EFFECT OF CONJUGATED LINOLEIC ACID OR OLEIC ACID ADDITION

ON FATTY ACID COMPOSITION PROFILES OF POULTRY MEAT

A Dissertation

by

DAE KEUN SHIN

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

May 2010

Major Subject: Poultry Science

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ABSTRACT

Effect of Conjugated Linoleic Acid or Oleic Acid Addition

on Fatty Acid Composition Profiles of Poultry Meat. (May 2010)

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Two different studies were conducted to reduce the overall amount of omega-6 fatty acids in broiler chickens. The first experiment was performed to determine the effects of dietary conjugated linoleic acid (CLA) and omega-3 fatty acid combination on the omega-6 fatty acid accumulation in broiler chicken breast and thigh meat. Eight broilers from each treatment were processed at 4 and 6 weeks of age, respectively. Regarding the diets containing five different fat sources, broiler chickens fed CLA and fish oil diet had a lower C20:4 (arachidonic acid, AA, n-6) deposition but showed a higher n-3/n-6 ratio in breast and thigh meat than those fed a flaxseed oil diet and CLA and flaxseed oil diet ($P < 0.05$). The C20:4 and n-3/n-6 ratio of breast and thigh samples from fish oil diet was similar to those of the conjugated linoleic acid and fish oil combination diet ($P > 0.05$). However, the addition of CLA and fish oil to the diet resulted in a increase of polyunsaturated fatty acid (PUFA) concentration in broiler chicken breast and thigh meat when compared to that of fish oil diet ($P < 0.05$).

The second experiment was conducted based on six different combination of n-3 and n-9 fatty acids. One bird per pen was processed, and each bird was weighed, and blood, liver, breast and thigh samples from the bird were collected. Although the generation of prostaglandin E_2 (PGE_2) was not affected due to combination of n-3 and n-9 fatty acids in our diets, the deposition of n-6 fatty acids including C18:2 and C20:4 was decreased in broiler chicken breast and/or thigh muscles as n-3 fatty acids were

supplied to broiler chickens for 9 weeks. Eicosapentaenoic acid (C20:5, EPA, n-3) addition to poultry diet (FEO) did not reduce the deposition of C18:2 and/or C20:4 as much as C22:6 (FDO) did. When C20:5 and C22:6 were blended to poultry diet (FHO) and fed to broiler chickens for 9 weeks, synergistic effects were observed. Reduction of C20:4 was obtained when FHO diet was fed to broiler chickens, and it may be induced due to decreased expression of delta-6 desaturase mRNA.

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CHAPTER I

INTRODUCTION

In the United States, meat, dairy, seed oils and their derived products are the major sources of calories.   Due to the rapid improvement and recent developments in fat and oil processing technologies, new dairy- and oil-based products enter the food chain with modifications in their fatty acid composition.   An important goal is to increase the amount of 'good' fatty acids in poultry and to maintain meat quality.   One of the most efficient ways to induce the deposition of 'good' fatty acids in chicken meat has been achieved through the modification of poultry feed fatty acid composition.

The use of oils derived from sunflower, cottonseed, safflower, corn or soybean is a common practice in commercial poultry production settings.   These oils contain high amounts of linoleic acid (C18:2, LA, n-6) (Schreiner et al., 2005; Cleland et al., 2006), which can induce an over-supply of omega-6 (n-6) fatty acids in poultry diets, and consequently, a higher deposition of these fatty acids in the meat.   It has been suggested that in western societies, the consumption of high proportions of n-6 fatty acids has contributed to a higher incidence of health problems such as cardiovascular diseases, obesity, and type-2 diabetes, thus prompting the development of alternative food products with lower levels of n-6 fatty acids that could help in preventing or reducing the incidence of these diseases.   The addition of omega-3 (n-3) fatty acids as a substitute for conventional n-6 fatty acids in poultry diets, and the effect on fatty acid deposition in poultry meat has been evaluated.   However, a different approach other than conventional substitution is necessary due to intrinsic poultry production disadvantages of n-3 fatty acid addition, such as increased bleeding and hemorrhagic stroke (Duttaroy, 2006).

This dissertation follows the style of Poultry Science.

Conjugated linoleic acids (CLA) and omega-9 (n-9) fatty acids have been associated with the reduction of cardiovascular diseases and/or some types of cancers. However, despite the potential for enhanced functional and nutritional properties that can be achieved by the inclusion of CLA or n-9 fatty acids in human diets, enrichment of CLA and n-9 fatty acids in poultry meats has yet to be commercially pursued. The lack of commercial consideration for this enrichment is basically due to some concerns about the potential inactivation of delta-9 desaturase when CLA and/or n-9 fatty acids are consumed by some animals, an outcome that may be considered nutritionally negative and commercially impractical. The addition of CLA or n-9 fatty acid components as a combined fat source of n-3 fatty acids in poultry diets would be of great advantage for consumers and the poultry industry, if appropriate and commercially acceptable levels of their dietary combinations and ratio of supplementation in the diet are established. A commercially formulated diet based on the addition of n-3 and CLA/n-9 as fatty acid sources may minimize the deposition of n-6 fatty acids in poultry meats, while contributing to minimizing the disadvantages associated with the direct supplementation of poultry diets with n-3, n-9 or CLA, respectively.

The main objective of this project is to determine an appropriate ratio and supplementation levels of n-3 and CLA/n-9 lipid sources in poultry diets that will provide enhanced deposition of n-3 and CLA/n-9 fatty acids, without significantly affecting productivity parameters. To achieve this objective, two consecutive experiments have been considered based on the following: (a) higher CLA/n-9 and n-3 fatty acids deposition in poultry meats is associated with significant presence of CLA/n-9 and n-3 fatty acid in the feed; (b) the activities of delta-6 and -9 desaturases may depend on the available combined levels of CLA/n-9 and n-3 fatty acids in the feed; (c) higher pro-inflammatory responses may be closely related to the accumulation of n-6 fatty acids in poultry; and (d) it would be beneficial to the poultry industry if more n-3, n-9 and CLA, but less n-6 fatty acids could be deposited in poultry meat, because it may add value by creating new markets for poultry products aimed at health conscious consumers.

1. HYPOTHESIS AND OBJECTIVES

By replacing n-6 fatty acid sources in the poultry diet with sources richer in n-3 and CLA/n-9, we will increase the deposition of n-3 and CLA/n-9 in the chicken meat. Higher levels of ingested n-3 and CLA/n-9 will promote desaturation and elongation of linoleic acid (C18:2, LA, n-6) to arachidonic acid (C20:4, AA, n-6) and improve the desaturation and elongation of linolenic acid (C18:3, LNA, n-3) to eicosapentaenoic acid (C20:5, EPA, n-3) and docosahexaenoic acid (C22:6, DHA, n-3) in the liver. Since n-3 fatty acids have a higher affinity to delta-6 desaturase, the enzyme responsible for desaturation of long-chain fatty acids, the production of n-3 polyunsaturated fatty acids for muscle deposition will be enhanced, thus increasing the n-3 to n-6 ratio in the muscle to the 2~4:1 recommended levels that have been shown to be beneficial in the human diet. Additionally, lower deposition and availability of C20:4 may in turn reduce the severity of pro-inflammatory responses in the chickens. To test this hypothesis the following specific objectives have been planned:

1) Effects of conjugated linoleic acid (CLA) and omega-3 fatty acids combination on the deposition of linoleic acid in two different types of broiler muscles; to increase the deposition of n-3 fatty acids but to decrease the amount of n-6 fatty acids in chicken meat,n-3 fatty acids (flaxseed and/or fish oil) and CLA combinations will be fed to chickens for 6 weeks, and the fatty acid composition of two different muscles will be traced after 4 and 6 weeks of growth. Also, the activities of each elongase and desaturase will be calculated based on the accumulation of n-3, -6 and -9 fatty acids in liver.

2) Effects of dietary supplementation of n-3 and -9 fatty acid combination on inflammation responses using broilers as an animal model; to evaluate the effects of n-9 fatty acid supplementation on n-3 and/or n-6 fatty acid metabolism and inflammation, broilers will be fed diets supplemented with a combination of olive and soybean, flaxseed, flaxseed and eicosapentaenoic acid (C20:5, n-3, EPA) combination, flaxseed and docosahexaenoic acid (C22:6, n-3, DHA) combination, or fish oils for 9 weeks of growth, and then individual fatty acid accumulation, gene expression related to *de novo*

lipogenesis, fatty acids oxidation, as well as prostaglandin E_2 (PGE_2) accumulation and cyclooxygenase2 (COX2) gene expression will be determined.

CHAPTER II

LITERATURE REVIEW

1. BACKGROUND

Fatty acids in the human and chicken diets are essential nutrients needed for a series of metabolic interactions in addition to their caloric contribution to a balanced nutritional regime. The type of fatty acids utilized in metabolic processes is dependent on their source, which could be exogenous (i.e., dietary ingestion); and endogenous, (i.e., generated in the body by metabolic processes). Unsaturated fatty acids (UFA) are categorized as either n-9, -7, -6 or -3 fatty acids, dependent on the double bond where the unsaturation is present. Among these, linoleic acid (C18:2, n-6, LA) and linolenic acid (C18:3, n-3, LNA) are essential fatty acids (EFA) that need to be included in the diet, in contrast to oleic acid (C18:1, n-9, OA) and palmitoleic acid (C16:1, n-7, PA), which can be synthesized in the body through metabolic pathways.

Not only C18:2 and C18:3 contribute to enhance the nutritional functionality in a balanced dietary regime, but also, the synthesis of polyunsaturated fatty acids (PUFA) with 20 or more carbons is based on the metabolic availability of LA and LNA. Exogenous fatty acids ingested in the diet are absorbed into the body through the formation of chylomicrons (pro-microns in bird) in the small intestine, while endogenous fatty acid biosynthesis occurs in the cytosol and the endoplasmic reticulum (ER) of the cell. In the body, the carbons derived from glucose, amino acids, and ethanol catabolism may produce acetyl-CoA (Schutz, 2004) and this metabolite can be converted to fats through a series of reactions mediated by acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS). Despite the fact that adipose tissue is involved in fatty acid biosynthesis, the liver is considered as a major site of *de novo* lipogenesis in birds. The major site of lipogenesis is species dependent.

Elongation and Desaturation of n-3 and n-6 FAs

The majority of the ingested and absorbed C18:2 and C18:3fatty acids are distributed to adipose tissue and other tissues in the body; however, a fraction of

C18:2andC18:3 will be elongated and further desaturated to form either arachidonic acid (C20:4, n-6, AA), or eicosapentaenoic acid (C20:5, n-3, EPA) and docosahexaenoic acid (C22:6, n-3, DHA), respectively. During the conversion of C18:2 to C20:4, and C18:3 to C20:5 or C22:6, elongation and desaturation of the respective precursors occur in the presence of elongation-of-very-long-chain-fatty acids (Elovl)-2 and/or Elovl-5 elongases and delta-5 and -6 desaturases (Leonard et al., 2002; Jump, 2004).

Both Elovl-2 (C20~22) and Elovl-5 (C16~20) are involved in the synthesis of n-3 and n-6 PUFAs in mammals (Leonard et al., 2002; Wang et al., 2005; Igarashi et al., 2007), but only Elovl-2 is activated during the conversion of very-long-chain PUFA (C≥20) (Igarashi et al., 2007). Therefore, Elovl-2 and/or Elovl-5 may be used by both C18:2 and C18:3 during the conversion to C20:4 or C20:5, and compete with each other during the process. For the synthesis of 22 carbon PUFA, both or either Elovl-2 and/or Elovl-5 would be involved until 20 carbons of elongation, or a final round of elongation (24 carbons), which is a step previous of peroxisomal β-oxidation (retro-conversion) to obtain the end products (e.g. C22:5, n-6 and C22:6, n-3) (Leonard et al., 2002; Jakobsson et al., 2006).

To synthesize C20:4, and C20:5 or C22:6 from the two respective fatty acid precursors (C18:2 and C18:3, respectively), the use of delta-5 and/or delta-6 desaturases can be overlapped (Willis et al., 1998; Simopoulos, 2000; Nakamura and Nara, 2004) (Figure 1). Delta-6 desaturase is involved in the production of major PUFAs (C≥20) in mammals. Although delta-6 has a higher affinity for C18:3 than for C18:2 (Watkins, 1995) under a normal ratio of C18:3:C18:2 (1:1~4) (Vessby et al., 2002; El-badry et al, 2007), the activity of delta-6 desaturase could become a rate-limiting factor in the biosynthesis of very-long-chain PUFAs when the C18:3:C18:2 ratio is very high (Watkins, 1995).

As a result, due to competition for delta-6 desaturase between C18:2 and C18:3 (Nakamura et al., 2000; Nakamura and Nara, 2003), the production of C20:4, C20:5 and C20:6 are closely related to each other. Thus, when there is a higher intake of C18:2 in relation to that of C18:3, a higher quantity of C20:4 will be biosynthesized compared wi-

n-3 Fatty Acids	Enzyme	n-6 Fatty Acids
α-Linolenic Acid (C18:3; 9,12&15) ↓	Δ6-desaturase	Linoleic Acid (C18:2; 9&12) ↓
Stearidonic Acid (C18:4; 6,9,12&15) ↓	Elongase (ELOVL5)	γ-Linolenic Acid (C18:3; 6,9&12) ↓
Eicosatetraenoic Acid (C20:4; 8,11,14&17) ↓	Δ5-desaturase	Dihomo-γ-linolenic Acid (C20:3; 8,11&14) ↓
Eicosapentaenoic Acid (C20:5; 5,8,11,14&17) ↓	Elongase (ELOVL5/2)	Arachidonic Acid (C20:4; 5,8,11&14) ↓
Docosapentaenoic Acid (C22:5; 7,10,13,16&19) ↓	Elongase (ELOVL2)	Docosatetraenoic Acid (C22:4; 7,10,13&16) ↓
Tetracosapentaenoic Acid (C24:5; 9,12,15,18&21) ↓	Δ6-desaturase	Tetracosatetraenoic Acid (C24:4; 9,12,15&18) ↓
Tetracosahexaenoic Acid (C24:6; 6,9,12,15,18&21) ↓	Peroxisomal β-oxidation	Tetracosapentaenoic Acid (C24:5; 6,9,12,15&18) ↓
Docosahexaenoic Acid (C22:6; 4,7,10,13,16&19)		Docosapentaenoic Acid (C22:5; 4,7,10,13&16)

Figure 1. The activity of desaturases and elongases on linoleic and linolenic acids synthesizing polyunsaturated fatty acids.

-th the biosynthesis of C20:5 and C22:6.   The principal functional role for C20:4 is as a substrate for synthesis of the family of bioactive mediators known as eicosanoids (Calder, 2002), which are involved in modulating the intensity and duration of inflammatory responses.   Furthermore, C20:5 competitively inhibits the oxygenation of C20:4 by the cyclooxygenase2 (COX2), preventing the formation of prostaglandin E_2 (PGE_2), a very potent pro-inflammatory agent (Obata et al., 1999).

Effects of Fatty Acids on Gene Expression Regulating de novo *Lipogenesis*

Fatty acids regulate *de novo* lipogenesis through their effects on gene expression. Essential regulators such as peroxisome proliferator activated receptors (PPARs) and sterol regulatory element binding proteins (SREBPs) are controlled by the relative amount of very-long-chain PUFAs ingested.   Due to an activation of PPRE or SRE, by PPARs or SREBPs, respectively, fatty acid oxidation- or lipogenesis-induced enzymes may be initiated and limited in peroxisomes or mitochondria (Sessler and Ntambi, 1998; Nakamura and Nara, 2004).   Peroxisome proliferator activated receptor subtypes (PPARα, PPARγ and PPARδ) and SREBP isoforms and subforms (SREBP-1 and SREBP-2, and SREBP-1a and SREBP-1c) are activated and/or limited by PUFAs (Clarke et al., 2002; Sampath and Ntambi, 2004; Sanayl, 2005; Jump et al., 2008). Docosahexaenoic acid, one of the major PUFAs, suppresses the SREBP-1c nuclear abundance, reducing fatty acid biosynthesis (Jump, 2008; Jump et al., 2008).   However, C22:6, which has 22 carbons must be β-oxidized and retro-converted to EPA to initiate all PPAR subtypes (Jump, 2008).

