc</sup>	24.55 <sup>d</sup>	19.12 <sup>e</sup>	29.26 <sup>ab</sup>	28.60 <sup>bc</sup>	27.42 <sup>c</sup>	0.78	<0.0001	<0.0001	0.000	<0.0001				

**Table 3.10** Fatty acid profile (g/100 g total FA) of thigh meat of the chickens (cont.)

Fatty acid <sup>1</sup>	Treatment <sup>2</sup>										SEM	P-value	P-value of contrast			
	Control	TO2	TO4	TO6	LO2	LO4	LO6	C <sup>3</sup> vs TO	C vs LO	TO vs LO						
C18:3n-6	0.06	0.07	-	0.06	0.07	0.03	0.04	0.06	0.649	0.707	0.916					
C20:2n-6	0.39	0.34	0.34	0.23	0.33	0.30	0.21	0.08	0.077	0.115	0.565					
C20:3n-6	0.19	0.20	0.24	0.32	0.22	0.20	0.37	0.11	0.253	0.409	0.354					
C20:4n-6	4.21 <sup>a</sup>	1.76 <sup>b</sup>	2.84 <sup>ab</sup>	2.34 <sup>b</sup>	2.81 <sup>ab</sup>	2.74 <sup>ab</sup>	2.44 <sup>b</sup>	0.55	0.002	<0.0001	0.001	0.174				
C18:3n-3	1.08 <sup>d</sup>	1.28 <sup>d</sup>	1.00 <sup>d</sup>	0.75 <sup>d</sup>	6.66 <sup>c</sup>	11.39 <sup>b</sup>	17.04 <sup>a</sup>	1.09	<0.0001	0.923	<0.0001	<0.0001				
C20:3n-3	0.02 <sup>c</sup>	0.01 <sup>c</sup>	0.00 <sup>c</sup>	0.07 <sup>bc</sup>	0.04 <sup>c</sup>	0.33 <sup>a</sup>	0.20 <sup>ab</sup>	0.06	<0.0001	0.948	0.001	<0.0001				
C20:5n-3	0.03 <sup>d</sup>	0.64 <sup>bc</sup>	1.25 <sup>a</sup>	0.69 <sup>b</sup>	0.07 <sup>d</sup>	0.25 <sup>cd</sup>	0.67 <sup>bc</sup>	0.15	<0.0001	<0.0001	0.008	<0.0001				
C22:6n-3	0.49 <sup>e</sup>	3.84 <sup>b</sup>	7.97 <sup>a</sup>	3.88 <sup>b</sup>	1.22 <sup>c</sup>	1.37 <sup>c</sup>	1.46 <sup>c</sup>	0.46	<0.0001	<0.0001	0.012	<0.0001				
C22:2n-2	-	-	-	-	-	-	0.01	0.005	0.592	1.000	0.560	0.392				
SFA	26.93 <sup>cd</sup>	29.05 <sup>bc</sup>	31.44 <sup>b</sup>	36.98 <sup>a</sup>	25.91 <sup>cd</sup>	25.46 <sup>cd</sup>	24.20 <sup>d</sup>	1.58	<0.0001	<0.0001	0.117	<0.0001				
MUFA	35.57 <sup>a</sup>	34.53 <sup>a</sup>	30.37 <sup>b</sup>	35.55 <sup>a</sup>	33.41 <sup>a</sup>	29.32 <sup>b</sup>	25.93 <sup>c</sup>	1.15	<0.0001	0.013	<0.0001	<0.0001				
PUFA	37.50 <sup>d</sup>	36.42 <sup>d</sup>	38.19 <sup>d</sup>	27.47 <sup>c</sup>	40.68 <sup>c</sup>	45.22 <sup>b</sup>	49.87 <sup>a</sup>	0.95	<0.0001	<0.0001	<0.0001	<0.0001				
n-6	35.88 <sup>a</sup>	30.66 <sup>b</sup>	27.97 <sup>c</sup>	22.08 <sup>d</sup>	32.69 <sup>b</sup>	31.89 <sup>b</sup>	30.49 <sup>b</sup>	1.06	<0.0001	<0.0001	<0.0001	<0.0001				
n-3	1.62 <sup>f</sup>	5.76 <sup>de</sup>	10.22 <sup>e</sup>	5.39 <sup>e</sup>	7.99 <sup>cd</sup>	13.34 <sup>b</sup>	19.38 <sup>a</sup>	0.99	<0.0001	<0.0001	<0.0001	<0.0001				
n-6/n-3	22.23 <sup>a</sup>	5.40 <sup>b</sup>	2.74 <sup>c</sup>	4.22 <sup>b</sup>	4.13 <sup>b</sup>	2.41 <sup>c</sup>	1.60 <sup>e</sup>	0.55	<0.0001	<0.0001	<0.0001	<0.0001				

<sup>a-f</sup> Means within row carrying no common superscripts are significantly different at  $P < 0.05$ .

<sup>1</sup> -, - - mean proportion of FA was <0.01% of total FA or undetectable.

<sup>2</sup> Treatments consisted of 6% RBO (Control), 2% TO + 4% RBO (TO2), 4% TO + 2% RBO (TO4), 6% TO (TO6), 2% LO + 4% RBO (LO2), 4% LO + 2% RBO (LO4), 6% LO (LO6). <sup>3</sup> C: Control

**Table 3.11** The amount (mg/100g fresh meat) of some major n-3 fatty acids of breast and thigh meat

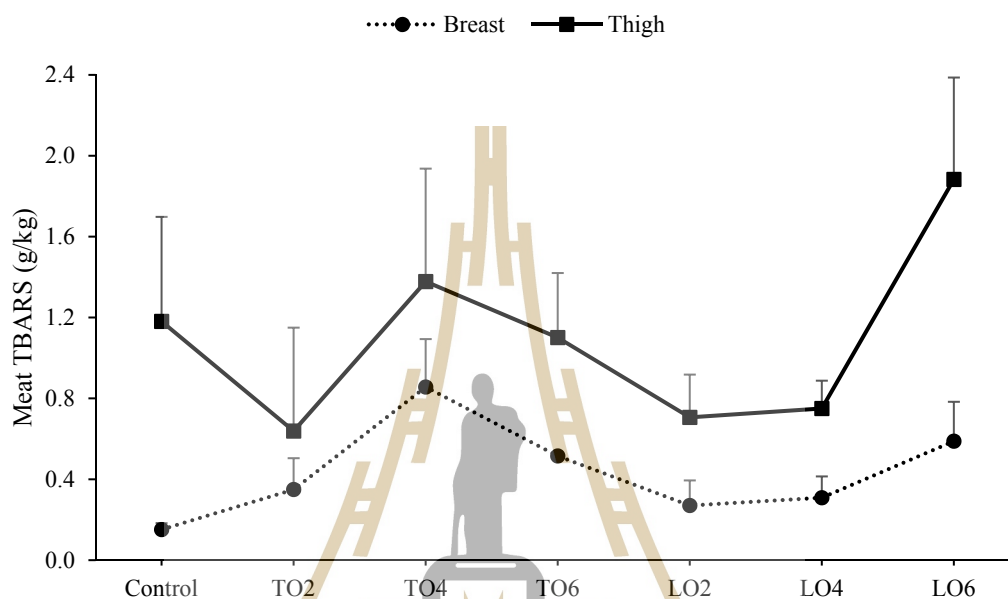
Items	Treatment <sup>1</sup>						
	Control	TO2	TO4	TO6	LO2	LO4	LO6
<b>Breast</b>							
ALA	13.31	26.98	6.42	6.18	83.34	144.84	228.61
EPA	-	22.93	42.95	37.80	8.00	26.66	27.07
DHA	33.35	252.88	377.53	225.94	80.57	92.39	84.23
EPA+DHA	33.35	275.81	420.48	263.75	88.57	119.06	111.31
Total n-3	46.66	306.38	426.90	269.93	174.57	277.19	344.70
<b>Thigh</b>							
ALA	39.80	47.09	37.02	27.59	245.50	419.94	628.40
EPA	1.21	23.63	46.00	25.46	2.70	9.21	24.81
DHA	18.01	141.42	293.92	143.15	45.08	50.51	53.90
EPA+DHA	19.23	165.05	339.92	168.61	47.78	59.72	78.70
Total n-3	59.87	212.35	376.94	198.83	294.58	491.74	714.53

<sup>1</sup> Treatments consisted of 6% RBO (Control), 2% TO + 4% RBO (TO2), 4% TO + 2% RBO (TO4), 6% TO (TO6), 2% LO + 4% RBO (LO2), 4% LO + 2% RBO (LO4), 6% LO (LO6).

### 3.4.6 Meat TBARS

The TBA method, and its different variations is the most widely used test for measuring the extent of lipid oxidation in the muscle foods (Ladeira et al., 2014). The oxidative status of the chicken meat as indicated by the TBARS values was found to be no significant difference between treatments (Figure 3.3). The TBARS concentrations of the breast meat ranged from 0.15 to 0.86 g/kg meat and those in the thigh meat from 0.64 to 1.88 g/kg meat. In general, thigh meat had the higher level of TBARS than breast meat owing to its higher fat content. In fact, the formation of TBARS and its accumulation in foodstuffs depend on the degree of unsaturation of PUFA and other factors (Raharjo and Sofos, 1993 cited by Wrolstad et al. (2003). In each meat type, the total PUFA content was different between treatments; therefore, their TBARS values

would also be expected to be different. Unfortunately, in the present study, other measurements, for example, of the derived volatile aldehydes, were not performed, thus not allowing a definite conclusion to be made on the oxidative status of the chicken meat.



**Figure 3.3** Meat TBARS value of experimental chickens (mean  $\pm$  SEM; n=4/treatment).

#### 3.4.7 Plasma lipoprotein and meat cholesterol

High-density lipoprotein cholesterol (HDL-C) exhibited higher in the LO4 diets and inverse effect ( $P < 0.05$ ) in the TO4 and TO6 diets. Supplementing tuna or linseed oils did not influence on triglyceride and VLDL-C (Table 3.12). In the present study, low-density lipoprotein cholesterol (LDL-C) was analysis by direct LDL method which cannot give the value below measuring (reportable) range ( $<30$  mg/dL) in 14 out of 28 samples. In summary, the trend of LDL-C seems opposite with HDL-C where the higher level was found in the TO4 and TO6 diets and lower level in the linseed oil group.

**Table 3.12** Plasma lipoprotein cholesterol of Thai crossbred chicken

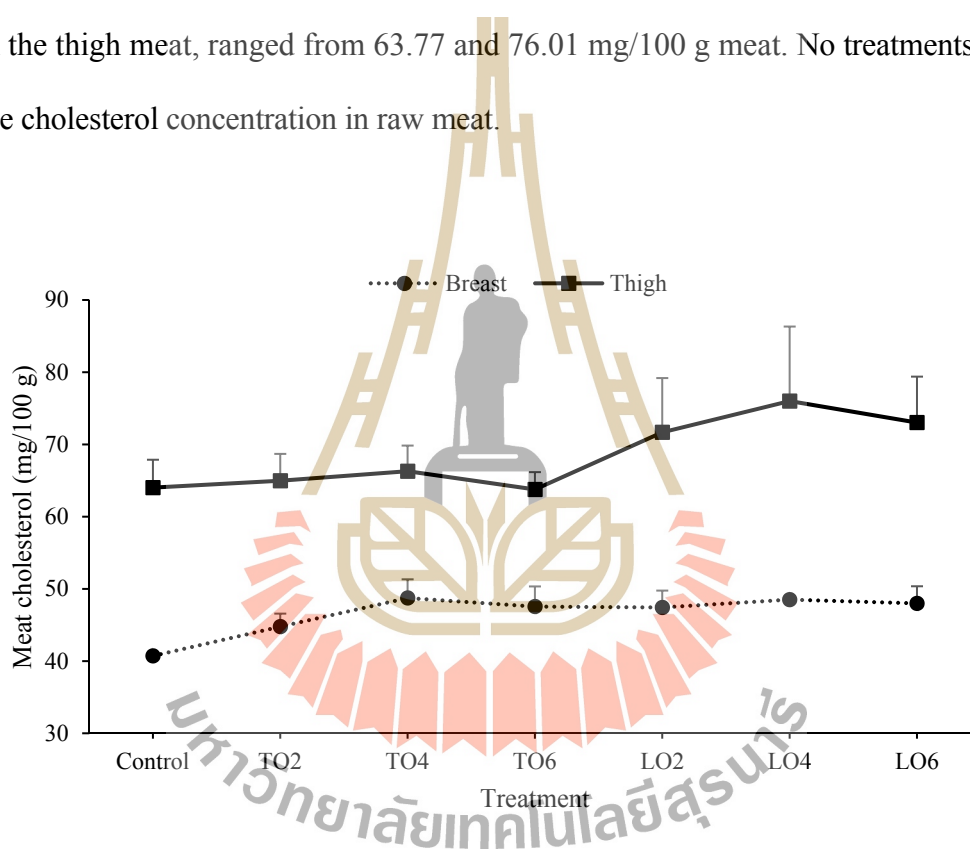
Treatment <sup>1</sup>	Triglyceride (mg/dL)	HDL-C (mg%)	VLDL-C (mg/dL)
Control	35.50	55.00 <sup>ab</sup>	7.10
TO2	28.25	46.00 <sup>ab</sup>	5.65
TO4	31.50	42.75 <sup>b</sup>	6.30
TO6	31.67	43.75 <sup>b</sup>	6.33
LO2	26.75	55.75 <sup>ab</sup>	5.35
LO4	28.67	59.00 <sup>a</sup>	5.73
LO6	28.50	46.67 <sup>ab</sup>	5.70
SEM	4.36	5.76	0.87
<i>P</i> -value	0.151	0.003	0.151

<sup>a-b</sup> Means within column carrying no common superscripts are significantly different at  $P < 0.05$

<sup>1</sup> Treatments consisted of 6% RBO (Control), 2% TO + 4% RBO (TO2), 4% TO + 2% RBO (TO4), 6% TO (TO6), 2% LO + 4% RBO (LO2), 4% LO + 2% RBO (LO4), 6% LO (LO6).

The plasma triglyceride and VLDL-C level in the present study were relatively lower than that of broiler found elsewhere (Abdulkarimi et al., 2011; Rahimi et al., 2011) and similar with the study of Daneshyar et al. (2011). Supplementation TO or LO did not affect the triglyceride and VLDL-C but influenced on the plasma HDL-C ( $P < 0.01$ ) and LDL-C. Tuna oil with high DHA concentration caused reduction HDL-C and increase LDL-C in chickens. It was not agreed with the findings in human (Kromhout et al., 2012) showing the n-3 fatty acids in fish oils (DHA and EPA) had a powerful action to reduce plasma VLDL-C levels and did not have a major effect on fasting total cholesterol and the LDL-C levels. The difference can cause by SFA in TO4 and TO6 diets, including higher C16:0 and C18:0 content compared with other diets, where C16:0 and C18:0 make the increase in LDL-C (Willett, 2012).

In general, including different sources and levels of oil in the chicken diet did not affect the cholesterol concentration in either the breast or thigh meat (Figure 3.4). In particular, the breast of chickens fed the control diet had a lower ( $P < 0.05$ ) cholesterol content than the groups fed TO or LO (by orthogonal contrast analysis). However, this trend was not found in the thigh meat. The cholesterol content in the breast meat ranged from 40.73 to 48.73 mg/100g meat while higher levels were found in the thigh meat, ranged from 63.77 and 76.01 mg/100 g meat. No treatments affected the cholesterol concentration in raw meat.



