ผลของวิตามินอีในน้ำมันปาล์มดิบต่อประสิทธิภาพการเจริญเติบโต การเกิดกระบวนการออกซิเดชั่นของไขมัน และความเข้มข้นของวิตามินอีในเนื้อเยื่อของไก่เนื้อ

นายบรรจง อุรา

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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THE EFFECTS OF VITAMIN E IN CRUDE PALM OIL ON GROWTH PERFORMANCE, LIPID PEROXIDATION AND TISSUE VITAMIN E CONCENTRATION OF BROILERS.

Mr. Banjong Ura

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บรรจง อุรา : ผลของวิตามินอีในน้ำมันปาล์มดิบ ต่อประสิทธิภาพการเจริญเติบโต การเกิดกระบวนการออกซิเดชั่นของไขมัน และความเข้มข้นของวิตามินอีในเนื้อเยื่อของไก่เนื้อ. (THE EFFECTS OF VITAMIN E IN CRUDE PALM OIL ON GROWTH PERFORMANCE, LIPID PEROXIDATION AND TISSUE VITAMIN E CONCENTRATION OF BROILERS) อ. ที่ปรึกษา: รศ. สุวรรณา กิจภากรณ์, 69 หน้า.

การวิจัยครั้งนี้มีวัตถุประสงค์เพื่อศึกษาผลของวิตามินอีในน้ำมันปาล์มดิบต่อประสิทธิภาพการเจริญเติบโต การเกิดกระบวนการออกซิเดชั่นของไขมัน และความเข้มข้นของวิตามินอีในเนื้อเยื่อของไก่เนื้อ โดยใช้ไก่เนื้อ เพศผู้ พันธุ์ ROSS 308 อายุ 1 วัน จำนวน 576 ตัว แบ่งออกเป็น 6 กลุ่ม กลุ่มละ 6 ซ้ำ ซ้ำละ 16 ตัว โดยใช้ข้าวโพด และกากถั่วเหลืองเป็นวัตถุดิบพื้นฐานร่วมกับไขมันหมูในระดับ 8% และปรับระดับวิตามินอีในสูตรอาหารด้วย α-tocopheryl acetate เพื่อให้ได้วิตามินอีเท่ากับความต้องการของไก่เนื้อที่ระดับ 50 มก/กก. อาหาร เป็นกลุ่มควบคุม จากนั้นใช้น้ำมันปาล์มดิบทดแทนไขมันหมูในระดับ 0, 2, 4, 6 และ 8 % ตามลำดับ และกลุ่มควบคุมที่เสริมวิตามินอี (α-tocopheryl acetate) 100 มก/กก. ของอาหาร เพื่อใช้ประเมินประสิทธิภาพของวิตามินอีในน้ำมันปาล์มดิบ อาหารทุกสูตรถูกคำนวนให้มีสารอาหารตามความต้องการของพันธุ์ บันทึกน้ำหนักไก่และปริมาณอาหารที่กินในวันที่ 21 และ 42 ของการทดลอง สุ่มไก่ทดลองมากลุ่มละ 6 ตัว เก็บตัวอย่างเลือด และ ตับในวันที่ 21 และเก็บตัวอย่างเลือด ตับ เนื้ออกและเนื้อสะโพกในวันที่ 42 เพื่อตรวจวัดการเกิดกระบวนการออกซิเดชั่นของไขมัน(TBARS) และความเข้มข้นของวิตามินอีทุกรูปแบบ หาความสัมพันธ์ระหว่างความเข้มข้นของวิตามินอีในเนื้อเยื่อกับอาหาร ตรวจวัดค่าTBARS ในเนื้ออกและเนื้อสะโพกในวันที่ 4 และ 7 และหาค่าการสูญเสียน้ำในเนื้ออกและเนื้อสะโพกในวันที่ 2, 4, 6 และ 8 ในระหว่างการเก็บรักษาที่อุณหภูมิ 8°C

จากการศึกษาพบว่าอัตราการเจริญเติบโตและอัตราการแลกเนื้อทั้ง 2ระยะของทุกกลุ่มไม่มีความแตกต่างกัน($P>0.05$) การใช้น้ำมันปาล์มดิบเพิ่มขึ้น สามารถเพิ่มปริมาณโทโคฟีรอลและโทโคไตรอีนอลในตับ เนื้ออก และเนื้อสะโพกเมื่อเปรียบเทียบกับกลุ่มควบคุม($P<0.05$) ไก่เนื้อที่ได้รับน้ำมันปาล์มดิบในระดับ 8% มีการสะสมวิตามินอีโทโคไตรอีนอลสูงสุดในทุกเนื้อเยื่อ ขณะที่กลุ่มที่เสริมวิตามินอี อะซิเตทให้ค่าการสะสมวิตามินอีโทโคฟีรอลสูงที่สุด สำหรับรูปแบบของวิตามินอีที่สะสมในเนื้อเยื่อพบว่าแอลฟา-โทโคฟีรอลสูงที่สุด ตามด้วย แอลฟา-โทโคไตรอีนอล แกรมม่า-โทโคฟีรอล และ แกรมม่า-โทโคไตรอีนอล ตามลำดับ และความเข้มข้นของวิตามินอีในตับ เนื้ออก และเนื้อสะโพกมีความสัมพันธ์เชิงบวกกับปริมาณน้ำมันปาล์มดิบในอาหาร($P<0.05$) การใช้น้ำมันปาล์มดิบเป็นแหล่งวิตามินอีที่ระดับตั้งแต่ 4% ขึ้นไป ในอาหารสามารถช่วยลดการเกิดการออกซิเดชั่นของไขมันในน้ำเลือดไก่ที่อายุ 21 และ42วัน ($P<0.001$) เมื่อเปรียบเทียบกับกลุ่มควบคุม และที่ระดับน้ำมันปาล์มดิบ 6 และ 8 % ให้ผลไม่แตกต่างจากกลุ่มที่เสริมวิตามินอี ในส่วนของตับและเนื้ออกไม่พบความแตกต่างขณะที่เนื้อสะโพกของกลุ่มที่ได้รับน้ำมันปาล์มดิบทุกระดับให้ค่าTBARSไม่แตกต่างจากกลุ่มที่เสริมวิตามินอี แต่แตกต่างจากกลุ่มควบคุม ($P<0.001$) การใช้น้ำมันปาล์มดิบเป็นแหล่งวิตามินอีสามารถช่วยลดการเกิดการออกซิเดชั่นของไขมัน และการใช้น้ำมันปาล์มดิบที่ระดับตั้งแต่ 4%ขึ้นไปลดการสูญเสียน้ำในเนื้ออกและเนื้อสะโพกระหว่างเก็บรักษา เมื่อเปรียบเทียบกับกลุ่มควบคุมและไม่แตกต่างจากกลุ่มที่เสริมวิตามินอี

ภาควิชาสัตวบาล
สาขาวิชาอาหารสัตว์
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ลายมือชื่อนิสิต.....
ลายมือชื่ออาจารย์ที่ปรึกษา.....

4975563331 : MAJOR ANIMAL NUTRITION

KEY WORD: CRUDE PALM OIL / GROWTH PERFORMANCE / ANTIOXIDANT / TISSUE VITAMIN E CONCENTRATION / TBARS / DRIPLOSS / BROILER

BANJONG URA: THE EFFECTS OF VITAMIN E IN CRUDE PALM OIL ON GROWTH PERFORMANCE, LIPID PEROXIDATION AND TISSUE VITAMIN E CONCENTRATION OF BROILERS. THESIS ADVISOR: ASSOC. PROF. SUWANNA KIJPARKORN, M.S. 69 pp.

An experiment was studied to investigate the effects of vitamin E in crude palm oil on growth performance, lipid peroxidation and tissue vitamin E concentration of broilers. Total 576 day old male Ross 308 broiler chicks were randomly allocated into 6 treatments which composed of 6 replicates of 16 birds each. Corn-soybean meal basal diets with 8% lard which was adjusted vitamin E level according to the recommended requirement (50 mg/kg diet) by adding α-tocopheryl acetate, was used as a control diet. Crude palm oil (CPO) was substituted to lard at the level of 0, 2, 4, 6, and 8 % respectively. Positive control was conducted to evaluate vitamin E efficacy in CPO by supplementation of α-tocopheryl acetate 100 mg/kg control diet. Diets were calculated to meet Ross requirement. On day 21 and 42 of the experiment, body weight and feed intake were recorded and six broilers in each treatment group of each period were randomly selected. On day 21, blood and liver were collected while blood, liver, breast and thigh meat were collected on day 42. Thiobarbituric acid-reactive substances (TBARS) and vitamin E concentration of all forms were analyzed in plasma, liver, breast and thigh meat. Relationship between vitamin E concentration in diet and tissues were calculated by linear regression. TBARS were determined on days 4 and 7 and drip loss was measured on day 2, 4, 6 and 8 day in breast and thigh meat in controlled-chilled room at 8°C.

The results demonstrated that growth rate and feed conversion ratio were not significant difference among treatment groups in both periods ($P>0.05$). An increasing of CPO level increased tocopherol and tocotrienol concentration in liver breast and thigh meat when compared to control group ($P<0.05$). Broilers fed with CPO 8% had highest deposition of tocotrienol while the positive control group has highest tocopherol concentration in tissue. Form of vitamin E deposition in tissues, α-tocopherol had highest follow by α-tocotrienol, γ-tocopherol and γ-tocotrienol respectively. The tocopherols, tocotrienols and total vitamin E concentration in all tissues showed the positive relationship with crude palm oil level in diet ($P<0.05$). Inclusion of CPO as a vitamin E source at the level of 4% or more in diet decreased TBARS in plasma at the age of 21 and 42 day ($P<0.001$) when compare to control and CPO at the level of 6 and 8% fed group were not difference with positive control group. No significant difference of TBARS was found in liver and breast meat while thigh meat of CPO fed groups were significant difference when compared to control group($P<0.001$) but not significant difference with positive control group. During storage, inclusion of CPO as a vitamin E source in diet decreased TBARS and CPO from the level of 4% and onward decreased drip loss in breast and thigh meat when compare to control group and not significant difference with positive control group.

Department Animal Husbandry

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Advisor's signature A. Kijparkorn

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ABBREVIATION

CPO	=	crude palm oil
Fe	=	iron
h	=	hour
H_2O	=	water
H_2O_2	=	hydrogen peroxide
HDL	=	high density lipoprotein
$HO^{\cdot}$	=	hydroxyl radical
HOCl	=	hypochlorous acid
IU	=	international unit
kg	=	kilogram
$L^{\cdot}$	=	lipid radical
LDL	=	low density lipoprotein
$LOO^{\cdot}$	=	lipoperoxyl radical
LOOH	=	lipohydroperoxide
m^2	=	square meter
Me	=	methyl group
ME	=	metabolizable energy
mg	=	milligram
mg/g	=	milligram per gram
mg/kg	=	milligram per kilogram
min	=	minute
mL	=	milliliter
ml/min	=	milliliter per minute
mm	=	millimeter
N	=	nitrogen
nm	=	nanometer
nmol	=	nanomole
nmol/L	=	nanomole per liter
O_2	=	oxygen

$O_2^{-\bullet}$	=	superoxide anion radical
$^1O_2$	=	singlet oxygen
OH	=	hydroxyl
ppm	=	part per million
PUFA	=	polyunsaturated fatty acid
$RO^{\bullet}$	=	alkoxyl
$ROO^{\bullet}$	=	peroxyl
ROS	=	reactive oxygen species
rpm	=	round per minute
S	=	sulfur
sec	=	section
SR-BI	=	scavenger receptor class B type I
TBARS	=	thiobarbituric acid-reactive substances
VLDL	=	very low density lipoprotein
v/v	=	volume per volume
µg/g	=	microgram per gram
µL	=	microliter
µm	=	micrometer
α-TTP	=	alpha-tocopherol transfer protein

CHARPTER I

INTRODUCTION AND AIMS

Lipids, especially phospholipids present in cell membranes, are particularly susceptible to oxidative damage, being positively correlated with the degree of unsaturation of its fatty acids. This process, known as lipoperoxidation in live systems and as oxidative rancidity in food, represents an oxidative chain reaction. The absence of antioxidants becomes autopropagative and lead to lipohydroperoxide (LOOH) production. These LOOH are easily decomposed into aldehydes, ketones, alcohols, and lactones, with some of these being potentially cytotoxic to live systems. The accumulation of these productions in poultry meat can affect its organoleptic characteristics (Fellenberg and Speisky, 2006). Lipid oxidation in muscle systems is initiated at the membrane level in the intracellular phospholipid fractions (Gray et al., 1996). Lipid oxidation is one of the primary mechanisms of quality deterioration in foods and especially in meat products. The changes in quality are manifested by adverse changes in flavor, color, texture and nutritive value, and the possible production of toxic compounds (Gray et al., 1996; Fellenberg and Speisky, 2006).

Vitamin E is a lipid-soluble antioxidant that is present in the membranes of intracellular organelles and plays an important role in the suppression of free radical-induced lipid peroxidation (Dutta-Roy et al, 1994). Vitamin E in the form of tocopherols and tocotrienols are well recognized for their effective inhibition of lipid peroxidation in biological systems and delay oxidative deterioration of meat (Kamal-Eldin and Appelqvist, 1996; Wood and Enser, 1997). Since vitamin E is only synthesized by plants, it is a very important dietary nutrient for humans and animals (Kamal-Eldin and Appelqvist, 1996; McDowell, 2000). Numerous studies have reported the positive effect of vitamin E enriched diets on the susceptibility to lipoperoxidation of plasma (Sheehy et al., 1994; Fellenberg and Speisky, 2006) and tissues such as muscle (Bartov and Bornstein, 1981; Sheehy et al., 1994; Woodall et al., 1996; Fellenberg and Speisky, 2006), liver (Sheehy et al., 1994; Woodall et al., 1996; Surai and Sparks, 2000;

Fellenberg and Speisky, 2006), heart (Sheehy et al., 1994; Surai and Sparks, 2000; Fellenberg and Speisky, 2006) and adipose tissue (Bartov and Bornstein, 1981; Fellenberg and Speisky, 2006). Vitamin E is the major antioxidant in meat post-mortem and its addition to poultry feeds to increase the oxidative stability of the meat has been established for many years (Marusich et al., 1975; O'Neill et al., 1998; Enser, 1999).