Peroxisome proliferator activated receptor α (PPARα) accelerates fatty acid oxidation in the liver, and regulates Elovl-2 and -5 elongases (Kersten et al., 2000; Yoshikawa et al., 2003; Jakobsson et al., 2006).   In contrast to PPARα, acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS) synthesize fatty acids and are generally up-regulated by liver SREBP-1c (Kersten et al., 2000; Sampath and Ntambi, 2005).   Also, SREBP-1c regulates the activity of Elovl-1 and -6 but does not regulate the activity of the Elovl-2 and -5 (Jakobsson, et al., 2006; Wang et al., 2006; Kumadaki

et al., 2008).   The regulation of Elovl-2 and -5 by PPARα is dependent on the concentration of PUFAs absorbed and/or synthesized (Igarashi et al., 2007).

Thus, a higher level of PUFAs increases the activity of the PPARα transcription factor thus increasing fatty acid oxidation, and at the same time reducing *de novo* synthesis of fatty acids through down-regulation of the SREBP-1c receptor (Jump, 2008; Jump et al., 2008).   Furthermore, it has been shown that the PUFAs of the n-3 family, rather than those of the n-6 family, activate the PPARα (Clarke, 2001; Videla et al., 2004).   Therefore, very-long-chain n-3 PUFAs ($C \geq 20$) may diminish the elongation of C18:2 to C20:4 by reducing the activity of Elovl-2 and/or -5, and/or by increasing the affinity of Elovl-2 and/or -5 to C18:3.   A higher supply of n-3 PUFAs may increase the flux of glucose and/or fatty acids into the citric acid cycle.

Effects of n-3 and n-6 Fatty Acids on Prostaglandin E (PGE) Biosynthesis

During excessive intake of n-6 fatty acids, more n-6 UFAs will be incorporated into the cell membrane, resulting in a low n-3 and n-6 ratio.   As n-6 FAs are deposited, the opportunity to release C20:4s from cell membrane is increased.   Phospholipase A_2, an enzyme that acts more on C20:4 than C20:5 or C22:6 (Sumida et al., 1993), releases C20:4s, C20:5s and/or C22:6s to lead the formation of eicosanoids (20 carbons metabolites) including prostaglandins (PGs), prostacyclins (PGIs) and thromboxanes (TXs).   Among the eicosanoids, PGs are bioactive lipids and are formed using either C20:4 or C20:5 as a main substrate when both C20:4 and C20:5 are released from the cell membrane.   The C20:4 is converted to PGE_2 due to the activity of cyclooxygenase (COX) and other related enzymes.   Prostaglandin E_2 is a key metabolite in both acute and chronic inflammation as compared to 1- or 3-series of PG which is derived from C20:3, C20:5 and/or C22:6 (Bagga et al., 2003; Cherian, 2007) (Figure 2).

Membrane Phospholipid

Eicosapentaenoic Acid (C20:5, n-3, EPA)	Enzyme Phospholipase A$_2$	Dihomo-γ-linolenic Acid (C20:3, n-6, DGLA)	Enzyme Phospholipase A$_2$	Arachidonic Acid (C20:4, n-6, AA)
↓	Cyclooxygenase (COX)	↓	Cyclooxygenase (COX)	↓
Prostaglandin G$_3$	Cyclooxygenase (COX)	Prostaglandin G$_1$	Cyclooxygenase (COX)	Prostaglandin G$_2$
↓		↓		↓
Prostaglandin H$_3$	Prostaglandin E Synthase	Prostaglandin H$_1$	Prostaglandin E Synthase	Prostaglandin H$_2$
↓		↓		↓
Prostaglandin E$_3$ (Anti-inflammation)		Prostaglandin E$_1$ (Anti-inflammation)		Prostaglandin E$_2$ (Pro-inflammation)

Figure 2. Biosynthesis of eicosanoid from arachidonic acid, dihomo-γ-linolenic acid and eicosapentaenoic acid (Simmons et al., 2004).

Excessive production of PGE$_2$ may be harmful and it contributes to chronic diseases. COX is a bifunctional protein that has both COX and peroxidase active sites (van Ryn et al., 2000) and catalyzes the formation of PGG$_2$ or PGG$_3$ to PGH$_2$ or PGH$_3$ from C20:4 or C20:5. COX is functionally present as two different isomers, cyclooxygenase1 (COX1) and cyclooxygeanse2 (COX2). Although both are structurally similar (Ringbom et al., 2001), the main functions of COXs differ. COX1 is the enzyme responsible for multiple biological activities including the regulation of kidney functions, stomach acid secretion and inhibition of platelet aggregation (Mello et al., 2000). On the other hand, COX2 induces the expression of pain, fever and other inflammatory responses. Additionally, COX1 is constitutively formed, but COX2 expression is associated to the concentration of PGE$_2$ that is synthesized by COX2, itself (Kim et al., 2006). During PG biosynthesis, COX2 catalyzes the production of PGH$_1$, PGH$_2$ and PGH$_3$ using C20:3, C20:4 and C20:5 as substrates. COX2 converts C20:4 to PGG$_2$ by providing two molecules of oxygen to a C20:4 radical and finally to PGH$_2$ due to the reduction of PGG$_2$. PGE$_2$ is now formed by the action of PGE synthase. Since

less effect of PGE_1 and PGE_3 when compared to PGE_2, C20:4is considered to be a more pro-inflammatory agent (Bagga et al., 2003). Therefore, over concentration of PGE_2should be avoided.

Oxidative Stress and PUFA Metabolism

Oxygen is necessary as a metabolic fuel generation system in aerobic organisms, but reactive oxygen species (ROS) are inevitably formed during cellular energy production. Most ROS are generated and released in mitochondria and peroxisomes. In particular, oxygen is reduced to superoxide (O_2^-) and released from complexes I, II and III of mammalian and/or broiler mitochondria (Staniek and Nohl, 2000; Nohl et al., 2004; Nohl et al., 2005; Ojano-Dirain et al., 2007). All superoxide released must be converted to hydrogen peroxide (H_2O_2), which is a non-radical derivative, and finally forms water to eliminate superoxide molecules from the body (Choe and Min, 2006). However, due to the restricted capacity of the body's defense systems (superoxide dismutase, glutathione peroxidase, catalase, etc.), only limited amount of ROS can be converted to water when high levels of ROS are generated.

When PPARα is highly activated and increases fatty acid oxidation, there is an increased production and release of unstable electrons which can overwhelm the body's antioxidant capacity. When high amounts of ROS are produced and not properly neutralized, the endoplasmic reticulum may increase the release of sterol regulatory element binding proteins-1 (SREBP1), leading to increased fatty acids synthesis. Then, when PPARα is highly activated we may expect an increased production of ROS, and in order to protect the endoplasmic reticulum and other organelles from ROS damage, it may be necessary to reduce the formation of ROS by incorporating less unsaturated chain fatty acids to cell membranes. Omega-9 fatty acids possess only one double-bond and may release the lowest number of ROS, as compared to n-6 and/or n-3 FAs, thus becoming a valid alternative to minimize ROS formation. Therefore, incorporation of n-3 and n-9 fatty acids may be more beneficial than n-3 fatty acids alone.

Approach to Provide a Nutritionally Enhanced Chicken Meat Source to Consumers

Modern diets in western societies are characterized by increased intake of saturated fat, n-6 fatty acids and *trans* fatty acids, with a concomitant decreased intake of n-3 fatty acids (Simopoulos, 2009). In the 1990's, the Food and Nutrition Board of the National Academy of Sciences recommended that more than 3% of daily calories on a balanced diet must be from C18:2. The recent recommendation for average daily intake of C18:2 was adjusted down to 1~2% (Sardesai, 1992). However, the average daily intake of linoleate in typical western diets is about 10 g (Sardesai, 1992), which is higher than the recommended level, and it may be responsible for causing serious health problems in consumers, such as inflammatory disorders. Omega-3 and -6 fatty acids compete for elongases (Elovl-2 and/or -5), desaturases (delta-5 and -6), and cyclooxygenase (COX) during the biosynthesis of long chain polyunsaturated fatty acids. Under high n-6 FA intake conditions, the eicosanoid metabolic products from C20:4, specifically prostaglandins, thromboxanes, leukotrienes, hydroxyl fatty acids, and lipoxins are formed in larger quantities than those formed from n-3 fatty acids, specifically C20:5.

The eicosanoids derived from C20:4 are biologically active in very small quantities and if they are formed in large amounts due to high intake of n-6 FAs they contribute to several pathophysiological responses, including allergic and inflammatory responses. Therefore, to reduce pro-inflammatory substances, the supply of n-3 and n-6 fatty acids must be controlled through the diet, *de novo* lipogenesis and/or fatty acid oxidation. In this regard, an elevated CLA/n-9 intake could be a key regulator for increasing the n-3/n-6 ratio in broilers and potentially reduce inflammatory response. Moreover, such a process might be expected to diminish inflammatory effects in consumers, due to lower intake of n-6 fatty acids and higher intake of n-3 and CLA/n-9 fatty acids.

CHAPTER III

EFFECTS OF DIETARY CONJUGATED LINOLEIC ACID (CLA) AND OMEGA-3 FATTY ACID COMBINATIONS ON THE DEPOSITION OF LINOLEIC ACID IN TWO DIFFERENT TYPES OF BROILER MUSCLES

1. OVERVIEW

This study was conducted to determine the effects of dietary CLA and omega-3 fatty acids (n-3 FAs) combination on the omega-6 fatty acids (n-6 FAs) accumulation of broiler chicken breast and thigh meats. Five hundred and twenty, one day old broiler chicks were purchased and raised up to 6 weeks. All chicks were fed with a basal corn-soybean meal diet containing five different fat sources with 2% total fat content: 1) conjugated linoleic acid (CLA), 2) flaxseed oil (FXO), 3) fish oil (FHO), 4) CLA and flaxseed oil (CXO) and 5) CLA and fish oil (CHO). Eight broilers from each treatment were processed at 4 and 6 weeks of age, respectively. During two different processing weeks, liver, breast and thigh samples were collected and analyzed for fatty acids profiles and total fat content. Elongation, delta-6 and delta-9 desaturase activities and overall n-3 fatty acids index were calculated using the fatty acid profiles of the liver.

Regarding the diets containing five different fat sources, broiler chickens fed CHO diet had a lower C20:4 deposition but showed a higher n-3/n-6 ratio in breast and thigh meats than those fed FXO and CXO diets ($P < 0.05$). The C20:4 and n-3/n-6 ratio of breast and thigh samples from FHO diet containing 2% fish oil only, was similar to those of CHO diet ($P > 0.05$). However, the addition of CLA and fish oil combination (CHO) to the diet resulted in an increase of polyunsaturated fatty acids (PUFA) concentration in broiler chicken breast and thigh meats when compared to that of FHO ($P < 0.05$). In conclusion, feeding broiler chickens with CHO diet, in contrast to FXO and CXO diets reduced the amount of C20:4 but increased the ratio of n-3/n-6. Moreover, the inclusion of PUFA to broiler chicken breast and thigh meats of CHO significantly improved when compared to that of a diet containing fish oil, only.

2. INTRODUCTION

The increasing consumer demand for healthier foods enriched with unsaturated fatty acids (UFA), generates an important growth opportunity for the poultry industry. Most UFAs are from dietary fat sources shown to provide beneficial effects in human health beyond the natural effects of conventional lipid sources. The poultry industry has the opportunity to fulfill this market need by producing customized poultry meat and its derived products to be rich in UFAs. Commonly, fat is a naturally occurring component of poultry food products, its inherent nutritional and functional properties depend on the lipid biosynthesis pathways in the bird's liver and the source and type of fatty acids present in the diet. In birds, as in humans, dietary fats and oils are important sources of energy and are absorbed with little modification of the fatty acids structure during this process. Absorbed fatty acids are deposited and accumulated in intra- and inter-muscular tissues in broilers.

Omega-3 fatty acids (n-3) are one of the common UFAs recognized as 'good' fat, with a variety of products in the market enriched with these fatty acids including n-3 fatty acids-enriched eggs. Omega-3 enhanced eggs and related products have revitalized the shell-eggs category at grocery stores nationwide, and there is some impact reported worldwide (Surai and Sparks, 2001). Omega-3 rich products have been shown to have significant acceptance by the health conscious consumer and are responsible for significant growth in egg consumption (Surai and Sparks, 2001). However, n-3 enriched broiler meats have not been commercially produced and are not yet available in retail settings. Reasons vary widely from productivity issues, to the relatively minor fat deposition in the muscle, which may make this addition non-commercially acceptable. However, the potential exist to enhance the nutritional composition of chicken meat products but more information is needed about competition with omega-6 fatty acids during elongation and desaturation of n-3 fatty acids after ingestion.

Due to potential drawbacks caused by the ingestion of n-3 fatty acids in poultry, the amount of dietary n-3 fatty acids is limited in commercial settings. Such drawbacks include rapid oxidation and unacceptable flavor generation of n-3 FA enriched poultry

meat and eggs (Hargis and Van Elswyk, 1993). However, to increase the potentially beneficial effects of n-3 fatty acids, one of the possible alternatives is to reduce competition of n-3 and -6 fatty acids in poultry taking into consideration that n-6 fatty acids are the most abundant nutrient in commercial poultry diets. Therefore, a new approach is necessary to increase n-3 fatty acids usage commercially. Conjugated linoleic acid (CLA) is a group of geometrical and positional isomers of linoleic acid (C18:2, n-6, LA) that has shown positive effects on reducing fat deposition in animal models mainly caused by increased metabolic rates (Corino et al., 2002; Zabala et al., 2006; Suksombat et al., 2007). The average daily intake of CLA is about 150 to 210 mg (Schmid et al., 2006); however, these levels would be considered insufficient to meet the 3.0 g per day recommended to promote human health (Aydin, 2005). Therefore, enriching poultry meat with CLA would represent an important source of CLA to consumers with easy accessibility and low cost and will in turn represent a significant growth opportunity for the poultry industry in the health conscious market.

Conclusively, to increase the benefits of n-3 fatty acid deposition in poultry meat in contrast to n-6 fatty acids, CLA is a possible alternative to bring enough energy to poultry when mixed to n-3 fatty acids and when supplied in the diet to broilers. Less n-6 fatty acids deposition could be achieved when CLA replaces n-6 fatty acids required in the diet due to the limited supplementation with n-3 fatty acids. Particularly, reduced competition between n-3 and -6 fatty acids during elongation and desaturation and more long chain n-3 polyunsaturated fatty acids accumulation as a result are expected when CLA is supplied in combination with n-3 fatty acids to broilers.

Hypothesis and Objective

The combined use of n-3 fatty acids and CLA combinations in broiler diets may lead the less competition between fatty acid sources during elongation and desaturation of n-3 and -6 fatty acids, thus potentially increasing the accumulation of long chain n-3 polyunsaturated fatty acids (PUFAs) but decreasing the long chain n-6 fatty acids deposition in broilers (Figure 3). Conjugated linoleic acid and n-3 fatty acids combinations may also minimize the synthesis of saturated fatty acids (SFAs) induced

by CLA presence due to a higher affinity to deposit n-3 fatty acids in the muscle.