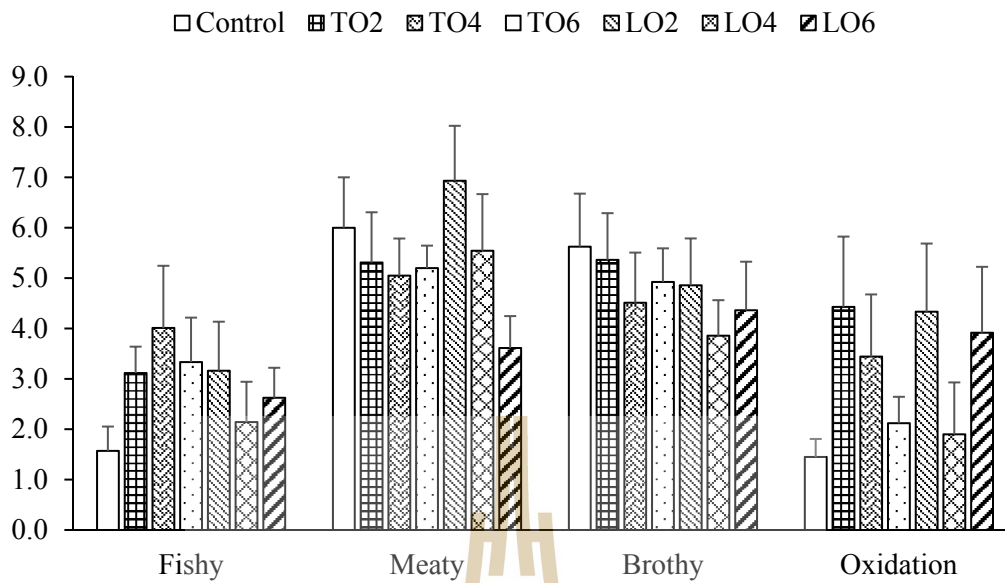
**Figure 3.4** Cholesterol content (Mean  $\pm$  SEM;  $n=4$ /treatment) of the breast and thigh meat of experimental chickens

In fact, Dinh et al. (2011) found contradictory results in their review of various studies, suggesting that unless there are pronounced changes in muscle structure and composition, the cholesterol content is unlikely to be affected. In the present study, the

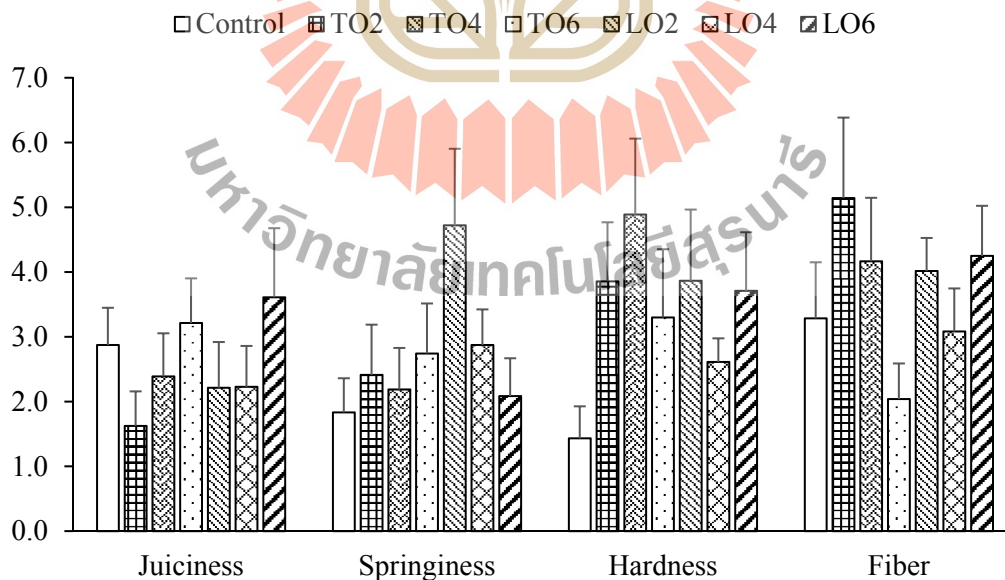
cholesterol of breast meat was higher than that reported by Jaturasitha et al. (2008), who found only 10.9-15.1 mg/100 g meat. However, their results for the thigh meat of crossbred chickens were similar to the present study. This difference could have been caused by the very low percentage of fat in the breast meat of these crossbred chickens (0.43-0.59%) was detected by Jaturasitha et al. (2008). The range of cholesterol in the present study was also higher than that obtained from Thai indigenous crossbred chicken by Molee et al. (2012). However, when compared with the value for broiler chicken meat reported by Dinh et al. (2011), the cholesterol of the Thai crossbred chickens in the present study was lower.

#### **3.4.8 Quantitative sensory test**

The score of major attributes evaluated by trained panellists was illustrated in the Figures 3.5 and 3.6. The data of sensory test followed non-normal distribution; therefore non-parametric test was performed. There was no significant difference between treatments in all of the attributes. The fishy odor was numerically highest in the TO4 treatment. However, the oxidation odor (the reference was oxidized vegetable oil) was numerically lowest in the control. The hardness was slightly different ( $P = 0.105$ ) which the TO groups was found more hardness. The overall aroma in the control seems favorable than the other diets. This might explain by the oxidation occurred during storage, thawing and cooking meat in the TO and LO diets. The fishy odor was more likely detectable in the TO group than the others. Notably, the LO2 treatment had more meaty flavor and springiness. The results were inconsistent in the TO group.



**Figure 3.5** The score (Mean ± SEM; n=9/treatment) of aroma test of the breast meat of Thai crossbred chickens



**Figure 3.6** The difference (Mean ± SEM; n=9/treatment) of texture of the breast meat of Thai crossbred chickens



### 3.4.9 Return over feed cost

The calculation based on the practical condition where the present study was conducted. Including TO or LO in the chicken diets increased feed cost (Table 3.13). When partly replaced RBO by every 2%, the feed cost increased 15% in the TO groups and 17% in the LO groups compared with the control. The selling price of oils was higher in LO (4.98 USD/kg) than TO (4.56 USD/kg), which in turn it was more expensive than RBO (1.03 USD/kg). The differences in feed cost per kg live body weight were 17%, 32%, and 63% for TO2, TO4, and TO6, respectively). The LO treatments had higher feed cost per kg than that of TO groups at the same level of supplementation, excluding LO6 and TO6.

**Table 3.13** Price of the treatment diets, feed cost, and return over feed cost

Items		Treatment <sup>1</sup>						
		Control	TO2	TO4	TO6	LO2	LO4	LO6
Feed price <sup>2</sup> (USD/kg)	d22 – 42	0.49	0.56	0.63	0.70	0.57	0.64	0.72
	d 43 – 84	0.47	0.54	0.61	0.68	0.55	0.63	0.71
Feed cost <sup>3</sup> (USD/bird)		1.91	2.23	2.50	2.73	2.33	2.53	2.89
Feed cost <sup>3</sup> (USD/kg BW)		1.21	1.42	1.60	1.98	1.48	1.66	1.80
<i>Return over feed cost<sup>4</sup></i>								
USD/bird		1.41	1.07	0.78	0.17	0.98	0.67	0.49
USD/kg		0.89	0.68	0.50	0.12	0.62	0.44	0.30

<sup>1</sup> Treatments consisted of 6% RBO (Control), 2% TO + 4% RBO (TO2), 4% TO + 2% RBO (TO4), 6% TO (TO6), 2% LO + 4% RBO (LO2), 4% LO + 2% RBO (LO4), 6% LO (LO6).

<sup>2</sup> Price of starter diet: 0.51 USD/kg

<sup>3</sup> Including feed cost of d 1 - 21 where feed intake was 0.29 kg/bird, then feed cost was 0.15 USD/bird for all treatments.

<sup>4</sup> Selling price of normal slow-growing chicken was 2.1 USD/kg live weight.

Return over feed cost was the lowest in TO6 and highest in the control. Return per kg live body weight ranged from 0.12 USD to 0.89 USD if the selling price was equal to that of slow-growing chicken in the normal market. If the cost of 1-d old chicks (0.53 USD) was counted, it could be seen that the TO6 and LO6 showed a negative benefit. From the economic point of view, TO2 was a good option for modifying chicken meat with n-3 PUFA, following by LO2, TO4, and LO4. The return over feed cost was just for comparison between treatment diets. However, the cost of production was not only for feed but also for 1 d old chicks, labor, water bill, bedding materials, and so on. And the price of functional chicken meat is expected to be higher than normal market's price. Therefore, this calculation did not intend to be the exactly financial investment for large scale production.

### 3.5 Conclusions

The breast meat accumulated most DHA in the TO2 and TO4 treatments, but the best response for thigh meat was the TO4 diet. In addition, TO4 also modified meat to provide the highest proportion of EPA in both the breast and thigh meat. ALA in meat increased linearly with the concentration of linseed oil in the diets. From the economic point of view, tuna oil was a good option for modifying chicken meat with n-3 PUFA. Therefore, the best treatment for incorporating long-chain n-3 PUFA into Thai crossbred chicken meat was to supplement the diet with 4% tuna oil.

### 3.6 References

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# CHAPTER IV

## COMBINATIONS OF CURCUMINOIDS AND TUNA OIL AFFECT PERFORMANCE, OXIDATION STATUS, AND MEAT QUALITY OF THAI CROSSBRED CHICKENS

### 4.1 Abstract

The aim of the present study was to determine the effects of dietary curcuminoids combined with tuna oil on growth performance, meat quality, thiobarbituric acid reactive substances (TBARS) of plasma and raw meat, and fatty acid profile of chicken meat. A total of 480 21-d-old mixed-sex Thai crossbred chickens were assigned to a CRD model with six treatment diets and four replicates. The basal diets based on corn-soybean with 2% rice bran oil and 4% tuna oil were used as the negative control. The experimental treatment diets supplemented with dl- $\alpha$ -tocopheryl acetate at 200 ppm was used as positive control diet (E-200). Other treatment diets were an addition of 20, 40, 60 or 80 ppm curcuminoids from curcumin removed turmeric oleoresin into basal ration (CUR-20, CUR-40, CUR-60, and CUR-80, respectively). At the end of the present study, vacuum-packed carcasses were stored in a freezer (-20°C) over a three-month period to examine the effect of curcuminoids on the change of TBARS and fatty acid composition of the breast and thigh meat. Increasing levels of curcuminoids tend to improve feed conversion ratio (linear,  $P = 0.065$ ) and significant increased breast fillet proportion (linear,  $P = 0.037$ ) and yellowness of the skin of either breast (linear,  $P = 0.016$ ) or thigh (linear,  $P = 0.023$ ). The curcuminoids exhibited the antioxidant properties but its effect

did not depend on dose supply. The CUR-20 and CUR-40 increased linoleic acid and decreased C22:6n-3 (DHA) of the breast meat, implying the inhibition fatty acid synthesis or activation  $\beta$ -oxidation in chicken body by dietary curcuminoids. The CUR-60 exhibited oxidation inhibition (TBARS measurement) in the chicken meat similar to dl- $\alpha$ -tocopheryl acetate and no effect on DHA proportion of the breast and thigh meat. The auto-oxidation during three-month storage found in the breast but not in the thigh. Further studies should focus on the bioavailability of curcuminoids and the combination curcuminoids and DHA and linolenic acid in the chicken diets. The suitable level in the present study of curcuminoids in the slow-growing chicken diets was 60 mg/kg.

## 4.2 Introduction

Slow-growing chicken meat contains favorable high in n-3 polyunsaturated fatty acids (PUFA) and less fat was preferred in the niche market. The n-3 PUFA enriched meat leads to more susceptibility to lipid oxidation (Cortinas et al., 2004) which causes an adverse effect by deterioration in flavor, color, texture, nutritive value and the production of toxic compounds (Kanner, 1994). Therefore, an antioxidant is employed to limits the rate of oxidation in the feed, in the bird, and subsequently poultry products (Leeson, 2012). The antioxidants from natural sources are usually considered as GRAS - generally recognized as safe and can be alternatives to synthetic antioxidant.

The antioxidant properties of curcumin and its derivatives was intensively studied (Barclay et al., 2000; Fujisawa et al., 2004; Masuda et al., 1999). In fact, curcumin help stabilizes free radicals (Barzegar and Moosavi-Movahedi, 2011) and increase the activities of antioxidant enzymes (Aggarwal, 2010). Its derivatives, demethoxycurcumin and bisdemethoxycurcumin also have antioxidant effects

(Chattopadhyay et al., 2004). Therefore, it is hypothesized that curcuminoids could prevent oxidation in the enriched n-3 PUFA meat.

Dietary turmeric powder in broiler diets decreased the triglycerides and saturated fatty acid (SFA) in thigh meat (Daneshyar et al., 2011). Curcumin (from turmeric) inhibited  $\Delta 5$ -*desaturases* of rat hepatocytes (Fujiyama-Fujiwara et al., 1992) and microsomal  $\Delta 5$  and  $\Delta 6$  *desaturases* of rat liver (Shimizu et al., 1992). The combination of curcuminoids and long-chain n-3 PUFA might have synergetic effects and benefit the accumulation of C22:6n-3 (DHA) in the chicken meat. To the best of our knowledge, the present study was the first study about the effect of different levels of curcuminoids from the curcumin removed turmeric oleoresin combined with tuna oil supplementation in slow-growing chicken diet.

Combination curcuminoids with tuna oil (rich in n-3 PUFA) is expected to protect the meat from lipid oxidation and influence on the fatty acid content of chicken meat. The aim of the present study was to determine the effects of dietary curcuminoids combined with tuna oil on growth performance, carcass yield, meat quality, thiobarbituric acid reactive substances (TBARS) of plasma and raw meat, cholesterol, and fatty acid profile of chicken meat. It also evaluated the effect of curcuminoids on TBARS and fatty acid composition of meat over three-month period in frozen (-20°C) storage condition.

## 4.3 Materials and methods

### 4.3.1 Birds, experimental design, and diets of feeding trial

All procedures used in the present study were approved by the Ethics Committee on Animal Use of the Suranaree University of Technology (SUT). A type of Thai crossbred chicken, known as the “Korat meat chicken” (male Lueng Hang Khao and female SUT line), was used in the study. Four hundred and eighty 1-d-old unsexed chicks were housed and cared for following the SUT Farm guidelines. The chicks were fed a corn-soybean meal-based diet (21% CP and 3,100 kcal ME/kg) from days 1 to 21.

On day 21, the chicks, with an average BW of  $289.05 \pm 36.46$  g, were randomly allotted to six experimental diets with four replications of 20 birds each in a CRD. The study lasted for 63 days, from days 22 to 84. Chicks were fed *ad libitum* throughout the experimental period. For all treatments, the birds were reared in floor pens (8 birds/m<sup>2</sup>) in an open-sided house.

A basal ration (Table 4.1) containing 2% rice bran oil (RBO; Thai Ruam Jai Korat Co., LTD, Nakhon Ratchasima 30000, Thailand) and 4% tuna oil (TO; T. C. Union Agrotech Co., LTD, Bangkok 10400, Thailand) were formulated as negative control diet, whereas positive control diet (E-200) was supplemented with vitamin E at 200 mg/kg into the basal ration. Other treatment diets, namely CUR-20, CUR-40, CUR-60, and CUR-80, were added curcumin removed turmeric oleoresin into basal ration to provide the diets with 20, 40, 60 or 80 mg/kg curcuminoids, respectively.

The turmeric oleoresin used in the present study was taken from Thai Government Pharmaceutical Organization (Bangkok 10400, Thailand). Total curcuminoids content of curcumin removed turmeric oleoresin was  $21.6 \pm 0.62\%$  by weight.

**Table 4.1** Ingredients and nutrient composition of the basal diets (as-fed basis)

Items	Grower (d 22 to 42)	Finisher (d 43 to 84)
Soybean meal (44% CP)	33.55	26.55
Corn (8% CP)	50.36	51.50
De-oil rice bran	6.11	12.48
Tuna oil	4.00	4.00
Rice bran oil	2.00	2.00
DL-Methionine	0.25	0.19
L-Lysine	0.13	0.10
L-Threonine	0.10	0.06
Salt	0.35	0.28
CaCO <sub>3</sub>	1.57	1.42
Monocalcium phosphate (21% P)	1.08	0.92
Premix <sup>1</sup>	0.50	0.50
<b>Calculated nutrients (% unless stated otherwise)</b>		
ME (kcal/kg)	3,100	3,100
Crude protein	19.00	17.00
Digestible lysine	1.00	0.85
Digestible methionine	0.51	0.43
Digestible Met + Cys	0.76	0.65
Calcium	0.90	0.80
Available phosphorus	0.35	0.30
<b>Analyzed nutrient level (%)</b>		
Total lipid (%)	7.30	7.74
Fatty acid profile (% of total fatty acid)		
SFA	31.98	30.33
MUFA	32.93	32.56
PUFA	35.09	37.11
n-6	26.58	26.89
n-3	8.51	10.22

<sup>1</sup> Premix (0.5%) provided the following per kilogram of diet: 15,000 IU of vitamin A; 3,000 IU of vitamin D3; 25 IU of vitamin E; 5 mg of vitamin K3; 2 mg of vitamin B1; 7 mg of vitamin B2; 4 mg of vitamin B6; 25 µg of vitamin B12; 11.04 mg of pantothenic acid; 35 mg of nicotinic acid; 1 mg of folic acid; 15 µg of biotin; 250 mg of choline chloride; 1.6 mg of Cu; 60 mg of Mn; 45 mg of Zn; 80 mg of Fe; 0.4 mg of I; 0.15 mg of Se.

**Table 4.2** Fatty acid profile (%) of rice bran oil and tuna oil used in the diets

Items	Rice bran oil	Tuna oil
C14:0	-	5.17
C16:0	22.98	28.73
C18:0	-	8.28
C21:0	-	0.32
C24:0	-	0.21
C16:1	-	5.42
C17:1	-	0.93
C18:1n-9	45.74	17.70
C20:1	0.18	0.19
C22:1n-9	-	0.37
C24:1	-	2.16
C18:2n-6	29.74	2.42
C18:3n-6	0.86	0.58
C20:2n-6	-	0.41
C20:4n-6	-	1.00
C18:3n-3	0.50	1.12
C20:3n-3	-	1.63
C20:5n-3	-	4.77
C22:6n-3	-	18.32
SFA	22.98	42.85
MUFA	45.92	26.78
PUFA	31.10	30.37
n-6	30.60	4.53
n-3	0.50	25.84
n-6/n-3	61.52	0.18

“-” not detectable; C13:0 (0.08%), C15:0 (0.06%), and C20:3n-6 (0.13%) of tuna oil were detected.

### 4.3.2 Sampling and storage condition

At d 84, chickens, which have  $\pm$  10% average body weight per pen, were harvested similar to commercial procedure (as the description in Chapter III). A total of 48 birds (four males and four females per treatment) were measured carcass



composition and pH value. After chilling 24 h at 4°C, breast and thigh meat was sampling to evaluate color characteristic [lightness ( $L^*$ ), redness ( $a^*$ ), and yellowness ( $b^*$ )], some functional properties (drip loss, cooking loss, and shear force).