Crude palm oil is rich in natural antioxidants such as tocotrienols, tocopherols and carotenoids (Goh et al., 1985). Natural antioxidants may also supplement the antioxidant effects at cellular level. In addition, recently there has been increased interest in role of antioxidant nutrients due to their health benefits in disease conditions such as cancer, coronary heart disease and immune functions (Kang et al., 2001). Therefore, incorporation of crude palm oil rich in natural antioxidants by dietary means may be more effective and economical in controlling post slaughter lipid peroxidation and an alternative way of increasing these health enhancing nutrients in human diets. The purposes of the present study were to evaluate the effect of vitamin E in crude palm oil on growth performance, lipid peroxidation in plasma and tissues, all forms of vitamin E deposit in tissues, relationship between vitamin E in the diet and tissues lipid peroxidation and drip loss in tissues during storage.

CHARPTER II

BACKGROUND INFORMATION

2.1 Lipid peroxidation

Under normal physiological conditions, animal cells are continuously challenged by stressors arising from both internal and external sources. The most important of these are reduced derivatives of oxygen called reactive oxygen species (ROS). These include free radicals having one or more unpaired electrons which can exist independently for a brief period (Morrissey et al., 1998). Examples are hydroxyl radical ($HO^{\bullet}$) (the most potent oxidant encountered in biological systems), superoxide anion radical ($O_2^{-\bullet}$), and oxygencentred radicals of organic compounds (peroxyl, $ROO^{\bullet}$ and alkoxyl, $RO^{\bullet}$). Other ROS include hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl) and hydroperoxide and epoxide metabolites of endogenous lipids. These are not free radicals but contain chemically reactive oxygen-containing functional groups (Keher, 1993; Morrissey et al., 1998).

Reactive oxygen species can either be produced accidentally or deliberately (Keher, 1993; Morrissey et al., 1998). During normal aerobic metabolism, mitochondria consume molecular oxygen and reduce it sequentially to produce H_2O. During this process, $O_2^{-\bullet}$, H_2O_2 and $HO^{\bullet}$ are produced accidentally at a low rate. Peroxisomal enzymes and cytochrome P_{450} mixed-function oxidases also produce ROS accidentally. On the other hand, phagocytes generate $O_2^{-\bullet}$, H_2O_2, and HOCl deliberately and use them to inactivate bacteria or viruses. ROS can oxidize lipids, proteins, nucleic acids, and other macro-molecules leading to cell death and tissue injury. Lipid peroxidation is probably still the most widely used measure of oxidative stress in living animals (Morrissey et al., 1998).

Lipids, especially phospholipids present in cell membranes, are particularly susceptible to oxidative damage, being positively correlated with the degree of

unsaturation of its fatty acids. In the case of free radical, the attack begins by removal of an H atom (generally adjacent to a double bond of a polyunsaturated fatty acid (PUFA)), leading to a lipid radical ($L^{\bullet}$). On the other hand, the peroxidative action of singlet oxygen ($^1O_2$) is start by addition to a double bond of fatty acid, leading to a lipoperoxyl radical ($LOO^{\bullet}$). During the lipoperoxidative process started by a free radical, in O_2 presence the radical $L^{\bullet}$ generates free radical $LOO^{\bullet}$. The latter is able to remove a new atom of H from an adjacent fatty acid. As a result, $LOO^{\bullet}$ looses its radical character, becoming a lipohydroperoxide (LOOH) and generating a new radical $L^{\bullet}$. This process, known as lipoperoxidation in live systems and as oxidative rancidity in food, represents an oxidative chain reaction, which in absence of antioxidants becomes autopropagative, leading to the production of lipohydroperoxide (LOOH). These LOOH are easily decomposed into aldehydes, ketones, alcohols, and lactones, with some of these being potentially cytotoxic to live systems and if accumulating in poultry meat they can affect its organoleptic characteristics (Fellenberg and Speisky, 2006). Lipid oxidation in muscle systems is initiated at the membrane level in the intracellular phospholipid fractions (Gray et al., 1996). Lipid oxidation is one of the primary mechanisms of quality deterioration in foods and especially in meat products. The changes in quality are manifested by adverse changes in flavor, color, texture and nutritive value, and the possible production of toxic compounds (Gray et al., 1996; Fellenberg and Speisky, 2006).

In the case of meat, haemoglobin can perform at the beginning of lipoperoxidative process. It has been found that the haeme group (contains iron, Fe) present in some proteins would have an important catalytic effect in the oxidative decomposition of PUFA. Due to the fact that poultry leg and breast contain significant concentration of haemoglobin, 0.67 mg/g and 0.24 mg/g, respectively (Kranen et al., 1999) and when animals are slaughtered, the biochemical processes that turn the muscle into meat allow haemoproteins to control the lipoperoxidative processes that definitively accelerate the deterioration of the meat (Johns et al., 1989; Andersen and Skibsted, 1991; Fellenberg and Speisky, 2006).

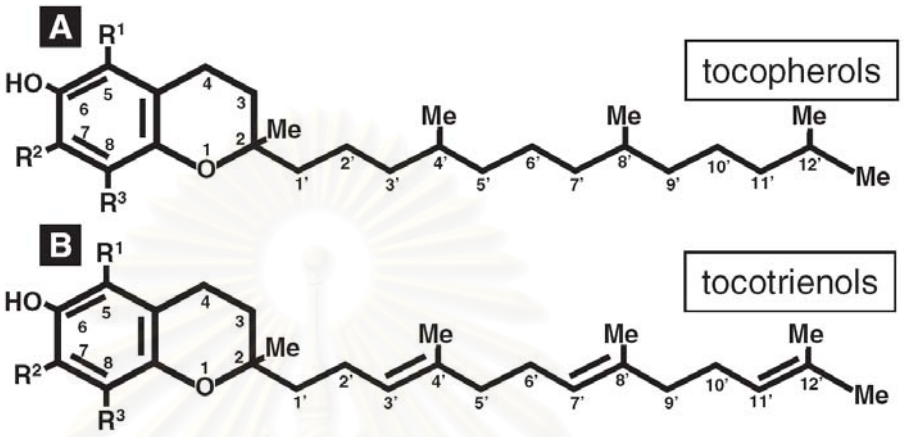
2.2 Protein, peptide and amino acid oxidation

Interactions between lipids and proteins have a significant effect on the progress of oxidative reactions in foods. Due to strong interactions, the oxidation reactions can easily transfer from lipids to proteins. Oxidation reactions affect the quality of food, but they also have an impact on the charge and conformation of the protein, loss of enzyme activity, and changes in the nutritive value (loss of essential amino acids) (Karel et al., 1975; Howell et al., 2001). The most sensitive amino acids toward oxidation are heterocyclic amino acids. In addition, amino and phenolic groups of amino acids are susceptible to oxidation. Their structure tryptophan, histidine, proline, lysine, cysteine, methionine and tyrosine are prone to oxidation where the hydrogen atom is abstracted either from OH-, S or N-containing groups (Doorn and Petersen, 2002). In addition, the modified proteins will have different functional properties from those of their unmodified molecules; their emulsifying, foaming, gelling, and water binding properties may be affected as well as the texture of food will be changed (Leaver et al., 1999).

2.3 Vitamin E isoforms and their natural sources.

Vitamin E occurs in nature in at least eight different isoforms: α-, β-, γ- and δ-tocopherols and α-, β-, γ- and δ-tocotrienols. Tocotrienols differ from the corresponding tocopherols only in their aliphatic tail. Tocopherols have a phytyl side chain attached to their chromanol nucleus, whereas the tail of tocotrienols is unsaturated and forms an isoprenoid chain (Fig. 1). The various isoforms of tocotrienols differ in their methyl substituents on the chromanol nucleus. The α-form contains 3 methyl groups, whereas the β- and γ- have two and the δ-form only one methyl group. Each of these forms of vitamin E has a reportedly different biopotency (Azzi and Stocker, 2000, Brigelius-Flohe and Traber, 1999, Traber and Packer, 1995). Humans absorb all forms of vitamin E, but the body maintains only α-tocopherol. Lipid-rich plant products and vegetable oils are the main natural sources of vitamin E. Tocotrienols are found in high concentration in palm oil and rice bran (Theriault et al., 1999). Other natural sources

include coconut oil, cocoa butter, soybeans, barley and wheat germ. Moreover, tocotrienols were also detected in meat and eggs. Sunflower, peanut, walnut, sesame and olive oils, however contain only tocopherols (Heinonen and Piironen, 1991).



A) R1=R2=R3=Me, known as α- tocopherol.
R1=R3=Me; R2=H, known as, β-tocopherol.
R1=H; R2=R3=Me, known as γ-tocopherol.
R1=R2=H; R3=Me, known as δ –tocopherol.

B) R1=R2=R3=Me, known as α-tocotrienol.
R1=R3=Me; R2=H, known as β-tocotrienol.
R1=H; R2=R3=Me, known as γ-tocotrienol.
R1=R2=H; R3=Me known as δ-tocotrienol

Fig. 1. Vitamin E: variations and nomenclature (Sen et al., 2006).

2.4 Absorption and transport of vitamin E

Absorption of vitamin E is related to intestinal fat digestion and is facilitated by bile and pancreatic lipase. Dietary vitamin E from natural sources is directly solubilized into mixed micelles by bile salts and amphipatic lipids present in the intestinal lumen before uptake into intestinal epithelial cells (Traber et al., 1990; Rigotti, 2007). Pancreatic lipase secretion also facilitates intestinal vitamin E absorption, however this effect is most likely due to increased formation of mixed micelles as a consequence of hydrolysis of non-vitamin E lipids rather than a direct effect on vitamin E digestion (Rigotti, 2007). Uptake of vitamin E into enterocytes depends on the prior incorporation of free vitamin E into mixed micelles. This means that a small amount of fat has to be absorbed along with vitamin E. A very low-fat meal or poor fat digestion effectively minimize vitamin E absorption (Kohlmeier, 2003).

The major steps and molecular mediators of vitamin E transport from the luminal micellar phase into the enterocyte have not been well defined (McDowell, 2000; Kohlmeier, 2003; Rigotti, 2007). However, vitamin E uptake is likely to involve at least some of the actors involved in fatty acid uptake (Kohlmeier, 2003; Rigotti, 2007). Even though intestinal cellular uptake of vitamin E from mixed micelles was traditionally assumed as a simple process of passive diffusion, recent studies have suggested the role of the scavenger receptor class B type I (SR-BI) (Reboul et al., 2006; Rigotti, 2007). Indeed, previous work had indicated that SR-BI was involved in cellular uptake on vitamin E in extraintestinal tissues. In addition, SR-BI was found in the apical surface of intestinal epithelial cells, suggesting a potential role in intestinal lipid absorption. While some initial in vitro evidence supported the role of SR-BI in cholesterol absorption (Rigotti, 2007). The primary site of absorption appears to be the medial small intestine. Whether presented as free alcohol or as ester, most vitamin E is absorbed as the alcohol (McDowell, 2000). The detailed molecular and cellular mechanisms involved in the intracellular trafficking, assembly and/or efflux of dietary vitamin E in intestine-derived lipoproteins have not been well established. After protein-mediated uptake into the apical plasma membrane of intestinal epithelial cells, hydrophobic α-tocopherol molecules will likely require carrier-mediated cytosolic transport during its intracellular redistribution towards the sites involved in chylomicron assembly and/or direct vitamin E efflux from the intestinal mucosa (Rigotti, 2007). Vitamin E absorption in the intestine is thought to occur predominantly through chylomicron secretion into the lymphatic system in mammals (Kohlmeier, 2003; Rigotti, 2007). However, in birds the lymphatic system is poorly developed and does not appear to participate in lipid absorption (Pearce, 1980; Sklan et al., 1984). The portal system is the major absorption route of lipid in birds (Sklan et al., 1984). Regardless of the specific cellular mechanisms underlying the transport of vitamin E from the intestinal lumen into the bloodstream, the overall fractional intestinal absorption of vitamin E ranges from 20 to 80%. The highly significant interindividual variability in vitamin E absorption may be determined by intrinsic differences in expression and activity of candidate intestinal vitamin E transporters such as SR-BI. On

the other hand, absorptive efficiency of this nutrient is also influenced by the amount and quality of dietary fat as well as the food matrix (Rigotti, 2007).

2.4.1 Hepatic transport and secretion of vitamin E:

After feeding, the majority of the vitamin E incorporated into chylomicrons ultimately reaches the liver parenchymal cells transported in remnant lipoprotein particles. Little chylomicron vitamin E is stored in endothelial, stellate and Kupffer cells (Bjorneboe et al., 1987). Under fasting conditions, LDL and HDL transport most of plasma α-tocopherol and may also be an important source of plasma vitamin E for hepatic uptake. Whether intestine-derived HDL can also directly deliver α-tocopherol into liver cells remains to be established. Regardless on the plasma source of vitamin E, the liver is a major storage site of α-tocopherol, accounting for one-third of the total body content of this vitamin (Bjorneboe et al., 1986).

After hepatic uptake, the α-tocopherol form of vitamin E is preferentially resecreted into the circulation. α-Tocopherol transfer protein (α-TTP), a small cytoplasmic hepatic protein with differential affinity for various vitamin E forms, is responsible for the biodiscrimination process underlying the selective resecretion of α-tocopherol from the liver into plasma (Traber and Arai, 1999). Besides its function in vitamin E recycling into circulation, the liver plays a critical role in α-tocopherol disposal from the body. In fact, α-tocopherol and its oxidized metabolites as well as other vitamin E-derived compounds are mainly secreted into bile in mice (Mustacich et al., 1998). In addition, enterohepatic circulation of α-tocopherol seems to occur under physiological conditions (Lee-Kim et al., 1988; Bjorneboe et al., 1986).

2.4.2 Plasma lipoprotein transport of vitamin E:

Lipoproteins are the major, if not the only, carriers of plasma lipid-soluble antioxidants, including vitamin E. Indeed, plasma α-tocopherol levels are very well correlated with plasma lipid levels. In humans, relative lipoprotein distribution analysis

indicates that tocopherols are mostly transported in LDL and HDL at similar proportions with less than 20% carried in VLDL and other lipoproteins (Rigotti, 2007). However, VLDL accounts for one-third of total α-tocopherol levels after vitamin supplementation. This heterogeneous distribution of plasma vitamin E among different lipoprotein classes and the differential response to increased dietary intake suggest that measurements of total α-tocopherol levels may not be reliable to establish relationships of significant predictive value between plasma vitamin E concentration and a variety of physiological and disease conditions. Besides consumption due to oxidation as well as biotransformation and excretion, plasma total and lipoprotein vitamin E levels are determined by different metabolic processes, such as: (1) assembly and secretion in intestine and liver-derived lipoproteins (2) transfer between lipoproteins during remodeling in plasma, and (3) net uptake in different tissues. Thus, plasma vitamin E homeostasis is intimately connected to mechanisms underlying normal lipoprotein metabolism in vivo (Rigotti, 2007).