↑Omega-3 FAs	⬛	↑CLAs		↓Omega-6 FAs
⊕		⊖		⊖
↑Omega-3 FAs		↑SFAs		↑Omega-6 FAs
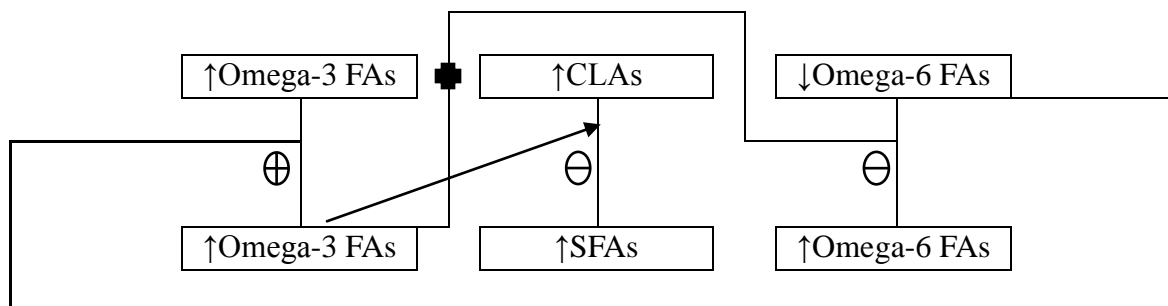
Figure 3. Simplified diagram of hypothesis.

To increase the deposition of n-3 fatty acids while decreasing the amount of n-6 fatty acids deposited in chicken meat during commercial production, different combinations of n-3 fatty acids (flaxseed and/or fish oil) and CLA were fed to chickens for up to 6 weeks. Fatty acids composition of two different muscles (breast and thigh) was determined at different stages during growth. In addition, the activities of elongases and desaturases were determined based on the accumulation of n-3, -6 and -9 fatty acids in the liver.

3. MATERIALS AND METHODS

Five hundred and twenty, one day old broiler chicks (*Gallus gallus domesticus*) were secured (26 × 4 × 5 = birds × replications × treatments) and raised up to 6 weeks of age at the Poultry Science Center of Texas A&M University.   All chicks were fed with a basal corn-soybean meal diet containing five different lipid sources based on 2% total fat content (Table 1);

1) Conjugated linoleic acid (50% c9t11 + 50% t10c12, CLA)

2) Flaxseed oil (FXO)

3) Fish (menhaden) oil (FHO)

4) CLA and flaxseed oil combination (CLA + FXO 1:1 combination, CXO)

5) CLA and fish oil combination (CLA + FHO 1:1 combination, CHO)

Fatty acids composition of basal diet and experimental diets were determined as in Tables 2, 3 and 4.   Sufficient essential fatty acids such as linoleic acid were provided through ingredients in the basal diet, only.   All birds were raised under commercial-like conditions.   A total of eight broilers from each treatment (5 × 4 = treatments × replications) were processed at 4 and 6 weeks of age.   During two different processing weeks, liver, breast and thigh samples were collected and stored at -80°C until analyzed. Individual liver, breast and thigh samples were analyzed for fatty acid composition and fat content.   Elongation, delta-6, and -9 desaturase activities and over-all n-3 fatty acids index were calculated using the fatty acid profiles of the liver.

Crude Fat Determination

Crude fat content of liver, breast and thigh was determined using a CEM auto-analyzer (Smart Trac System, CEM Co., Matthews, NC).   Each sample was trimmed, ground, and approximately 3 g of the sample were used as programmed.   Average crude fat content of sample is reported as a percent of fat.

Table 1. Composition of experimental basal diets of broiler chickens. Experiment I

Ingredient (%)	Starter (0~3 wk)	Grower (4~5 wk)	Finisher (6 wk)
Corn	58.81	63.97	68.84
Soybean meal	34.81	29.94	25.32
Biophos	1.67	1.59	1.51
Limestone	1.52	1.45	1.38
Oil	2.00	2.00	2.00
Salt	0.51	0.45	0.31
Vitamin Premix[1]	0.25	0.25	0.25
DL-Methionine	0.20	0.07	-
Choline 60	0.10	0.10	0.10
Coban 60	0.08	0.08	-
Mineral Premix[2]	0.05	0.05	0.05
Sodium bicarbonate	-	0.05	0.21
Calculated Nutrient Content (%)			
Crude Protein	22.0	20.00	18.15
ME energy (Kcal/lb)	3007.00	3056.22	3105.14
Calcium	0.95	0.90	0.85
Available Phosphorous	0.47	0.45	0.42
Methionine	0.53	0.38	0.32
Methionine + Cystine	0.90	0.72	0.63
Lysine	1.18	1.05	0.92
Threonine	0.82	0.75	0.68
Sodium	0.22	0.21	0.20

[1]Vitamin Premix (lb): vitamin A 2,000,000 IU, vitamin D3 700,000 IU, vitamin E 8,333 IU, vitamin B12 3.0 mg, riboflavin 1,083 mg, niacin 8,333 8,333 mg, d-pantohenic acid 3,667 mg, choline 86,667 mg, K 267 mg, folic acid 317 mg, vitamin B6 1,3000 mg, thiamine 533 mg, biotin 100. Breeder turkey, DSM Nutritional Products, Inc., Parsippany, NJ;[2]Mineral Premix: Ca 1.20%, Mn 30.0%, Zn 21.0%, Cu 8500 ppm, I 2100 ppm, Se 500 ppm, Mo 1670 ppm, Tyson Poultry 606 Premix.

Table 2. Fatty acid profile of basal and starter diets (%). Experiment I

	Starter[1]					
	BAS	CLA	FXO	FHO	CXO	CHO
C14:0	0.717	0.401	0.495	2.882	0.452	1.197
C16:0	14.172	10.245	11.028	15.270	9.243	11.749
C16:1	0.266	0.375	0.469	3.651	0.463	1.650
C18:0	2.874	3.533	3.024	3.035	3.391	3.260
C18:1c9	25.376	23.771	23.435	20.205	26.023	21.781
C18:1c11	0.772	0.745	0.787	1.622	0.838	1.091
C18:2	50.400	27.656	36.951	33.390	34.915	30.723
C18:3	2.418	2.342	20.703	2.286	15.697	5.109
c9t11 CLA[2]	-	12.800	-	-	4.216	6.358
t10c12 CLA	-	12.851	-	-	4.131	6.494
C20:1	-	0.264	0.098	1.099	0.151	0.660
C20:4	0.261	0.453	0.260	0.542	0.325	0.507
C20:5	-	-	0.432	4.414	0.368	2.119
C22:0	-	0.327	-	0.334	0.131	0.216
C22:1	-	-	-	0.581	-	0.280
C22:5	-	-	0.077	0.771	0.061	0.390
C22:6	-	0.194	0.336	3.458	0.276	1.764

[1]BAS: basal diet (no fat source), CLA: 2% conjugated linoleic acid(50% c9t11 + 50% t10c12 CLA), FXO: 2% flaxseed oil, FHO: 2% fish oil, CXO: 1% conjugated linoleic acid+ 1%flaxseed oil, CHO: 1% conjugated linoleic acid+1% fish oil;[2]CLA: conjugated linoleic aicd.

Table 3. Fatty acid profile of grower diets (%). Experiment I

	Grower[1]				
	CLA	FXO	FHO	CXO	CHO
C14:0	0.111	0.094	2.562	0.401	1.126
C14:1	-	-	0.222	-	0.109
C16:0	11.464	10.976	15.110	10.735	12.169
C16:1	0.487	0.328	3.560	0.703	1.739
C18:0	3.553	3.128	3.138	3.324	3.265
C18:1c9	26.569	25.295	21.312	24.255	22.950
C18:1c11	4.077	0.700	1.626	0.799	1.074
C18:2	32.484	36.776	33.424	31.946	31.328
C18:3	1.594	14.847	3.379	11.146	6.297
c9t11 CLA[2]	10.001	2.787	-	5.730	4.787
t10c12 CLA	10.169	2.829	-	5.773	4.885
C20:1	5.194	-	0.979	0.261	0.639
C20:4	0.369	0.306	0.576	0.377	0.493
C20:5	0.067	0.093	4.282	0.722	2.243
C22:0	0.274	-	0.476	0.172	0.174
C22:1	-	-	0.605	0.088	0.275
C22:5	-	-	0.774	0.120	0.402
C22:6	-	-	2.956	0.527	1.769

[1]CLA: 2% conjugated linoleic acid(50% c9t11 + 50% t10c12 CLA), FXO: 2% flaxseed oil, FHO: 2% fish oil, CXO: 1% conjugated linoleic acid+ 1%flaxseed oil, CHO: 1% conjugated linoleic acid+1% fish oil;[2]CLA: conjugated linoleic aicd.

Table 4. Fatty acid profile of finisher diets (%). Experiment I

	Finisher[1]				
	CLA	FXO	FHO	CXO	CHO
C14:0	0.094	0.085	2.288	0.073	1.038
C14:1	-	-	0.216	-	0.107
C16:0	10.262	10.858	14.155	10.266	11.917
C16:1	0.217	0.330	3.147	0.218	1.581
C18:0	3.445	3.123	3.142	3.259	3.310
C18:1c9	25.126	24.582	20.023	24.636	22.431
C18:1c11	0.699	0.725	1.540	0.704	1.082
C18:2	31.295	37.736	31.586	35.264	31.152
C18:3	2.386	20.681	2.760	15.855	6.039
c9t11 CLA[2]	11.097	0.142	2.368	3.634	5.712
t10c12 CLA	11.164	0.141	2.402	3.650	5.873
C20:1	0.189	-	1.077	0.062	0.615
C20:4	0.378	0.247	0.537	0.294	0.482
C20:5	0.116	-	3.998	0.072	1.947
C22:0	0.289	-	0.132	0.105	0.199
C22:1	-	-	0.539	-	0.260
C22:5	-	-	0.718	-	0.347
C22:6	-	-	3.221	-	1.569

[1]CLA: 2% conjugated linoleic acid(50% c9t11 + 50% t10c12 CLA), FXO: 2% flaxseed oil, FHO: 2% fish oil, CXO: 1% conjugated linoleic acid+ 1%flaxseed oil, CHO: 1% conjugated linoleic acid+1% fish oil;[2]CLA: conjugated linoleic aicd.

Fatty Acid Composition Determination

Total lipid extraction: to determine the fatty acids profile of diet, liver, breast and thigh, the fatty acids methyl ester (FAME) methodology was performed using a method described by Smith et al. (2002).Briefly, 1.5 g of sample were used to extract total lipid by a method described by Folch et al. (1957).   Approximately five milliliters of chloroform:methanol (CHCl$_3$:CH$_3$OH, 2:1, v/v) was added to the sample before being homogenized it for 30 sec. using a Polytron homogenizer (Tissumizer, Tekmar Co., Cincinnati, OH).Additional chloroform:methanol mixture was added to make up for a final volume of 15 mL.   Each sample was set for 30 min and filtered (Glass Microfibre Filter 691, 2.4 cm, VWR International, UK).   Eight milliliters of 0.74% potassium chloride (KCl) was added to the sample and vortexed for 1 min.   The sample was further centrifuged (International Centrifuge Universal Model UV, International Equipment Co., Needham HTS, MASS) at 1620$\times$ g and the upper phase was discarded. The remaining sample was transferred into a 20 mL glass tube and dried using nitrogen gas in a warm water bath.

Saponification and methylation of lipids: after evaporation of the sample, 1 mL of 0.5 N potassium hydroxide (KOH) in methanol (MeOH) was added to the sample, and the mix was heated in a water bath maintained at 70°C up to 10 min.   Approximately one milliliter of 14% boron trifluoride (BF$_3$) was added, and each tube was flushed with nitrogen gas, loosely capped, and placed in 70 ^{o}C water bath for 30 min.   At the end of 30 min the tube was removed from the water bath and cooled.   After cooling, approximately 2 mL of hexane and 2 mL of sodium chloride (NaCl) were added, the sample mix was vortexed and set for separation of phases.   The upper layer was transferred to another 20 mL glass tube containing 800 mg of sodium sulfate (Na$_2$SO$_4$). The sample was vortexed briefly, and then hexane was transferred to a scintillation vial.

Injection of sample: hexane was removed completely using nitrogen gas, and the lipid was reconstituted with the appropriate amount of hexane to obtain a final concentration of approximately 50 mg/mL.   Around 0.4 mL of the sample was transferred into a 2 mL auto-sampler vial containing 1.6 mL of HPLC-grade hexane. The composition of the FAME was determined by a gas chromatography (GC) fixed with a CP-8200 Auto-Sampler (Varian Chromatography System, Walnut Creek, CA) (Table 5).   Each fatty acid profile was expressed as percentage (%) of total known FAME.

Elongation, Delta-6 and -9 Desaturase Activities and Overall n-3 Fatty Acids Index

Elongation, delta-6 and -9 desaturase activities and overall n-3 fatty acids index were calculated using profiles of fatty acids from liver as established by Jula et al. (2005), Okada et al. (2007), Smith et al. (2002) and Agostoni et al. (2008) and expressed as follows;

$$Elongation = (C18{:}0) / (C16{:}0)$$

$$Delta\text{-}6 \text{ desaturase index} = (C20{:}4) / (C18{:}2)$$

$$Delta\text{-}9 \text{ desaturase index} = (C16{:}1) / (C18{:}0)$$

$$Over\text{-}all \text{ n-3 index} = (C22{:}6) / (C18{:}3)$$

Overall n-3 fatty acids index indicates the n-3 biosynthetic pathway including elongations, delta-5 and -6 activities and peroxisomal ß-oxidation.

Table 5. Gas chromatograph conditions for fatty acid analysis of broiler diet, liver, breast and thigh muscle

	Condition
Instrument	Varian Chrompack, CP-3800 Gas Chromatograph
Column	WCOT Fused Silica Capillary Column, 100 m $\times$ 0.25 mm i.d., CP-7420
Detector	Flame Ionization Detector (FID)
Oven Temperature	Initial Temperature: 185°C (hold for 32 min)
	Increase Rate: 20°C/min
	Final Temperature: 235°C (hold for 15.50 min)
Injector Temperature	270°C
Detector Temperature	270°C
Carrier Gas	Helium (He)
Split Ratio	100

Statistical Analysis

All data were analyzed as a factorial arrangement by Analysis of Variance using the generalized linear model (GLM) procedure of SAS (Version 6.12, Cary, NC, 1998) with a predetermined significance level of $P < 0.05$. Main effects of treatment and age and two--way interactions (treatment by age) were included in the initial model. Two-way interactions for all main effects were analyzed and remained in the final model if they were significant ($P < 0.05$). Least squares means were estimated and separated using the stderr pdiff function when differences were determined by Analysis of Variance. All final models included significant two-way interactions or main effects were stayed if two-way interaction was not significant ($P > 0.05$).

4. RESULTS

Fatty Acid Profiles of Broiler Chicken Liver

Omega-3 (n-3) and -6 (n-6) fatty acid profiles of broiler chicken livers from broilers fed with five different diets during two different ages are summarized in Tables 6 and 7. There was a significant difference in the treatment by age interaction of docosahexaenoic acid (C22:6, DHA) and n-3/n-6 ($P < 0.05$). However, the main effect observed on either treatment or age was significantly related to the fatty acids content including linoleic acid (C18:2, LA), linolenic acid (C18:3, LNA), dihomo-γ-linolenic acid (C20:3, DGA), arachidonic acid (C20:4, AA), eicosapentaenoic acid (C20:5, EPA) and docosapentaenoic acid (C22:5, DPA) ($P < 0.05$). The conjugated linoleic acid (CLA) induced the deposition of C18:2 and saturated fatty acid (SFA) (13.84 and 53.40%, respectively) but diminished the overall content of C18:3, C20:3, C20:4, C20:5, C22:5, monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) to 0.31, 0.81, 3.60, 1.16, 0.86, 18.95 and 25.53%, respectively.