The breast and thigh muscle (without skin) were collected right after de-feathering and eviscerating from randomized 48 chickens (one male and one female per pen). They were vacuum-packed and chilled 24 h at 4°C, followed by freezing at -20°C for 15 days to determine cholesterol (one sample/pen), fatty acid (FA) composition (one sample/pen) and TBARS (two samples/pen) of the first month. The total of 96 whole carcasses divided into two groups (one male and one female per experimental unit per group) were vacuum-packed and chilled 24 h at 4°C, followed by freezing at -20°C for the second (60 d) and third month (90 d) of frozen storage. Before analysing, the carcasses were thawed in ice-slurry, following manually separated breast and thigh meat (without skin). The breast and thigh meat were chopped and blended individually right after separation on condition that the meat still chilled. The individually minced meat was sampling for determination of TBARS and fatty acid (FA) composition.

#### 4.3.3 Measurements and chemical analysis

**Growth performance and carcass composition.** Body weight and feed intake (FI) were monitored once a week on a pen basis, whereas weight gain and feed conversion ratio (FCR) values were subsequently calculated. The health status was also recorded daily in each pen. The occurrence of mortality was also recorded. Carcass composition was evaluated similarly to previous experiment (Chapter III).

**pH Measurement.** The pH value was measured at 45 min and 24 h post-mortem in the *pectoralis major* muscle at a depth of 0.5 to 1.0 cm. The breast fillet pH was measured directly using a pH meter with a precision of 0.01 pH units (UltraBasic pH

meter, Model UB10A, Denver Instrument, Bohemia, NY, USA) coupled to a probe inserted into the center of the muscle.

**Water-holding capacity.** After chilling for 24 h, pieces of breast and thigh muscles from the same location were cut to a shape of 1.5 x 3.0 x 0.5 cm<sup>3</sup>. The water-holding capacity was determined as drip loss (hung in an airtight container, chilled 24 h at 4°C) and cooking loss (boiled in a water bath in open plastic bags until an internal temperature of 80°C was reached). The drip loss and cooking loss were expressed as a percentage of the initial weight.

**Skin and meat color measurement.** The skin color was measured using a Hunter Lab ColorQuest XE spectrophotometer, with QC Software (Reston, VA, USA) calibrated against black and white reference tiles. L\* (lightness), a\* (redness), and b\* (yellowness) values were obtained by an illuminant/observer D65/10°. The measurements were performed through the transparent packaging material. Three random readings were averaged on a sample surface for statistical analysis. The meat samples were ground and mixed before color measurement (Honikel, 1998).

**Shear force measurement.** Cooked meat samples were used to determine shear force value. At least 3 subsamples were removed (2.0×1.0×0.5 cm) by cutting parallel to the muscle fibers from each cooked sample. A Texture Analyzer (TA-XT2, Texture Technologies Corp., Scarsdale, NY, USA) equipped with a Warner-Bratzler shear force (WBSF) apparatus was used to determine the peak shear force values of subsamples. The crosshead speed was set at 20 cm/min. Peak WBSF values were determined by averaging the values of three subsamples (Froning et al., 1978).

**Plasma TBARS measurement.** Blood samples were collected from 8 chickens per treatment (1 male and 1 female per pen) into EDTA-treated tubes, gently shaken

and kept and handle on wet ice. Plasma was separated by centrifugation the blood at  $1,000 \times g$  for 10 min at  $4^{\circ}\text{C}$ , then transferred to 1.5-ml microcentrifuge tubes and stored in  $-80^{\circ}\text{C}$ . Measurement of TBARS in EDTA plasma were done by a modified method in accordance with Grotto et al. (2007). A standard curve with 1,1,3,3-tetramethoxypropane (Sigma-Aldrich Pte Ltd., Singapore 118222) was used, and the concentration was expressed as nmol MDA/ml solution. Assays were performed in the centrifuge tube to which were added 300  $\mu\text{L}$  plasma in duplicate (or standard or  $\text{H}_2\text{O}$  blank) and 200  $\mu\text{L}$  of 1.5 M NaOH. The tubes were capped, vortexed, and placed in a  $60^{\circ}\text{C}$  water bath for 30 min. Following alkaline hydrolysis, 0.5 ml of 6%  $\text{H}_3\text{PO}_4$  and 0.5 ml of 0.8% TBA were added. The tubes were vortexed and then heated to  $90^{\circ}\text{C}$  for 45 min. Upon cooling, 200  $\mu\text{l}$  of 10% sodium dodecyl sulfate were added and extraction with 2 ml of *n*-butanol was carried out by vortex-mixed for 1 min and centrifuged at 2,500 rpm for 5 min. Two hundred microliters of the *n*-butanol layer were transferred into 96 well plate (in triplicate). The determination of TBARS was then performed by Bio-Rad Benchmark Plus<sup>®</sup> Microplate Spectrophotometer (Bio-Rad Laboratories Inc., Hercules, CA, USA).

**Meat TBARS.** Duplicate 5-g samples of raw meat were used to analysis TBARS following the protocol of Leick et al. (2010). Samples, blanks, and standards were read at 530 nm using a Bio-Rad Benchmark Plus<sup>®</sup> Microplate Spectrophotometer.

**Meat Cholesterol.** The cholesterol content was measured on the raw breast and thigh meat as describe by Rowe et al. (1999). The cholesterol was analyzed using gas chromatography (Hewlett-Packard 6890, Agilent Technologies, Santa Clara, CA, USA) with a capillary column (HP 19091A-112, 25 m  $\times$  0.32 mm  $\times$  0.52  $\mu\text{m}$  film thickness) and a flame ionization detector. The temperatures of injector and detector were 260 and

300°C, respectively. Separation was carried out isocratically at 300°C with a helium gas flow rate of 1 ml/min.

**Meat fatty acid content.** The lipids were extracted from approximately 5 g of each muscle samples using 90 ml of chloroform:methanol (2:1, v/v) (Folch et al., 1957). Then, 20 to 25 mg of extracted fat were methylated (Metcalf et al., 1966). The fatty acid methyl esters were analyzed using gas chromatography (Hewlett-Packard 7890A; Agilent Technologies, Santa Clara, CA, USA) with a capillary column (SP 2560, Supelco Inc., Bellefonte, PA, USA, 100 m × 0.25 mm i.d., 0.20-μm film thickness) and a flame ionization detector. The carrier gas was helium at a flow rate of 0.95 ml/min. The temperatures of the injector and detector were 260°C. The initial column temperature was 70°C, which was then raised to 175°C at a rate of 13°C/min, and finally raised to 240°C at a rate of 4°C/min.

#### 4.3.4 Statistical analysis

If the measurements conducted with more than one sample in each experimental unit, the arithmetic mean of each experimental unit ( $n = 4$ ) was calculated before statistical analysis. Analysis of variance was performed by GLM procedure using SAS® University Edition (SAS Institute Inc., Cary, NC, USA) with the following statistical model:  $y_{ij} = \mu + \tau_i + \varepsilon_{ij}$ , where  $y_{ij}$  = the dependent variable,  $\mu$  = the overall mean,  $\tau_i$  = the treatment effect, and  $\varepsilon_{ij}$  = the random residual error.

The data of fatty acid profile and TBARS content of chicken meat over the time of storage were analyzed by PROC MIXED and REPEATED statement of SAS® University Edition as the statistical model:  $y_{ijk} = \mu + \tau_i + t_k + (\tau*t)_{jk} + \varepsilon'_{ijk}$  where  $y_{ijk}$  = observation  $ijk$ ,  $\mu$  = the overall mean,  $\tau_i$  = the effect of treatment  $i$ ,  $t_k$  = the effect of time,  $(\tau*t)_{jk}$  = the effect of interaction between treatment  $i$  and the time  $k$ , and  $\varepsilon'_{ijk}$  = the

random error which is of the first autoregressive structure. Kenward-Roger method was chosen for computing the denominator degrees of freedom for the tests of fixed effects resulting from the model.

In addition, orthogonal polynomial contrasts were used to test the linear and quadratic effects of increasing levels of curcuminoids (0, 20, 40, 60, 80 ppm) in the diet. Differences from the curcuminoids treated groups to the control group were tested by orthogonal contrast while Dunnett's test was employed to examine the effects of each curcuminoids level compared with the controls if necessary. Pairwise treatment differences were determined with the Tukey-Kramer adjustment. Overall differences between treatment means were considered to be significant at  $P < 0.05$ , meanwhile a tendency toward significance was declared at  $0.05 < P \leq 0.1$ . Data are expressed as a mean  $\pm$  SEM, which represents the pooled SEM for the model.

#### **4.3.5 Site and period of the study**

Feeding trial was conducted at University Farm, SUT from 16 October 2015 to 9 January 2016. Chemical analysis was done at Facility Building 3, 9, 10 (F3, F9, F10) of SUT from January to May 2016.

## **4.4 Results and discussion**

### **4.4.1 Growth performance**

The mortality of chickens observed in negative control (2.50%), CUR-60 (3.75%), and CUR-80 (2.50%). Body weight gain and FCR of chicken receiving curcuminoids were not affected compared to the controls (Table 4.3). However, CUR-40 showed the highest FI ( $P < 0.01$ ) compared with the other treatments during the period of day 21-42, occurring from 28 to 42 days of age ( $P < 0.05$ ) but this trend did not reflect in the next phases. When

analyzing the effect of curcuminoids excluded the positive control treatment, there were an influence (cubic,  $P = 0.014$ ) on FI in the final period from the day 64 – 84 and a decreased tendency (linear,  $P = 0.091$ ) of the overall FI. Subsequently, overall FCR tended to decrease (linear,  $P = 0.065$ ) by supplementation of curcuminoids.

The growth performance in the present study was in line with those reported elsewhere (Emadi and Kermanshahi, 2006; Mehala and Moorthy, 2008; Rahmatnejad et al., 2009). The growth promoting effects of curcuminoids was observed on certain conditions such as toxin-contaminating feed (Gowda et al., 2008) or heat stress (Sahin et al., 2012; Swathi et al., 2012). The tendency of FCR in the present study agreed with Rajput et al. (2013b) who demonstrated that supplementing 200 mg/kg pure curcumin improved feed efficiency of broiler chickens.

#### **4.4.2 Carcass composition**

Carcass characteristic of chicken meat is an important measurement for poultry enterprise (Abdulla et al., 2017). The proportions of carcass portions were not significantly affected by different treatment diets, excluded heart (Table 4.4) as reported by Al-Mashhadani (2015) who used turmeric powder in broiler diets. The breast fillet was slightly increase (linear,  $P = 0.037$ ) in curcuminoids treated chickens when E-200 was not accounted in the analysis, agreeing with Wang et al. (2015).

**Table 4.3** Growth performance of Thai crossbred chicken fed experimental diets

Items	Treatment <sup>1</sup>								SEM	P-value	Contrast <sup>2</sup>	
	Control	E-200	CUR-20	CUR-40	CUR-60	CUR-80	L	Q				
BW d21	273.00	271.88	273.25	275.75	271.25	271.75	5.68	0.889	NS	NS		
BW d84	1,793.28	1,777.00	1,752.57	1,802.25	1,790.40	1,765.38	53.61	0.781	NS	NS		
<b>Body weight gain (g/b)</b>												
d21-42	403.13	391.88	399.88	420.88	400.36	386.75	21.86	0.372	NS	NS		
d43-63	558.29	557.13	540.63	546.75	552.66	539.47	35.46	0.954	NS	NS		
d64-84	558.86	556.13	538.82	558.88	563.79	564.22	28.38	0.820	NS	NS		
Overall	1,520.28	1,505.13	1,479.32	1,526.50	1,519.15	1,493.63	52.86	0.792	NS	NS		
<b>Feed intake (g/b)</b>												
d21-42	979.63 <sup>b</sup>	964.38 <sup>b</sup>	993.50 <sup>b</sup>	1071.25 <sup>a</sup>	947.21 <sup>b</sup>	962.25 <sup>b</sup>	33.35	0.001	NS	**		
d43-63	1,640.97	1,591.88	1,560.38	1,606.38	1,555.38	1,602.91	73.38	0.596	NS	NS		
d64-84	1,851.98	1,735.25	1,746.69	1,789.88	1,867.91	1,670.87	96.05	0.076	NS	NS		
Overall	4,472.57	4,291.50	4,300.56	4,467.50	4,370.50	4,236.03	132.77	0.103	†	NS		
<b>Feed conversion ratio</b>												
d21-42	2.43	2.46	2.49	2.55	2.37	2.49	0.12	0.452	NS	NS		
d43-63	2.95	2.86	2.90	2.95	2.81	2.97	0.16	0.729	NS	NS		
d64-84	3.31	3.12	3.25	3.21	3.32	2.97	0.19	0.135	NS	NS		
Overall	2.94	2.85	2.91	2.93	2.88	2.84	0.07	0.309	†	NS		

<sup>1</sup> Treatments consisted of basal diet without antioxidant supplement (Control); basal diet added 200 ppm alpha-tocopherol acetate (E-200); basal diet added turmeric oleoresin to provide 20 ppm (CUR-20), 40 ppm (CUR-40), 60 ppm (CUR-60), and 80 ppm (CUR-80) curcuminoids.

<sup>2</sup> Contrast: orthogonal polynomial contrast where L=linear, Q=Quadratic, and NS, †, \* or \*\* means no significance (P > 0.1), tending significance (0.05 < P ≤ 0.1), significance (P ≤ 0.05), or highly significance (P ≤ 0.01).

<sup>a,b</sup> Means within row carrying no common superscripts are significantly different at P < 0.05.

**Table 4.4** Carcass composition of Thai crossbred chicken fed experimental diets

Item	Treatment <sup>1</sup>						SEM	P-value	Contrast <sup>2</sup>	
	Control	E-200	CUR-20	CUR-40	CUR-60	CUR-80			L	Q
Live weight (g)	1,673.8	1,692.5	1,657.5	1,682.5	1,696.7	1,647.5	54.58	0.789	NS	NS
Dressing (%)	68.61	68.19	68.28	67.73	69.35	68.17	1.19	0.535	NS	NS
<b>% of chilled carcass weight</b>										
Thigh	17.43	16.76	17.05	17.61	17.42	17.42	0.71	0.577	NS	NS
Drumstick	16.28	16.01	16.18	15.86	15.59	15.74	0.56	0.513	†	NS
Wings	13.78	13.19	14.02	12.91	13.59	13.58	0.43	0.053	NS	NS
Breast fillet	16.09	16.82	16.38	17.09	17.49	16.79	0.69	0.694	*	†
Inner fillet	5.54	5.69	5.93	6.05	5.96	5.90	0.35	0.338	NS	***
<b>% of hot carcass weight</b>										
Heart	0.49 <sup>ab</sup>	0.53 <sup>a</sup>	0.46 <sup>b</sup>	0.48 <sup>ab</sup>	0.52 <sup>ab</sup>	0.50 <sup>ab</sup>	0.03	0.045	NS	NS
Spleen	0.25	0.33	0.23	0.21	0.20	0.28	0.08	0.292	NS	NS
Liver	1.89	1.79	1.82	1.84	1.75	1.80	0.11	0.519	NS	NS
Gizzard	2.23	1.98	2.14	2.01	2.15	2.21	0.33	0.845	NS	NS
Abdominal fat	0.41	0.72	0.75	0.66	0.87	0.88	0.30	0.312	*	NS
Organ fat	0.13	0.16	0.08	0.20	0.12	0.07	0.14	0.726	NS	NS

<sup>1</sup> Treatments consisted of basal diet without antioxidant supplement (Control); basal diet added 200 ppm alpha-tocopherol acetate (E-200); basal diet added turmeric oleoresin to provide 20 ppm (CUR-20), 40 ppm (CUR-40), 60 ppm (CUR-60), and 80 ppm (CUR-80) curcuminoids.

<sup>2</sup> Contrast: orthogonal polynomial contrast where L=linear, Q=Quadratic, and NS, †, \* or \*\* means no significance ( $P > 0.1$ ), tending significance ( $0.05 < P \leq 0.1$ ), significance ( $P \leq 0.05$ ), or highly significance ( $P \leq 0.01$ ).

<sup>a-b</sup> Means within row carrying no common superscripts are significantly different at  $P < 0.05$ .



A linear ( $P = 0.039$ ; by polynomial contrast) effect of different levels of curcuminoids on abdominal fat in the present study. Some previous studies exhibited the decrease of abdominal fat of broiler fed turmeric powder (Emadi and Kermanshahi, 2006; Nouzarian et al., 2011). The study of Nouzarian et al. (2011) supplemented turmeric powder (38.28 mg/kg curcumin content) and found a statistical decrease of abdominal fat while the study of Rajput et al. (2013a) which 150 mg/kg pure curcumin did not show the such effect. There were inconsistent results because of the differed form of supplements, and abdominal fat might be influenced by turmeric essential oils or synergetic effect of the essential oil and curcuminoids rather than the pure curcumin itself as reported by Honda et al. (2006).