2.4.3 Lipoprotein lipase-mediated vitamin E metabolism:

Metabolic studies have shown a short half-life (about 12 min) for plasma α-tocopherol in circulation with predominant accumulation in liver, adipose tissue and skeletal muscle after 24 h intravenous injection of intestinal lymph labeled with radioactive vitamin E in rats (Bjorneboe et al., 1987). As chylomicron triglycerides are metabolized in the adipose tissue, muscle, and other organs, a fraction of α-tocopherol is released and taken up by these extrahepatic tissues. Lipoprotein lipase (LPL) seems to play a critical role in the delivery of α-tocopherol carried in triglyceride-rich lipoproteins to peripheral tissues. LPL may facilitate cellular α-tocopherol delivery due to direct LPL binding to the cell surface as a molecular bridge between the lipoprotein donor particle and the plasma membrane of the cells. Cellular uptakes vitamin E by LDL receptor superfamily and SR-BI.

2.5 Effect of vitamin E on lipid peroxidation

Vitamin E is incorporated into cellular membranes in which it effectively inhibits the peroxidation of lipids. Both tocopherols and tocotrienols scavenge the chain-propagating peroxyl radical. In membranes, the mobility of the molecule also becomes important, and this depends on the structure of the hydrophobic side chain. Vitamin E does not work in isolation from other antioxidants; rather it is part of an interlinking set of redox antioxidant cycles, which has been termed the “antioxidant network” (Fig. 2). Vitamin C can regenerate vitamin E directly and thiol antioxidants such as glutathione and lipoic acid can regenerate vitamin E indirectly via vitamin C.

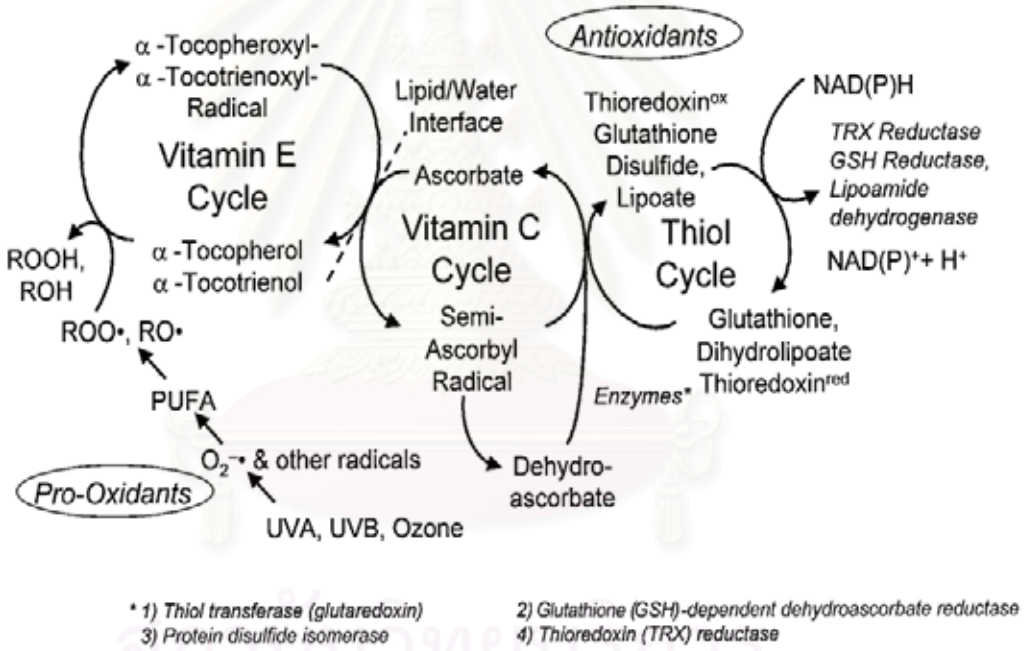


Fig. 2. The antioxidant network showing the interaction among vitamin E, vitamin C and thiol redox cycles (Packer et al., 2001).

Vitamin E is one of the most abundant lipid-soluble antioxidant agents found in plasma and cells of higher mammals. This vitamin exists as tocopherol and tocotrienol forms, which differ significantly in their biological activities due to variations in bioavailability as well as intrinsic antioxidant properties. Alpha-tocopherol is the most

relevant vitamin E form for human physiology, accounting for the majority of this vitamin present in mammalian tissues.

The antioxidant function of vitamin E is closely related selenium (Se). Selenium has been shown to act in aqueous cell media (cytosol and mitochondrial matrix) by destroying hydrogen peroxide and hydroperoxides via the enzyme glutathione peroxidase (GSH_{px}). In this capacity, it prevents oxidation of unsaturated lipid materials within cell, thus protecting fats within the cell membrane from breaking down. While the vitamin E prevents oxidation of other lipid materials to free radicals and per oxides within cells, thus protecting the cell membrane from damage (McDowell, 2000).

The activity of α-tocotrienol in scavenging peroxyl radicals is 1.5-fold higher in liposomes compared with α-tocopherol (Serbinova et al., 1991). In rat liver microsomes, the efficacy of α-tocotrienol to protect against Fe (II) NADPH-induced lipid peroxidation was 40 times higher than that of α-tocopherol. α-Tocotrienol also was 6.5 times more effective in the protection of cytochrome P-450 against oxidative damage. Several reasons have been suggested for the increased antioxidant activity of α-tocotrienol vs. α-tocopherol, focusing on the differences in the tail structure. The chromanoxyl radical of α-tocotrienol (α-tocotrienoxyl) has been found to be recycled in membranes and lipoproteins more quickly than the corresponding α-tocopheroxyl radical (Serbinova et al., 1991). Furthermore, α-tocotrienol has a stronger disordering effect on membranes than α-tocopherol and is distributed more uniformly within the membrane. These properties likely enhance the interaction of chromanols with lipid radicals (Serbinova et al., 1991; Suzuki et al., 1993).

The antioxidant efficacy of tocotrienols in membranes is higher than that of tocopherols, although their uptake and distribution after oral ingestion are less than that of α-tocopherol. In hamsters fed a mixture of vitamin E isoforms also containing tocotrienols, α-tocopherol was absorbed preferentially. However, tocotrienols could still be detected in the postprandial plasma of humans, and tocotrienols were found in all

classes of lipoproteins (Hayes et al., 1993). The liver contains a transfer protein that preferentially enriches VLDL with α-tocopherol (Arita et al., 1995). Therefore, α-tocopherol is secreted preferentially by the liver in a manner that discriminates between tocopherols and tocotrienols. Interestingly, the α-tocopherol transfer protein (α-TTP) preferentially selects α-tocopherol seems to explain why all other forms of vitamin E have a lower biological activity in the gestation-resorption assay compared with α-tocopherol.

The numerous studies have investigate and found the positive effect of vitamin E enriched diets on the susceptibility to lipoperoxidation in plasma (Sheehy et al., 1994; Fellenberg and Speisky, 2006) and tissues such as muscle (Bartov and Bornstein, 1981; Sheehy et al., 1994; Woodall et al., 1996; Fellenberg and Speisky, 2006), liver (Sheehy et al., 1994; Woodall et al., 1996; Surai and Sparks, 2000; Fellenberg and Speisky, 2006), heart (Sheehy et al., 1994; Surai and Sparks, 2000; Fellenberg and Speisky, 2006) and adipose tissue (Bartov and Bornstein, 1981; Fellenberg and Speisky, 2006).

Oxidation of lipid components in muscle tissues is the major cause of quality deterioration and short shelf life after slaughter. Thiobarbituric acid reactive substances (TBARS) value, expressed as MDA concentration, is a good indicator reflecting the degree of oxidation (Guo et al., 2001). Supplementation of α-tocopherol acetate decreased TBARS values of the breast meat (Nam et al., 1997; Galvin et al., 1998; Higgins et al., 1998; Chae et at., 2006) the thigh meat (Nam et al., 1997; Galvin et al., 1998; O'Neill et al., 1998; Guo et al., 2003) and the hepatic tissue (Guo et al., 2003) during storage at 4 ^{o}C and the breast meat (Higgins et al., 1998) and the thigh meat (O'Neill et al., 1998) during storage at -20 ^{o}C. Supplementation of Vitamin E significantly improved meat stability substantially by preventing oxidative deterioration during storage (Guo et al., 2001).

2.6 Effect of vitamin E on growth performance and vitamin E deposition in tissues.

Supplementation of α-tocopherol acetate improved weight gain significantly ($p<0.05$), with higher values in the 100 and 200 mg/kg α-tocopherol acetate fed group than the control (Chae et al., 2006) or tended to improve growth and feed utilization (Guo et al., 2001) but feed intake remained unaffected (Hsieh et al., 2002; Guo et al., 2001; Chae et al., 2006). Dietary supplementation with α-tocopherol acetate increased α-tocopherol concentration in breast meat (Galvin et al., 1998; O'Neill et al., 1998; Chae et al., 2006) thigh meat (Galvin et al., 1998; O'Neill et al., 1998) egg yolk (Cherian et al., 1996; Cherian and Sim, 1997; Sahin et al., 2006) adipose tissue (Cherian et al., 1996) liver and plasma (Guo et al., 2001) and showed a positive linear trend with the dietary levels supplemented (Cherian et al., 1996). Vitamin E concentration in breast and thigh meat increase about three times in supplemented of α-tocopherol acetate group than the control (Galvin et al., 1998; O'Neill et al., 1998). However, the levels of tocopherol in plasma and breast meat were not significant difference when supplementation of α-tocopheryl acetate at levels 100 and 200 mg/kg of feed (Chae et al., 2006). Supranutritional supplementation with a mixture of tocopherols (70%) and tocotrienols (30%) increased tocopherol deposition in muscle but lower than supplemented tocopherol only (Lanari et al., 2004).

2.7 Effect of vitamin E on drip loss of meat.

Supplementation of α-tocopheryl acetate in diet reduced drip loss in breast fillets (O'Neill et al., 1998), fresh pork (Monahan et al., 1994), fresh beef (Mitsumoto et al., 1995) and thawed pork (Asghar et al., 1991). Evidence suggests that the ability of α-tocopherol to reduce drip loss is related to its membrane stabilizing effects.

2.8 Crude palm oil

Palm oil (*Elaeis guineensis*) is currently the second largest traded edible oil and accounts for about one quarter of the world's fats and oil supply. The palm gives rise to

two distinct oils; palm oil from the mesocarp of the fruit, and palm kernel oil from the kernel. Crude palm oil and refined palm oil contain vitamin E at the level of 600-1000 and 470-670 ppm, respectively. The carotenoid content of crude palm oils varies between 500 and 700 ppm. A typical analysis of the carotenoids composition in crude palm oil shows that α- and β-carotenes are the major components (36 and 54%, respectively), and the rest are γ-carotene, lycopene and xanthopylls (Goh et al., 1985). Vitamin E in palm oil consists of various isomers of tocopherols and tocotrienols (Ng et al., 2004) as show in Table 2.1.

Table 2.1 Composition of vitamin E in crude palm oil.

Compound	%
α- tocopherol	36
α- tocomonoenol	4
α- tocotrienol	22
γ- tocotrienol	31
δ- tocotrienol	7

Source: Ng et al. (2004)

Vitamin E in the forms of α-tocopherol and γ-tocotrienol are predominant composition in crude palm oil. Tocopherol predominates in certain oil such as corn oil, soybean oil, and olive oil and tocotrienol predominates in palm oil and rice bran oil whereas coconut oil and lard are low in vitamin E (Table 2.2). Tocotrienols are found in the aleurone and subaleurone layers of cereal seeds and in palm oil. (Qureshi and Qureshi, 1993). The comparative level of vitamin E in crude palm oil and 2 sources of refined palm oil (Table 2.3) indicated that the crude palm oil and refined palm olein had higher average level of vitamin E than refined palm stearin (Gapor et al., 1983).

Table 2.2 Tocopherols(T_p) and tocotrienols(T_t) in different oils and fat.

Oil and Fat	Tocopherols (ppm)				Tocotrienols (ppm)				Total $T_p + T_t$ (ppm)
	α	β	γ	δ	α	β	γ	δ	
Corn oil	112	50	602	18	-	-	-	-	782
Soybean oil	101	-	593	264	-	-	-	-	958
Rice oil	124	40	50	-	184	21	570	-	989
Palm oil	279	-	61	-	274	-	398	69	1082
Olive oil	51	-	-	-	-	-	-	-	51
Coconut oil	5	-	-	6	5	1	19	-	36
Lard	12	-	7	-	7	-	-	-	26

Source: Qureshi and Qureshi (1993)

Table 2.3 Tocopherols(T_p) and tocotrienols(T_t) in crude palm oil and refined palm oil.

Oil	Total T_p and T_t (ppm)	
	Average	Range
Crude Palm Oil	3973	744 - 8191
Palm olein, refined	3391	1081 - 7122
Palm stearin, refined	1379	162 - 2408

Source: Gapor et al. (1983)

2.9 Effect of palm oil on lipid peroxidation.

Kang et al. (2001) evaluated the antioxidant potency of palm oil when fed laying hens a high polyunsaturated fatty acid (PUFA) diet with three levels of palm oil (PO) [low (LPO, 0%), medium (MPO, 1.5%), and high (HPO, 3.5%)] or a PUFA diet with tocopherol mix (control). Flax and fish oils were used as PUFA sources in all of the diets. Inclusion of tocopherols resulted in a lower ($P<0.05$) thiobarbituric acid reactive substances

(TBARS) in the control eggs, in white and dark meats. Among the PO treatments, eggs from HPO diet had the lowest TBARS ($P<0.05$). A higher ($P<0.05$) TBARS was observed for eggs, meat, and liver from hens on the LPO diet. No significant difference was observed between the TBARS of LPO and MPO dark meat. Supplementation of PO (MPO and HPO) also resulted in an increase in tocopherol and tocotrienol contents in egg, liver, and meat of MPO and HPO (Kang et al., 1998). Thus, the lower ($P<0.05$) TBA values for MPO and HPO coincided with the content of intrinsic antioxidants available to the bird.