Flaxseed oil (FXO) and fish oil (FHO) diets significantly lowered the overall content of C18:2 and SFA but showed a higher deposition of C20:3, C20:4, C20:5, C22:5 and MUFA when compared to that of CLA ($P < 0.05$). Overall content of PUFA was not significantly influenced when FXO was fed to broilers ($P > 0.05$) in contrary to FHO which significantly affected the PUFA content ($P < 0.05$). Conjugated linoleic acid and flaxseed oil combination (CXO) treatment had a higher deposition of C18:2 and lower deposition of MUFA compared to broilers raised on FXO and FHO diets ($P < 0.05$) but similar to that of CLA diet ($P > 0.05$). Additionally, the deposited amount of C20:4 generated in CXO treatment was similar to that of CLA but was not similar to that of FXO. The C18:2 and C20:3 of CLA and fish oil combination (CHO) diet were similar to that of CLA only. Individual CLA and fish oil supplementation affected the overall content of C18:2 and C20:3 when CHO diet was fed to broilers. However, CHO increased the deposition of C20:5 and C22:5 to 2.11 and 1.36%, respectively but decreased the overall content of SFA to 50.43% when compared to that of CLA (1.16 and 53.40%, respectively) ($P < 0.05$). The overall content of C20:4, MUFA and PUFA

Table 6. Omega-3 and -6 fatty acid profiles of broiler chicken livers fed with different fat source diets and processed at 4 and 6 weeks of growth (%)

Effect	C18:2	C18:3	C20:3	C20:4	C20:5	C22:5
TRT*WKS[1]						
P-value	0.369	0.016	0.056	0.004	0.384	0.049
TRT[2]						
P-value	0.017	0.001	0.001	0.001	0.001	0.001
CLA	13.84[a]	0.31[d]	0.81[c]	3.60[c]	1.16[c]	0.86[c]
FXO	11.84[b]	1.33[a]	1.36[a]	5.91[a]	1.89[b]	1.27[b]
FHO	12.19[b]	0.36[d]	1.05[b]	4.55[b]	3.31[a]	1.83[a]
CXO	13.83[a]	0.89[b]	1.04[b]	4.08[bc]	1.94[b]	1.39[b]
CHO	12.60[ab]	0.59[c]	0.94[bc]	3.26[c]	2.11[b]	1.36[b]
WKS[3]						
P-value	0.093	0.092	0.067	0.193	0.204	0.055
4	13.24	0.67	1.09	4.46	2.17	1.42
6	12.50	0.77	0.99	4.10	1.96	1.25
ROOT MSE[4]	2.047	0.263	0.245	1.238	0.618	0.383

[1]TRT*WKS = treatment by age interaction; [2]Treatment: CLA = 2% conjugated linoleic acid (50% c9t11 + 50% t10c12 CLA), FXO = 2% flaxseed oil, FHO = 2% fish (menhaden) oil, CXO = 1% conjugated linoleic acid + 1% flaxseed oil, CHO = 1% conjugated linoleic acid + 1% fish (menhaden) oil; [3]WKS = age; [4]ROOT MSE = Root Mean Square Error; [a,b,c,d]Mean values within a row followed by the same letter are not significantly different ($P > 0.05$).

Table 7. Omega-3 and total fatty acid[1] profiles of broiler chicken livers fed with different fat source diets and processed at 4 and 6 weeks of growth (%)

Effect	C22:6	SFA	MUFA	PUFA	n3/n6
TRT*WKS[2]					
P-value	**0.009**	0.828	0.060	0.094	**0.001**
TRT[3]					
P-value	0.001	0.001	0.001	0.042	0.001
CLA	3.35	53.40[a]	18.95[c]	25.53[b]	0.31
FXO	3.19	41.28[c]	30.52[a]	26.79[b]	0.40
FHO	7.80	42.26[c]	25.39[b]	31.39[a]	0.74
CXO	4.07	50.03[b]	20.49[c]	27.91[ab]	0.45
CHO	5.21	50.43[b]	21.67[c]	26.80[b]	0.55
WKS[4]					
P-value	0.001	0.058	0.169	0.014	0.022
4	5.31	46.84	22.78	29.09[a]	0.51
6	4.03	48.21	24.03	26.18[b]	0.46
ROOT MSE[5]	1.450	2.851	4.534	5.163	0.083

[1]SFA = saturated fatty acid (C14:0 + C16:0 + C18:0), MUFA = monounsaturated fatty acid (C16:1 + C18:1c9), PUFA = polyunsaturated fatty acid (C18:2 + C18:3 + c9t11 + t10c12 + C20:3 + C20:4 + C20:5 + C22:5 + C22:6), n3/n6 = ratio of omega-3 fatty acids (C18:3 + C20:5 + C22:5 + C22:6) and omega-6 fatty acid (C18:2 + C20:3 + C20:4); [2]TRT*WKS = treatment by age interaction; [3]Treatment: CLA = 2% conjugated linoleic acid (50% c9t11 + 50% t10c12 CLA), FXO = 2% flaxseed oil, FHO = 2% fish (menhaden) oil, CXO = 1% conjugated linoleic acid + 1% flaxseed oil, CHO = 1% conjugated linoleic acid + 1% fish (menhaden) oil; [4]WEEK = age; [5]ROOT MSE = Root Mean Square Error; [a,b,c,d]Mean values within a row followed by the same letter are not significantly different ($P > 0.05$).

of CHO treatment was apparently closely influenced but by CLA not by FHO.

The main effect of age did not affect the overall content of C18:2, C18:3, C20:3, C20:4, C20:5, C22:5, SFA and MUFA ($P > 0.05$) but had an effect on the deposition of PUFA during 4 and 6 weeks of age ($P < 0.05$).   As expected, an increased content of PUFA (29.09%) at 4 weeks of growth was determined.

Least squares means of the treatment by age interaction for C22:6 (Docosahexaenoic acid, DHA, n-3) are reported in Figure 4.   Within 4 weeks of feeding, CLA, CHO and CXO diets (4.19, 4.62 and 5.08%, respectively) did not influence the deposition of C22:6 ($P > 0.05$), but FHO (9.00%) affected C22:6 deposition levels ($P < 0.05$).   Additionally, the deposition of C22:6 in FXO (3.96%) was neither more nor less than C22:6 content of CLA ($P > 0.05$) but significantly differed to that of CHO and CXO ($P < 0.05$).   The C22:6 of FXO, CLA and CXO treatment was not significantly different up to 6 weeks of feeding with values of 2.41, 2.61 and 3.07%, respectively.   The overall content of C22:6 was similar when both CHO (5.79%) and FHO (6.59%) diets were supplied to broiler chickens for 6 week.   During 4 to 6 weeks of feeding, the deposition of C22:6 significantly diminished when FXO, CLA, CXO and FHO diets were fed to broiler chickens ($P < 0.05$).   Only, C22:6 of CHO (4.62 and 5.79%) increased and finally reached to that of FHO at 6 week ($P > 0.05$).

Differences in the omega-3 and -6 fatty acid ratio were not significant due to CLA and FXO diets (0.37 and 0.42, respectively) ($P > 0.05$), however, these levels had significant differences when compared to that of CHO (0.51) and CXO (0.52) at 4 weeks of growth (Figure 4).   The n-3 and -6 ratio of CHO and CXO was even closer to 1 than CLA and FXO; however, the highest n-3 and n-6 ratio was observed with broilers raised on FHO (0.74) diets for 4 weeks ($P < 0.05$).   For full grown broilers up to 6 weeks of age, CLA treatment (0.25) had the lowest n-3 and n-6 ratio when compared to that of other treatments, and the n-3 and -6 ratio of CLA differed as compared to CXO (0.37) and FXO (0.38) treatments ($P < 0.05$).   A non-significant n-3 and -6 ratio was determined when both CXO and FXO diet was provided to broilers for 6 weeks ($P > 0.05$), and the highest n-3 and -6 ratio at 6 weeks of feeding was observed when boilers
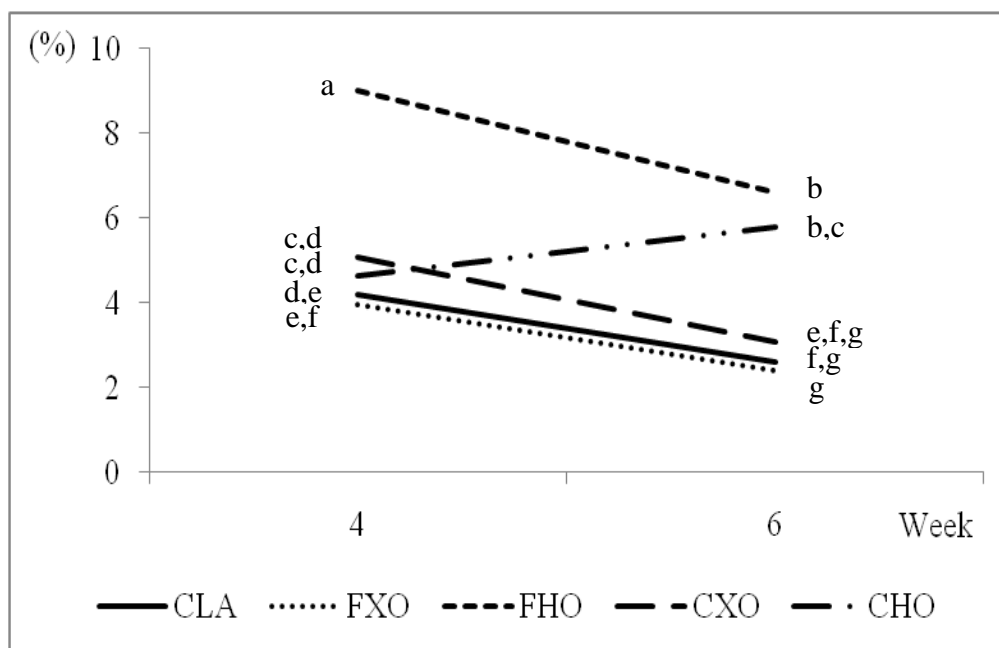
Docosahexaenoic Acid (C22:6, DHA, n-3; $P = 0.009$)
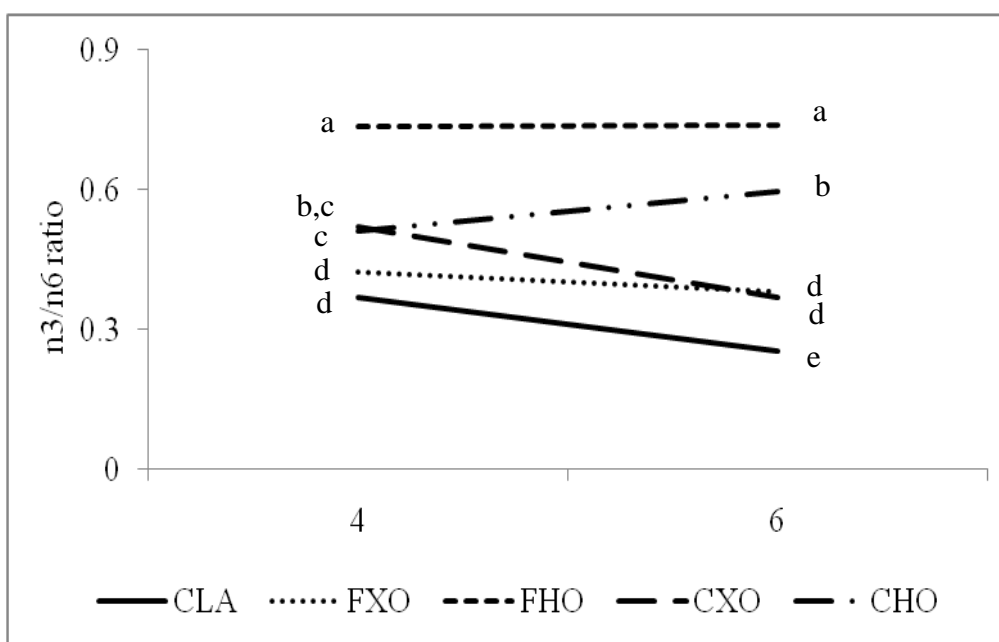
n-3/n-6 Ratio ($P = 0.001$)

Figure 4. Least squares means for treatment by age interaction for docosahexaenoic acid (DHA) and omega-3 to -6 ratio of broiler chicken liver fed with different fat source diets.

CLA = 2% conjugated linoleic acid (50% c9t11 + 50% t10c12 CLA), FXO = 2% flaxseed oil, FHO = 2% fish (menhaden) oil, CXO = 1% conjugated linoleic acid + 1% flaxseed oil, CHO = 1% conjugated linoleic acid + 1% fish (menhaden) oil.

[a-g]Mean values within a row followed by the same letter are not significantly different ($P > 0.05$).

were fed with the FHO diet.   Omega-3 and -6 ratio of CLA and CXO decreased to 0.25 and 0.37 respectively ($P < 0.05$), but FXO and FHO preserved the n-3 and -6 ratio when each diet was supplied to broilers during the 4 to 6 weeks ($P > 0.05$).   However, CXO diet had a negative influence on n-3 and n-6 ratio, but a positive effect was observed when CHO was fed to broilers for 6 weeks of grow-out.

Enzyme Activity Ratios of Broiler Chicken Liver

The influence of five different fat sources on the changes in the ratio of fatty acids was estimated and presented in Table 8.   There was not a significant two-way interaction (treatment by age) ($P > 0.05$).   A main effect, due to treatment and/or age, was determined in fatty acid ratios including C18:0 to C16:0, C20:4 to C18:2, C16:1 to C18:0 and C22:6 to C18:3 ratios ($P < 0.05$).   The five different dietary fat sources did not significantly influence the ratio of C18:0 to C16:0, indicating that the activities of elongation-of-very-long-chain-fatty acid (Elovl)-2 and -5 elongase was not affected by CLA, flaxseed oil, fish oil and their combination in the diet ($P > 0.05$).   However, the five different fat sources had effects on the ratio of C20:4 to C18:2, C16:1 to C18:0 and C22:6 to C18:3.   Three different ratios of C20:4 to C18:2 (delta-6 desaturase activity index) due to CLA, CXO and CHO diets were not significant, and neither CLA and CXO nor CHO was similar to that of FHO and FXO treatments.   The FXO treatment showed the highest C20:4 to C18:2 ratio as compared to the ratio of other four treatments ($P < 0.05$).