#### 4.4.3 Meat quality

Curcuminoids are yellow colorant (Chattopadhyay et al., 2008) and similar to lutein, greenish-yellow (Mortensen, 2006). Therefore, supplementation curcuminoids was expected to improve yellowness of chicken skin because consumers prefer such trait in certain region of the world. The yellowness ( $b^*$ ) of the breast and thigh skin linearly ( $P = 0.016$  and  $P = 0.023$ ) increased by increasing level of dietary curcuminoids in the present study (Table 4.5). However, the  $L^*$  and  $a^*$  values of skin color showed no effect. The effect of curcuminoids in the present study on skin color similar to the effect of marigold flower extract (lutein) (Wang et al., 2017). In this study, there was no significant effect on meat color (Table 4.6) of the breast and thigh meat. The redness of thigh meat reduced (linear,  $P = 0.004$ ) by increasing level of curcuminoids in the diets.

Shifts of meat quality were detected by adding curcuminoids from curcumin removed turmeric oleoresin (Table 4.7). The pH value of the breast meat at 45 min exhibited the strong effect of the treatment, the highest value was in the control group

compared to the lower values ( $P < 0.001$ ) in the curcuminoids groups; indeed, pH of curcuminoids group was similar to E-200. At 24 h, the pH value showed a numbered decrease of most of the treatments, except CUR-40 and CUR-80. The pH value had almost no change in the aging process because of an increase of radical oxygen species, oxidation stress, and other factors (Beauchercq et al., 2016).

Drip loss of the breast meat showed no statistical difference; although the value obtained in the present study was higher than in the report of Maliwan et al. (2017) who fed the same chicken breed with standard diets. Drip loss of the thigh meat tended toward a decrease ( $P = 0.06$ ), showing the lower loss in antioxidant-treated groups. Increasing levels of curcuminoids exhibited significant (linear,  $P = 0.035$ ) effect on the drip loss of the thigh meat but no influence the breast meat. The higher fat content of thigh might result in higher amount of accumulated curcuminoids or its derivatives into the muscle. The drip loss results were in agreement with Wang et al. (2015) because the oxidative defense system of muscle has a direct effect on water holding capacity (Huff-Lonergan and Lonergan, 2005). Curcuminoids might have a potential to inhibit oxidation in membrane lipid as stated in the study of Menon and Sudheer (2007), resulting in an increase of the integrity of muscle cell membrane; therefore, entrapped water of the meat can be retained.

There was significantly different ( $P < 0.01$ ) in cooking loss of the thigh meat with the highest value in E-200, and the effect of curcuminoids treated group was similar to the control (by orthogonal contrast analysis). The shear force value of the thigh meat showed the significant difference ( $P < 0.05$ ) from the control to the curcuminoids group (by contrast analysis). The results of the present study partly agreed with Zhang et al. (2015) who supplemented 50, 100 and 200 mg/kg curcumin in broiler diets and revealed

that there were a quadratic decrease of drip loss at 48 h but pH (45 min and 24 h), yellowness (b\*), drip loss at 24 h, and cooking loss of the breast meat were not influenced. Attia et al. (2017) also found no significant difference in pH, tenderness and water-holding capacity of meat of broilers fed turmeric up to 2 g/kg in comparison with oxytetracyclines and mannan oligosaccharides.

**Table 4.5** Skin color value of the breast and thigh of Thai crossbred chickens

Treatment <sup>1</sup>	Breast skin			Thigh skin		
	L*	a*	b*	L*	a*	b*
Control	69.92	4.62	10.01	70.83	3.07	6.19
E-200	70.54	4.27	10.18	69.95	3.62	7.56
CUR-20	68.54	4.46	10.00	70.75	3.55	7.30
CUR-40	68.87	4.33	10.71	70.52	3.58	8.12
CUR-60	68.36	4.98	11.56	70.44	3.88	8.52
CUR-80	69.42	4.60	11.59	70.12	3.87	8.76
SEM	1.15	0.65	1.17	1.95	0.63	1.58
P-value	0.104	0.682	0.206	0.985	0.51	0.268
Contrast <sup>2</sup> for the effect of different levels of curcuminoids						
Linear	NS	NS	*	NS	†	*
Quadratic	†	NS	NS	NS	NS	NS

<sup>1</sup> Treatments consisted of basal diet without antioxidant supplement (Control); basal diet added 200 ppm alpha-tocopherol acetate (E-200); basal diet added turmeric oleoresin to provide 20 ppm (CUR-20), 40 ppm (CUR-40), 60 ppm (CUR-60), and 80 ppm (CUR-80) curcuminoids.

<sup>2</sup> Contrast: orthogonal polynomial contrast where L=linear, Q=Quadratic, and NS, †, \* or \*\* means no significance ( $P > 0.1$ ), tending significance ( $0.05 < P \leq 0.1$ ), significance ( $P \leq 0.05$ ), or highly significance ( $P \leq 0.01$ ).

**Table 4.6** Color value of the breast and thigh meat of Thai crossbred chickens

Treatment <sup>1</sup>	Breast meat			Thigh meat		
	L*	a*	b*	L*	a*	b*
Control	60.25	9.09	17.61	57.32	11.60	17.22
E-200	61.52	8.58	17.58	58.78	10.67	17.03
CUR-20	60.86	8.80	17.84	57.50	10.67	16.59
CUR-40	60.67	8.98	18.15	58.32	10.62	16.83
CUR-60	60.13	9.19	17.82	58.17	10.14	16.77
CUR-80	60.24	8.38	17.25	58.05	10.07	15.88
SEM	1.46	0.59	0.95	1.88	0.67	1.15
P-value	0.757	0.398	0.839	0.890	0.059	0.656
Contrast <sup>2</sup> for the effect of different levels of curcuminoids						
Linear	NS	NS	NS	NS	**	NS
Quadratic	NS	NS	NS	NS	NS	NS

<sup>1</sup> Treatments consisted of basal diet without antioxidant supplement (Control); basal diet added 200 ppm alpha-tocopherol acetate (E-200); basal diet added turmeric oleoresin to provide 20 ppm (CUR-20), 40 ppm (CUR-40), 60 ppm (CUR-60), and 80 ppm (CUR-80) curcuminoids.

<sup>2</sup> Contrast: orthogonal polynomial contrast where L=linear, Q=Quadratic, and NS, †, \* or \*\* means no significance ( $P > 0.1$ ), tending significance ( $0.05 < P \leq 0.1$ ), significance ( $P \leq 0.05$ ), or highly significance ( $P \leq 0.01$ ).

**Table 4.7** Effects of supplementing antioxidants on meat quality

Item	Treatment <sup>1</sup>						SEM	P-value	Contrast <sup>2</sup>	
	Control	E-200	CUR-20	CUR-40	CUR-60	CUR-80			L	Q
<i>Breast</i>										
pH 45 min	5.84 <sup>a</sup>	5.62 <sup>ab</sup>	5.49 <sup>b</sup>	5.46 <sup>b</sup>	5.58 <sup>b</sup>	5.54 <sup>b</sup>	0.10	0.001	**	***
pH 24 h	5.46 <sup>b</sup>	5.48 <sup>b</sup>	5.47 <sup>b</sup>	5.57 <sup>ab</sup>	5.49 <sup>b</sup>	5.61 <sup>a</sup>	0.05	0.003	**	NS
Drip loss (%)	8.97	8.65	7.41	7.33	8.55	8.17	1.33	0.428	NS	NS
Cooking loss (%)	16.96	17.25	16.71	16.44	16.75	16.68	1.30	0.966	NS	NS
Shear force <sup>2</sup>	3.38	2.77	2.59	2.83	3.40	3.14	0.56	0.244	NS	†
<i>Thigh</i>										
Drip loss (%)	6.14 <sup>a</sup>	5.04 <sup>ab</sup>	4.45 <sup>ab</sup>	4.26 <sup>b</sup>	4.66 <sup>ab</sup>	4.34 <sup>b</sup>	0.87	0.060*	*	†
Cooking loss (%)	15.27 <sup>b</sup>	18.93 <sup>a</sup>	14.35 <sup>b</sup>	17.25 <sup>ab</sup>	15.16 <sup>b</sup>	16.70 <sup>ab</sup>	1.47	0.004	†	NS
Shear force <sup>3</sup>	2.27	2.72	2.55	2.56	2.66	3.03	0.35	0.122	*	NS

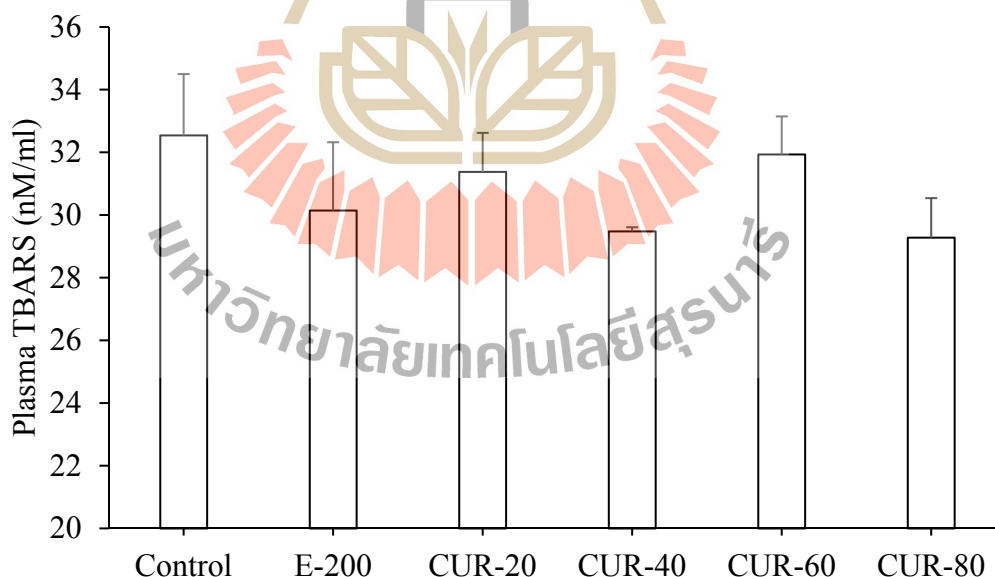
<sup>1</sup> Treatments consisted of basal diet without antioxidant supplement (Control); basal diet added 200 ppm alpha-tocopherol acetate (E-200); basal diet added turmeric oleoresin to provide 20 ppm (CUR-20), 40 ppm (CUR-40), 60 ppm (CUR-60), and 80 ppm (CUR-80) curcuminoids.

<sup>3</sup> WBS: Warner-Bratzler shear force expressed as kgf/0.5 cm<sup>2</sup>; \* The difference was test with alpha = 0.1

<sup>a-b</sup> Means within row carrying no common superscripts are significantly different at  $P < 0.05$ .

#### 4.4.4 TBARS value of plasma and meat

Plasma TBARS showed no statistical difference (Figure 4.1) when basal ration supplemented with either vitamin E or different levels of curcuminoids. The TBARS value in plasma of the present study was in contrast to the study of Akhavan-Salamat and Ghasemi (2016) which dietary 0.2% turmeric powder in heat stress condition and of Hosseini-Vashan et al. (2012) which 0.4 and 0.8% turmeric powder in normal condition from d 0-28 and in heat stress condition afterward. In particular, E-200, CUR-40, and CUR-80 exhibited the lower value of plasma TBARS compared to control by 7.37, 9.41, and 10.03%. To some extent, the plasma TBARS results accorded with the pattern of drip loss of the breast and thigh meat, implying the antioxidant effect of dietary curcuminoids in the chicken.



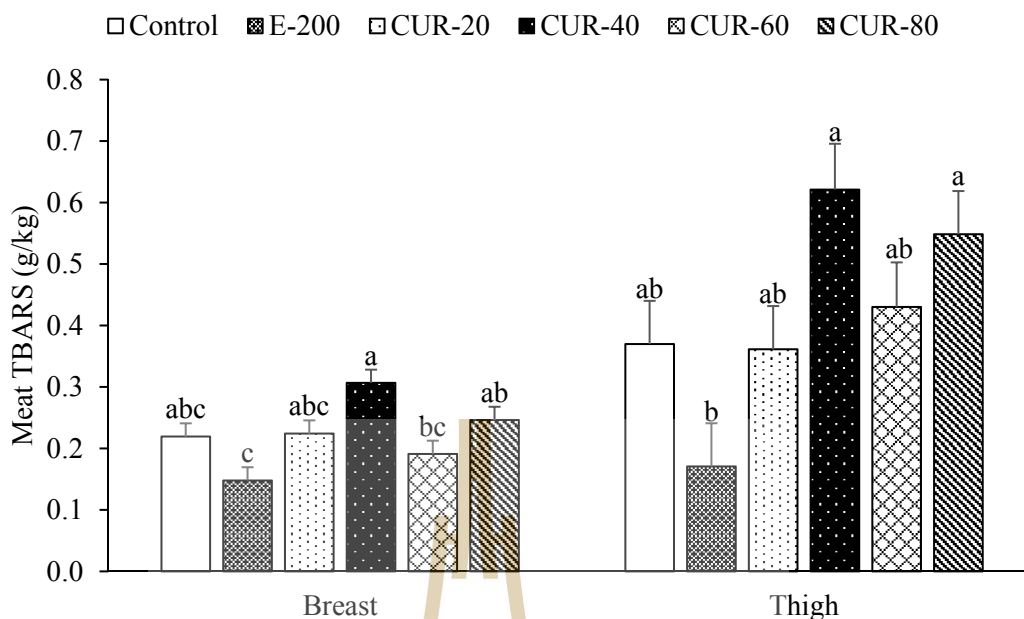
**Figure 4.1** Plasma TBARS (Mean  $\pm$  SEM; n=4/treatment) of Thai crossbred chickens

The TBARS concentration of the chicken meat showed a noticeable difference ( $P < 0.01$ ) (Figure 4.2). TBARS of the breast and thigh shared the same trend, exhibiting the lowest value in E-200. In the present study, the TBARS value was obtained from

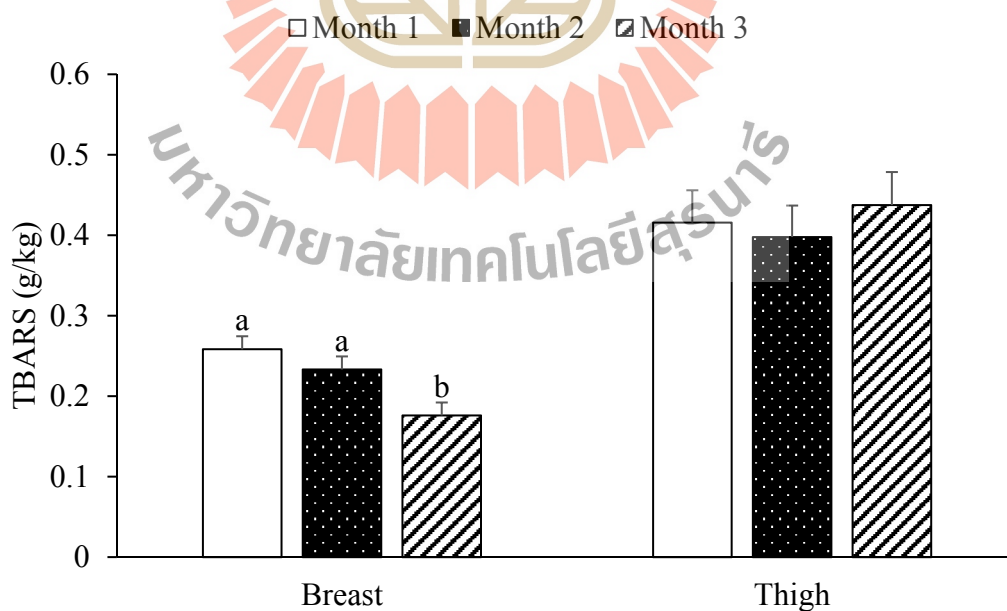
repeated measurement over the three-month period (from different carcasses). There was no interaction between dietary treatment and time, which meant that dietary curcuminoids in the present study could not preserve the breast and thigh meat from oxidation over the time of frozen storage; indeed, the difference of curcuminoids group from the negative control was no statistical significance (by orthogonal contrast).

The increasing curcuminoids supplementation caused no dose-dependence response, according to those reported recently (Daneshyar, 2012; Zhang et al., 2015). There was an opposite pattern of the TBARS value of meat to that of plasma. The lower plasma TBARS concentration found in CUR-40 and CUR-80 as compared with CUR-20 and CUR-60 but the TBARS value of the breast and thigh meat was in reverse order. On certain condition, curcumin express pro-oxidant properties (Galati et al., 2002; Kelly et al., 2001) depending on its concentration and the presence of metal ions but the mechanism *in vivo* is not clearly determined (Joe et al., 2004). In the present study, it is thought that curcuminoids might switch from anti-oxidative activity in the living birds to become a pro-oxidative agent during post-mortem aging of the meat.

The change of TBARS value over the time of frozen storage (-20°C) was showed in Figure 4.3. There was a statistical difference ( $P < 0.05$ ) in TBARS of the breast meat over three months while that of the thigh meat showed no significant influence. The pattern of TBARS of the thigh meat agreed with Botsoglou et al. (2003) who found that MDA values, measuring in breast and thigh muscle of chicken fed 6.06% soybean oil, exhibited a lag phase by month 6, followed by a more rapid increase at longer storage times (1, 3, 6 or 9 months of frozen storage). Flavia et al. (2014) also found the TBARS value of poultry fat did not significantly differ from day 1 until day 90 of frozen storage (-18°C).