2.10 Effect of crude palm oil on growth performances.

Valencia et al. (1993) evaluated the utilization of crude palm and palm kernel oil compare with poultry oil in broiler diet at levels 0, 2, 4, 6 and 8% at 0-21 day and 22-42 day of age. They found that no significant differences in 21 day body weight or feed conversion ratio among broilers fed diets but not for 42 day. Broilers fed diets supplemented with crude palm kernel oil were significantly heavier and utilized their feed more efficiently than broilers fed diets supplemented with crude palm oil or poultry oil. There were not significant differences in mortality rate and dressing percentage of broilers fed the different oils, however, the weight and percentage of abdominal fat of broilers fed the crude palm kernel oil were significantly greater than the broilers fed crude palm oil or poultry oil. Increasing the level of supplemental oil resulted in a significant improvement in both 21 and 42 day body weight. There were significant improvements in feed utilization at both 21 and 42 days as the level of supplemental oil increased. Level of supplemental oil had no effect on mortality during the study. Dressing percentage was not significantly affected by level of supplemental oil; however, the weight and percentage of abdominal fat was significantly increased as level of supplemental oil increased.

2.11 Effect of crude palm oil on vitamin E concentration in blood and tissues.

Areerob P. (2004) evaluated crude palm oil (CPO) on vitamin E concentration in blood, adipose tissue, liver and egg yolk. Diets included CPO at the level of 2, 3 and 4% can enhance ($p<0.05$) total tocopherols and total tocotrienols, especially 3% CPO and 4% CPO groups. Hens fed on 4% CPO had the lowest total tocopherols in egg yolk and adipose tissue but had the highest tocotrienol in plasma, egg yolk and adipose tissue when compared to the other groups. Similar results were reported by Kang et al. (1998) when the treatment diets included palm oil (PO) at the level of 0, 1.5 and 3.5% respectively. Incorporation of PO increased the α-and γ-tocotrienol content of eggs, liver, meat and adipose tissue. Total tocotrienols were higher in eggs, liver and adipose tissue of hens fed 1.5% PO than those fed 3.5% PO. Dark meat contained higher levels of tocopherols and tocotrienols than white meat. Addition of tocopherol mix, contained 17.3, 36.3 and 7.3% of δ-, γ- and α-tocopherol respectively, in the control diet increased total tocopherol concentration in all tissues.

CHARPTER III

MATERIALS AND METHODS

This study was approved by Animal Care and Use Committee of the Faculty of Veterinary Science, Chulalongkorn University.

3.1 Animals and management

Total 576 day old male (Ross 308) broiler chicks were randomly allocated into 6 treatments which composed of 6 replicates of 16 birds each. Initial weights of chicks in each group were not significant difference and raised on the pen size 150 x 100 x 90 centimeters or 10 chicks/m^2 in open-sided housing. Size of pen was adjusted to maintain constant density when chicken died. Chicks were subjected to artificial fluorescent illumination for 11 hour/day. All chicks received Newcastle-bronchitis vaccine (BIO LA SOTA + H120®) and infectious bursal disease vaccine (Izovac GUMBORO 3®) on day 1 and day 14, respectively. Diets and water were fed *ad libitum* through out the experimental period.

3.2 Feed and feeding

Corn-soybean meal basal diets with 8% lard (LO) was used as a control diet. Crude palm oil (CPO) was substituted for LO at the level of 2, 4, 6 and 8%. Positive control was conducted by supplementation of vitamin E synthesis, α-tocopheryl acetate, at the level of 100 mg/kg control diet (Table 3.1). Diets were calculated to meet Ross requirement (Ross, 1999). Metabolizable energy (ME) value of LO and CPO used for formulation were the same value, 8,600 kcal/g (Brake et al., 2002). Corn-soy based diet without fat, control diet with 8%LO, and diet with 8%CPO were analyzed for vitamin E concentration (Ikeda et al., 2001) in both starter and finisher periods (Table 3.2 and 3.3). Analysis data of vitamin E in 8% LO(control diet) was used for adjusting the level of vitamin E according to recommended requirement (50 mg/kg diet) by supplementation

of α-tocopheryl acetate. Vitamin E levels in the remained diets of both periods were calculated base on analytical data. The ingredients composition, nutritional contents, calculated vitamin E in both periods were showed in Tables 3.4 and 3.5. The birds were fed with starter and finisher diets for a period of 0-3 and 4-6 weeks, respectively.

Table 3.1 The experiment diets.

Treatments	Description
1. Control (C)	Corn-soybean diet containing 8 %lard
2. Positive control (C+E)	Control diet plus α-tocopheryl acetate 100 mg/kg
3. Crude palm oil 2 (CPO2)	Corn-soybean diet containing lard 6 % and crude palm oil 2 %
4. Crude palm oil 4 (CPO4)	Corn-soybean diet containing lard 4 % and crude palm oil 4 %
5. Crude palm oil 6 (CPO6)	Corn-soybean diet containing lard 2 % and crude palm oil 6 %
6. Crude palm oil 8 (CPO8)	Corn-soybean diet containing crude palm oil 8 %

Table 3.2 Analytical composition of vitamin E in basal diet without fat, lard, crude palm oil, 8%LO diet and 8%CPO diet in starter period before supplemented with α-tocopheryl acetate[1].

Compound	Basal diet without fat (mg/kg)	LO (mg/kg)	CPO (mg/kg)	Starter diets	
				8% LO (mg/kg)	8% CPO (mg/kg)
Tocopherol					
α	10.47	7.43	242.89	10.59	26.23
β	0.27	0.00	2.94	0.25	0.51
γ	14.40	0.47	1.21	12.97	15.97
δ	1.19	0.00	0.43	1.08	1.32
Total tocopherol	26.33	7.90	247.47	24.89	44.03
Tocotrienol					
α	2.28	0.43	215.29	2.12	16.11
β	0.00	0.00	14.77	0.00	1.31
γ	2.81	0.44	296.65	2.43	23.28
δ	0.29	0.00	104.53	0.30	8.86
Total tocotrienol	5.38	0.87	631.24	4.85	49.56
Total vitamin E	31.71	8.77	878.71	29.74	93.59

[1] Experimental diets were supplemented with α-tocopheryl acetate at the levels of 20.26 mg/kg diet.

Table 3.3 Analytical composition of vitamin E in basal diet without fat, lard, crude palm oil, 8%LO diet and 8%CPO diet in finisher period before supplemented with α-tocopheryl acetate[1].

Compound	Basal diet without fat (mg/kg)	LO (mg/kg)	CPO (mg/kg)	Finisher diets	
				8% LO (mg/kg)	8% CPO (mg/kg)
Tocopherol					
α	14.59	12.98	222.91	11.99	29.45
β	0.41	0.04	2.91	0.34	0.58
γ	18.30	0.77	1.49	15.15	15.59
δ	1.43	0.00	0.00	1.21	1.31
Total tocopherol	34.73	13.79	227.30	28.69	46.93
Tocotrienol					
α	3.28	0.89	203.02	2.78	20.12
β	0.00	0.00	20.11	0.00	1.48
γ	3.53	1.30	276.23	2.83	26.87
δ	0.31	0.00	99.00	0.35	11.50
Total tocotrienol	7.12	2.19	598.36	5.96	59.97
Total vitamin E	41.85	15.98	825.66	34.65	106.90

[1] Experimental diets were supplemented with α-tocopheryl acetate at the levels of 15.35 mg/kg diet.

Table 3.4 Ingredients and composition of the experimental diet in the starter period.

Ingredient	Amount (kg/100 kg diet)					
	C	C+E	CPO2	CPO4	CPO6	CPO8
Corn	40.7	40.7	40.7	40.7	40.7	40.7
Soybean meal	42.5	42.5	42.5	42.5	42.5	42.5
Defatted rice barn	2.0	2.0	2.0	2.0	2.0	2.0
Zeolite	2.4	2.4	2.4	2.4	2.4	2.4
Limestone	1.8	1.8	1.8	1.8	1.8	1.8
Mono-dicalcium phosphate	1.6	1.6	1.6	1.6	1.6	1.6
Salt	0.5	0.5	0.5	0.5	0.5	0.5
DL-methionine	0.2	0.2	0.2	0.2	0.2	0.2
Premix[1]	0.4	0.4	0.4	0.4	0.4	0.4
Lard	8.0	8.0	6.0	4.0	2.0	0.0
Crude palm oil	0.0	0.0	2.0	4.0	6.0	8.0
Chemical analysis (%)						
Dry matter	91.53	92.21	92.11	92.17	91.79	92.08
Crude protein	22.91	22.95	22.94	23.00	22.90	22.93
Crude fat	9.97	9.99	9.96	9.97	9.95	10.00
Crude fiber	3.70	3.68	3.73	3.67	3.65	3.74
Ash	8.55	8.67	8.72	8.70	8.78	8.66
Calcium	1.11	1.12	1.09	1.07	1.05	1.10
Phosphorus	0.76	0.76	0.77	0.76	0.74	0.74
ME (kcal/kg)[2]	3100	3100	3100	3100	3100	3100
Calculated vitamin E[3] (mg/kg)	50.00	150.00	67.53	84.93	102.33	113.85
Tocopherol(T_p) : Tocotrienol(T_t)	9.0:1	28.9:1	2.8:1	1.8:1	1.4:1	1.2:1

[1] Supplied per kilogram of diet: retinol, 14,000 IU; cholecalciferol, 5,000 IU; menadione, 4 mg; thiamin, 3 mg; riboflavin, 8 mg; nicotinic acid, 70 mg; D-calcium pantothenate, 20 mg; pyridoxine, 4 mg; biotin, 0.15 mg; folic acid, 2.00 mg; cyanocobalamin, 0.016 mg; choline chloride, 1,800 mg; Cu, 8 mg; Fe, 80 mg; Mn, 100 mg; Mo, 1 mg; Se, 0.15 mg; Zn, 80 mg.

[2] Calculated analysis.

[3] Calculated vitamin E base on chemical analysis data.

Table 3.5 Ingredients and composition of the experimental diet in the finisher period.

Ingredient	Amount (kg/100 kg diet)					
	C	C+E	CPO2	CPO4	CPO6	CPO8
Corn	47.4	47.4	47.4	47.4	47.4	47.4
Soybean meal	36.2	36.2	36.2	36.2	36.2	36.2
Defatted rice barn	4.0	4.0	4.0	4.0	4.0	4.0
Limestone	1.9	1.9	1.9	1.9	1.9	1.9
Mono-dicalcium phosphate	1.4	1.4	1.4	1.4	1.4	1.4
Salt	0.4	0.4	0.4	0.4	0.4	0.4
DL-methionine	0.2	0.2	0.2	0.2	0.2	0.2
Premix[1]	0.4	0.4	0.4	0.4	0.4	0.4
Lard	8.0	8.0	6.0	4.0	2.0	0.0
Crude palm oil	0.0	0.0	2.0	4.0	6.0	8.0
Chemical analysis (%)						
Dry matter	91.33	91.14	91.00	91.62	90.85	91.01
Crude protein	20.83	20.89	20.96	21.02	20.95	20.85
Crude fat	10.33	10.32	10.30	10.29	10.30	10.30
Crude fiber	3.69	3.69	3.66	3.71	3.69	3.66
Ash	6.96	6.77	6.80	6.99	6.73	6.76
Calcium	1.09	1.07	1.10	1.08	1.09	1.10
Phosphorus	0.75	0.74	0.73	0.75	0.75	0.74
ME (kcal/kg)[2]	3200	3200	3200	3200	3200	3200
Calculated vitamin E[3] (mg/kg)	50.00	150.00	71.33	87.52	103.72	122.25
Tocopherol(T_p) : Tocotrienol(T_t)	7.2:1	22.1:1	2.8:1	1.9:1	1.4:1	1.2:1

[1] Supplied per kilogram of diet: retinol, 11,000 IU; cholecalciferol, 5,000 IU; menadione, 3 mg; thiamin, 2 mg; riboflavin, 6 mg; nicotinic acid, 70 mg; D-calcium pantothenate, 20 mg; pyridoxine, 3 mg; biotin, 0.15 mg; folic acid, 1.75 mg; cyanocobalamin, 0.016 mg; choline chloride, 1,600 mg; Cu, 8 mg; Fe, 80 mg; Mn, 100 mg; Mo, 1 mg; Se, 0.15 mg; Zn, 80 mg.

[2] Calculated analysis.

[3] Calculated vitamin E base on chemical analysis data.

3.3 Data and Sample Collection

The temperature and relative humidity were recorded at 8:00 am, 12:00 am 16:00 pm. The average temperatures were 29.3 ± 1.31°C, 33.7 ± 1.85°C and 31.2 ± 2.33°C, respectively and relative humidity were 68.9 ± 9.56%, 49.0 ± 7.74% and 57.8 ± 10.99%, respectively. The range of the temperature and relative humidity in period of experimental period were 35.3 ±1.73°C; 85.1 ± 7.74% and 26.3 ± 0.67°C; 41.6 ± 6.03% for maximum and minimum, respectively. The broilers were weighed at 0, 21 and 42 days old. The feed intake was recorded between days 0 to 21 and days 22 to 42. Mortality were recorded daily of experimental period. The body weight and feed intake were used to calculate growth performance.

Six broilers in each treatment group (1 chick/replicate) at 21 and 42 days old were randomly selected. Schedule for sample collection were shown in Figure 3.1.

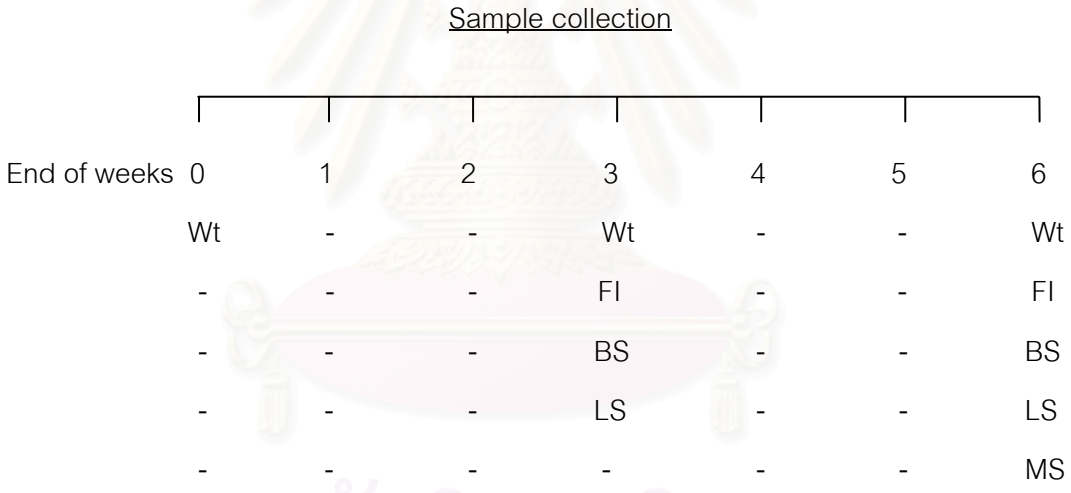


Fig. 3.1. Diagram showing the whole period of the experiment.