The FXO treatment which contains 2% of flaxseed oil as a dietary fat source, significantly influenced C16:1 to C18:0 ratio (delta-9 desaturase activity index) ($P < 0.05$), while the other fat sources had no effects on C16:1 to C18:0 ratio ($P > 0.05$).   It is important to indicate that FXO led to a higher C16:1 and less C18:0 deposition or *de novo* lipogenesis in the liver when compared to that of other fat sources including CLA, FHO, CXO and CHO.   Fish oil addition to broiler diet was effective and had the highest C22:6 to C18:3 ratio at 26.88 ($P < 0.05$).   On the contrary, fish oil and CLA combination (CHO) diet showed similar effect as that of CLA ($P > 0.05$), however, the

Table 8. Estimate of changes in the ratios of liver fatty acids[1] when broiler chickens were fed with different fat source diets and processed at 4 and 6 week of growth

Effect	C18:0/C16:0	C20:4/C18:2	C16:1/C18:0	C22:6/C18:3
TRT*WKS[2]				
P-value	0.162	0.001	0.143	0.564
TREAT[3]				
P-value	0.866	0.001	0.001	0.001
CLA	0.76	0.26^c	0.08^b	12.46^b
FXO	0.79	0.49^a	0.16^a	2.49^c
FHO	0.79	0.37^b	0.12^b	26.88^a
CXO	0.76	0.29^c	0.10^b	7.84^{bc}
CHO	0.77	0.26^c	0.10^b	12.12^b
WEEK[4]				
P-value	0.010	0.658	0.253	0.018
4	0.81^a	0.34	0.10	13.97^a
6	0.74^b	0.33	0.12	9.63^b
ROOT MSE[5]	0.116	0.063	0.054	8.458

[1]C18:0/16:0 indicates the activity of Elovl-2 and/or -5 elongase,C20:4/C18:2 Indicates the activity of delta-6 desaturase,C16:1/C18:0 indicates the activity of delta-9 desaturase,C22:6/C18:3 indicates the overall omega-3 related enzymes and β-oxidation of peroxisome;[2]TRT*WKS = treatment by processing week interaction; [3]Treatment: CLA = 2% conjugated linoleic acid (50% c9t11 + 50% t10c12 CLA), FXO = 2% flaxseed oil, FHO = 2% fish (menhaden) oil, CXO = 1% conjugated linoleic acid + 1% flaxseed oil, CHO = 1% conjugated linoleic acid + 1% fish (menhaden) oil; [4]WEEK = age; [5]ROOT MSE = Root Mean Square Error; [a,b,c]Mean values within a row followed by the same letter are not significantly different ($P > 0.05$).

C22:6 to C18:3 ratio of CXO was similar to that of FXO, and it had the lowest C22:6 to C18:3 ratio when compared to CLA, CHO and FHO treatments ($P < 0.05$). A main effect, due to age, was observed in C18:0 to C16:0 and C22:6 to C18:3 ratios but was not present in C20:4 to C18:2 and C16:1 to C18:0 ratios ($P < 0.05$). Both C18:0 to C16:0 and C22:6 to C18:3 ratios were higher at 4 weeks of growth when compared to those at 6 weeks, and they were 0.81 and 13.97, and 0.74 and 9.63, respectively.

Total crude fat content of broiler chicken liver, breast and thigh muscles were evaluated and summarized in Table 9. Two-way interaction which is treatment by age for crude fat was not significant in liver, breast and thigh ($P > 0.05$). Also, the main effect of treatment and age did not influence the total crude fat of liver, breast and thigh samples ($P > 0.05$). These results suggest that both dietary fat source and feeding period may not be important factors that contribute to total fat accumulation in liver, breast and thigh muscles during the 6 weeks of growth evaluated.

Fatty Acid Profiles of Broiler Chicken Breast Muscle

Overall content of n-3 and -6 fatty acid profiles of broiler chicken breast due to five different fat diets was studied and summarized in Tables 10 and 11. Only, C20:5 (Eicosapentaenoic acid, EPA, n-3) and saturated fatty acids (SFA) had a treatment by age interaction ($P < 0.05$). However, a main effect due to treatment or age significantly influenced the rest of n-3 and -6 fatty acids, MUFA, PUFA and the ratio of n-3 to -6 fatty acids ($P < 0.05$). Conjugated linoleic acid (CLA) induced the deposition of C18:2 but minimized the accumulation of C20:3, C20:4, C22:5 and the ratio of n-3 to n-6 fatty acids when compared to other four treatments. The overall content of C18:3, C22:6, MUFA and PUFA of CLA treatment was similar to that of FHO, CXO and/or CHO, respectively ($P > 0.05$). The FXO diet increased the overall content of C18:3 and MUFA, however, provided a similar deposition of C20:3, C20:4 and C22:6 to that of CHO, CXO and/or CLA.

Table 9. Crude fat of broiler chicken liver, breast and thigh muscle samples when fed with different fat source diets and processed at 4 and 6 weeks of growth (%)

Effect	LIVER	BREAST	THIGH
TRT*WKS[1]			
P-value	0.271	0.724	0.879
TREAT[2]			
P-value	0.845	0.066	0.651
CLA	4.79	1.16	2.76
FXO	5.21	1.00	3.02
FHO	5.34	0.77	2.78
CXO	4.81	0.97	2.74
CHO	4.88	0.78	2.66
WEEK[3]			
P-value	0.063	0.150	0.709
4	4.64	0.87	2.82
6	5.38	1.00	2.77
ROOT MSE[4]	1.777	0.428	0.687

[1]TRT*WKS = treatment by age interaction; [2]Treatment: CLA = 2% conjugated linoleic acid (50% c9t11 + 50% t10c12 CLA), FXO = 2% flaxseed oil, FHO = 2% fish (menhaden) oil, CXO = 1% conjugated linoleic acid + 1% flaxseed oil, CHO = 1% conjugated linoleic acid + 1% fish (menhaden) oil; [3]WEEK = age; [4]ROOT MSE = Root Mean Square Error.

Table 10. Omega-3 and -6 fatty acid profiles of broiler chicken breast fed with different fat source diets and processed at 4 and 6 weeks of growth (%)

Effect	C18:2	C18:3	C20:3	C20:4	C20:5	C22:5
TRT*WKS[1]						
P-value	0.117	0.076	0.876	0.430	**0.041**	0.500
TRT[2]						
P-value	0.001	0.001	0.001	0.001	0.001	0.001
CLA	18.70[a]	0.79[d]	0.59[c]	1.57[d]	0.99	1.04[c]
FXO	17.48[b]	5.30[a]	0.89[a]	2.92[a]	1.14	2.06[b]
FHO	16.49[c]	1.09[cd]	0.75[b]	2.30[bc]	2.13	2.71[a]
CXO	17.42[b]	3.32[b]	0.74[b]	2.54[ab]	1.37	1.97[b]
CHO	15.62[c]	1.29[c]	0.70[ab]	2.11[c]	2.05	2.74[a]
WKS[3]						
P-value	0.004	0.001	0.001	0.430	0.001	0.307
4	16.73[b]	2.14[b]	0.79[a]	2.41	1.73	2.15
6	17.57[a]	2.58[a]	0.64[b]	2.16	1.33	2.05
ROOT MSE[4]	1.238	0.481	0.144	0.538	0.280	0.427

[1]TRT*WKS = treatment by age interaction; [2]Treatment: CLA = 2% conjugated linoleic acid (50% c9t11 + 50% t10c12 CLA), FXO = 2% flaxseed oil, FHO = 2% fish (menhaden) oil, CXO = 1% conjugated linoleic acid + 1% flaxseed oil, CHO = 1% conjugated linoleic acid + 1% fish (menhaden) oil; [3]WKS = age; [4]ROOT MSE = Root Mean Square Error; [a,b,c,d]Mean values within a row followed by the same letter are not significantly different ($P > 0.05$).

Table 11. Omega-3 and total fatty acids[1] profiles of broiler chicken breast fed with different fat source diets and processed at 4 and 6 weeks of growth (%)

Effect	C22:6	SFA	MUFA	PUFA	n3/n6
TRT*WKS[2]					
P-value	0.419	**0.001**	0.774	0.385	0.166
TRT[3]					
P-value	0.001	0.001	0.001	0.010	0.001
CLA	1.56[b]	42.63	24.04[d]	30.13[c]	0.21[d]
FXO	1.76[b]	31.31	34.80[a]	31.55[ab]	0.48[b]
FHO	5.35[a]	34.16	32.47[b]	30.59[bc]	0.59[a]
CXO	2.01[b]	36.60	25.98[c]	31.72[ab]	0.41[c]
CHO	4.88[a]	37.71	23.69[d]	32.37[a]	0.60[a]
WKS[4]					
P-value	0.001	0.001	0.001	0.346	0.001
4	3.49[a]	35.38	27.20[b]	31.46	0.49[a]
6	2.71[b]	37.58	29.45[a]	31.03	0.43[b]
ROOT MSE[5]	0.794	1.807	1.990	1.849	0.065

[1]SFA = saturated fatty acid (C14:0 + C16:0 + C18:0), MUFA = monounsaturated fatty acid (C16:1 + C18:1c9), PUFA = polyunsaturated fatty acid (C18:2 + C18:3 + c9t11 + t10c12 + C20:3 + C20:4 + C20:5 + C22:5 + C22:6), n3/n6 = ratio of omega-3 fatty acids (C18:3 + C20:5 + C22:5 + C22:6) and omega-6 fatty acid (C18:2 + C20:3 + C20:4); [2]TRT*WKS = treatment by age interaction; [3]Treatment: CLA = 2% conjugated linoleic acid (50% c9t11 + 50% t10c12 CLA), FXO = 2% flaxseed oil, FHO = 2% fish (menhaden) oil, CXO = 1% conjugated linoleic acid + 1% flaxseed oil, CHO = 1% conjugated linoleic acid + 1% fish (menhaden) oil; [4]WEEK = age; [5]ROOT MSE = Root Mean Square Error; [a,b,c,d]Mean values within a row followed by the same letter are not significantly different ($P > 0.05$).

In contrast to the deposition of C18:2 and C18:3 of FXO treatment, lower deposition of C18:2 and C18:3 was determined when broilers were raised on FHO diet for 4 and 6 weeks ($P < 0.05$).   Also, FHO treatment negatively induced the deposition of PUFA but positively influenced the overall content of C22:5 and C22:6 and the ratio of n-3 and -6 fatty acids ($P < 0.05$).   Neither C20:3 and C20:4 nor MUFA of FHO was highly deposited when compared to that of FXO.   Overall content of C20:3 and PUFA was similar when both CXO and CHO diet was supplied to broilers even though each diet contains 1% flaxseed oil and 1% fish oil, respectively ($P > 0.05$).   However, more C18:2, C18:3, C20:4 and MUFA but less C22:5 and C22:6 were deposited in CXO when compared to that of CHO ($P < 0.05$).

Flaxseed oil when combined with CLA (1:1 ratio), positively induced C18:2 and C20:4 accumulations as compared to the fish oil with CLA in the diet.   The CXO diet provided a similar overall content of C18:2 and C20:4 even though less flaxseed oil was added to CXO diet when compared to 2% flaxseed oil addition of FXO.   Therefore, not C18:3 but C20:5, C22:5 and/or C22:6 of n-3 fatty acids of diets negatively affected the accumulation of C18:2 and C20:4 in broiler chicken breast muscle when they are provided as fish oil, itself or as a combined form with CLA in the poultry diet.   The overall content of C20:4 and C22:5 and PUFA was not significantly affected by age ($P > 0.05$).   However, the deposition of C18:2 and C18:3 and MUFA were increased from 16.73, 2.14 and 27.20 to 17.57, 2.58 and 29.45%, respectively.   Significant reduction to 0.64, 2.71 and 0.43 was determined in C20:3, C22:6 and n-3/n-6 when five different diets were supplied to broilers for 6 weeks ($P < 0.05$).

The overall content of C20:5 (Eicosapentaenoic acid, EPA, n-3) in CLA (1.18%) was neither more nor less than that of FXO (1.26%) treatment ($P > 0.05$), but less C20:5 was deposited when compared to that of CXO, FHO and CHO (1.59, 2.22 and 2.41%, respectively) at 4 weeks of growth ($P < 0.05$) (Figure 5).   However, the C20:5 of FHO and CHO treatment were not significant ($P > 0.05$).   Both FHO and CHO diet positively induced the deposition of C20:5 when compared to that of CXO during 4 weeks of feeding ($P < 0.05$).   When broilers were raised on five different fat diets for 6 weeks, C20:5 of CLA and FXO (0.78 and 1.02%, respectively) were insignificant ($P > 0.05$), but only C20:5 of CLA had a significant difference when compared to that of CXO treatment ($P < 0.05$).   In addition, more C20:5 was deposited but less C20:5 was accumulated in CHO treatment when compared to that of CXO and FHO, respectively ($P < 0.05$).   The FXO and FHO maintained the overall content of C20:5 ($P > 0.05$), but significant reduction of C20:5 was observed when CLA, CXO and CHO diets were supplied to broilers for 4 to 6 weeks of age ($P < 0.05$).

Treatment by age interaction for saturated fatty acids (SFA) due to FXO diet significantly differed when compared to other four diets at 4 weeks of age ($P < 0.05$) (Figure 5).   On the other hand, CXO and CHO had a similar overall content of SFA ($P > 0.05$), and both CXO and CHO diet showed a significant difference of SFA as compared to that of FXO, FHO and CLA ($P < 0.05$).   Within 6 weeks of feeding to broilers, only SFA of FHO and CXO (35.79 and 36.22, respectively) did not differ ($P > 0.05$) but had a significant difference when compared to that of FXO, CXO and CLA treatments (32.16, 36.22 and 45.10, respectively) ($P < 0.05$).   The lowest accumulation of SFA was induced in FXO diet, however, the highest SFA was deposited when CLA diet was supplied to broilers for 6 weeks ($P < 0.05$).   The FXO, CXO and CHO diets did not influence the accumulation of SFA ($P > 0.05$), but SFA of FHO and CLA was positively increased from 32.53 and 40.16 to 35.79 and 45.10 during 4 to 6 weeks of feeding ($P < 0.05$).
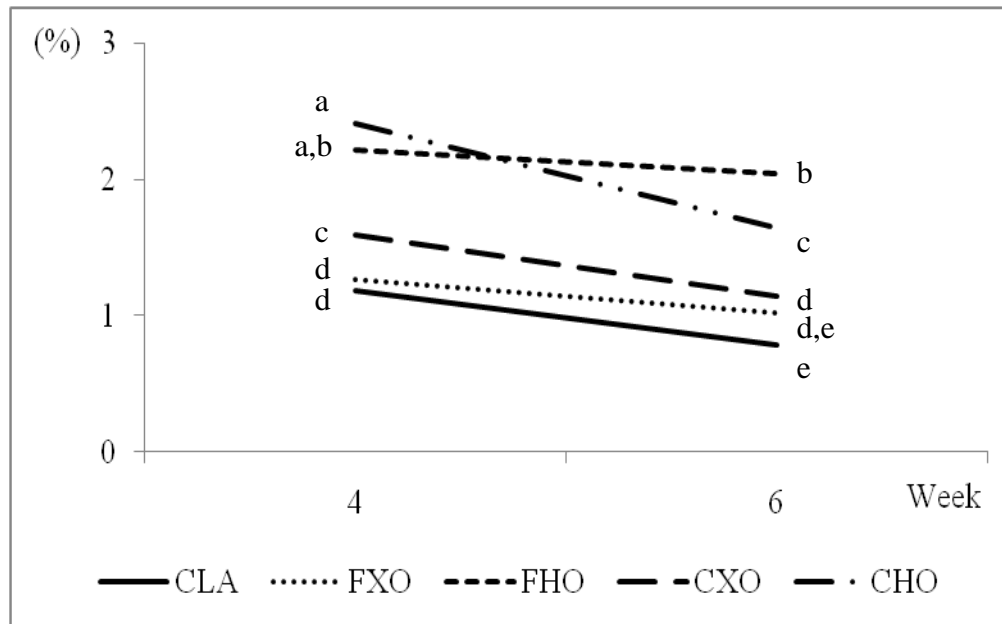
Eicosapentaenoic Acid (C20:5, EPA, n-3; $P = 0.041$)
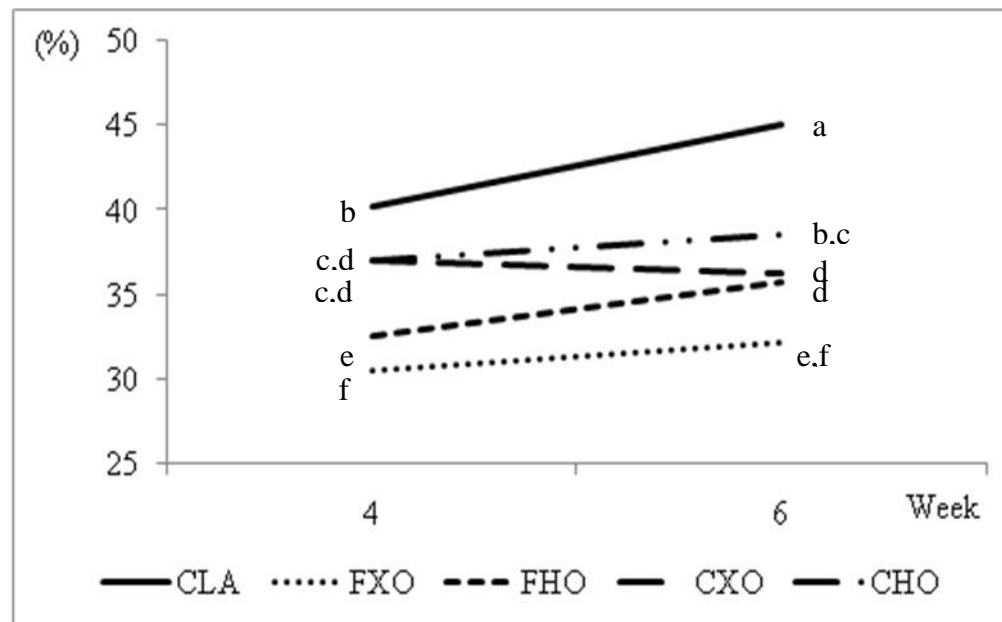
Saturated Fatty Acid (SFA; $P = 0.001$)

Figure 5. Least squares means for treatment by age interaction for eicosapentaenoic acid (EPA) and total saturated fatty acid of broiler chicken breast fed with different fat diets.