**Figure 4.2** Effects of treatment on TBARS value (Mean  $\pm$  SEM; n=4/treatment) of the meat kept over three months ( $-20^{\circ}\text{C}$ ). The letters, a-c, mean significant difference ( $P < 0.05$ ) between the treatments within each type of meat.



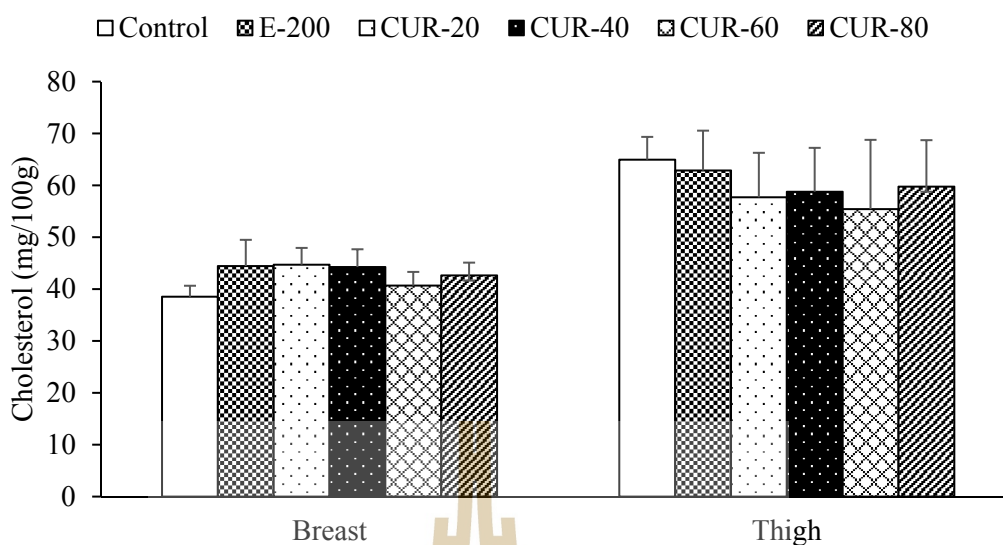
**Figure 4.3** The change of TBARS value (Mean  $\pm$  SEM; n=4/treatment) of the frozen meat over three months



To evaluate the extent of lipid oxidation, TBARS value is often employed; however, lipid oxidation is a dynamic process, and the meat TBARS curve is likely to increase over the storage period, reach a peak, and then go down (Larick and Parker, 2001). In the present study, a steady decrease of the TBARS value of breast meat suggested that the greater breakdown of hydroperoxides probably took place before the first measurement. The variation influence of antioxidant between the breast and thigh meat agreed with Delles et al. (2016) who suggested that dietary antioxidants can protect broiler meat from dietary oxidized oil, and this effect in the thigh was more pronounced than breast muscle. In fact, the total PUFA proportion, especially n-3 PUFA, in the breast meat (14.88% of n-3 PUFA) was higher than the thigh meat (8.44% of n-3 PUFA) in the present study, and probably antioxidant such as vitamin E incorporated more amount in thigh meat as reported by Botsoglou et al. (2003).

#### **4.4.5 Cholesterol content of meat**

The cholesterol content in the breast and thigh meat was no significant difference between treatments (Figure 4.4). Konjufca et al. (1997) also found no effect of garlic powder on breast muscle cholesterol of broiler (21 day-old) although Zdanowska-Sasiadek et al. (2016) reported a 4.5% decrease of cholesterol in either breast or thigh meat by supplementing 200 mg/kg vitamin E. In fact, Dinh et al. (2011) suggested that unless there are pronounced changes in muscle structure and composition, the cholesterol content is unlikely to be affected. The concentration of cholesterol in the breast meat ranged from 38.54 to 44.71 mg/100 g and the thigh meat from 55.42 to 64.96 mg/100 g meat. In the present study, cholesterol content in the breast and thigh meat were respective in between that of Korat chickens (37.88 and 40.13 mg/100 g) and commercially broilers (79.44 and 82.32 mg/100 g) as reported by Pongjanla et al. (2014).



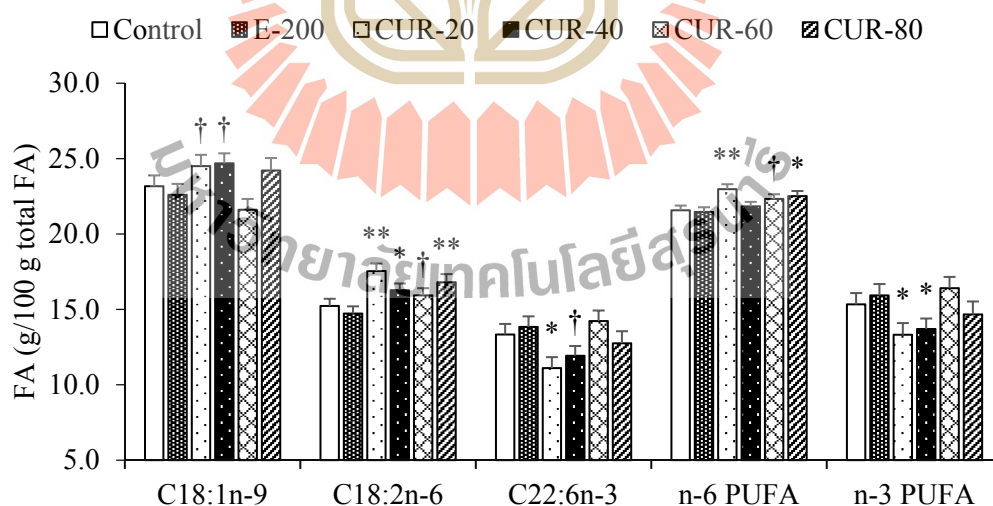
**Figure 4.4** Cholesterol content of the chicken meat (Mean  $\pm$  SEM; n=4/treatment)

#### 4.4.6 Fatty acid profile of the breast and thigh meat

The curcuminoids supplementation showed remarkable effects on certain fatty acids of the breast meat (Table 4.8). The Dunnett's test was conducted to compare the difference of curcuminoids-treated groups from either control or E-200 as can be seen partly in Figure 4.5. The CUR-60 brought out the highest proportion of favorable C22:6n-3 and total n-3 PUFA although there was no significant improvement. The dietary CUR-80 influenced on C22:6n-3 and total n-3 PUFA similar to CUR-60. CUR-20 led to the highest percent of C18:2n-6 and total n-6 PUFA, strongly differed from E-200 ( $P < 0.01$ ). CUR-40 had a tendency toward an increase of C18:1n-9 ( $P < 0.10$ ) while its effect on aforementioned FA was in between CUR-20 and CUR-60. A study of Daneshyar et al. (2011) determined 0.75% turmeric powder caused a significant decrease of total saturated fatty acid (SFA) of thigh meat. Curcumin inhibited microsomal  $\Delta 5$  and  $\Delta 6$  desaturases of rat liver (Shimizu et al., 1992); therefore, curcumin is likely to involve in the regulation of biosynthesis of PUFA in chicken. In fact, the C18:2n-6, which constituted proximately 25% in the diets, accumulated in the

breast meat of curcuminoids group was significant higher ( $P < 0.05$ ) than of E-200. Supplementing 20 mg/kg curcuminoids exhibited the more down-regulation effect of FA synthesis than other curcuminoids-added diets by inhibiting longer chain PUFA synthesis in breast meat, expressing the highest content of C18:2n-6 and lowest percent of C22:6n-3 in comparison with either control or vitamin E. The treatments, namely CUR-40 and CUR-60, had C18:2n-6 and C22:6n-3 proportion similar to control. The CUR-60 can be applied because of the best response in breast DHA content.

The almost fatty acids of the thigh meat were displayed in Table 4.9, showing no meaningful change in the proportion of all FA caused by either vitamin E or curcuminoids. The fatty acids of the thigh meat of the present study were in agreement with the report of Daneshyar et al. (2011). To the best of our knowledge, the present study provides the first observation about meat fatty acids content of Thai crossbred chickens fed a combination of curcumin removed turmeric oleoresin and tuna oil.

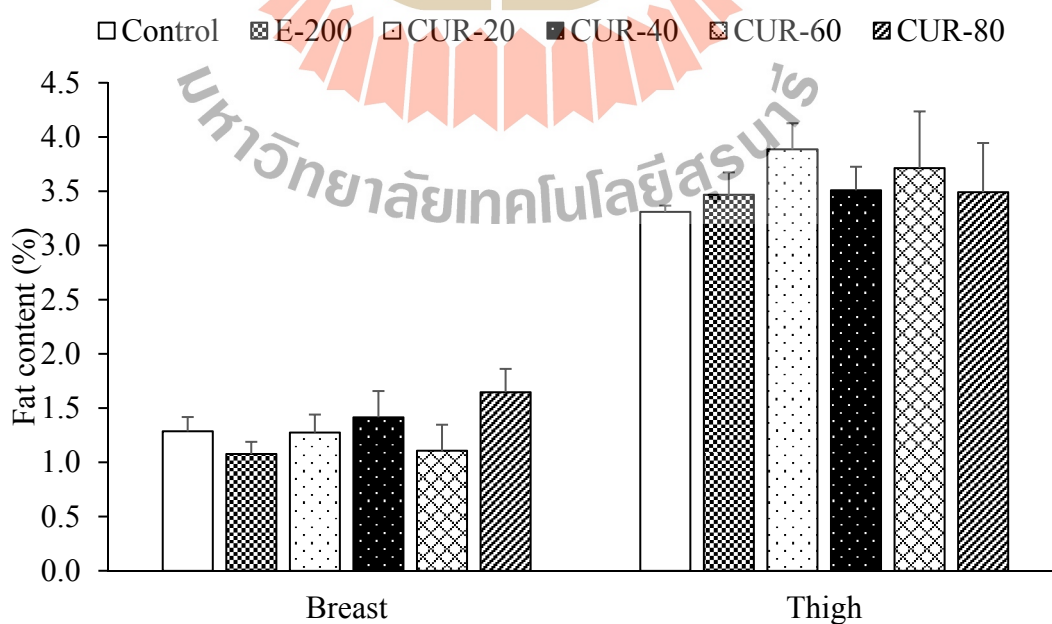


**Figure 4.5** The remarked fatty acids in the breast meat were influenced by dietary curcuminoids.

The label expressed the difference from the treatments to E-200 within each type of fatty acid, where where “†”, “\*” or “\*\*” means no significance ( $P > 0.1$ ), tending significance ( $0.05 < P \leq 0.1$ ), significance ( $P \leq 0.05$ ), or highly significance ( $P \leq 0.01$ ) by Dunnett’s Test.

Vitamin E affected the FA content of chicken meat was reported elsewhere (Coetzee and Hoffman, 2001; Zdanowska-Sasiadek et al., 2016); however, it showed no effect in the present study because of the probably higher level of dietary long-chain n-3 PUFA. Coetzee and Hoffman (2001) found the significant influence of vitamin E on SFA (increase) and total PUFA (decrease) at the dietary level 120 mg/kg but either lower or higher its supplement than 120 mg/kg showed no effect. A recently study of Zdanowska-Sasiadek et al. (2016) showed 200 mg/kg vitamin E led to a significant increase of C18:3n-3 and total C20-22 n-3 in leg muscle.

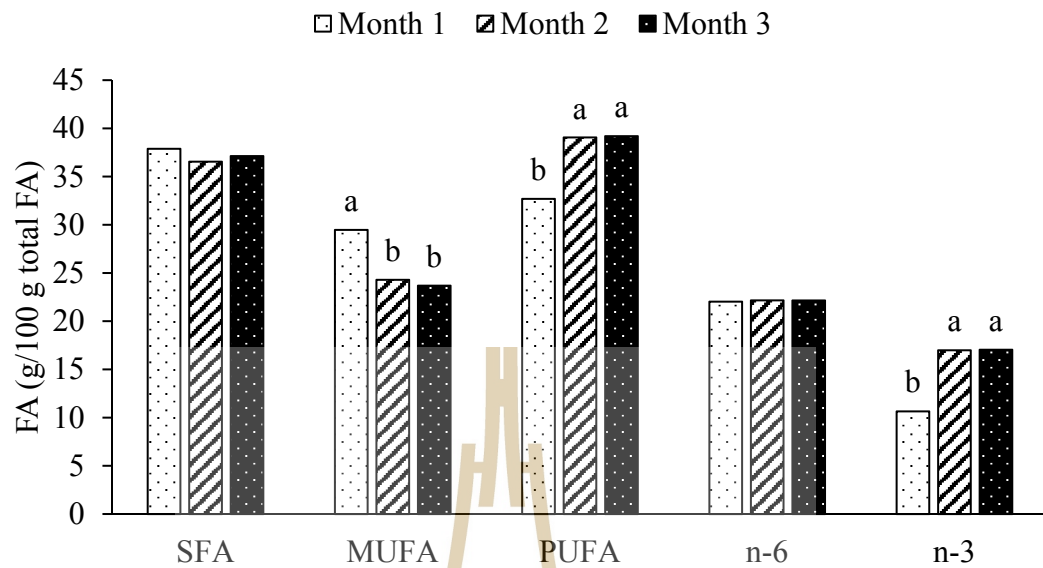
The combination of curcumin and long-chain n-3 PUFA might have synergetic effects, then caused a decrease of fat accumulation in tissue (Forman et al., 1997) and the effect of curcumin or long-chain n-3 PUFA on fatty acid metabolism was reported recently (Fan et al., 2016; Kang et al., 2013; Thota et al., 2016). However, dietary curcuminoids did not significantly affect intramuscular fat in the breast and thigh meat in the present study (Figure 4.6).



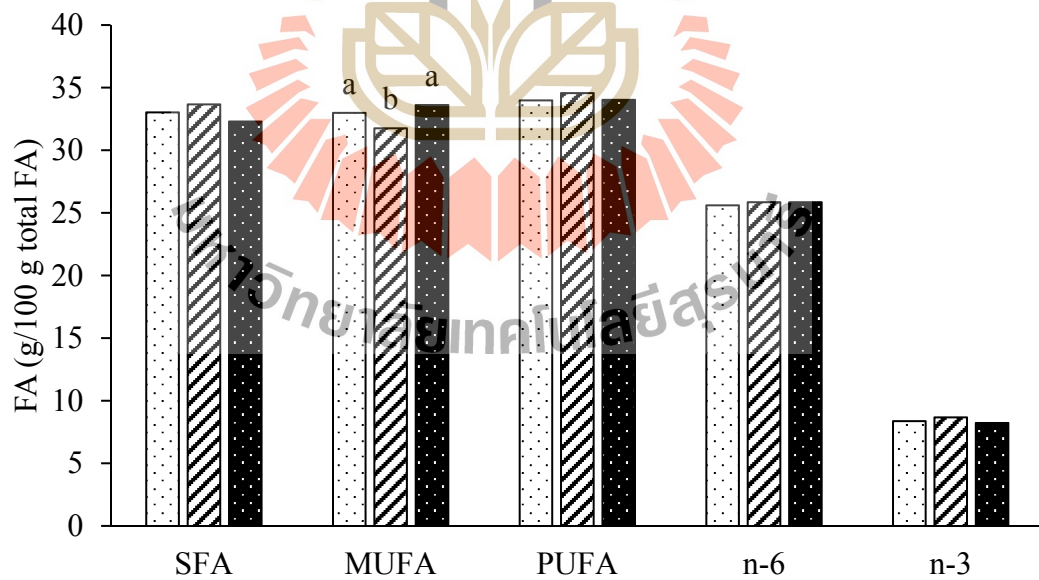
**Figure 4.6** Total lipid content in the breast and thigh meat

As reported in TBARS of the breast and thigh meat, the individual treatment exhibited no different impact on FA proportion during the period of frozen storage (Figure 4.7). In the breast meat, MUFA decreased ( $P < 0.0001$ ) while PUFA, especially n-3 PUFA, increased ( $P < 0.0001$ ) over three-month storage ( $-20^{\circ}\text{C}$ ). The change of PUFA of the thigh meat did not exhibit. The MUFA proportion of the thigh meat decreased in the second month and recovered in the third month but the major MUFA composition, C18:1n-9, remained virtually unchanged. The report of Horbanczuk et al. (2015) showed the meat DHA and total PUFA content of ostrich fed 4 or 8% linseed oil (especially from 61 to 120 days of storage) decreased; however, storage ( $-20^{\circ}\text{C}$ ) did not influence the fatty acid profile of ostrich meat up to 60 days, and different muscle had differed response. A study on stored different animal fat for 210 days at  $-18^{\circ}\text{C}$  revealed no significant change in fatty acids or total fatty acid content of poultry fat (Flavia et al., 2014). Therefore, it was hypothesized that the PUFA will be lost during storage because of lipid auto-oxidation. However, the present study found an increase of n-3 PUFA while n-6 PUFA was nearly the same at day 90 of frozen storage. The higher proportion of EPA and DHA of frozen breast meat agreed with Zymon et al. (2007) who found a tendency towards a higher content of EPA and DHA of frozen veal ( $-18^{\circ}\text{C}$ , 3 months). A comparison study between the “Korat meat chickens” (similar breed to the present study) and commercial broilers revealed that the PUFA content (mg/g) of breast and thigh meat of “Korat meat chickens” increased while that of broilers decreased after eight-month storage (Yongsawatdigul and Molee, 2016). It may suggest that there was certain change during the frozen storage rather than autooxidation and hydrolysis.