Where:

Wt = broiler weighing

FI = feed intake record

BS = blood sampling

LS = liver sampling

MS = meat sampling

- breast meat
- thigh meat

At the end of weeks 3rd, Blood sampling were collected via wing vein into a heparinized graduated centrifuge tube and sacrificed with an overdose of sodium pentobarbital (100 mg/kg BW) using 22G, 1.5 inch needle by intravenous injection into the jugular vein. Then, carcass was opened and liver was collected. At the end of weeks 6th, blood sample were collected into a heparinized graduated centrifuge tube and centrifuged at 1,500 rpm for 5 minutes and plasma was collected and stored at -80 °C. Chicks were transported to the local slaughter house, which was certified by Department of Livestock Development, Ministry of Agriculture and Cooperatives, Thailand. Chicks were fasted for 8 hour and slaughtered by conventional methods. Liver, breast and thigh meat were collected. The right of breast and thigh meat were cut to four sections and each section placed on polystyrene tray, under layed with an absorbent pad and wrapped with PVC film. The samples were stored at 8 °C for up to 8 day. At day 2, 4, 6 and 8 breast and thigh meat were weighed and the drip loss at each time point was calculated as the percentage reduction in weight relative to the initial weight (day 0). The left of breast and thigh meat were separated to four sections. One section of the left of breast and thigh meat and liver were collected in plastic bag and kept at -20 °C until an analysis for vitamin E. Three sections of the left of breast and thigh meat were placed on polystyrene trays, layed with an absorbent pad and wrapped with PVC film. The samples were stored at 8 °C for up to 7 day. At day 0, 4 and 7 breast and thigh meat sample were take off from storage room and kept in freezer at -80 °C until an analysis for lipid peroxidation.

3.4 Sample analysis

Diets were analyzed for nutritional content by proximate analysis (AOAC, 1990). Plasma was analyzed for the TBARS value (Feix et al., 1991). Tissues (liver, breast and thigh meat) were analyzed for the TBARS value (Salih et al., 1978) and vitamin E concentration (Ikeda et al., 2001).

Determination of thiobarbituric acid-reactive substances (TBARS) in plasma.

The TBARS values were determined on plasma samples as described by Feix et al. (1991) as follows: 600 µL of sample, 120 µL of Butylate Hydroxytuluene (BHT, 50 nmol/L) and 1,800 µL of trichloric acetic acid (TCA 10%) were mixed and incubated for 10 min at 4 °C and then centrifuged for 10 min at 600 rpm and 1,500 µL of the supernatant were mixed with 1,500 µL of thiobarbituric acid 5%. The mixture was heated for 15 min in boiling water, cooled and read at the wavelength at 532 nm using UV-VIS spectrophotometer (Shimudsu® UV-160 A, double beam). The TBARS values were expressed as nmols of MDA per milliliter. The standard curve was plotted using the 1, 1, 3, 3– tetraethoxypropane at 0, 2, 4, 6, 8 and 10 nmols/mL.

Determination of thiobarbituric acid-reactive substances (TBARS) in tissue.

The TBARS values of tissue samples were determined as described by Salih et al. (1987), with some modifications by Cherian et al (1996). Tissue samples (2g) were weighed into 50 mL test tubes, and 18 mL of 3.86% perchloric acid was added. The samples were homogenized with a Polytron (Polytron PT-MR3100, KINEMATICA AG, Littau-Switzerland) for 15 s at high speed (5000 rpm). The BHT solution (1mL) was added to each sample during homogenization to control lipid oxidation. The homogenate was filtered through Whatman filter paper NO 1. Two mL of filtrate was mixed with 2 mL of 20 mM TBA in distilled water, and incubated in a boiling water bath for 30 min and cooled for 45 min. Absorbance was determined at 531 nm using UV-VIS spectrophotometer (Shimudsu® UV-160 A, double beam). The standard curve was plotted using the malondialdehyde tetrabutylammonium salt. The TBARS values were expressed as milligrams of malondialdehyde per kilogram of tissue and calculated as follows:

TBARS values = (MDA concentration from standard curve) x K

where K = molecular weight of MDA x 100/%recovery x 10^{-2}/1

= 72 x 100/93 x 10^{-2}

= 0.77

Determination of tocopherols and tocotrienols in feed and oil.

The tocopherols and tocotrienols of feed samples were determined as described by Ikeda et al. (2001). Feed samples (0.2 g) were weighed into 15 mL centrifuge tube, 0.1 mL of sodium chloride and 1 mL of pyrogallol in ethanol (60g/L) were added and vortexed for 1 min followed by 0.2 mL of 2,2,5,7,8-pentamethyl-6-chromanol (PMC; internal standard, 25 µg/mL) and vortexed for 1 min. Then, 0.2 mL of potassium hydroxide (600 g/L) was added, vortexed for 1 min, sonicated 5 min and saponified at 70°C for 30 min. Afterwards, 4.5 mL of sodium chloride (20 g/L) was added and tocopherols and tocotrienols were extracted with 3 mL of 10% ethyl acetate in hexane (v/v) and vortexed for 1 min. the sample tubes were centrifuged at 4,000 rpm for 15 min at 4°C. The upper layer (1mL) was pipetted into test tube and evaporated under a stream of nitrogen. The residue samples were redissolved with hexane (1mL), vortexed and filtrated through 0.45 µm PTFE syringe filter. Concentration of tocopherols and tocotrienols were determined using High Performance Liquid Chromatography (HPLC) (Waters Corporation, Milford, MA, USA). All samples were detected using fluorescence (Waters 2475 Multi fluorescence detector) at 290 nm excitation and 330 nm emission. The analytical column was Mightysil Si 60 (250 x 4.6 mm), the mobile phase was hexane containing 4 % (v/v) dioxane and 0.2 % (v/v) isopropanol, flow rate was 2 mL/min and run time was 18 min (Ueda and Igarashi, 1987). Recovery of tocopherol and tocotrienol concentration in this study was 95%.

To determine vitamin E concentration in oil, 0.1 g of oil was employed with vitamin E assay describes above.

Determination of tocopherols and tocotrienols in tissues.

The tocopherols and tocotrienols of tissue samples were determined as described by Ikeda et al. (2001). In brief: tissue were ground into small pieces (<ca. 1 mm) and weighed (0.2 g) into 15 mL centrifuge tube, and added 0.1 mL of sodium chloride and vortexed for 30 second. Then, 1 mL of pyrogallol in ethanol (60g/L) was added and vortexed for 1 min, 0.1 mL of 2,2,5,7,8-pentamethyl-6-chromanol (PMC; internal standard, 30 µg/mL) and 0.1 mL of ethanol were added and vortexed for 1 min. Then, 0.2 mL of potassium hydroxide (600 g/L) was added, vortexed for 1 min, sonicated 5 min and saponified at 70°C for 30 min and cooled 15 min in room temperature. Afterwards, 4.5 mL of sodium chloride (20 g/L) was added and vortexed for 1 min. Tocopherols and tocotrienols were extracted with 3 mL of 10% ethyl acetate in hexane (v/v) and vortexed for 1 min. The sample tubes were centrifuged at 4,000 rpm for 15 min at 4°C. The upper layer was pipetted into test tube and evaporated under a stream of nitrogen. The residue samples were redissolved with hexane (1mL), vortexed and filtrated through 0.45 µm PTFE syringe filter. Concentration of tocopherols and tocotrienols were determined using HPLC as described in feed assay (Ueda and Igarashi, 1987). Recovery of tocopherol and tocotrienol concentration in this study was 101%.

3.5 Statistical analysis

The growth performance data, the TBARS values in plasma, tissue and vitamin E concentration in tissues were analyzed using one-way analysis of variance (ANOVA) to determine the effects of treatments. Significant differences among means were compared with Duncan's New Multiple Range Test. The level of significant difference was set at $P<0.05$. The level of vitamin E in crude palm oil was linear regressed on vitamin E concentration in tissue. Statistical analysis was performed using the GLM procedure of SAS software (SAS Institute Inc., 2002).

CHARPTER IV

RESULTS

4.1 Effect of vitamin E in crude palm oil on growth performance.

The effects of various treatments on growth performance of broilers were shown in Table 4.1 and 4.2. Body weight, both initial and final body weight, was not significant differences. In starter and finisher periods (day 0-21 and day 22-42), there were not treatment effect on the weight gain, feed intake, average daily gain and feed conversion ratio among groups in each period. However, CPO2 group had higher the weight gain and average daily gain than the other groups, but was not significant difference.

At the overall period (day 0 - 42), there was also not significant differences in weight gain, feed intake, average daily gain feed conversion ratio and mortality rate among groups of the broilers.

Table 4.1 Effect of vitamin E in crude palm oil on growth performance of the broilers[1].

Item	Treatments[2]						Pooled	P-value
	C	C+ E	CPO2	CPO4	CPO6	CPO8	SE	
Body weight (g/bird)								
Initial body weight	47.16	47.26	47.65	47.26	47.65	47.06	0.119	0.6110
Final body weight	2759.48	2769.30	2830.68	2722.16	2753.69	2673.29	14.910	0.0544
Weight gain (g/bird)								
Day 0 - Day 21	826.33	832.00	832.50	840.33	841.33	807.50	4.112	0.1778
Day 22 - Day 42	1868.50	1849.00	1880.50	1830.17	1814.00	1791.67	14.137	0.4751
Day 0 - Day 42	2694.50	2681.00	2713.00	2670.50	2655.50	2599.17	14.341	0.2820
Feed intake (g/bird)								
Day 0 - Day 21	984.83	1000.17	992.17	1012.33	1018.33	1003.50	3.909	0.1248
Day 22 - Day 42	3411.50	3422.17	3474.50	3369.33	3378.83	3386.17	17.995	0.6006
Day 0 - Day 42	4396.50	4422.17	4467.00	4381.50	4397.50	4389.67	18.908	0.8282

[1]The number of birds per replicate = 16.

[2] Treatments were C: control; C+E: control + α-tocopheryl acetate100 mg/kg; CPO2: crude palm oil 2%; CPO4: crude palm oil 4%; CPO6: crude palm oil 6%; CPO8: crude palm oil 8%.

Table 4.2 Effect of vitamin E in crude palm oil on growth performance of the broilers[1] (continuous).

Item	Treatments[2]						Pooled SE	P-value
	C	C+ E	CPO2	CPO4	CPO6	CPO8		
Average daily gain (g/bird/day)								
Day 0 - Day 21	39.35	39.63	39.64	40.01	40.07	38.46	0.196	0.1824
Day 22 - Day 42	89.60	88.05	90.71	87.38	86.38	85.32	0.626	0.1169
Day 0 - Day 42	64.16	63.84	64.60	63.58	63.22	61.89	0.342	0.2818
Feed conversion ratio								
Day 0 - Day 21	1.19	1.20	1.20	1.22	1.22	1.25	0.007	0.3560
Day 22 - Day 42	1.83	1.83	1.83	1.84	1.87	1.89	0.010	0.3505
Day 0 - Day 42	1.63	1.65	1.65	1.65	1.66	1.69	0.007	0.3457
Mortality rate (%)								
Day 0 - Day 21	0.00	1.04	3.13	2.08	1.04	3.13	0.640	0.6921
Day 22 - Day 42	1.11	1.11	4.45	2.22	4.45	2.22	0.764	0.6841
Day 0 - Day 42	1.11	2.22	7.78	4.45	5.56	5.56	1.029	0.4797

[1]The number of birds per replicate = 16.

[2]Treatments were C: control; C+E: control + α-tocopheryl acetate100 mg/kg; CPO2: crude palm oil 2%; CPO4: crude palm oil 4%; CPO6: crude palm oil 6%; CPO8: crude palm oil 8%.

4.2 Effect of vitamin E in crude palm oil on tocopherols and tocotrienols concentration in liver.

The effect of vitamin E in crude palm oil on tocopherols and tocotrienols concentration in liver of the broilers was shown in Table 4.3. Broilers fed on C+E had higher α-tocopherol concentration in liver ($P<0.001$) than other groups. While, broilers fed on CPO8 had higher ($P<0.05$) β-tocopherol concentration in liver than other groups. However, the concentration of γ- and δ-tocopherol in the liver were not significant difference among groups. Total tocopherol concentration of C+E group was highest ($P<0.001$) compared to other groups whereas CPO2, CPO4 and CPO6 had higher total tocopherol concentration than C group ($P<0.001$). Alpha-tocotrienol ($P<0.05$) and total tocotrienol concentration ($P<0.01$) were highest in broilers fed CPO8 group, but the concentration of β, γ and δ forms of tocotrienol in liver were not significant difference among groups. An increasing of total vitamin E in the form of tocopherol increased the total tocopherol concentration in liver while increasing in tocotrienol from CPO also increased the total tocotrienol concentration in liver compared to control group.

For total vitamin E concentration in liver, broiler fed on C+E was highest value(12.36 μg/g) compared to other groups ($P<0.001$), but it was not significant difference from CPO8 (11.36 μg/g) groups. CPO2, CPO4 and CPO6 had higher total vitamin E concentration than C ($P<0.001$). An increasing of total vitamin E in diet by inclusion CPO and supplementation α–tocopheryl acetate was increased total vitamin E concentration in liver compared to control group. Alpha form of both tocopherol and tocotrienol was major form followed by γ-form of vitamin E deposited in liver of broiler.

Table 4.3 Effect of vitamin E in crude palm oil on tocopherols (T_p) and tocotrienols (T_t) concentration in liver[1].