CLA = 2% conjugated linoleic acid (50% c9t11 + 50% t10c12 CLA), FXO = 2% flaxseed oil, FHO = 2% fish (menhaden) oil, CXO = 1% conjugated linoleic acid + 1% flaxseed oil, CHO = 1% conjugated linoleic acid + 1% fish (menhaden) oil.

[a-f]Mean values within a row followed by the same letter are not significantly different ($P > 0.05$).

Fatty Acid Profiles of Broiler Chicken Thigh Muscle

Two-way interaction due to treatment by age was performed in C18:2 and SFA and presented in Tables 12 and 13 ($P < 0.05$). The rest of n-3 and -6 fatty acids, MUFA and PUFA, and the ratio of n-3 and -6 fatty acids had a main effect due to treatment and/or age, only ($P < 0.05$). Broiler chicken thigh muscle raised on CLA diet had the lowest deposition of C18:3, C22:5 and the ratio of n-3/n-6, and the overall content of C20:3, C20:4, MUFA and PUFA of CLA was similar to that of FHO and/or CHO ($P < 0.05$). In contrast to CLA diet, more C18:3, C20:3, C20:4, C22:5, MUFA, PUFA and n-3/n-6 accumulated when FXO was supplied to broilers ($P < 0.05$), however, no significant deposition of C20:5 and C22:6 in FXO was determined when compared to that of CLA treatment ($P > 0.05$). The 2% flaxseed oil of FXO treatment effectively induced the deposition of C20:4 within the broiler thigh muscle and had higher overall C20:4 content when compared to broilers fed by CLA, FHO and CHO diets for 6 weeks ($P < 0.05$).

The diet containing 2% fish oil (FHO) had a positive relation to C20:5, C22:5, C22:6 and n-3/n-6 but negatively induced the overall content of C20:4 when compared to that of FXO ($P < 0.05$). As a result of n-3 fatty acid accumulation due to FHO diet, 0.59 of n-3 and n-6 ratio was determined, and it exceeds the 1:2, n-3 and n-6 ratio recommended. A similar percentage of C20:3, C20:4 and C22:5 was confirmed when broilers were fed with CXO as compared to the diet containing flaxseed oil (FXO), only. However, less of C18:3, MUFA and n-3 and n-6 ratio of CXO but more of C20:5 of CXO was determined when compared to that of FXO diet ($P < 0.05$).

Similar to CLA diet in MUFA and to FHO diet in n-3 and -6 ratio was shown when fish oil and CLA (CHO) was combined and provided to broilers for 4 and 6 weeks. Neither effects of C18:3, C20:5, C22:5 and C22:6 nor PUFA was similar to that of CLA or CHO. The accumulation of n-3 PUFA including C20:5, C22:5 and C22:6 seems to be influenced by fish oil rather than CLA of CHO diet, however, C20:3 and C20:4 of n-6 PUFAs were closely affected due to both CLA and fish oil of CHO diet. Only C18:3, C20:5 and PUFA were not influenced by age effect ($P > 0.05$), but C20:3, C20:4, C22:5,

Table 12. Omega-3 and -6 fatty acid profiles of broiler chicken thigh fed with different fat source diets and processed at 4 and 6 weeks of growth (%)

Effect	C18:2	C18:3	C20:3	C20:4	C20:5	C22:5
TRT*WKS[1]						
P-value	**0.039**	0.001	0.323	0.532	0.360	0.203
TRT[2]						
P-value	0.001	0.001	0.001	0.001	0.001	0.001
CLA	17.54	1.10[e]	0.28[c]	1.36[b]	0.70[d]	0.68[d]
FXO	17.89	6.74[a]	0.45[a]	1.99[a]	0.71[d]	1.09[c]
FHO	17.10	1.40[d]	0.39[ab]	1.60[b]	1.88[a]	1.45[a]
CXO	17.35	4.20[b]	0.43[a]	2.00[a]	0.96[c]	1.05[c]
CHO	16.36	1.84[c]	0.34[bc]	1.53[b]	1.37[b]	1.26[b]
WKS[3]						
P-value	0.007	0.774	0.002	0.001	0.340	0.001
4	16.96	3.05	0.34[b]	1.51[b]	1.10	0.96[b]
6	17.57	3.05	0.42[a]	1.89[a]	1.13	1.25[a]
ROOT MSE[4]	1.033	0.323	0.105	0.509	0.199	0.215

[1]TRT*WKS = treatment by age interaction; [2]Treatment: CLA = 2% conjugated linoleic acid (50% c9t11 + 50% t10c12 CLA), FXO = 2% flaxseed oil, FHO = 2% fish (menhaden) oil, CXO = 1% conjugated linoleic acid + 1% flaxseed oil, CHO = 1% conjugated linoleic acid + 1% fish (menhaden) oil; [3]WKS = age; [4]ROOT MSE = Root Mean Square Error; [a,b,c,d]Mean values within a row followed by the same letter are not significantly different ($P > 0.05$).

Table 13. Omega-3 and total fatty acid[1] profiles of broiler chicken thigh fed with different fat source diets and processed at 4 and 6 weeks of growth (%)

Effect	C22:6	SFA	MUFA	PUFA	n3/n6
TRT*WKS[2]					
P-value	0.697	**0.001**	0.774	0.385	0.166
TRT[3]					
P-value	0.001	0.001	0.001	0.010	0.001
CLA	1.01[c]	42.63	24.04[d]	30.13[c]	0.21[d]
FXO	0.90[c]	31.31	34.80[a]	31.55[ab]	0.48[b]
FHO	3.06[a]	34.16	32.47[b]	30.59[bc]	0.59[a]
CXO	1.00[c]	36.60	25.98[c]	31.72[ab]	0.41[c]
CHO	2.08[b]	37.71	23.69[d]	32.37[a]	0.60[a]
WKS[4]					
P-value	0.026	0.001	0.001	0.346	0.001
4	1.51[b]	35.38	27.20[b]	31.46	0.49[a]
6	1.71[a]	37.58	29.45[a]	31.03	0.43[b]
ROOT MSE[5]	0.406	1.807	1.990	1.849	0.065

[1]SFA = saturated fatty acid (C14:0 + C16:0 + C18:0), MUFA = monounsaturated fatty acid (C16:1 + C18:1c9), PUFA = polyunsaturated fatty acid (C18:2 + C18:3 + c9t11 + t10c12 + C20:3 + C20:4 + C20:5 + C22:5 + C22:6), n3/n6 = ratio of omega-3 fatty acids (C18:3 + C20:5 + C22:5 + C22:6) and omega-6 fatty acid (C18:2 + C20:3 + C20:4); [2]TRT*WKS = treatment by age interaction; [3]Treatment: CLA = 2% conjugated linoleic acid (50% c9t11 + 50% t10c12 CLA), FXO = 2% flaxseed oil, FHO = 2% fish (menhaden) oil, CXO = 1% conjugated linoleic acid + 1% flaxseed oil, CHO = 1% conjugated linoleic acid + 1% fish (menhaden) oil; [4]WEEK = age; [5]ROOT MSE = Root Mean Square Error; [a,b,c,d]Mean values within a row followed by the same letter are not significantly different (*P* > 0.05).

C22:6 and MUFA of 6th week thigh samples were observed when compared to that of fourth week, in contrast, 0.43 of n-3 and n-6 ratio was determined while diets were supplied to broilers for 6 weeks.   It was 0.06 lower than that of fourth week.

Figure 6 shows the least squares means for treatment by age interaction for linoleic acid (C18:2, LA, n-6) and saturated fatty acid (SFA) ($P < 0.05$).   At 4 weeks of age, C18:2 of CHO, FHO and CXO (16.37, 16.48 and 16.56%, respectively) were not significant ($P > 0.05$) but differed to that of CLA and FXO (17.60 and 17.77%, respectively) ($P < 0.05$) even though overall content of C18:2 was insignificant in both CLA and FXO treatments ($P > 0.05$).   However, broilers fed by CHO diet had 16.36% of C18:2, and it was the lowest C18:2 among treatments at 6 weeks of growth.   The deposition of C18:2 in CLA, FHO, FXO and CXO treatments had a significant difference when compared to that of CHO ($P < 0.05$).   Neither CHO and CLA nor FXO increased the overall content of C18:2 during 4 to 6 weeks of feeding, in contrast, both FHO and CXO altered the C18:2 depositions to 17.82 and 18.41%, respectively ($P < 0.05$).

The saturated fatty acid (SFA) due to CXO and CHO diet (36.99 and 36.97%, respectively) was insignificant ($P > 0.05$) but differed with that of other three diets ($P < 0.05$).   The FXO (30.47%) induced the least deposition of SFA, however, SFA accumulated when CLA (40.16%) was supplied to broilers for 4 weeks ($P < 0.05$). Moreover, minimum amount of SFA was observed when FXO (32.16%) was supplied to broilers for 6 weeks, and it was significantly different when compared to other treatments ($P < 0.05$).   Not only SFA of FHO (35.79%) but SFA of CXO (36.22%) differed with that of CHO (38.56%) and CLA (45.10%) even though both FHO and CXO were not significantly different to one another.   However, CLA maintained the highest deposition of SFA at 6 weeks.   The SFA significantly increased from 32.53 and 40.16% to 35.79 and 45.10%, respectively, when broilers were raised on FHO and CLA diets, but other treatments were not significant ($P > 0.05$).
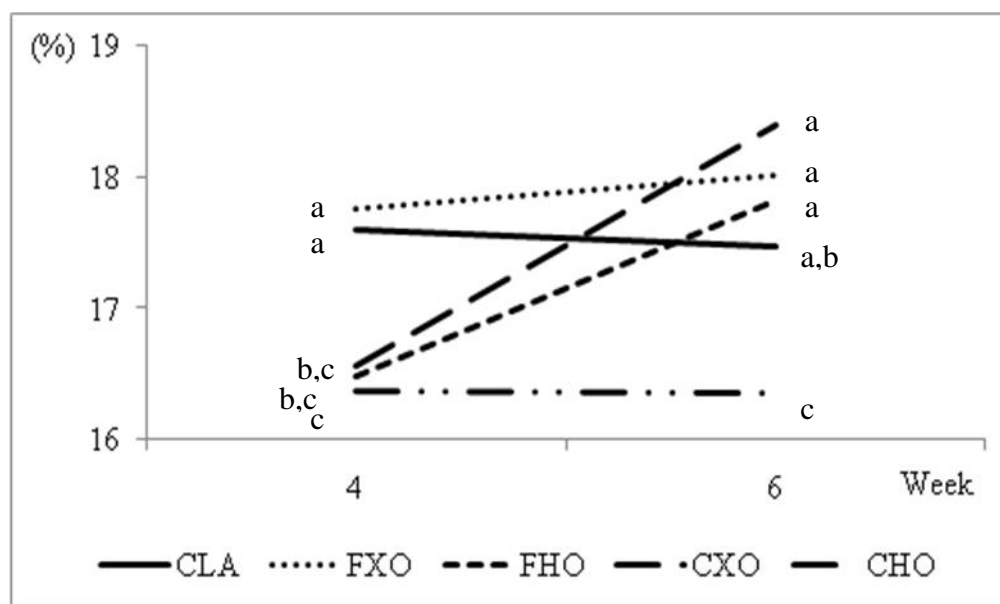
Linoleic Acid (C18:2, LA, n-6; $P = 0.039$)
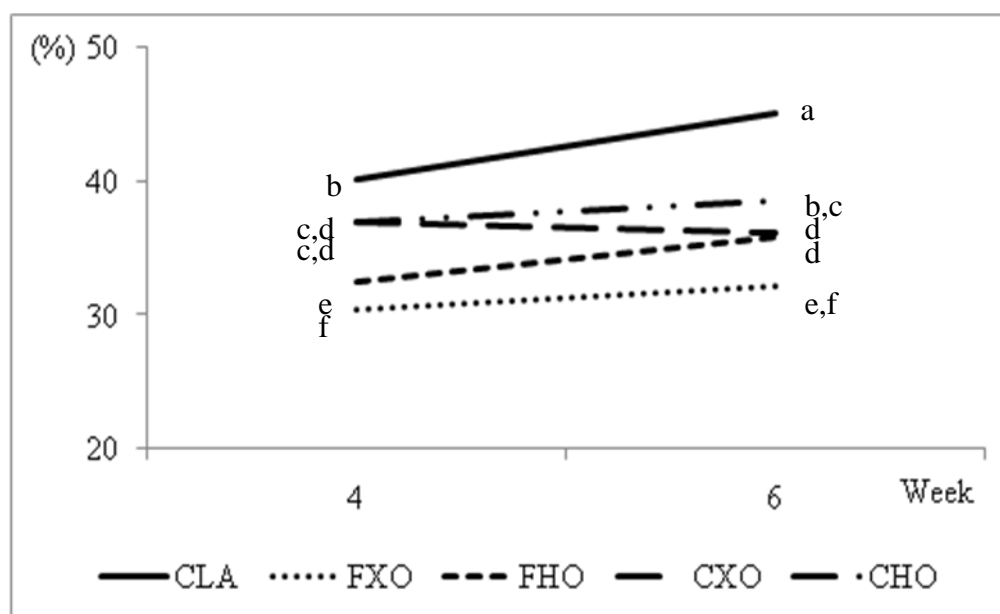
Saturated Fatty Acid (SFA; $P = 0.001$)

Figure 6. Least squares means for treatment by age interaction for linoleic acid and total saturated fatty acid of broiler chicken thigh fed with different fat diets.

CLA = 2% conjugated linoleic acid (50% c9t11 + 50% t10c12 CLA), FXO = 2% flaxseed oil, FHO = 2% fish (menhaden) oil, CXO = 1% conjugated linoleic acid + 1% flaxseed oil, CHO = 1% conjugated linoleic acid + 1% fish (menhaden) oil.
[a-f]Mean values within a row followed by the same letter are not significantly different ($P > 0.05$).