A



B



**Figure 4.7** Change of fatty acids profile of the breast (A) and thigh meat (B) over time of freezing storage ( $-20^{\circ}\text{C}$ ). The letters, a-b, showed the significant difference within each fatty acid ( $P < 0.05$ ).

**Table 4.8** Fatty acid profile (g/100 g total FA) of the breast meat of the chickens

Items	Treatment <sup>1</sup>						SEM	P-value		
	Control	E-200	CUR-20	CUR-40	CUR-60	CUR-80		Diet	Time	D*T
C14:0	0.88	0.93	1.14	1.03	0.83	0.95	0.09	0.282	0.003	0.290
C16:0	23.91	23.83	23.28	24.01	23.80	23.15	1.29	0.547	<0.0001	0.569
C18:0	11.70	11.86	11.14	11.43	11.36	11.12	1.64	0.753	<0.0001	0.124
C20:0	0.31	0.28	0.25	0.23	0.41	0.23	0.08	0.699	<0.0001	0.402
C22:0	0.51	0.49	0.40	0.41	0.62	0.44	0.08	0.250	0.000	0.951
C16:1	1.18	1.14	1.32	1.51	1.24	1.47	0.31	0.540	0.003	0.043
C17:1	0.90	1.00	0.86	0.67	0.88	0.63	0.33	0.596	<0.0001	0.795
C18:1n-9	23.15 <sup>ab</sup>	22.56 <sup>ab</sup>	24.47 <sup>a</sup>	24.68 <sup>a</sup>	21.57 <sup>b</sup>	24.33 <sup>a</sup>	6.56	0.059*	<0.0001	0.589
C20:1	0.07	0.05	0.27	0.12	0.16	0.21	0.04	0.302	0.001	0.137
C18:2n-6	15.23 <sup>cd</sup>	14.73 <sup>d</sup>	17.53 <sup>a</sup>	16.28 <sup>abc</sup>	15.92 <sup>bcd</sup>	16.87 <sup>ab</sup>	2.69	0.010	<0.0001	0.155
C20:4n-6	5.93	6.63	5.29	5.48	6.24	5.47	1.51	0.212	<0.0001	0.424
C18:3n-3	0.37	0.33	0.37	0.25	0.33	0.38	0.06	0.752	<0.0001	0.960
C20:5n-3	1.63	1.77	1.81	1.52	1.83	1.65	0.15	0.571	<0.0.001	0.424
C22:6n-3	13.34 <sup>ab</sup>	13.89 <sup>ab</sup>	11.17 <sup>c</sup>	11.93 <sup>bc</sup>	14.27 <sup>a</sup>	12.42 <sup>abc</sup>	7.31	0.044	<0.0001	0.739
SFA	37.74	37.70	36.63	37.43	37.35	36.29	3.44	0.498	0.065	0.148
MUFA	25.36	24.86	27.04	27.04	23.90	26.76	8.85	0.106	<0.0001	0.305
PUFA	36.90	37.44	36.33	35.54	38.76	36.95	6.26	0.141	<0.0001	0.872
Total n-6	21.56 <sup>bc</sup>	21.46 <sup>c</sup>	22.98 <sup>a</sup>	21.84 <sup>bc</sup>	22.32 <sup>abc</sup>	22.49 <sup>ab</sup>	0.97	0.021	0.884	0.153
Total n-3	15.34 <sup>ab</sup>	15.98 <sup>a</sup>	13.35 <sup>b</sup>	13.70 <sup>b</sup>	16.43 <sup>a</sup>	14.45 <sup>ab</sup>	7.55	0.061*	<0.0001	0.736
n-6/n-3 ratio	1.51	1.42	2.01	1.77	1.46	1.77	0.33	0.262	<0.0001	0.727

<sup>1</sup> Treatments consisted of basal diet without antioxidant supplement (Control); basal diet added 200 ppm alpha-tocopherol acetate (E-200); basal diet added turmeric oleoresin to provide 20 ppm (CUR-20), 40 ppm (CUR-40), 60 ppm (CUR-60), and 80 ppm (CUR-80) curcuminoids; <sup>ab</sup> Means within row carrying no common superscripts are significantly different at  $P < 0.05$ . \* The difference was tested with alpha = 0.10; Data of some detectable fatty  $\leq 0.4\%$  for all treatments was not showed (C15:0, C23:0, C20:2n-6)

**Table 4.9** Fatty acid profile (g/100 g total FA) of thigh meat of the chickens

Item	Treatment <sup>1</sup>								SEM	P-value		
	Control	E-200	CUR-20	CUR-40	CUR-60	CUR-80	Diet	Time		D*T		
C14:0	1.42	1.47	1.50	1.49	1.49	1.45	0.02	0.926	0.089	0.638		
C16:0	22.17	21.96	21.56	21.86	21.74	22.15	2.60	0.983	0.480	0.566		
C18:0	8.90	9.70	8.28	8.95	8.75	8.15	1.29	0.302	<0.001	0.450		
C22:0	0.22	0.29	0.24	0.25	0.22	0.25	0.90	0.832	0.011	0.119		
C16:1	3.16	2.63	2.87	3.08	2.97	3.57	0.44	0.354	0.001	0.135		
C17:1	0.47	0.71	0.51	0.49	0.30	0.34	0.13	0.180	0.008	0.061		
C18:1n-9	28.31	27.45	28.61	28.76	28.34	29.86	2.24	0.088	0.091	0.637		
C20:1	0.53	0.43	0.54	0.47	0.47	0.46	0.01	0.222	<0.0001	0.399		
C18:2n-6	22.35	22.37	23.56	22.57	23.33	22.30	1.84	0.448	0.011	0.192		
C18:3n-6	0.10	0.14	0.11	0.08	0.06	0.09	0.01	0.536	<0.0001	0.417		
C20:4n-6	2.75	2.95	2.47	2.54	2.63	2.20	0.35	0.404	0.001	0.557		
C18:3n-3	0.35	0.36	0.34	0.37	0.35	0.36	0.04	0.991	<0.0001	0.999		
C20:5n-3	1.37	1.30	1.47	1.34	1.40	1.34	0.05	0.687	0.266	0.755		
C22:6n-3	6.70	6.95	6.83	6.69	6.60	6.36	1.63	0.954	0.995	0.549		
SFA	33.41	34.08	32.28	33.17	32.87	32.72	4.79	0.788	0.057	0.573		
MUFA	32.69	31.41	32.77	33.02	32.30	34.45	4.03	0.247	0.001	0.656		
PUFA	33.90	34.51	34.95	33.81	34.83	32.83	5.26	0.455	0.568	0.521		
Total n-6	25.41	25.90	26.30	25.40	26.46	24.77	2.46	0.419	0.801	0.441		
Total n-3	8.52	8.61	8.65	8.41	8.37	8.06	1.74	0.929	0.528	0.437		
n-6/n-3 ratio	3.03	3.08	3.11	3.06	3.20	3.16	0.22	0.971	0.602	0.467		

<sup>1</sup> Treatments consisted of basal diet without antioxidant supplement (Control); basal diet added 200 ppm alpha-tocopherol acetate (E-200); basal diet added turmeric oleoresin to provide 20 ppm (CUR-20), 40 ppm (CUR-40), 60 ppm (CUR-60), and 80 ppm (CUR-80) curcuminoids.

Data of some detectable fatty  $\leq 0.50\%$  for all treatments was not showed (C15:0, C24:1, C20:2n-6).



#### 4.5 Conclusions and suggestions

The curcuminoids exhibited the antioxidant effect but its effect did not depend on dose supply. Either vitamin E or curcuminoids could not improve the n-3 PUFA in the meat of Thai crossbred chickens. The auto-oxidation during three-month storage found in the breast but not in the thigh; therefore, thigh meat can be stored for 90 days without adverse effects. Further studies are suggested to enhance the bioavailability of the oleoresin and/or deeply focus on molecular level to determine the activity of curcuminoids on antioxidative defense system and on n-3 PUFA accumulation by the combination of curcuminoids and n-3 PUFA in the chicken diet.

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# CHAPTER V

## EFFECTS OF DIETARY ANTIOXIDANTS AND COOKING ON OXIDATION AND FATTY ACIDS CONTENT OF ENRICHED MEAT

### 5.1 Abstract

The present study conducted to determine the effects of dietary antioxidants on oxidative susceptibility and fatty acid content of cooked meat, and find out an optimum heating method which preserves more n-3 PUFA when cooking enriched chicken meat. The experimental design was a factorial arrangement in CRD with two factors, sources of meat (the chickens fed either 200 ppm vitamin E or 80 ppm curcuminoids) and heating method (boiling, steaming, deep-fat frying, or roasting), eight treatments, and four replicates. The source of meat showed no influence on cooking yield and fatty acid content of ready-to-eat chicken meat. Meat from chickens received dietary curcuminoids expressed higher TBARS value than those of birds fed vitamin E ( $P < 0.05$ ). Boiling yielded more weight after cooking than frying, roasting, and steaming. The highest TBARS value ( $P < 0.05$ ) was found in roasted meat of either breast or thigh at the first and fourth day storage (4°C) compared to other heating treatments. When cooking without culinary fat, the fatty acid profile showed a noticeable loss of n-3 PUFA of the breast and thigh meat. Roasting in an oven showed less effect on FA profile of breast meat, having a little loss of n-3 PUFA but caused a remarkable increase of total PUFA of the thigh, in particular, n-6 PUFA. Roasting, frying or steaming demonstrated

the similar content of DHA and total n-3 PUFA of the breast meat and better than boiling. There was no effect of boiling, frying, roasting, or steaming on the n-6/n-3 ratio of the thigh meat. The fishy odor was strongly detected in moist heating. Dry heating recorded higher umami taste in compared with moist heating method. In conclusion, roasting and steaming can be the good choices for cooking n-3 PUFA enriched breast meat. Although there was more favorable amount of EPA and DHA found in steamed thigh meat, other heating such as boiling, frying, or roasting was an optional method without influence on n-3 PUFA of thigh meat.

## 5.2 Introduction

The end product of present project was slow-growing chicken meat which enriched n-3 PUFA content by dietary tuna oil and either vitamin E or curcuminoids as dietary antioxidants. Because chicken meat is consumed after cooking, the nutrient content of raw meat does not fully provide convincing information. The effects of cooking methods on the change in values of nutrients of chicken meat can vary (Al-Khalifa and Dawood, 1993). It is hypothesized that the change of fatty acid (FA) during heat treatment may relate to the level of antioxidant content in the salmon fish (Gladyshev et al., 2006). The level of vitamin E supplementation (0, 100 to 200 ppm) reduced lipid oxidation of cooked thigh meat (Cortinas et al., 2005). Studies of supplementation n-3 PUFA in chicken diet have predominantly reported the fatty acid composition of ready-to-cook meat (Kartikasari et al., 2012; López-Ferrer et al., 2001; Shin et al., 2012), only some data about FA of cooked chicken meat (Cortinas et al., 2004; Gibbs et al., 2010). The food traceability in some reports was sometimes ignored. Therefore, the cooking study following feeding trial could bring more valuable information for marketing the n-3 PUFA enriched

chicken meat. The aim of the present trial was determination the effects of dietary antioxidant on oxidative susceptibility and fatty acid content of cooked meat, and examination four heating methods to preserve more n-3 PUFA when cooking enriched chicken meat.

## **5.3 Materials and methods**

### **5.3.1 Experimental design**

The raw meat used in the present study was taken from two groups of the Thai crossbred chickens of the previous study. They were fed different diets which supplementation either 200 mg/kg vitamin E or 80 mg/kg curcuminoids and 4% tuna oil, respectively labeled as TE and TC. The experimental model was a factorial arrangement of completely randomized design. Two factors were meat sources (TE and TC) and heating methods [boiling (B), steaming (S), roasting (R), and deep-fat frying (F)]. There will be 8 combination treatments with 4 replicates, namely BTE, STE, RTE, FTE, BTC, STC, RTC, and FTC. Total 32 boneless breasts ( $77.50 \pm 13.03$  g) and 32 thigh meat ( $72.17 \pm 15.24$  g) were cooked entirely (whole intact muscle).

### **5.3.2 Method of cooking**

The meat was not marinated with any food additive. Common heating method was used such as boiling, steaming, deep fat frying in soybean oil, and roasting similar to household practice. Soybean oil was chosen because it is the popular cooking oil. Sample meat was cooked until the core temperature of the meat reaches  $80^{\circ}\text{C}$ . Before conducting with the experimental samples, a small trial was carried out to determine suitable time and temperature of heating with available equipment. The results were showed in Table 5.1.

**Table 5.1** The cooking condition of the present study

Methods	Temperature	Time	Note
Boiling	100°C	10 min	
Deep-frying	160-170°C	5 min	
Roasting	180°C	20 min	Turn the meat pad upside down after 10 min
Steaming	95°C	10 min	

### 5.3.3 Sampling, measurements, and analytical method

Total 32 chickens (two males and two females per pen) were selected, then harvesting similar to commercial procedure (described in Chapter III). The breast without skin and thigh with skin was deboned, vacuum-packed, chilling 24h, then stored in a freezer (-20°C) for cooking. Before cooking, the meat was defrosted in the refrigerator (4°C) overnight. The meat of the different chickens in each pen was cooked by four heating method, considered as one replicate. The other side of carcasses in each pen were pooled and measured dry matter (DM) of the raw meat. The FA content of raw meat was taken from feeding study which underwent similar storage time and condition.

To determine cooking yield, the meat before and after cooking was removed water or fat in the surface by filter papers, then weighted. The cooked meat was weighted after cooking 5 minutes (hot weight). Each cooked breast and thigh were divided into four parts for further analysis of DM, FA, TBARS, and sensory test.

Moisture content in raw and cooked meat was analyzed by a conventional oven. The frozen meat (about 5 g, recorded weight) was added 5 ml ethanol 95% and then mixed the meat and sea sand (acid treated and purified) by a glass rod (known weight container+sea sand+glass rod). (ISO 1442-1973). The containers of meat were placed in the oven for 3 hours at 103°C.

To determine fatty acid content, cooked meat of each treatment was pooled according to chicken gender; therefore, there were two samples of the breast meat and two samples of the thigh meat per each treatment. The analytical procedures of FA, TBARS, and sensory test were described in the previous chapter (Chapter III). The TBARS value (n=4) was determined at two time points, once right after cooked (d0) and another at the fourth day of storage (4°C) cooked meat (d4). The sensory test was cut into parties and randomly mixed in each treatment before providing to panelists.

#### 5.3.4 Calculation and statistical analysis

Analysis of variance was performed by GLM procedure for a completely randomized design using SAS University Edition (SAS Institute Inc., Cary, NC, USA). The statistical model:  $y_{ijk} = \mu + D_i + H_j + (D*H)_{ij} + \varepsilon_{ijk}$  where  $y_{ijk}$  = observation  $ijk$ ,  $\mu$  = the overall mean,  $D_i$  = the effect of type  $i$  of dietary antioxidant (Diet),  $H_j$  = the effect of type  $j$  of heating method (Heat),  $(D*H)_{ij}$  = the effect of interaction, and  $\varepsilon_{ijk}$  = the random error. Tukey multiple range test will be used to detect significant differences between treatment means. The data of sensory test followed not non-normal distribution; therefore non-parametric test was performed. The differences between treatment means were considered to be significant at  $P < 0.05$ . Data are expressed as a mean  $\pm$  standard error of the mean (SEM), which represents the pooled SEM for the model.

The amount of FA in meat was calculated by the following equation where 0.945 was the conversion factors for poultry meat (Greenfield and Southgate, 2003).

$$[\text{FA in meat (mg/g)}] = [\% \text{ total lipid} * 0.945 * \% \text{ FA of total FA of meat}] / 10$$

#### 5.3.5 Site and period of the study

Cooking trial was conducted in Facility Building 10 (F10) of Suranaree University of Technology from 21 April to 30 June 2016.

## 5.4 Results and discussion

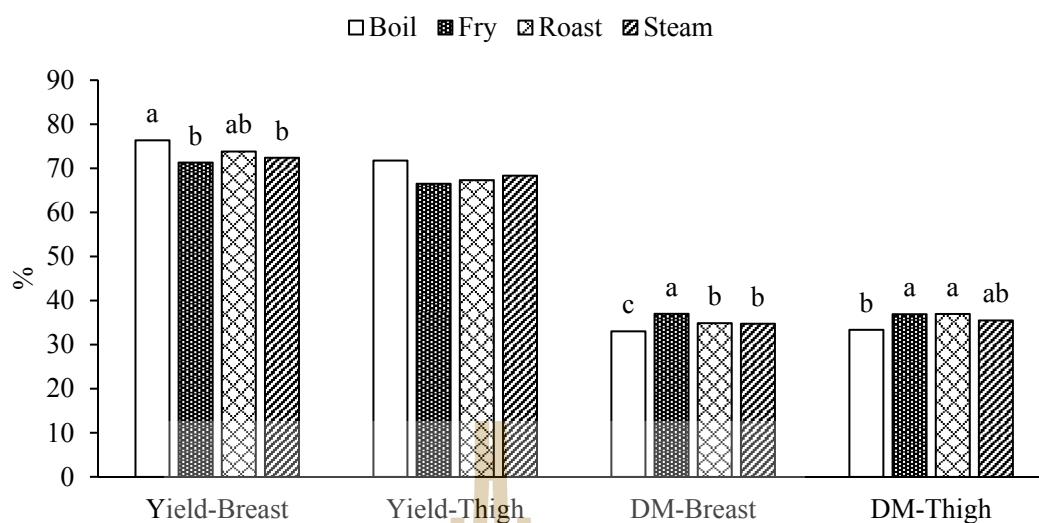
### 5.4.1 Cooking yield and dry matter of cooked meat

Dietary curcuminoids or vitamin E had no significant influence on cooking yield, resulting in average 73.48% for breast meat and 68.48% for thigh meat. Different heating method exhibited a significant difference in the water loss of cooked breast meat which the highest cooking yield found in boiling ( $P < 0.05$ ). The results of the present study agreed with Arguelo et al. (2016) who found the highest cooking weight loss of chicken inner fillets (the end point temperature 72°C) was in deep-frying, following by electric oven and the lowest in boiling in water bath. The frying, roasting and steaming treatments yielded similar results in the breast meat (Figure 5.1).