Item	Treatments[2]						Pooled SE	P-value
	C	C+E	CPO2	CPO4	CPO6	CPO8		
Tocopherol (μg/g)								
α	3.9390 ^{d}	11.5080 ^{a}	5.4170 ^{cd}	6.5160 ^{c}	5.7280 ^{cd}	8.4781 ^{b}	0.696	0.0002
β	0.0119 ^{bc}	0.0091 ^{c}	0.0200 ^{ab}	0.0240 ^{a}	0.0168 ^{abc}	0.0241 ^{a}	0.002	0.0294
γ	0.9916	0.6877	1.1564	1.1585	0.7494	0.9916	0.068	0.2865
δ	0.0390	0.0313	0.0349	0.0356	0.0507	0.0382	0.003	0.7009
total	4.9820 ^{d}	12.2360 ^{a}	6.6280 ^{c}	7.7341 ^{c}	6.5451 ^{c}	9.5170 ^{b}	0.569	<0.0001
Tocotrienol (μg/g)								
α	0.2635 ^{b}	0.1125 ^{b}	0.2960 ^{b}	0.5290 ^{b}	0.6583 ^{b}	1.7829 ^{a}	0.173	0.0389
β	0.0138	0.0110	0.0142	0.0280	0.0263	0.0317	0.004	0.5529
γ	0.0198	0.0038	0.0089	0.0474	0.0197	0.0259	0.005	0.1344
δ	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	-
total	0.2971 ^{b}	0.1272 ^{b}	0.3192 ^{b}	0.6044 ^{b}	0.7044 ^{b}	1.8405 ^{a}	0.162	0.0027
Total vitamin E	5.2788 ^{c}	12.3635 ^{a}	6.9473 ^{b}	8.3385 ^{b}	7.2495 ^{b}	11.3574 ^{a}	0.618	<0.0001

[1]The number of tissue sample per treatment = 3 (pool sample: 2 replicates = 1 sample).

[2] Treatments were C: control; C+E: control + α-tocopheryl acetate100 mg/kg; CPO2: crude palm oil 2%; CPO4: crude palm oil 4%; CPO6: crude palm oil 6%; CPO8: crude palm oil 8%.

[a,b,c,d] Means in a row with different superscripts are significantly different($P < 0.05$).

4.3 Effect of vitamin E in crude palm oil on tocopherols and tocotrienols concentration in breast meat.

The effect of vitamin E in crude palm oil on tocopherols and tocotrienols concentration in breast meat of the broilers was shown in Table 4.4. Broilers fed on C+E had the highest α-tocopherol ($P<0.05$) and total tocopherol concentration ($P<0.05$) in breast meat. The α-tocopherol and total tocopherol concentration of the diets included with CPO was not significant differences compared with C group, but tended to increase the total tocopherol concentration. However, the concentration of β-, γ-, δ-and total tocopherol in the breast meat were not significant difference among groups. Broilers fed on CPO8 had higher α-, γ-, δ-, total tocotrienol ($P<0.001$) and β- ($P<0.01$) tocotrienol concentration in breast meat than other groups. An increasing in tocotrienol from CPO increased total and all forms of tocotrienol in breast meat over control and significant difference was found in CPO8 fed group.

For total vitamin E concentration in the breast meat of broiler was significant difference ($P<0.05$) among groups. Broiler fed C+E had the highest ($P<0.05$) total vitamin E concentration, but it was not significant difference from CPO6 and CPO8 groups. An increasing of total vitamin E in diet by inclusion CPO and supplementation α–tocopheryl acetate was increased total vitamin E in breast meat compared to control group. Alpha form of tocopherol and tocotrienol was also the major forms followed by γ-form of vitamin E deposited in breast meat of broiler.

Table 4.4 Effect of vitamin E in crude palm oil on tocopherols and tocotrienols concentration in breast meat[1].

Item	Treatments[2]						Pooled SE	P-value
	C	C+E	CPO2	CPO4	CPO6	CPO8		
Tocopherol (µg/g)								
α	2.1856 ^{b}	4.6797 ^{a}	2.5205 ^{b}	2.5377 ^{b}	2.8416 ^{b}	3.2331 ^{b}	0.268	0.0235
β	0.0059	0.0010	0.0049	0.0069	0.0063	0.0075	0.001	0.0926
γ	0.5597	0.3154	0.5280	0.5136	0.3805	0.3887	0.030	0.0692
δ	0.0216	0.0033	0.0000	0.0223	0.0241	0.0112	0.004	0.4047
total	2.7727 ^{b}	4.9994 ^{a}	3.0534 ^{b}	3.0805 ^{b}	3.2526 ^{b}	3.6405 ^{b}	0.231	0.0340
Tocotrienol (µg/g)								
α	0.1696 ^{d}	0.1189 ^{d}	0.2274 ^{d}	0.4508 ^{c}	0.5942 ^{b}	0.7963 ^{a}	0.061	<0.0001
β	0.0064 ^{b}	0.0082 ^{b}	0.0068 ^{b}	0.0342 ^{a}	0.0363 ^{a}	0.0370 ^{a}	0.004	0.0013
γ	0.0367 ^{c}	0.0250 ^{c}	0.0570 ^{c}	0.1085 ^{b}	0.1401 ^{b}	0.1907 ^{a}	0.015	<0.0001
δ	0.0000 ^{b}	0.0000 ^{b}	0.0000 ^{b}	0.0000 ^{b}	0.0000 ^{b}	0.0434 ^{a}	0.004	<0.0001
total	0.2127 ^{de}	0.1522 ^{e}	0.2912 ^{d}	0.5935 ^{c}	0.7706 ^{b}	1.0675 ^{a}	0.081	<0.0001
Total vitamin E	2.9853 ^{c}	5.1515 ^{a}	3.3447 ^{bc}	3.6740 ^{bc}	4.0231 ^{abc}	4.7080 ^{ab}	0.238	0.0374

[1]The number of tissue sample per treatment = 3 (pool sample: 2 replicates = 1 sample).

[2] Treatments were C: control; C+E: control + α-tocopheryl acetate100 mg/kg; CPO2: crude palm oil 2%; CPO4: crude palm oil 4%; CPO6: crude palm oil 6%; CPO8: crude palm oil 8%.

[a,b,c,d] Means in a row with different superscripts are significantly different($P < 0.05$).

4.4 Effect of vitamin E in crude palm oil on tocopherols and tocotrienols concentration in thigh meat.

The effect of vitamin E in crude palm oil on tocopherols and tocotrienols concentration in thigh meat of the broilers was shown in Table 4.5. Broilers fed on C+E had higher α-tocopherol concentration ($P<0.05$), but lower β- ($P<0.01$) and γ- ($P<0.01$) tocopherol concentration in thigh meat than other groups. However, the concentration of δ-tocopherol in the thigh meat was not significant difference among groups. Total tocopherol concentration showed the same result as α-tocopherol.

The concentration of tocotrienol in thigh meat was significant difference. Broilers fed on CPO8, CPO6 and CPO4 had higher ($P<0.001$) α-, β-, γ- and δ-tocotrienol concentration in thigh meat than C, C+E and CPO2 and CPO2 had higher γ-tocotrienol than C and C+E groups. Total tocotrienol concentration of CPO8 (2.49 µg/g) group was highest ($P<0.001$) compared to other groups, but it was not significant difference from CPO4 (2.05 µg/g) and CPO6 (2.26 µg/g) groups. An increasing in tocotrienol from CPO increased total and all forms of tocotrienol in breast meat over control and significant difference was found in CPO8 fed group.

Total vitamin E concentration in thigh meat of broiler fed on C+E was highest ($P<0.0119$) compared to other groups, but it was not significant difference from CPO treatments. An increasing of total vitamin E in diet by inclusion CPO and supplementation α–tocopheryl acetate was increased total vitamin E in thigh meat compared to control group. Alpha form of tocopherol and tocotrienol was also the major form followed by γ-form of vitamin E deposited in thigh meat of broiler.

Table 4.5 Effect of vitamin E in crude palm oil on tocopherols and tocotrienols concentration in thigh meat[1].

Item	Treatments[2]						Pooled SE	P-value
	C	C+E	CPO2	CPO4	CPO6	CPO8		
Tocopherol (µg/g)								
α	3.7960 [b]	9.1350 [a]	5.4440 [b]	5.7010 [b]	5.9590 [b]	6.0480 [b]	0.533	0.0130
β	0.0104 [a]	0.0044 [b]	0.0153 [a]	0.0165 [a]	0.0150 [a]	0.0104 [a]	0.001	0.0068
γ	0.9891 [ab]	0.6818 [c]	1.1880 [a]	1.1305 [a]	0.8178 [bc]	0.6983 [c]	0.055	0.0016
δ	0.0363	0.0126	0.0360	0.0380	0.0324	0.0246	0.004	0.2758
total	4.8318 [b]	9.8339 [a]	6.6833 [b]	6.8857 [b]	6.8241 [b]	6.7853 [b]	0.426	0.0071
Tocotrienol (µg/g)								
α	0.4209 [b]	0.2743 [b]	0.7482 [b]	1.5275 [a]	1.7064 [a]	1.8731 [a]	0.167	0.0002
β	0.0000 [b]	0.0033 [b]	0.0059 [b]	0.0525 [a]	0.0589 [a]	0.0647 [a]	0.008	0.0006
γ	0.1114 [c]	0.0884 [c]	0.2384 [b]	0.4111 [a]	0.4342 [a]	0.4710 [a]	0.040	<0.0001
δ	0.0000 [b]	0.0000 [b]	0.0114 [b]	0.0618 [a]	0.0619 [a]	0.0833 [a]	0.009	<0.0001
total	0.5323 [b]	0.3660 [b]	1.0038 [b]	2.0529 [a]	2.2614 [a]	2.4922 [a]	0.220	<0.0001
Total vitamin E	5.3640 [b]	10.2000 [a]	7.6870 [ab]	8.9390 [a]	9.0860 [a]	9.2770 [a]	0.461	0.0119

[1]The number of tissue sample per treatment = 3 (pull sample: 2 replicates = 1 sample).

[2] Treatments were C: control; C+E: control + α-tocopheryl acetate100 mg/kg; CPO2: crude palm oil 2%; CPO4: crude palm oil 4%; CPO6: crude palm oil 6%; CPO8: crude palm oil 8%.

[a,b,c] Means in a row with different superscripts are significantly different($P < 0.05$).

4.5 Relationship of the crude palm oil in diet and vitamin E concentration in tissues.

Relationship between the crude palm oil in diet and vitamin E concentration in tissue was shown in Figure 4.1, 4.2 and 4.3. The total tocopherols concentration in breast meat ($P<0.0099$, r=0.95888) showed the positive relationship and high correlation coefficient with the crude palm oil in diet. But, the correlation coefficient of total tocopherols with the crude palm oil in diet was not found in liver and thigh meat ($P\geq0.05$).

The total tocotrienols concentration in liver ($P<0.0296$, r=0.91453), breast meat ($P<0.0018$, r=0.98665) and thigh meat ($P<0.0097$, r=0.95967) showed the positive relationship and high correlation coefficient with the crude palm oil in diet.

The total vitamin E concentration in liver ($P<0.0501$, r=0.87825), breast meat ($P<0.0019$, r=0.98659) and thigh meat ($P<0.0423$, r=0.89126) showed the positive relationship and high correlation coefficient with the crude palm oil in diet.

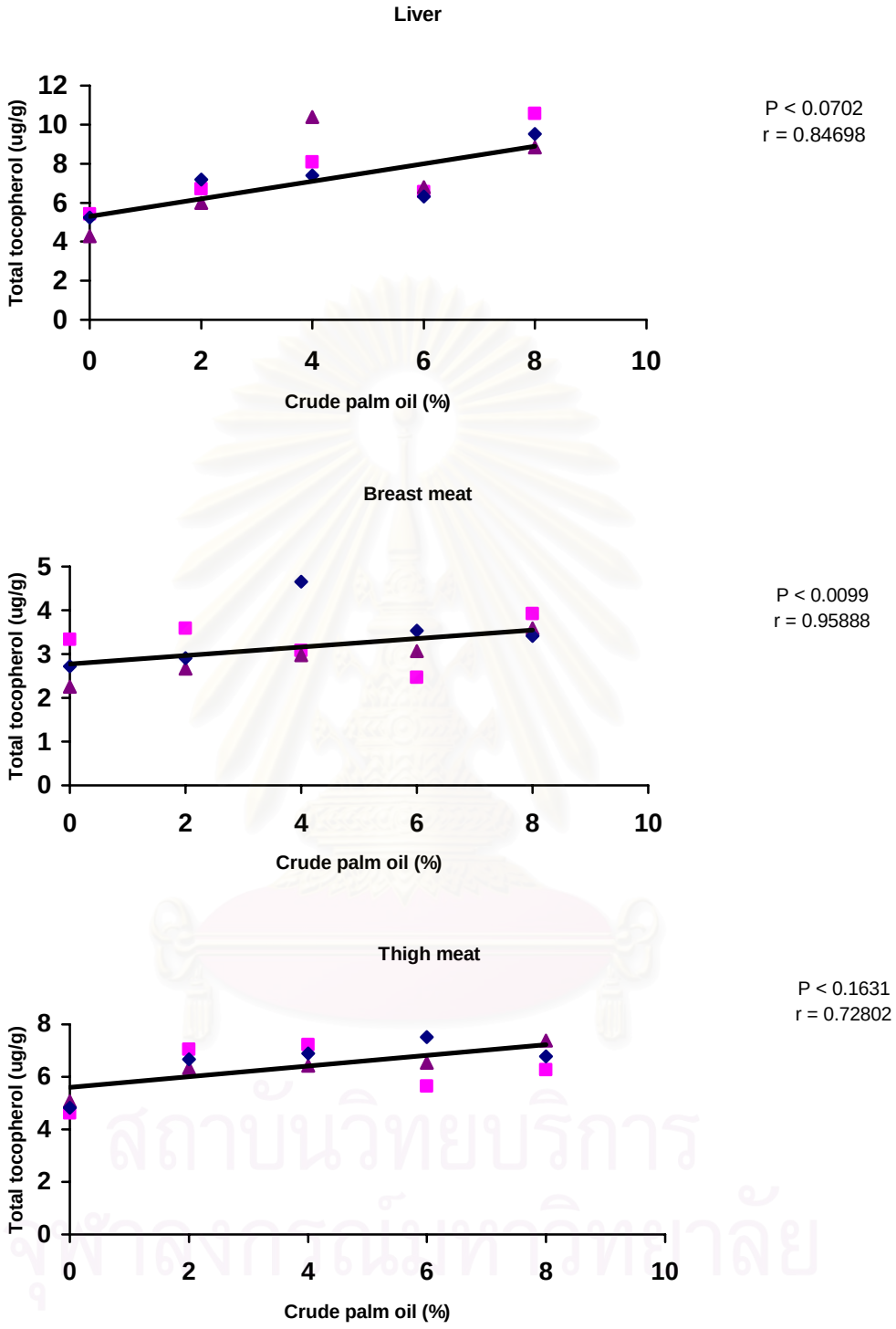


Figure 4.1 Relationship of the crude palm oil in diet with total tocopherol concentration in tissues.