5. DISCUSSION

      Omega-3 fatty acids and conjugated linoleic acid (CLA) have been reported to reduce the depositions of linoleic acid (C18:2, LA, n-6)) and/or arachidonic acid (C20:4, AA, n-6) which are plentiful in chickens and chicken products (Du et al., 2000; López-Ferrer et al., 2001; Sirri et al., 2003).   The reduction of C20:4 in chickens is a positive implication because it improves consumers' health due to decreased prostaglandin E_2 (PGE_2) production, a pro-inflammatory agent contributing to chronic diseases such as cardiovascular disease, type-2 diabetes, cancers and obesity.   Poultry meat is of low cost and easily accessible to the general population when compared to beef and pork. Therefore, enrichment with omega-3 (n-3) fatty acids and/or CLA in poultry meat is an excellent alternative to the poultry farmers and industry providing 'functional' chicken meat to consumers.

      Duttaroy (2006) warned of the possible health risks associated with high intake of fish oil (n-3 PUFAs), increased bleeding and hemorrhagic stroke.   Our study was undertaken to provide a new approach in not only reducing the deposition of C20:4 but minimizing the disadvantages of n-3 FAs when n-3 FAs and CLA were supplied to broilers for 6 weeks.   Generally, FAs are absorbed *via* small intestine by passive diffusion, but long-chain FAs containing carbons $\geq$ 18 are poorly soluble in aqueous environment.   They form micelles with other components and then bind to fatty-acid-binding-proteins (FABP) to be secreted into lymph (Niot et al., 2009) and finally reached to breast, thigh and liver.   During the uptake of fatty acids *via* small intestine, competitions for FABPs depending on the affinity of long chain FAs were generated (Cunningham and McDermott, 2009).

      According to one study conducted by Nemecz et al. (1991), FABPs had relatively higher affinity to unsaturated fatty acids (UFA) as compared to saturated fatty acids (SFA) of the same carbon numbers, but *cis*- or *trans*-configuration of fatty acids did not influence the affinity to FABPs.   It is important to indicate that the number of double bond is one of the important factors that influence the affinity of fatty acids to FABPs. Therefore, C18:2 or C20:4 which have two or four double bonds, may have less

opportunity to bind FABPs when they are blended with C18:3 or C20:5 and supplied to broilers.

Furthermore, it is assumed that rapid uptake, depending on the number of carbons, could be accomplished due to higher requirement of very-long-chain fatty acids (VLFA) of phosphatidylcholine (PC), a major component of animal cell membrane. Indirect evidence for the requirement of VLFAs was performed by Du et al. (2000). Du and his colleagues postulated that higher C20:4 and C20:5 were accumulated in PC when compared to that of triglycerides (TG). It implies that the cell membrane is a major place where C20:4 and C20:5 are deposited, thereby, selective uptake for VLFA *via* small intestine may be required to provide VLFAs to cell membrane, directly and rapidly. Due to our study, it seems that there was a competition during the absorption or deposition of n-3 and n-6 FAs when both CXO and CHO were supplied to broilers. The C18:2 in breast and thigh of CHO treatment was reduced when compared to that of CXO ($P < 0.05$) but was similar to that of FHO. Therefore, our findings suggested that competition between C18:2 and C18:3 may not be intensive when compared to that of C18:2 and n-3 VLFAs.

To reduce the accumulation of n-6 fatty acids in breast and thigh meats, another approach that have considered is the substitution of n-6 fatty acids with n-3 fatty acids and/or CLA in diets. Substitution of n-6 FAs by n-3 FAs may reduce the opportunities for absorption of C18:2 and/or C20:4 *via* small intestine. Igarashi et al. (2009) showed that omega-6 FAs deficient diets induced the reduction of n-6 FAs in five different organs including brain, liver, heart, testis and adipose tissue when n-6 deficient diet was provided to rats for 15 weeks. The amount of C18:2 and C20:4 reduced in liver by 70 and 84%, respectively. However, it was not consistent with our results when n-6 FAs were replaced due to CLA and flaxseed oil/fish oil combinations. Conjugated linoleic acid substitutes n-6 FAs in poultry diets and functions as a substrate of delta-6 desaturase, thereby, inhibiting the conversion of C18:2 to C20:4 leading to high overall content of C18:2 in broilers (Takahashi et al., 2003; Cherian et al., 2007; Javadi et al., 2007). With increased activity of delta-6 desaturase, CLA isomers are altered to C20:4 (Δ-5, 8,

12 and 14) or C20:4 (Δ-5, 8, 11 and 13) (Sebedio et al., 1997).

Additionally, Du et al. (2000) demonstrated that 4.1% flaxseed oil and 2.5% CLA combination in a laying hen diet induced the enzymes to promote the synthesis of n-3 PUFAs and finally had more n-3 PUFAs than other treatments including egg yolks of 8.2% soy oil, 4.1% soy oil + 2.5% CLA and 4.1% soy oil + 4.1% flaxseed oil. It implies that delta-6 desaturase has higher affinity to linolenic acids (C18:2, LNA, n-3) when compared to that of n-6 fatty acids. Therefore, desaturation of C18:2 to C20:4 decreased due to reduced affinity of delta-6 desaturase when flaxseed oil and CLA combination diet was supplied to layers up to 6 weeks of age. Our study was carried out to evaluate the effects of n-3 PUFAs containing either carbons = 18 (flaxseed oil) or carbons $\geq$ 20 (fish oil) on the affinity of delta-6 desaturase when each n-3 FAs group (flaxseed oil or fish oil, respectively) was combined with CLA. As mentioned above, CLA increases the affinity of delta-6 desaturase to CLA, itself (Bretillon, et al., 1999; Sirri et al., 2003) resulting in C18:2 accumulation when CLA is fed to broilers.

The effects of CLA on delta-6 desaturase may be similar in CXO and CHO of our treatments, but beneficial synergistic effects on delta-6 desaturase activity due to combination of CLA and n-3 FAs or VLFAs were unexpected. The C18:2 of thigh of chickens fed the CXO diet was insignificant at 4 weeks but significantly differed to that of CHO at 6 weeks of age. It indicates that CLA of CXO maintained a negative effect to delta-6 desaturase converting C18:2 to C20:4. At the same time, higher uptake of C18:2 may be accomplished, thereby, the deposition of C18:2 was increased in 6 weeks of growth.In contrast to C18:2 of thigh of broilers fed to CXO diet, 16.36 ~ 16.37% of C18:2 was maintained in CHO treatment since the competition of C18:2 and n-3 VLFAs due to high intake of n-3 VLFAs sustained. It seems that the competition of n-3 and n-6 FAs was effectively performed when n-3 VLFAs were combined to CLA as compared to that of C18:3. Additionally, delta-6 desaturase affinity to C18:2 was reduced when CLA and n-3 VLFAs were combined and supplied to broilers (Raz et al., 1998; Matsuzaka et al., 2002), and as seen in table 8, CHO did not significantly influence the conversion of C18:2 to C20:4 when compared to that of FXO and FHO.

In conclusion, the CXO diet increased the deposition of C18:2 and C20:4, however, had low percent of C20:5, C22:5 and C22:6 when compared to that of CHO. As a result, CXO provided only 0.41 of n-3/n-6 ratio in breast and thigh meat. It was lower than that of FXO, however, it may not be acceptable in commercial market if CXO has similar problems as that of FXO. The CHO which decreased the deposition of C18:2 and C20:4, decreased the SFA, and increased the PUFA in breast and thigh muscles, is recommendable, and it may provide 'functional' broiler chicken meats to consumers.

CHAPTER IV

EFFECTS OF DIETARY SUPPLEMENTATION OF OMEGA-3 AND -9 FATTY
ACID COMBINATION ON INFLAMMATION RESPONSES USING BROILERS
AS AN ANIMAL MODEL

1. OVERVIEW

To reduce the amount of omega-6 fatty acids in broiler chicken breast and thigh meats, two hundred and forty broiler chicks were purchased and randomly assigned to six different treatments. All birds of six different treatments were fed with a basal corn-soybean meal diet containing a fixed 5 % fat from five different lipid sources for the following treatments: 1) animal and vegetable combined oil (AVO), 2) soybean oil and olive oil combination (SYO), 3) flaxseed oil and olive oil combination (FXO), 4) flaxseed oil, C20:5 and olive oil combination (FEO), 5) flaxseed oil, C22:6 and olive oil combination (FDO) and 6) fish oil and olive oil combination (FHO). One bird per pen was processed at two different sampling periods including 6 and 9 weeks of age. Each bird was weighed, and blood, liver, breast and thigh samples from the bird were collected. Blood, liver, breast and/or thigh samples were used for fatty acids profiles, prostaglandin E_2 concentration and mRNA gene expressions.

Live weight of broiler chickens was affected by dietary fat source, in contrast to liver weight. Liver weight of broiler chickens raised on FXO and FHO diets was higher than that of AVO and SYO. Although the generation of PGE_2 was not affected due to combination of n-3 and n-9 fatty acids in our diets, the deposition of n-6 fatty acids including C18:2 and C20:4 was decreased in broiler chicken breast and/or thigh muscles as n-3 fatty acids were supplied to broiler chickens for 9 weeks. Eicosapentaenoic acid (C20:5, EPA, n-3) addition to poultry diet (FEO) did not reduce the deposition of C18:2 and/or C20:4 as much as C22:6 (FDO) did. However, C22:6 of FDO diet significantly reduced the overall content of C20:4 in broiler chicken breast muscle, as a result, increased the n-3 to n-6 ratio at 9 weeks. When C20:5 and C22:6

were blended to poultry diet (FHO) and fed to broiler chickens for 9 weeks, synergistic effects were observed. Reduction of C20:4 was obtained when FHO diet was fed to broiler chickens, and it may be induced due to decreased expression of delta-6 desaturase mRNA.

2. INTRODUCTION

Fatty acids are important components of energy metabolism, membrane formation and signaling processes (Jump et al., 2008). Linoleic acid (C18:2, LA, n-6) and α-linoleic acid (C18:3, ALA, n-3) are essential fatty acids, involved in many biological functions, and must be supplemented in the diets of mammalian and avian species (Simopoulos, 2009). However, previous studies have shown that an excessive intake of n-6 fatty acids can lead to the malfunctioning of lipogenic regulation and may be responsible, or contribute, to the development of chronic diseases due to an increased inflammatory response (Wood et al., 2003; Jump et al., 2008). The inflammatory response is associated with the production of reactive oxygen species (ROS) which accelerates the onset of chronic diseases as well (Wellen and Hotamisligil, 2005).

To prevent these adverse responses to excessive dietary n-6 FA, a proper balance between n-6 and n-3 fatty acids is required, however, an ideal n-6/n-3 ratio is difficult to maintain due to high proportion of n-6 fatty acids in both animal and human diets. A typical ratio of n-6 to n-3 in western diets ranges from 10:1 to 25:1 (Simopoulos, 2000), and it is far away from the ideal range of 3:1 to 6:1 (Simopoulos, 2000; Wijendran and Hayes, 2004; El-Badry et al., 2007). To reduce the inflammatory response and to provide a healthy and functional chicken meat to consumers, the regulation of n-6/n-3 fatty acid ratio deposition in chicken meat must be defined, and a possible intervention to reduce n-6/n-3 imbalances in broilers must be achieved. Oleic acid (C18:1, OA, n-9) belongs to the n-9 family of unsaturated fatty acids (UFA), containing a double bond at the C9 position from the terminal methyl carbon of the fatty acid chain.

Oleic acid is widely available and it is a safe source of energy. Oleic acid is found in large quantities in olive and canola oil, and it is recognized as a healthy fatty

acid by consumers of beneficial 'Mediterranean' diets. Oleic acid is stable to oxidation as compared to other UFAs and increases glucose conversion to glycogen, resulting in less fat accumulation in the organism (Clarke et al. 2002). Furthermore, OA has a neutral effect on n-3 PUFA metabolism (Cleland et al., 2006), and when compared to n-6 fatty acids, it produces less hydroperoxide (H_2O_2) in the mitochondria (Cocco et al., 1999). Thus, OA can be considered a safe and beneficial alternative to furnish energy in poultry diets instead of the common fat sources that are rich in n-6 fatty acids; replacing the n-6 fatty acids by n-9 fatty acids as a combination partner of n-3 fatty acids to ensure the right balance between n-6 and n-3 fatty acids.

Hypothesis and Objective

To manipulate the fat sources in the chicken diet to meet the requirements of n-3 and n-6 essential fatty acids, and to utilize an alternative source of energy that does not interfere with n-3 fatty acid metabolism, the absorption and incorporation of n-6 fatty acids must be limited and consequently reduce the synthesis of pro-inflammatory compounds in broiler chickens.

The specific objectives of this research project was to test the hypotheses that: (a) n-9 fatty acid supplementation improvesn-3/n-6 fatty acid ration; and (b) n-9 fatty acid supplementation improves metabolism and inflammation. To pursue this objective, broilers were fed diets supplemented with a combination of olive and soybean, flaxseed, flaxseed and eicosapentaenoic acid (EPA) combination, flaxseed and docosahexaenoic acid (DHA) combination, or fish oils. Individual fatty acid accumulation, gene expression related to *de novo* lipogenesis and fatty acid oxidation, prostaglandin E_2 (PGE_2) accumulation and cyclooxygenase2 (COX2) gene expression were determined.

3. MATERIALS AND METHODS

Two hundred and forty male broiler chicks (*Gallus gallus domesticus*) were purchased from a local commercial hatchery and transported to the Poultry Science Center at Texas A&M University. All chicks were randomly assigned to six different treatments that includes four replications ($10 \times 4 \times 6$ = birds × replications × treatments). Each bird was fed with a basal corn-soybean meal diet (Table 14) containing a fixed 5% fat from five different lipid sources (Tables 15 and 16) for the following treatments:

1) Animal and vegetable combined oil (control diet, AVO)

2) Soybean oil and olive oil combination (2.5% each, SYO)

3) Flaxseed oil and olive oil combination (2.5% each, FXO)

4) Flaxseed oil, C20:5 (EPA), and olive oil combination (2.45, 0.05, and 2.5%, FEO)

5) Flaxseed oil, C22:6 (DHA), and olive oil combination (2.45, 0.05, and 2.5%, FDO)

6) Fish (menhaden) oil and olive oil combination (2.5% each, FHO).

The C20:5 (EPA) (70% purity, Chemport Inc., Naju, Korea) or C22:6 (DHA) (80% purity, Chemport Inc., Naju, Korea) was added to the combination of flaxseed and olive oils to study their individual effects on the *de novo* lipogenesis. The fatty acid composition of each fat source and complete feed were analyzed in the laboratory (Tables 17, 18 and 19). The experimental diets may not contain additional n-6 fatty acids other than the one naturally contained in corn and soybean meal. One broiler per pen (4 birds per treatment) was slaughtered at two different sampling periods: 6 and 9 weeks of age, to evaluate the lipid biochemical components. Each bird was weighed, stunned, bled and eviscerated. The carcasses were pre-chilled (15 min at 4 ^{o}C) and post-chilled (45 min at 0 ℃). The breast and thigh muscles were collected right after chilling. Blood and liver samples were also collected during bleeding and evisceration, respectively. The liver sample was immediately frozen in a container with liquid nitrogen (N_2), and then finally stored at -80 ^{o}C until analyzed.