There was no statistical difference found in thigh meat and the interaction of meat sources and heat treatments. The lower cooking yield of thigh meat in compared to breast meat was also observed in agreement with the USDA table of cooking yields for meat and poultry (Showell et al., 2012).

Dry matter of the raw breast meat was 26.17% and 26.30% for TC and TE while such value of the raw thigh meat was 24.69% and 24.60%, respectively. Losses of water in the muscle were 8.67% of the breast and 11.03% of thigh meat. Boiling had the highest value of cooking yield, and its the DM was lower than other heating methods because of the uptake of water during cooking. The moisture content of raw meat in the present study similar to those of the same chicken genotype as stated by Yongsawasdigul (2016); however, the cooking loss was higher in slow-growing chicken compared to that of commercial broilers (Husak et al., 2008).





**Figure 5.1** Cooking yield (%) and DM (%) of cooked meat. The labels, a-c, mean the significant different with each group.

#### 5.4.2 TBARS value of cooked meat

In the present study, TBARS value of cooked meat increased when storage at 4°C for four days. (Table 5.2). The thigh meat generally had higher TBARS than that of breast meat because there were higher lipid content and might be more myoglobin which led to release of free ionic iron, a catalyst of peroxidation in meat (Min, 2006). Chickens meat from curcuminoids supplementation showed more sensitivity to oxidation agents than from vitamin E. The higher level of vitamin E supplementation (0, 100 to 200 ppm) the lower the lipid oxidation of cooked thigh meat was found (Cortinas et al., 2005).

Roasting exhibited the highest TBARS in either breast or thigh meat in agreement with the studies elsewhere (Broncano et al., 2009; Domínguez et al., 2014). Those studies reported that roasted pork or foal steaks led to the highest TBARS value in comparison with grilled, fried and microwave meat. Deep-fat frying was thought

to cause the highest rate of oxidation in cooked meat; however, its TBARS value was lower than roasting in breast meat and even the lowest value in thigh meat. The shorter time of heating the lower TBARS value was observed in the present study.

**Table 5.2** Effects of meat source (Diet) and cooking method (Heat) on TBARS value (mg/g) of cooked breast and thigh meat

	Breast		Thigh	
	Day 0	Day 4	Day 0	Day 4
<b>Source (Diet)<sup>1</sup></b>				
TC	3.37 <sup>A</sup>	3.57	4.07 <sup>A</sup>	5.26 <sup>A</sup>
TE	2.84 <sup>B</sup>	3.38	3.25 <sup>B</sup>	4.81 <sup>B</sup>
<b>Cooking method (Heat)</b>				
Boil	2.49 <sup>b</sup>	2.99 <sup>b</sup>	2.93 <sup>bc</sup>	4.35 <sup>c</sup>
Fry	2.92 <sup>b</sup>	3.19 <sup>b</sup>	2.83 <sup>c</sup>	3.87 <sup>c</sup>
Roast	4.16 <sup>a</sup>	4.33 <sup>a</sup>	5.44 <sup>a</sup>	6.98 <sup>a</sup>
Steam	2.84 <sup>b</sup>	3.38 <sup>b</sup>	3.43 <sup>b</sup>	4.93 <sup>b</sup>
SEM	0.33	0.33	0.38	0.42
Diet	0.0001	0.126	<0.0001	0.005
Heat	<0.0001	<0.0001	<0.0001	<0.0001
Diet*Heat	0.059	0.100	0.191	0.256

<sup>1</sup> Meat took from chicken fed different dietary supplementation which was 4% tuna oil with either 200 ppm vitamin E (TE) or 80 ppm curcuminoids (TC).

<sup>a-c, A-B</sup> the significant difference within column ( $P < 0.05$ ).

The meat used in the present experiment was vacuum-packed and stored for 102 days at -20°C which might lead to high TBARS value. From the previous study (Chapter IV) the TBARS values of raw breast meat (at 90 days of frozen storage) were 0.12 mg/kg and 0.19 mg/kg while of raw thigh meat were 0.19 mg/kg and 0.64 mg.kg in TE and TC, respectively.

In thigh meat, TBARS value highly correlated ( $P < 0.0001$ ) to the total lipid content but this correlation was not found in the breast meat. Obviously, the TBARS of either breast or thigh meat measuring at fourth day strongly ( $P < 0.0001$ ) depended on the initial TBARS at the first day. There was an increasing of TBARS in stored ready-to-eat meat in the present study, according to Samouris et al. (2007) who found boiled and roasted turkey meat significantly increased at the third day of refrigerated storage. The TBARS values of cooked meat increased significantly with storage, and the content of free ionic iron and heat-stable ferric iron reducing capacity (FRC) was responsible for the increase of cooked meat during storage (Min et al., 2008).

#### 5.4.3 Fatty acid content

The difference from before to after cooking in SFA, MUFA, and PUFA of breast meat and thigh meat were illustrated in Figure 5.2 and Figure 5.3, respectively. The change in the amount of FA (mg/g cooked, DM basis) exhibited different pattern from the profile of FA (g/100 g total lipid). The increase of DM and total lipid after cooking claimed responsibility for such discrepancy. The change of meat fatty acid composition during cooking might result from chemical reactions such as oxidation, hydrolysis, and polymerization (Hernández et al., 1999).

Regarded to breast meat, when cooking without culinary fat, the fatty acid profile (g/100g of total FA) of the breast meat exhibited an increase in SFA and MUFA and a decrease in PUFA, including both n-6 and n-3 PUFA. Deep-fat frying reduced EPA and DHA while embedded C18:2n-6 and C18:3n-3 from the fried oil to the breast meat. The culinary oil in the present study consisted in C18:2n-6 (54.35%) and C18:3n-3 (5.32%). Douny et al. (2015) revealed that fatty acid profile of n-3 PUFA enriched pork was modified according to the nature of the culinary fat used. In fact, there was a

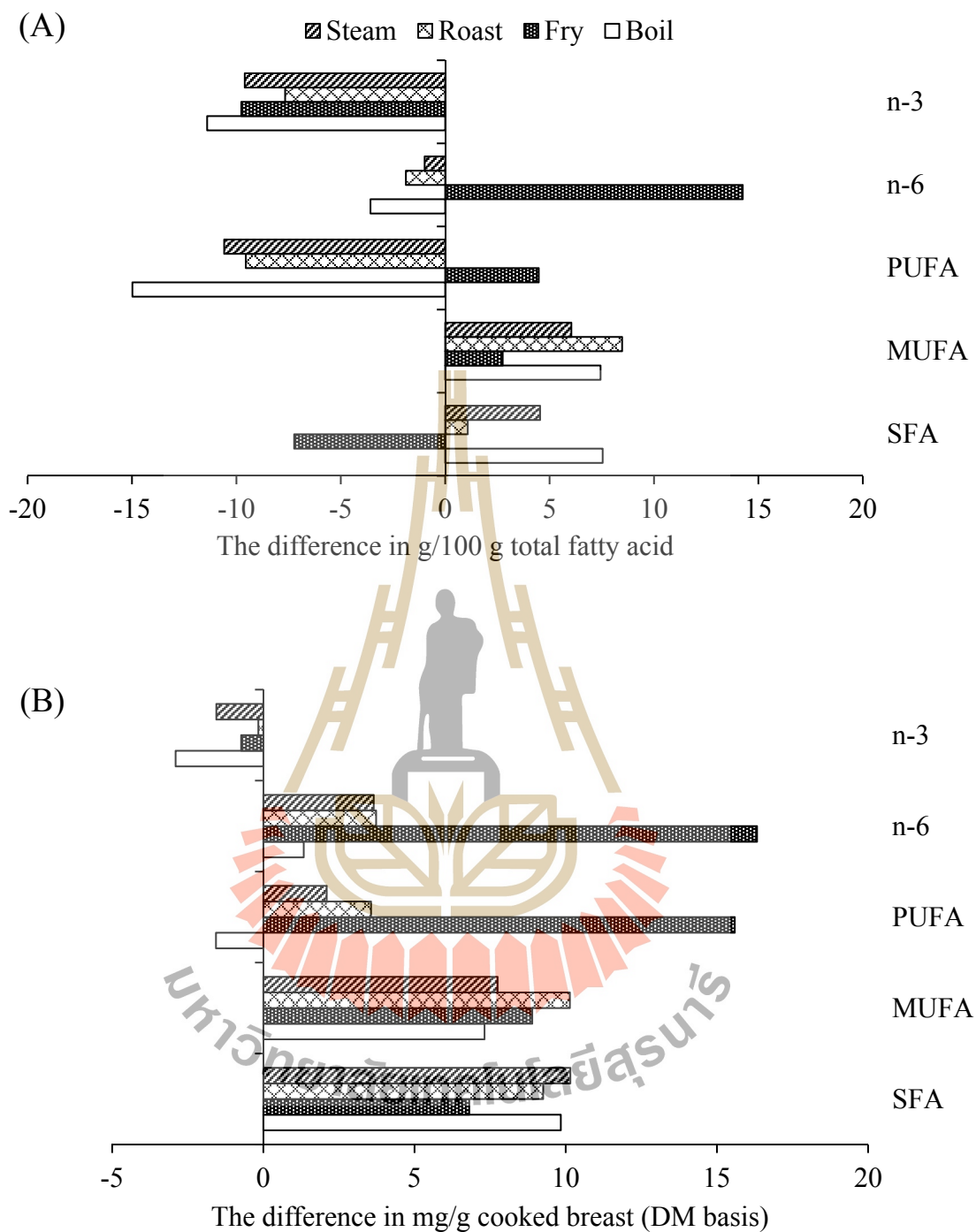
considerable increase in linoleic acid content, the main component of the sunflower oil used in frying breast meat (Candela et al., 1996). The number of detected fatty acids of meat after frying showed approximated 2-time much more than that of other heating methods, in particular *trans* fat in the present study. Roasting in an oven showed less effect on FA profile of breast meat, having a little loss in n-3 PUFA rather than other heating treatments. Moist heating had similar trend, increasing SFA and MUFA and decreasing PUFA which steaming preserved more n-3 PUFA than boiling.

Frying and boiling cause a reduction in the PUFA content of the thigh meat, both n-6 and n-3 PUFA. Roasting made a remarkable increase in total PUFA of the thigh meat, in particular, n-6 PUFA. The SFA of thigh lost at the highest rate when steaming. There was an increase of MUFA and a decrease of PUFA of the thigh meat in all cooking methods. The findings of the present study were disagreement with Cortinas et al. (2004) who reported that cooking of thigh meat led to a reduction in total FA content that affected SFA, MUFA, and PUFA in a similar proportion. The cooking condition, which variety time and the temperature were set up, might affect the FA content of cooked meat in different ways. The change of FA of enriched meat, containing a high level of EPA and DHA, in the present study was in agreement with the finding of Stephen et al. (2010) that found an increase of MUFA of cooking tuna fish and varied effect between different fatty acids. Cooking beef also reduced total PUFA content (Duckett and Wagner, 1998; Oliveira et al., 2015).

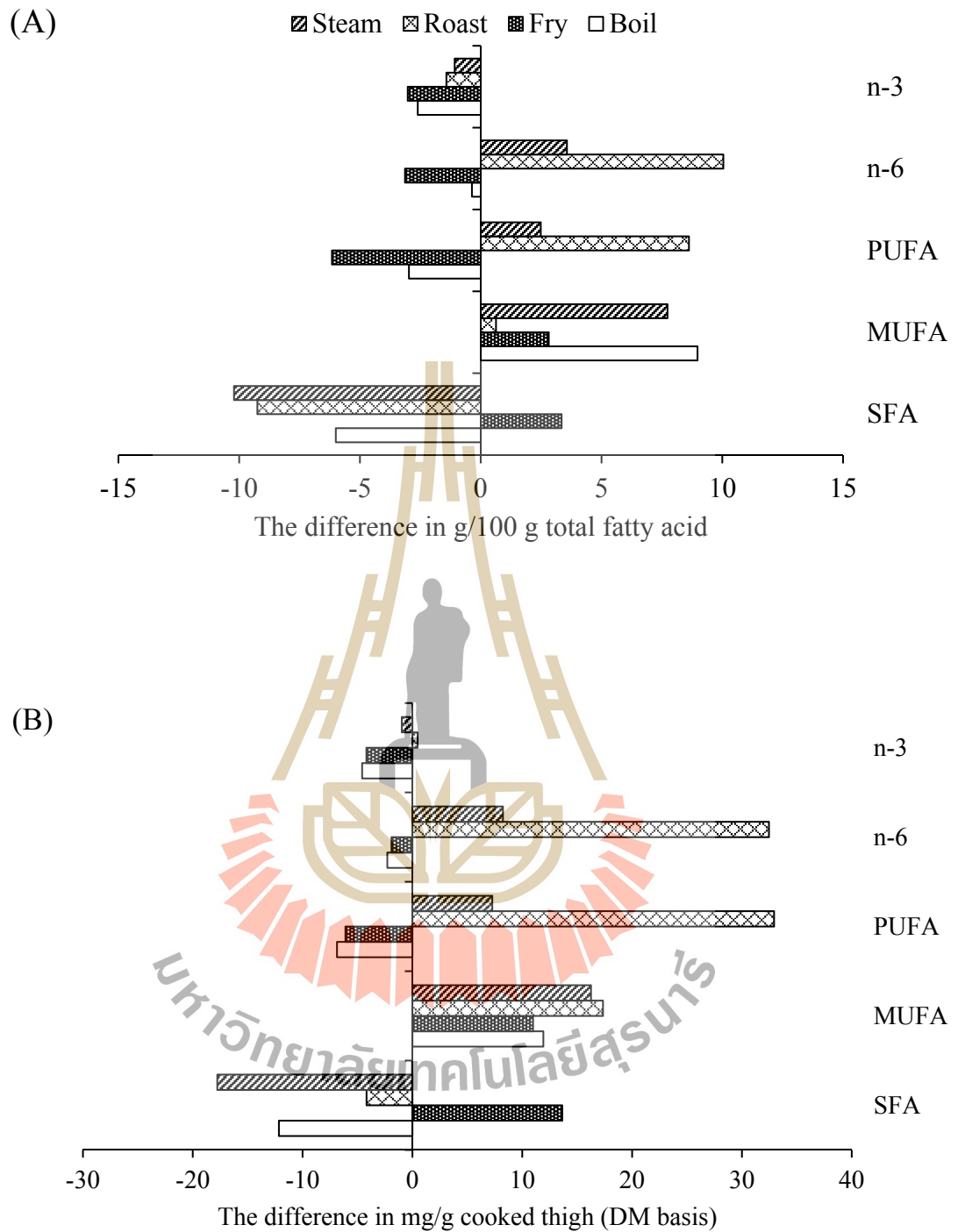
The cooking methods affected the total lipid content of either breast or thigh meat ( $P < 0.05$ ). The highest lipid content of breast meat found in frying (Table 5.3) while that of thigh meat exhibited in roasting (Table 5.4). Total lipid after cooking generally increased because of the decrease of moisture content (Alfaia et al., 2010).

It was observed that poultry meat no matter whether in a pan or by deep - frying, is associated with fat uptake (2 - 14 g per 100 g of raw food) while non-breaded high-fat food of animal origin loses fat during frying (2 - 30%) (Bognar, 1998). The thigh meat was cooked with skin; therefore, the diffusion of subcutaneous fat might incorporate into the muscle tissue during heating in the present study. In overall, the content of both EPA and DHA range were from 116.44 to 219.98 g/100g of cooked breast meat and from 252.28 to 371.39 g/100g of ready-to-eat thigh meat. The source of samples did not influence on the fatty acid content of both cooked breast and thigh, except for C18:1n-9 and C20:5n-3 of breast meat.

In the breast meat, roasting demonstrated the highest content of C18:1 n-9, C22:6n-3 and total n-3 PUFA but no statistical difference from frying or steaming. The n-6/n-3 ratio revealed that roasting and steaming can be good choices for cooking n-3 PUFA enriched breast meat. The thigh meat can be cooked by boiling, frying, roasting, or steaming without remarkable effect on the n-6/n-3 ratio of the thigh meat (removed skin after cooking). Alfaia et al. (2010) found a decrease in PUFA/SFA ratio of beef but did not change its n-6/n-3 index when cooking meat by boiling, deep-frying in soybean oil, baking or other heating methods. Roasting resulted in the highest ( $P < 0.05$ ) amount of C18:2n-6 and C18:3n-3 of thigh meat. Although there was no significant difference in the content of EPA and DHA, more favorable amount of them found in steamed thigh meat.