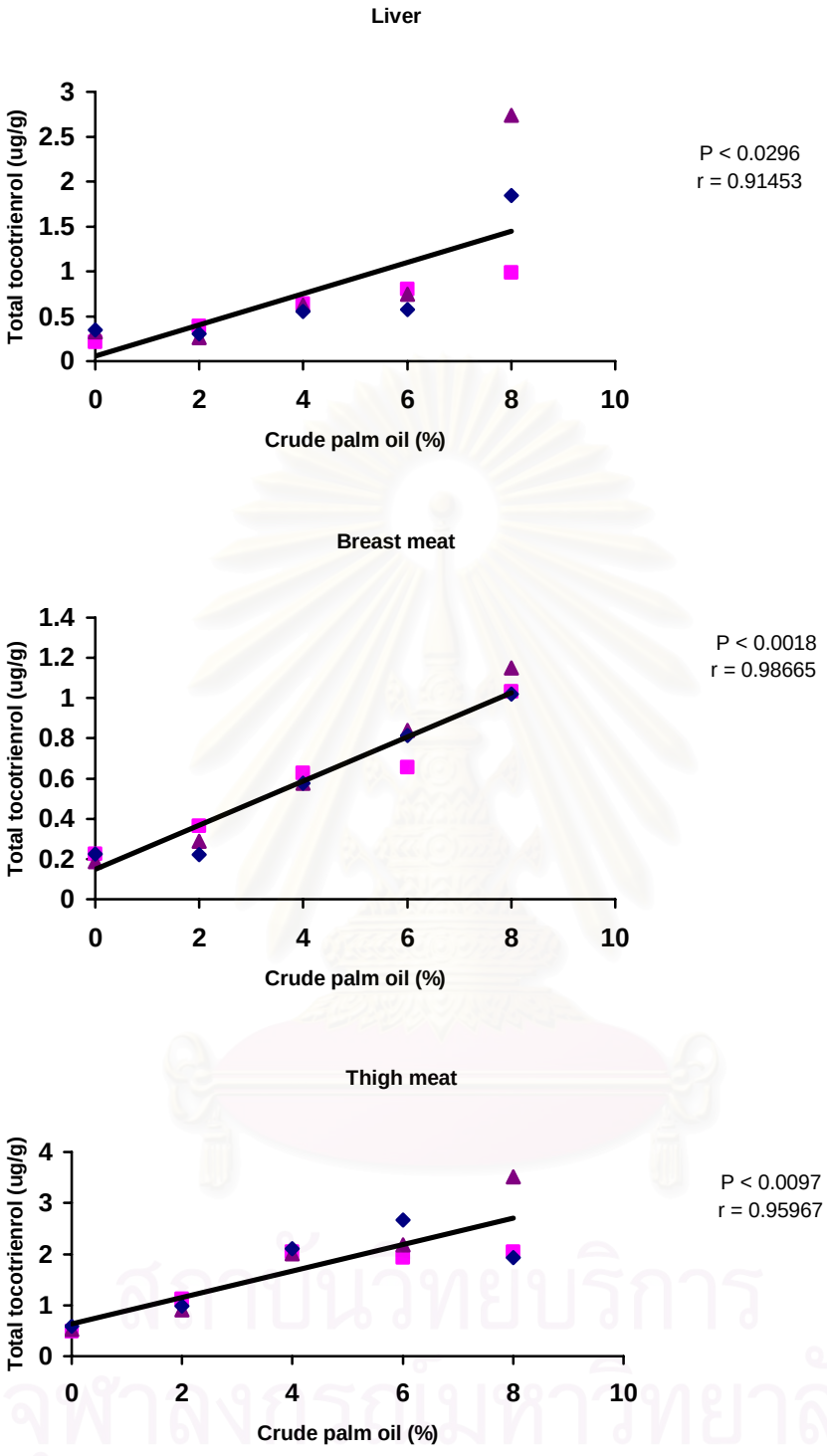


Figure 4.2 Relationship of the crude palm oil in diet with total tocotrienol concentration in tissues.

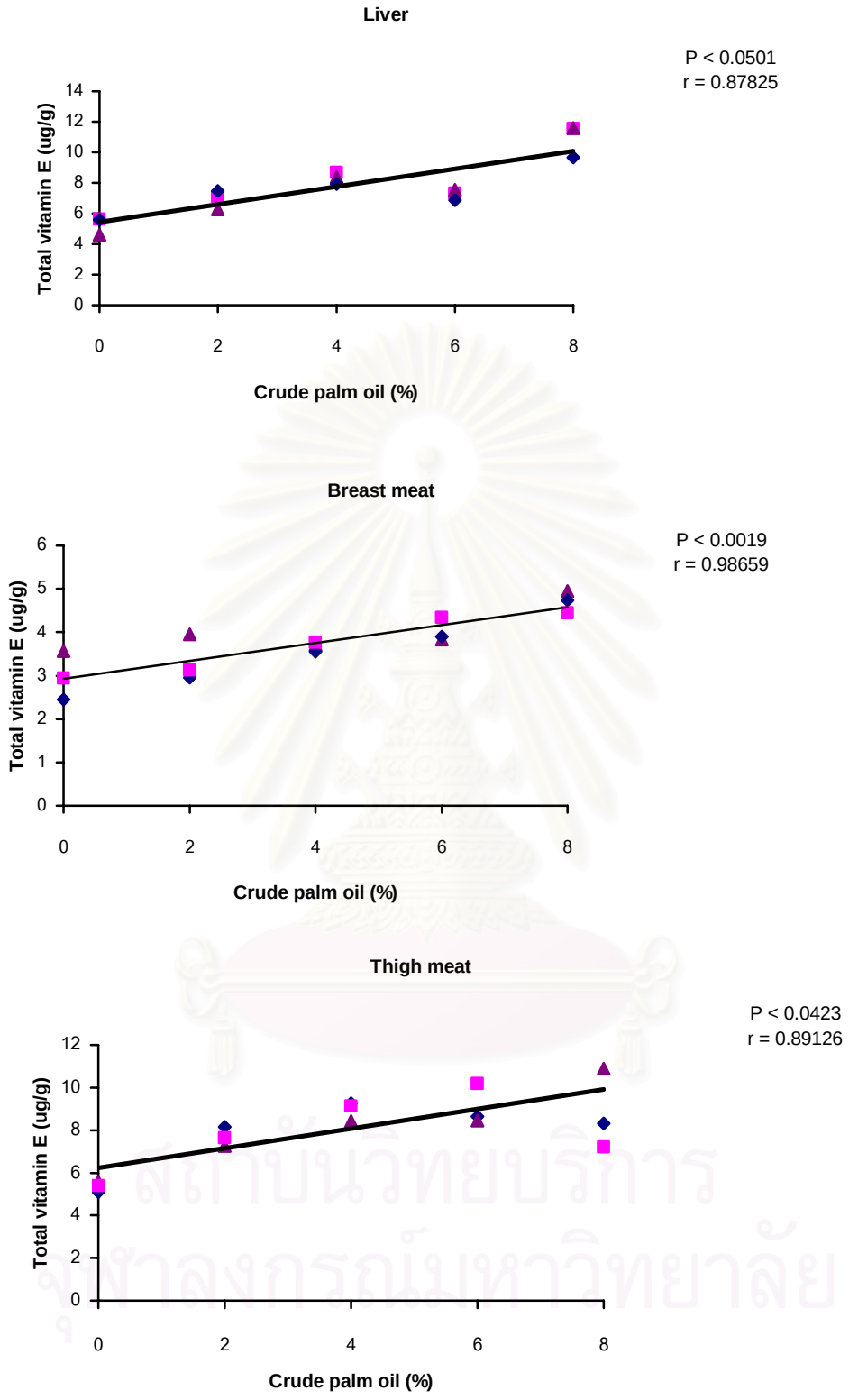


Figure 4.3 Relationship of the crude palm oil in diet with total vitamin E concentration in tissues.

4.6 Effect of vitamin E in crude palm oil on the thiobarbituric acid reactive substance (TBARS) value in plasma and tissues.

The effect of vitamin E in crude palm oil on the TBARS values of plasma and tissues of the broilers are shown in Table 4.6. Plasma TBARS values on day 21 were decreased in CPO4, CPO6, CPO8 and C+E groups compared with C ($P<0.001$) and no difference was observed in CPO6 and CPO8 groups compared with C+E. Similarly, the TBARS values of plasma on day 42 were decreased in CPO4, CPO6, CPO8 and C+E groups compared with C ($P<0.001$), but no difference was observed between CPO8 and C+E groups The TBARS values in CPO2 fed group was not difference with C group both on day. An increasing of total vitamin E in diet by inclusion CPO or supplementation with α–tocopheryl acetate was decreased TBARS values in plasma compared to control group on both days 21 and 42.

The TBARS values of liver were not significant difference ($P\geq0.05$) among groups in the 21 and 42 days. However an increasing of total vitamin E in diet by inclusion CPO or supplementation with α–tocopheryl acetate tended to decrease the TBARS values in liver.

The TBARS values of breast meat were not significant difference ($P\geq0.05$) among groups of broilers, but an increasing of total vitamin E in diet by inclusion CPO or supplementation with α–tocopheryl acetate tended to decrease the TBARS values. The TBARS values of thigh meat was decreased in CPO and C+E fed groups compared with C group ($P<0.001$).

When storage time was considered the TBARS values of breast meat was decreased in CPO and C+E fed groups compared with C on days 4 and 7 ($P<0.01$) after storage in chill room which was controlled temperature at 8°C, but not significant difference when compared to C+E. An increasing of total vitamin E in diet by inclusion CPO or supplementation with α–tocopheryl acetate was decreased TBARS values in breast meat compared to control group on days 4 and 7 after storage. While the TBARS

values of CPO or C+E fed groups gave the same result as breast meat. The C+E fed group had lowest value of TBARS but not significant difference with CPO8 fed group. An increasing of total vitamin E in diet by inclusion CPO or supplementation with α–tocopheryl acetate, the TBARS values was decreased for 4 and 7 days in breast meat after storage.

Table 4.6 Effect of vitamin E in crude palm oil on the thiobarbituric acid reactive substance (TBARS) values of plasma and tissues of the broilers[1].

Item	Treatments[2]						Pooled SE	P-value
	C	C+ E	CPO2	CPO4	CPO6	CPO8		
Plasma (nmol/mL)								
Day 21	4.04 [a]	1.69 [d]	3.39 [ab]	2.91 [bc]	2.42 [cd]	2.15 [cd]	0.171	<0.0001
Day 42	4.01 [a]	2.24 [d]	3.55 [ab]	2.97 [bc]	2.90 [c]	2.71 [cd]	0.124	<0.0001
Liver (mg of MDA/kg of liver)								
Day 21	1.08	0.94	1.07	1.00	0.95	0.94	0.024	0.3099
Day 42	1.02	0.88	0.93	0.92	0.91	0.88	0.021	0.3285
Breast meat (mg of MDA/kg of breast meat)								
Day 42	0.54	0.30	0.45	0.37	0.32	0.30	0.028	0.0554
Thigh meat (mg of MDA/kg of thigh meat)								
Day 42	0.63 [a]	0.31 [b]	0.40 [b]	0.33 [b]	0.31 [b]	0.31 [b]	0.024	<0.0001

[1]The number of birds per treatment = 6.

[2] Treatments were C: control; C+E: control + α-tocopheryl acetate100 mg/kg; CPO2: crude palm oil 2%; CPO4: crude palm oil 4%; CPO6: crude palm oil 6%; CPO8: crude palm oil 8%.

[a,b,c,d] Means in a row with different superscripts are significantly different($P < 0.05$).

Table 4.7 Effect of vitamin E in crude palm oil on the thiobarbituric acid reactive substance (TBARS) values of breast meat and thigh meat of the broilers[1] after storage.

Item	Treatments[2]						Pooled SE	P-value
	C	C+E	CPO2	CPO4	CPO6	CPO8		
Breast meat (mg of MDA/kg of breast meat)								
0 day	0.54	0.30	0.45	0.37	0.32	0.30	0.028	0.0554
4 day	0.63[a]	0.33[b]	0.46[b]	0.38[b]	0.39[b]	0.34[b]	0.025	0.0021
7 day	0.97[a]	0.52[b]	0.69[b]	0.62[b]	0.58[b]	0.57[b]	0.039	0.0054
Thigh meat (mg of MDA/kg of thigh meat)								
0 day	0.63[a]	0.31[b]	0.40[b]	0.33[b]	0.31[b]	0.31[b]	0.024	<0.0001
4 day	0.72[a]	0.35[c]	0.50[b]	0.41[c]	0.36[c]	0.36[c]	0.025	<0.0001
7 day	0.82[a]	0.54[b]	0.64[b]	0.58[b]	0.55[b]	0.58[b]	0.025	0.0047

[1]The number of birds per treatment = 6.

[2] Treatments were C: control; C+E: control + α-tocopheryl acetate100 mg/kg; CPO2: crude palm oil 2%; CPO4: crude palm oil 4%; CPO6: crude palm oil 6%; CPO8: crude palm oil 8%.

[a,b,c] Means in a row with different superscripts are significantly different ($P < 0.05$).

4.7 Effect of vitamin E in crude palm oil on drip loss in breast and thigh meat.

The effect of vitamin E in crude palm oil on drip loss of breast and thigh meat was shown in Table 4.8. The drip loss of breast meat were significantly difference among groups of broilers on day 2 ($P<0.05$) and 4 ($P<0.01$). The drip loss of breast meat was decreased in CPO4, CPO6 and CPO8 groups compared with C both on day 2 and 4, but not significant difference when compared with C+E. The drip loss of thigh meat was decreased in CPO4, CPO6 and CPO8 groups compared with C on day 2 and 8 ($P<0.05$), but not significant difference was found when compared with C+E. The drip loss of thigh meat in CPO8 were lowest compared with CPO groups, but not significant difference with CPO4 and CPO6. The drip loss in breast and thigh meat tended to decrease when CPO level was increased or α–tocopherol acetate was supplemented.

Table 4.9 Effect of vitamin E in crude palm oil on drip loss of breast meat and thigh meat of the broilers[1].