**Figure 5.2** The difference of fatty acids before and after cooking breast meat expressed in g/100 g of total FA (A) and mg/g DM basis (B). There was calculated based on the mean value of raw (n=4) and cooked meat (n=2/heating method).



**Figure 5.3** The difference of fatty acids before and after cooking thigh meat expressed in g/100 g of total FA (A) and mg/g DM basis (B). There was calculated based on the mean value of raw (n=4) and cooked meat (n=2/heating method).

**Table 5.3** Total lipid (%) and major fatty acids contents (mg/g sample) of cooked breast meat (without skin)

	Source (diet) <sup>1</sup>		Cooking method (heat)						SEM			P-value	
	TC	TE	Boil	Fry	Roast	Steam		Diet	Heat	Diet*Heat			
Lipid	2.26	2.34	1.94 <sup>b</sup>	2.87 <sup>a</sup>	2.22 <sup>b</sup>	2.16 <sup>b</sup>	0.28	0.425	<0.0001	0.680			
C16:0	5.07	5.41	5.09	4.93	5.41	5.53	0.64	0.321	0.561	0.561			
C18:0	2.47	2.68	2.63	2.40	2.63	2.66	0.26	0.144	0.502	0.221			
C16:1	0.04	0.00	0.00	0.00	0.07	0.00	0.07	0.347	0.441	0.441			
C17:1	1.08	1.08	1.52	1.23	1.21	0.36	0.81	1.000	0.279	0.502			
C18:1n-9	4.54 <sup>B</sup>	5.17 <sup>A</sup>	3.75 <sup>b</sup>	5.08 <sup>a</sup>	5.33 <sup>a</sup>	5.26 <sup>a</sup>	0.49	0.036	0.006	0.361			
C18:2n-6	3.78	4.32	2.04 <sup>b</sup>	8.09 <sup>a</sup>	3.09 <sup>b</sup>	2.99 <sup>b</sup>	0.96	0.294	<0.0001	0.822			
C20:4n-6	0.83	1.00	0.76 <sup>b</sup>	0.72 <sup>b</sup>	1.09 <sup>a</sup>	1.09 <sup>a</sup>	0.17	0.079	0.022	0.624			
C18:3n-3	0.11	0.18	0.00 <sup>b</sup>	0.57 <sup>a</sup>	0.00 <sup>b</sup>	0.00 <sup>b</sup>	0.15	0.394	0.001	0.522			
C20:5n-3	0.20 <sup>A</sup>	0.08 <sup>B</sup>	0.07	0.13	0.21	0.15	0.10	0.041	0.305	0.067			
C22:6n-3	1.38	1.57	1.09 <sup>b</sup>	1.35 <sup>ab</sup>	1.99 <sup>a</sup>	1.48 <sup>ab</sup>	0.29	0.238	0.015	0.652			
SFA	7.54	8.22	7.72	7.58	8.03	8.19	1.07	0.239	0.845	0.515			
MUFA	5.66	6.38	5.27	6.53	6.61	5.67	0.93	0.161	0.195	0.564			
PUFA	6.48	7.31	4.30 <sup>b</sup>	11.13 <sup>a</sup>	6.38 <sup>b</sup>	5.76 <sup>b</sup>	1.00	0.136	<0.0001	0.671			
n-6	4.78	5.43	3.13 <sup>b</sup>	9.03 <sup>a</sup>	4.18 <sup>b</sup>	4.07 <sup>b</sup>	0.74	0.117	<0.0001	0.603			
n-3	1.70	1.88	1.16 <sup>b</sup>	2.10 <sup>a</sup>	2.20 <sup>a</sup>	1.68 <sup>ab</sup>	0.34	0.321	0.010	0.644			
n-6/n-3	2.96	2.87	2.97 <sup>ab</sup>	4.36 <sup>a</sup>	1.91 <sup>b</sup>	2.44 <sup>b</sup>	0.69	0.808	0.005	0.506			

<sup>1</sup> Meat taken from chicken fed different dietary supplementation which were 4% tuna oil with either 200 ppm vitamin E (TE) or 80 ppm curcuminoids (TC).

<sup>a,b</sup>, <sup>A,B</sup> the significant difference within row ( $P < 0.05$ ).



**Table 5.4** Total lipid (%) and major fatty acids contents (mg/g sample) of cooked thigh meat (without skin)

	Source (diet) <sup>1</sup>		Cooking method (heat)					SEM		P-value	
	TC	TFE	Boil	Fry	Roast	Steam	Diet	Heat	Diet*Heat		
Lipid	6.97	6.44	5.25 <sup>c</sup>	6.95 <sup>ab</sup>	8.08 <sup>a</sup>	6.54 <sup>bc</sup>	0.94	0.126	<0.0001	0.728	
C16:0	11.79	9.74	7.62	16.94	12.56	5.95	6.76	0.562	0.169	0.495	
C18:0	5.58	5.58	5.67	6.02	4.62	5.99	2.23	0.998	0.794	0.509	
C16:1	1.50	1.25	1.75	1.65	0.63	1.46	0.86	0.573	0.308	0.295	
C17:1	1.29	1.36	1.36	1.19	2.11	0.63	1.03	0.894	0.314	0.127	
C18:1n-9	22.52	21.01	19.42	20.62	23.89	23.14	6.39	0.649	0.736	0.819	
C18:2n-6	16.31	16.87	11.85 <sup>b</sup>	13.35 <sup>b</sup>	25.34 <sup>a</sup>	15.81 <sup>b</sup>	4.03	0.789	0.006	0.918	
C20:4n-6	1.40	1.08	1.08	0.99	1.37	1.51	0.31	0.079	0.135	0.556	
C18:3n-3	0.44	0.47	- <sup>b</sup>	0.19 <sup>b</sup>	1.40 <sup>a</sup>	0.22 <sup>b</sup>	0.31	0.874	0.001	0.736	
C20:5n-3	0.40	0.37	0.18	0.44	0.30	0.63	0.44	0.889	0.545	0.757	
C22:6n-3	2.66	2.65	2.35	2.30	2.90	3.09	0.89	0.978	0.535	0.876	
SFA	18.34	15.52	13.36	24.56	17.62	12.18	7.20	0.455	0.142	0.544	
MUFA	25.89	23.62	22.53	24.61	26.62	25.24	6.83	0.525	0.861	0.953	
PUFA	21.30	21.44	15.45 <sup>b</sup>	17.45 <sup>b</sup>	31.31 <sup>a</sup>	21.26 <sup>ab</sup>	5.48	0.962	0.015	0.925	
n-6	17.78	17.95	12.93 <sup>b</sup>	14.50 <sup>b</sup>	26.71 <sup>a</sup>	17.33 <sup>ab</sup>	4.28	0.940	0.008	0.928	
n-3	3.52	3.49	2.52	2.95	4.60	3.93	1.30	0.963	0.181	0.911	
n-6/n-3	5.23	5.19	5.37	4.96	5.84	4.67	1.06	0.935	0.475	0.917	

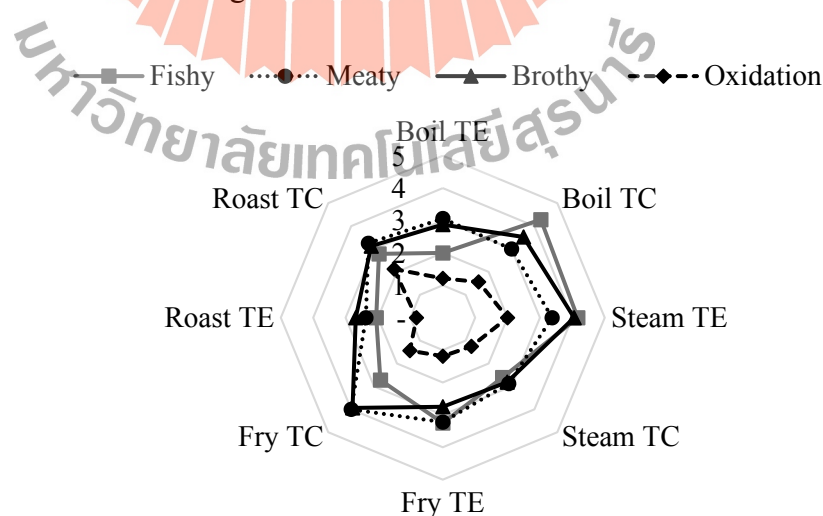
<sup>1</sup> Meat taken from chicken fed different dietary supplementation which were 4% tuna oil with either 200 ppm vitamin E (TE) or 80 ppm curcuminoids (TC).

<sup>a-b</sup> the significant difference within row ( $P < 0.05$ ).

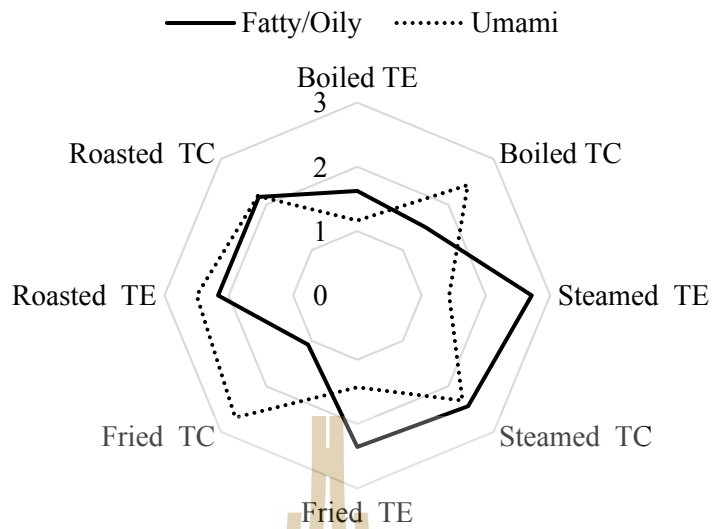
#### 5.4.4 Sensory evaluation for different cooking processing

The effect of meat source was no significant difference while the interaction of source and heating method exhibited in fishy odor ( $P < 0.05$ ). Notably, the effect of heating showed in an attribute of hardness ( $P < 0.05$ ). The flavor of steaming showed stronger in TE source than TC source while other heating method had the reverse pattern (Figure 5.4). The fishy odor was highly detected in TC and moist heating. Meaty and brothy flavor shared a similar pattern in most of the treatments. The difference between roasted TE and roasted TC in oxidative smell agreed with TBARS value found in that cooked meat.

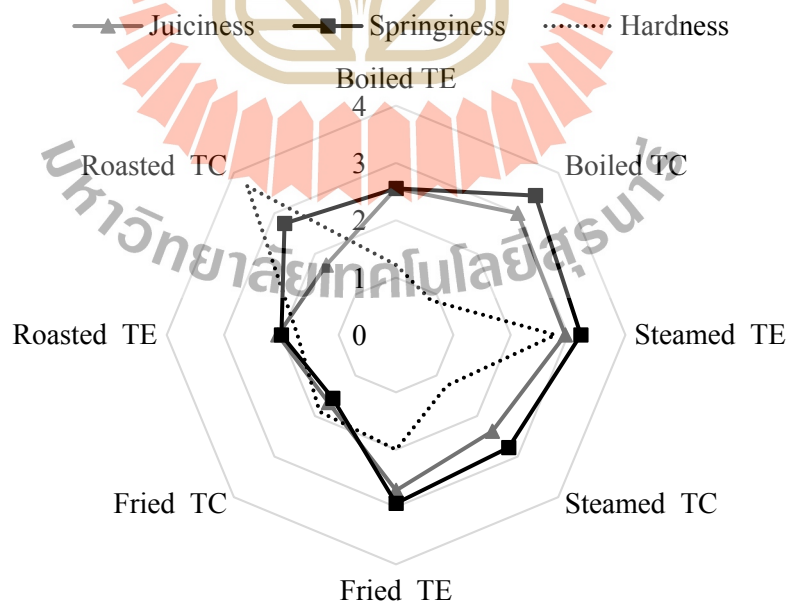
Although the more fat content was measured in frying, the mouthfeel of oily or fatty in steaming was numerically predominant (Figure 5.5). Umami taste seems stronger in dry heating compared with moist heating method ( $P > 0.05$ ). Juiciness was known for having a relationship with water-holding capacity of the meat. In the present study, juiciness felt downward in the following order from boiling, steaming to frying and roasting (Figure 5.6). The hardness of roasting gained the highest ( $P < 0.05$ ) score in comparison with other heating treatments.



**Figure 5.4** Aroma's scores of the cooked breast where TE or TC was meat from groups of chicken fed dietary vitamin E or curcuminoids, respectively.



**Figure 5.5** Taste's scores of the cooked breast where TE or TC was meat from groups of chicken fed dietary vitamin E or curcuminoids, respectively.



**Figure 5.6** Texture's scores of the cooked breast where TE or TC was meat from groups of chicken fed dietary vitamin E or curcuminoids, respectively.

## 5.5 Conclusions

Roasting and steaming were good choices for cooking n-3 PUFA enriched breast meat (without skin). Steamed thigh meat had the more favorable amount of EPA and DHA. Boiling, frying, roasting, or steaming was an optional method without effect on n-3 PUFA content of the thigh meat. The limitation of oxidation and fishy odor of roasting or steaming could be improved by marinating enriched meat with culinary herbs and spices.

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## CHAPTER VI

### CONCLUSIONS AND RECOMMENDATION

#### 6.1 Conclusion

In conclusion, the diet contained 4% of tuna oil and 2% rice bran oil can be applied to produce the n-3 PUFA enriched chicken meat, reaching the threshold for designating it as “high in n-3 PUFA” meat. In addition, the n-6/n-3 ratio was 1.20 in breast and 2.74 in thigh meat of crossbred chicken fed such diet. The curcuminoids applied in the present study showed no convincing data to prove its effective antioxidant in comparison with vitamin E. Although cooking cause loss of n-3 PUFA, the ready-to-eat chicken meat produced can supply a nutritionally valuable amount of EPA and DHA for health benefit.

#### 6.2 Impication and recommendation

The results from the present studies confirmed that Thai crossbred chicken can be value-added chicken meat for niche market. The current research benefits every sectors which take part in the process of production n-3 PUFA enriched meat from feed producers, chicken farm, meat retailer to restaurant.

Depend on which breast or thigh meat would like to be produced such as “source of n-3 PUFA” or “high in n-3 PUFA”, the level of tuna oil or linseed oil can be applied flexibly. In Thailand, the price of tuna oil was cheaper than linseed oil at the moment so that tuna oil was recommended. The present study evaluated meat quality at 84 day-



old chicken which the purpose of trading meat in portion cut; however, most of crossbred chicken, in particular “Korat” chicken, currently sell as a whole carcass. Therefore, the meat quality of “Korat” chicken at 63 days of age should be examine. In addition, the study on period of feeding supplementation oil before slaughter should be considered to reduce the feed cost.

The diet with n-3 PUFA riched oil should use antioxidant to preserve feed from rancidity. For this purpose, vitamin E was an good option for commercial application. Although curcuminoids from curcumin removed turmeric oleoresin did not show convincing antioxidant property in the present study, their potential utilization as feed additive is interested. Further research on the effect of the oleoresin as antioxidant should conduct with more measurement on free radical scavenging activity and antioxidant indices of meat. The combination of n-3 PUFA and curcuminoids was also predicted to affect animal immune and inflammatory system.

Last but not least, the appropriate food processing for n-3 PUFA enriched chicken meat should carry out. Firstly, such kind of studies eliminate or minimum the adverse effect of n-3 PUFA on meat quality. Secondly, the outcomes can bring more varieties and value of the enriched product.

## **BIOGRAPHY**

Mrs. Tran Thi Thuy Hang was born on 20<sup>th</sup> December 1985 in Tra Vinh, Viet Nam. In 2007, she graduated a Bachelor degree in Animal Husbandry at Can Tho University, Viet Nam. She was employed by the Department of Animal Sciences, College of Agriculture and Applied Biology, Can Tho University (CTU) to work as a researcher of poultry production. At the same time, she studied MSc program at CTU, Viet Nam in the field of Animal Nutrition and graduated in 2010. In May 2010, she worked for Department of Agricultural Research and Natural Resource, Office of Provincial People's Committee of Tra Vinh province, Viet Nam. From December 2011 she has been a lecturer of Department of Agricultural Technology, College of Rural Development, CTU, Viet Nam. In 2013, she got an award for a PhD program from Suranaree University of Technology (SUT), Thailand in the program of “SUT-PhD Scholarship Program for ASEAN”. She studied in the field of Animal Production Technology at School of Animal Production Technology, Institute of Agricultural Technology, Suranaree University of Technology from June 2013 to May 2017 with the thesis entitled “production of functional chicken meat by dietary supplementation of oil rich in n-3 polyunsaturated fatty acids and curcumin removed turmeric oleoresin”. The partly results of her Ph.D. thesis have been present in the 1<sup>st</sup> International Conference on Tropical Animal Science and Production (TASP 2016) on July 26-29, 2016 at Bangkok, Thailand and the 4<sup>th</sup> SUT International Agricultural Colloquium 2016 on June 28-29, 2016 at NakhonRatchasima, Thailand.