Tissues	Treatment[2]						Pooled	P-value
(%)	C	C+E	CPO2	CPO4	CPO6	CPO8	SE	
Breast meat								
Day 2	4.75 ^{a}	3.25 ^{b}	4.77 ^{a}	4.11 ^{ab}	4.13 ^{ab}	4.10 ^{ab}	0.140	0.0114
Day 4	6.47 ^{a}	4.38 ^{b}	6.04 ^{a}	4.84 ^{b}	4.80 ^{b}	5.30 ^{ab}	0.194	0.0053
Day 6	10.65	8.70	10.92	9.92	9.01	8.02	0.336	0.0686
Day 8	13.72	11.55	13.48	12.58	13.27	12.53	0.354	0.5405
Thigh meat								
Day 2	3.99 ^{a}	2.55 ^{b}	3.93 ^{a}	2.98 ^{ab}	3.27 ^{ab}	2.70 ^{b}	0.162	0.0206
Day 4	5.11	3.95	4.75	4.54	4.04	3.88	0.150	0.0881
Day 6	8.77	6.92	8.78	7.70	8.31	7.15	0.288	0.2597
Day 8	11.48 ^{a}	9.15 ^{c}	10.83 ^{ab}	10.16 ^{abc}	10.19 ^{abc}	9.86 ^{bc}	0.227	0.0495

[1]The number of birds per treatment = 6.

[2] Treatments were C: control; C+E: control + α-tocopheryl acetate100 mg/kg; CPO2: crude palm oil 2%; CPO4: crude palm oil 4%; CPO6: crude palm oil 6%; CPO8: crude palm oil 8%.

[a,b,c] Means in a row with different superscripts are significantly different ($P < 0.05$).

CHARPTER V

DISCUSSION

5.1 Effect of vitamin E in crude palm oil on growth performance.

Vitamin E requirement of Ross broiler in this study is 50 mg/kg of diet. More research work showed that a higher allowance of vitamin E had beneficial effects with chicks, such as improve performance, enhanced immune competence, especially under commercial production conditions (McDowell 1989, Sunder et al 1997, Flachowsky 2000, Guo et al., 2001). In the present study, there was not significant difference in body weight, feed intake and feed conversion ratio (FCR) both at day 21 and day 42. Neither the supplementation of α-tocopheryl acetate at 100 mg/kg feed nor vitamin E from with crude palm oil. Guo et al (2001) reported that supplementation of vitamin E tended to improve growth and feed utilization of birds during 0 to 3 weeks of age, but the performance from 0 to 6 weeks of age was not influenced. Similar result was reported by Valencia et al (1993) that no significant differences in body weight or feed utilization among broilers fed diets supplemented with crude palm oil, crude palm kernel oil or poultry oil. Brake et al., (2002) reported that lard and palm oil have similarly metabolizable energy and fatty acid compositions. Broilers fed lard and palm oil treatments were not significant difference in feed conversion ratio (Brake et al., 2002). Sanz et al (2000) reported that dietary fat sources (lard, tallow and sunflower oil) had no effect on intake, weight gain, final body weight or FCR. Several researchers have indicated that dietary fat source has no effect on productive characteristics as long as the ratios of energy to protein (E:P) or energy to amino acids and other nutrients are balanced (Bartov et al., 1974; Fuller and Rendon, 1977; Hulan et al., 1984; Pinchasov and Nir, 1992). Energy to protein ratio in all treatment diets were equal in each period. Thus, growth performances of all treatments were not significant difference. Although, no significant difference was found in the mortality rate but the CPO fed group tended to show higher than standard value in commercial production (4%). The sudden death was

found in high body weight broilers (average 2.37 kg) and scattered in all treatment groups and occurred in the same day (on day 38 of the experiment) which was high in temperature (38.4°C) and low relative humidity (30%).

5.2 Effect of vitamin E in crude palm oil on tocopherol and tocotrienol concentration in liver, breast meat and thigh meat.

The result demonstrated that broiler fed on CPO had higher α-tocopherol and tocotrienol concentration in all tissues than broiler fed on C diet but CPO groups had lower α-tocopherol than broiler fed C+E group. CPO groups had higher α- and γ-tocopherol in diet than C group while C+E group had highest α-tocopherol level. Cherian et al., (1996) reported that there was positive linear trend of α-tocopherol concentration in tissue (liver, adipose tissue, leg muscle and breast muscle) when the dietary level of vitamin E was supplemented. Alpha-tocopherol was a major form followed by γ-tocopherol form of vitamin E deposited in liver which was supported by Kang et al., (1998); Cherian et al., (1996). In contrast with Areerob et al., (2004) who reported that γ-tocopherol was a major form of vitamin E deposited in liver of hens. Inclusion of CPO8 and supplementation of α–tocopheryl acetate in diets, γ-tocopherol in tissues decreased lower than control group especially in thigh meat it might be because of the competitive absorption of each form of vitamin E (Kang et al., 1998). The high level of α-tocopherol in α–tocopheryl acetate group may interfere absorption of γ-tocopherol in this study.

Alpha-tocotrienol in liver were highest in broilers fed CPO8 group while tissue of chick fed CPO4, CPO6 and CPO8 had higher α-tocotrienol than C, C+E and CPO2 groups. The β-, γ- and δ-tocotrienol also increased in breast and thigh meat. Kang et al., (1998) reported that supplementation of 1.5 and 3.5% palm oil also resulted in an increase in tocopherol and tocotrienol contents in tissues ($P<0.05$). They also suggested that the concentration of vitamin E in the tissues was dependant upon their concentration in diet. The α-tocotrienol and total tocotrienol concentration in breast and thigh meat of broiler fed on CPO8 had higher than C and C+E about 5 and 7 times in

breast meat and 4.6 and 6.8 times in thigh meat. It demonstrated that, increasing in CPO in the diets increased tocotrienol in tissue. Similarly to Kang et al. (1998) who reported that supplemented with tocotrienols (as palm oil) in laying hens increased the total tocotrienols in breast and thigh meat and the major form tocotrienol deposited in breast meat and thigh meat were α and γ forms. Both forms increased markedly in breast meat and thigh meat from broilers fed on CPO diets compared to C in present study. The concentration of tocotrienols in C+E lower than C about 2 times. It was possibly that supplementation of α-tocopheryl acetate in diet interfered tocotrienol absorption and deposition in tissues. Qureshi et al. (1996) suggested that α-tocopherol may interfere with tocotrienol absorption and bind preferentially to the α-tocopherol transfer protein, resulting in a more rapid clearance of tocotrienol from plasma. The various levels of forms of vitamin deposited in tissues may result from the metabolism of vitamin E in liver on competitive absorption among forms of vitamin E (Kang et al., 1998). In contrast with Lanari et al., (2004) reported that the breast samples of chicken fed 92.4 mg/kg of tocotrienols as Oryza Tocotrienol, amounts of α-, β- and γ-tocopherol were detected but could not quantifiable amount of α–tocotrienol. Lanari et al., (2004) they also suggested that it was possibly that there was an interaction between α-tocopherol and tocotrienols or their metabolites on the α-tocopherol deposition in the muscle. Different fat soluble vitamins may compete with each other for binding sites in the miscelles and or in the lipoproteins, altering their final uptake or utilization by the tissues. Hosomi et al. (1997) determined the affinity of different vitamin E analogs for the α-tocopherol transfer protein in vitro, they found that the α-tocopherol's binding capacity with α-tocopherol was 8–11 times higher than γ-tocopherol and α-tocotrienol. The results from the presence of other tocopherol or tocotrienol forms reduce α-tocopherol deposition in the muscle (Hosomi et al., 1997). Pellett et al., (1994) reported that adding β-carotene (100 mg /kg diet) to a diet enriched in α-tocopherol resulted in substantially lower retention of hepatic α-tocopherol in chickens. The CPO has high level of β-carotene it might be diminish α-tocopherol in liver. However, absorption of vitamin E is related to intestinal fat digestion and is facilitated by bile and pancreatic lipase. A very low-fat meal or poor fat digestion effectively minimize vitamin E absorption (Kohlmeier, 2003). Abawi et al., (1985) showed that an increase in tissue concentration of vitamin E

with increasing dietary animal fat. Such increases could be explained possibly on the basis of enhanced solubility of the vitamins and other fat-soluble nutrients in the micellar phase, delayed gastric emptying with higher fat diets and also the enhanced efficiency of absorption and transport with moderate dietary fat. In present study, the fat level in all diets were constant level at 8%, this reason should be dismiss.

5.3 Relationship of the crude palm oil in diet with vitamin E concentration in tissues.

In the present study, the correlation analysis showed that the crude palm oil in diet had statistically significant association with the total tocopherol concentration, total tocotrienol and total vitamin E in liver, breast meat and thigh meat. Vitamin E content in poultry meat increases linearly as the dietary vitamin E increases (Barroeta, 2007). This may suggested that the dietary intake of vitamin E reflected vitamin E concentration in the tissues.

5.4 Effect of vitamin E in crude palm oil on the thiobarbituric acid reactive substance (TBARS) value in plasma and tissues.

Thiobarbituric acid reactive substances (TBARS) value, expressed as MDA concentration, is a good indicator reflecting the degree of oxidation (Guo et al., 2001). In the current study, broiler fed on C+E had lowest (P<0.001) TBARS value in plasma, however, no significant difference was observed in CPO6 and CPO8 groups compared with C+E. Inclusion of CPO in diets decreased plasma TBARS value. Similar result was reported by Sahin et al (2001, 2002a, 2002b) that broilers supplemented with dietary vitamin E had a significant reduction in malondialdehyde (MDA) values in serum and tissue. Unfortunately, this study was not examine vitamin E concentration in plasma but there are some previous research stated that dietary intake of vitamin E closely reflected serum levels of vitamin E (Willett et al., 1983; Schafer and Overvad, 1990). Suarna et al (1993) reported that plasma levels of tocotrienols and tocopherols increased in rats fed tocotrienol rich fraction of palm oil diet. Moreover, inclusion of 2, 3 and 4%crude palm oil increased vitamin E concentration in plasma of hens (Areerob et al., 2004). Although,

total vitamin E in diets of CPO6 and CPO8 were lower than C+E diet but the TBARS values in plasma of C+E group were not significant difference with CPO6 and CPO8 groups. It demonstrated that, the reduction of TBARS values in plasma might be because of the increasing of vitamin E in diets increased vitamin E concentration in blood incorporated with CPO had high level of tocotrienols, which is higher antioxidant efficacy than that of tocopherols (Serbinova et al., 1991; Suzuki et al., 1993).

The TBARS values in thigh meat decreased significantly when CPO and α-tocopheryl acetate were added. This may explain that inclusion of CPO and supplementation of α-tocopheryl acetate increased vitamin E concentration in thigh meat and effectively decreased the TBARS values in tissues. This similar result was not observed in breast meat because of oxidative changes are more extensive in the thigh meat than breast meat (Ajuyah et al, 1993; Kang et al., 2001; Botsoglou et al., 2002). The major contributing factors to poultry meat colour are myoglobin content, the chemical state of haem structure which has been shown to be primarily related to the species, muscle, and age of the animal. The white meat had the lower myoglobin content than dark meat (Fletcher, 2002). In addition, the rate of lipid peroxidation in microsomes and subcellular membranes isolated from dark muscle tissue of broilers was higher than white muscle (Rhee and Ziprin, 1987; Asghar et al., 1989). The TBARS values in liver and breast meat tended to decrease when CPO and α-tocopheryl acetate were added. Although vitamin E concentration in liver and breast meat increased significantly in C+E and CPO8 treatments, TBARS value was not significant difference. It might be because of the lower oxidative change thus the first level of antioxidant defensible enzymes; superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) are sufficient to completely prevent free radical formation and lipid peroxidation. On the other hand, if the first defense is not sufficient therefore the second level of oxidative defense is vitamin E which was the most effective natural free radical scavenger identified to date, is the main chain breaking antioxidant in the cell (Surai, 2002). In this study, the TBARS values in liver and breast meat of control group had total vitamin E 50 mg/kg diet, was not significant difference with the high level of vitamin E groups. It implied that, the requirement level of vitamin E in control diet is sufficient to

completely prevent lipid oxidation in liver and breast meat. However the antioxidant protection mechanisms in cell is not clear revealed (Surai, 2002; Guo et al., 2003).

During storage time, the results of the present study showed that the TBARS values in breast and thigh meat on day 4 and 7 in controlled chilled room at 8°C decreased significantly when CPO and α-tocopheryl acetate were added. The result supported by the previous studies that supplementation of α-tocopheryl acetate improved the antioxidant status of the meat, thereby improving oxidative stability (Nam et al., 1997; Galvin et al., 1998; Higgins et al., 1998; O'Neill et al., 1998; Guo et al., 2001; Guo et al., 2003; Chae et at., 2006). The rate and extent of lipid oxidation in meats depend on a number of factors including the vitamin E concentration and the degree of unsaturation of the fatty acids present in the muscle (Morrissey et al., 1998). Moreover, the presence or absence of vitamin E in animal tissues influences the stability of lipids in meats during storage (Gray et al., 1996).

From the present study, it can conclude that an increasing in the storage time increased the TBARS values in breast and thigh meat. Fortification of vitamin E with both natural source (CPO) and synthetic source effectively decreased the TBARS values in tissues during storage for 4 and 7 day.

5.6 Effect of vitamin E in crude palm oil on drip loss in breast and thigh meat.

The inclusion of CPO from the level of 4% and onward or supplementation of α-tocopheryl acetate in the diets decreased drip loss in breast and thigh meat. A similar effect of dietary α–tocopheryl acetate supplementation has been observed in breast fillets from refrigerated storage at 4°C (O'Neill et al., 1998). They also demonstrated that supplementation of vitamin E can reduce the drip loss from fresh pork (Monahan et al., 1994), thawed pork (Asghar et al., 1991) and beef (Mitsumoto et al., 1995). The evidence suggests that the ability of vitamin E to reduce drip loss is related to its membrane stabilizing effects. It is believed that vitamin E can maintain the integrity of cellular membranes, reduces leakage of sarcoplasmic components from muscle cells,

thereby reducing drip loss (Asghar et al., 1991; Mitsumoto et al., 1995; Gray et al., 1996).

In conclusion, the present study demonstrated that the inclusion of CPO as a natural source of vitamin E in the diet had no effect on growth performance in broiler. Increasing of CPO level increased tocopherol and tocotrienol concentration in tissues. Broilers fed CPO had higher vitamin E in the form of tocotrienol deposition in tissues. Form of vitamin E deposition in tissues, α-tocopherol was the highest follow by α-tocotrienol, γ-tocopherol and γ-tocotrienol respectively. The tocopherols, tocotrienols and total vitamin E concentration deposition in all tissues showed the positive relationship with crude palm oil level in diet. Inclusion of CPO as a vitamin E source in diet decreased TBARS value and also decreased drip loss in breast and thigh meat during storage.

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