Table 14. Composition of experimental basal diets of broiler chickens. Experiment II

INGREDIENT (%)	STARTER (0~3 wk)	GROWER (4~6wk)	FINISHER (7~9 wk)
Corn	52.81	60.88	65.81
Soybean meal	37.99	29.98	25.06
Biofos 16/21 P	1.55	1.40	1.29
Limestone	1.68	1.58	1.61
Oil	5.00	5.07	5.07
Salt	0.49	0.44	0.46
Vitamin Premix[1]	0.25	0.25	0.25
DL-Methionine	0.18	0.22	0.20
Mineral Premix[2]	0.05	0.05	0.05
Lysine	-	0.13	0.12
Coban 60	-	-	0.08
Calculated Nutrient Content (%)			
Crude Protein	23.02	20.00	17.98
ME energy (Kcal/lb)	3120.56	3209.07	3252.87
Calcium	1.00	0.92	0.90
Available Phosphorous	0.45	0.41	0.38
Methionine	0.53	0.52	0.48
Methionine + Cystine	0.90	0.85	0.79
Lysine	1.25	1.14	1.00
Threonine	0.87	0.74	0.67
Sodium	0.21	0.19	0.20

[1]Vitamin Premix (lb): vitamin A 2,000,000 IU, vitamin D3 700,000 IU, vitamin E 8,333 IU, vitamin B12 3.0 mg, riboflavin 1,083 mg, niacin 8,333 8,333 mg, d-pantohenic acid 3,667 mg, choline 86,667 mg, K 267 mg, folic acid 317 mg, vitamin B6 1,3000 mg, thiamine 533 mg, biotin 100. Breeder turkey, DSM Nutritional Products, Inc., Parsippany, NJ.   [2]Mineral Premix: Ca 1.20%, Mn 30.0%, Zn 21.0%, Cu 8500 ppm, I 2100 ppm, Se 500 ppm, Mo 1670 ppm.   Tyson Poultry 606 Premix.

Table 15. Fatty acid profiles of dietary ingredients and two different fat sources (mg/100g of diet). Experiment II

| | DIETARY SOURCE[1] | | | |
	CORN	SYM	SYO	OVO
C14:0	-	-	-	-
C14:1	-	-	-	-
C16:0	14030.87	14858.91	23821.10	613.71
C16:1	-	-	-	35.06
C18:0	2427.14	4499.25	10932.67	243.19
C18:1c9	30508.25	15172.51	54144.71	6353.31
C18:1c11	500.91	1542.86	2138.55	115.88
C18:2	42340.61	61749.24	89684.88	764.33
C18:3	1291.46	10514.55	20569.29	58.85
C20:3	-	-	-	-
C20:4	440.97	388.08	764.12	55.99
C20:5	449.56	751.81	327.27	15.79
C22:5	-	-	-	-
C22:6	855.70	-	-	-

[1]CORN=dried corn, SYM=soybean mill, SYO=soybean oil, OVO=olive oil.

Table 16. Fatty acid profiles of dietary fat sources (mg/100g of diet). Experiment II

| | DIETARY SOURCE[1] | | | |
	FXO	EPA	DHA	FHO
C14:0	-	-	-	431.72
C14:1	-	-	-	16.78
C16:0	9159.01	825.58	301.63	1107.33
C16:1	-	-	-	537.52
C18:0	7282.65	8059.84	-	252.82
C18:1c9	36578.99	9029.72	941.90	617.02
C18:1c11	1176.94	3783.65	-	212.40
C18:2	30180.21	1550.63	406.67	91.31
C18:3	90333.34	1626.60	-	56.62
C20:3	-	1143.64	-	-
C20:4	693.76	10793.93	2253.86	108.51
C20:5	392.66	98910.33	8354.66	1276.56
C22:5	-	-	5641.93	176.23
C22:6	-	-	134076.79	837.34

[1]FXO=flaxseed oil, EPA=eicosapentaenoic acid, DHA=docosahexaenoic acid, FHO=fish oil.

Table 17. Fatty acid profiles of basal and starter diets (mg/100g of diet). Experiment II

	DIET						
	STARTER[1]						
	BAS	AVO	SYO	FXO	FEO	FDO	FHO
C14:0	-	25.80	21.21	-	68.85	74.70	64.39
C14:1	-	-	-	-	-	-	-
C16:0	493.76	1207.20	601.74	549.85	600.29	726.30	639.86
C16:1	32.19	258.14	46.97	47.22	55.39	114.19	95.32
C18:0	100.87	292.59	165.92	161.66	169.95	187.48	154.41
C18:1c9	907.62	2380.42	1809.90	1643.45	1967.96	2121.25	1583.27
C18:1c11	28.39	98.52	61.74	48.24	61.44	81.51	59.69
C18:2	1643.80	1954.55	1707.84	1305.67	1393.21	1403.57	1594.20
C18:3	111.22	225.96	267.57	696.42	704.02	421.48	290.29
C20:3	-	-	-	-	-	-	-
C20:4	51.78	38.01	27.21	47.19	-	-	-
C20:5	33.54	20.67	53.83	40.09	93.94	198.80	116.79
C22:5	-	-	-	-	22.16	24.32	22.62
C22:6	-	-	29.67	-	98.62	136.44	122.92

[1]BAS: basal diet (no fat source), AVO: 5% animal fat & vegetable oil, SYO: 2.5% soybean oil + 2.5% olive oil combination, FXO: 2.5% flaxseed oil + 2.5% olive oil combination, FEO: 2.45% flaxseed oil + 0.05% eicosapentaenoic acid + 2.5% olive oil combination, FDO: 2.45% flaxseed oil + 0.05% docosahexaenoic acid + 2.5% olive oil combination, FHO: 2.5% fish oil + 2.5% olive oil combination.

Table 18. Fatty acid profiles of basal and grower diets (mg/100g of diet). Experiment II

	DIET						
	GROWER[1]						
	BAS	AVO	SYO	FXO	FEO	FDO	FHO
C14:0	25.20	17.96	-	9.27	-	42.09	56.97
C14:1	-	-	-	-	-	-	-
C16:0	441.72	737.55	555.38	564.16	456.47	375.56	312.80
C16:1	180.03	134.09	61.85	75.03	26.63	27.56	35.96
C18:0	95.39	181.81	147.09	150.67	131.29	100.19	42.32
C18:1c9	770.30	1662.31	1460.00	1478.76	1413.92	1115.25	645.26
C18:1c11	31.24	62.56	45.60	45.69	37.05	31.01	-
C18:2	1047.47	1498.33	1363.40	1193.45	1150.76	889.68	522.75
C18:3	89.17	162.08	283.13	556.22	637.40	485.90	76.16
C20:3	-	-	-	-	-	-	-
C20:4	-	-	16.05	21.62	14.15	-	44.72
C20:5	18.97	28.36	17.40	22.14	26.67	27.34	62.05
C22:5	-	-	-	-	-	-	-
C22:6	17.01	9.12	-	-	13.28	28.03	43.52

[1]BAS: basal diet (no fat source), AVO: 5% animal fat & vegetable oil, SYO: 2.5% soybean oil + 2.5% olive oil combination, FXO: 2.5% flaxseed oil + 2.5% olive oil combination, FEO: 2.45% flaxseed oil + 0.05% eicosapentaenoic acid + 2.5% olive oil combination, FDO: 2.45% flaxseed oil + 0.05% docosahexaenoic acid + 2.5% olive oil combination, FHO: 2.5% fish oil + 2.5% olive oil combination.

Table 19. Fatty acid profiles of basal and finisher diets (mg/100g of diet). Experiment II

	DIET						
	FINISHER[1]						
	BAS	AVO	SYO	FXO	FEO	FDO	FHO
C14:0	-	26.98	-	-	-	3.52	50.13
C14:1	-	-	-	-	-	-	-
C16:0	454.93	831.50	663.92	721.23	577.06	381.66	593.44
C16:1	-	176.81	75.82	87.95	53.04	23.18	76.35
C18:0	63.34	131.43	129.12	142.53	123.36	75.33	122.47
C18:1c9	711.92	1464.99	1597.51	1684.68	1561.45	1289.85	1452.70
C18:1c11	-	58.37	-	41.76	-	32.19	37.19
C18:2	899.84	1159.09	1273.38	1235.50	1076.18	696.11	889.68
C18:3	47.99	90.12	240.71	457.14	583.78	601.10	309.76
C20:3	-	-	-	-	-	-	-
C20:4	57.64	97.91	-	75.90	-	-	-
C20:5	-	66.64	-	42.39	70.27	34.96	73.91
C22:5	-	-	-	-	-	-	-
C22:6	-	-	-	-	-	21.88	54.19

[1]BAS: basal diet (no fat source), AVO: 5% animal fat & vegetable oil, SYO: 2.5% soybean oil + 2.5% olive oil combination, FXO: 2.5% flaxseed oil + 2.5% olive oil combination, FEO: 2.45% flaxseed oil + 0.05% eicosapentaenoic acid + 2.5% olive oil combination, FDO: 2.45% flaxseed oil + 0.05% docosahexaenoic acid + 2.5% olive oil combination, FHO: 2.5% fish oil + 2.5% olive oil combination.

Fatty Acid Composition Determination

For the fatty acid methyl ester (FAME) analysis of breast, thigh and liver samples, the fat was extracted, saponificated and methylated as described in experiment I.   After the methylation of each sample, the composition of the FAME was identified and quantified by a standard and an internal standard using a gas chromatograph with a flame-ionization detector.   Each fatty acid was expressed as mg/g tissue weight based on the area of internal standard methylated for each sample.

Prostaglandin E_2 (PGE_2) Determination

The PGE_2 content was determined using a commercial kit (Cayman Chemical Company, Ann Arbor, MI).   All plasma samples were transferred to a goat anti-mouse IgG coated plate, read in a plate reader and finally expressed as pg/mL.   Briefly, 100 or 50 µL of EIA buffers will be added to Non-specific binding (NSB) or Maximum binding (B_0) wells, respectively.   Also, 50 µL of prostaglandin E_2 EIA standard (S1 ~ S8), plasma sample, prostaglandin E_2 express AChE tracer and prostaglandin E_2 monoclonal antibody was transferred to wells where required.   The plate was covered with a plastic film and incubated for 60 min at room temperature on an orbital shaker.   After incubation, all wells were emptied and rinsed five times with a wash buffer.   The well was filled with a 200 µL of Ellman's Reagent or a 5 µL of tracer (total activity wells only), and final development on an orbital shaker was performed for 80 min.   As samples were developed in the dark place, the plate cover was carefully removed and read at 405 nm.

Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)

Tissue homogenization: the tissue was homogenized in 3 mL buffer containing 4 *M* guanidinium thiocyanate, 50 m*M* Tris pH7.5, 10 m*M* EDTA and 0.5% sodium N-lauroyl sarcosine.   Beta-mercaptoethanol (0.1 *M* BME) was added to a final concentration of 100 m*M* immediately prior to homogenization.

Preparation of total ribonucleic acid (RNA): three volumes of 4 M lithium chloride were added to homogenate, and the RNA was precipitated overnight at 4°C. Homogenate was centrifuged at $10,000 \times$ g at 4°C for 1.5 h.   Supernatant was discarded. The pellet was resuspended in 0.5 mL of protein digestion buffer (10 mM Tris pH 7.9, 5 mM EDTA, 1% sodium N-lauroyl sarcosine) containing 0.2 mg/mL proteinase K and incubated at 45°C for an hour.   After incubation, the samples were extracted several times with approximately one volume of phenol/chloroform, retaining the aqueous phase after each extraction, until the interphase was clear.   The RNA was ethanol precipitated with 0.2 M NaCl and resuspended the pellet in 400 µL of water.   The deoxyribonucleic acid (DNA) was removed by extracting twice with 1 volume of acidic phenol/chloroform and retaining the aqueous phase.   The RNA was ethanol precipitated, pellets was washed with 70% ethanol, air dried for 5 ~ 10 min and dissolved in 50 µL of RNAse free water, and the concentration of each sample was estimated by 260 nm/280 nm UV absorbance.

Reverse transcription: the cDNA was synthesized using 1.5 µg total RNA using random hexamer primers and Invitirogen M-MLV reverse transcriptase.   The manufacturer's instructions were followed except as follows: each reaction contained 1.5 µL M-MLV reverse transcriptase and 0.1 µL RNAse inhibitor.   The 1 min incubation at 37°C before adding reverse transcriptase was omitted.   The cDNA's was diluted to 135 µL with water.

Polymerase chain reaction: The PCR was performed on the cDNA using primers for peroxisome proliferator-activated receptor alpha (PPARα), peroxisome proliferator-activated receptor gamma (PPARγ), sterol regulatory element binding transcription factor 1 (SREBF1), phospholipase A$_2$ (PLA2G4A), steroyl-CoA desaturase (SCD), fatty acid desaturase 2 (FADS2) and prostaglandin-endoperoxide synthase 2 (PTGS2) (Tables 20 and 21).   The PCR reactions have two steps: (1) a 12 µL pre-amplification containing primers for the gene of interest, and (2) a 20 µL main amplification containing primers for the gene of interest and β-actin as an internal standard.   The PCR pre-amplification reactions contained 1 $\times$ Sigma ready mix, 10 pM forward primer,

10 p*M* reverse primer and 2 µL of cDNA, and the final volume were adjusted to 12 µL by water.

Next, 10 µL of the pre-amplification reaction was added to 10 µL β-actin master mix containing 1 × Sigma ready mix and 10 p*M* of each actin primer.   All samples were pre-amplified as follows; PPARα 5 pre-amplification, PPARγ 10 pre-amplification,SREBF1 6 pre-amplification, PLA2G4A 7 pre-amplification, SCD 2 pre-amplification, FADS2 2 pre-amplification and PTGS2 8 pre-amplification.   After pre-amplification of each tube, 20 more cycles were applied to tubes depending on the type of genes.   Reaction conditions were completed under 94°C for 20 sec (denaturation), 64°C for 30 sec (annealing), and 72°C for 40 sec (extension) as a cycle. Electrophoresis was performed in a 0.8 ~ 0.9% agarose gel containing ethidium bromide, and DNA bands were visualized by UV fluorescence.   Images were taken using a digital camera using exposure times long enough to clearly visualize the DNA bands but short enough that there are no saturated pixels.   Bands were quantified using Kodak 1D Image Analysis Software, Windows version 3.5.   The gene of interest was normalized to β-actin.I performed PCR reactions on −RT controls to show that there was no detectable signal under the conditions I used.

Statistical Analysis

All data were analyzed as a factorial arrangement by Analysis of Variance using the generalized linear model (GLM) procedure of SAS (Version 6.12, Cary, NC, 1998) with a predetermined significance level of $P < 0.05$.   Main effects of treatment and age and two--way interactions (treatment by age) were included in the initial model.   Two-way interactions for all main effects were analyzed and remained in the final model if they were significant ($P < 0.05$).   Least squares means were estimated and separated using the stderr pdiff function when differences were determined by Analysis of Variance.   All final models included significant two-way interactions or main effects were remained if two-way interaction was not significant ($P > 0.05$).

Table 20. Primers of genes for RT-PCR analysis

Gene[1]	Accession No.	Primer Sequence (5′ - 3′)
PPAR α	NM_001001464	TGGACGAATGCCAAGGTCTGAGAA(Forward)
		TCTCTGCCATGCACAAGGTATCCA(Reverse)
PPAR γ	NM_001001460	ACATAAAGTCCTTCCCGCTGACCA(Forward)
		ACAAACCTGGGCGATCTCCACTTA(Reverse)
SREBF1	NM_204126	ACCGCTCATCCATCAACGACAAGA(Forward)
		ATGCTTCTTCCAGGACCAGCAGTA(Reverse)
PLA2G4A	NM_205423	TTGGAGCTGTCTCTTGAAGTGTGCT (Forward)
		ACCAGCGATGTAAGTTGCACAGTCT (Reverse)
SCD	NM_204890	AAGTGGTGATGTTCCAGCGGAGAT (Forward)
		TTCTCCCGTGGGTTGATGTTCTGA (Reverse)
FADS2	NM_001160428	TGTCCTTGGCGAAAGTCAGCCTAT (Forward)
		TGACCCATACAAACCAGTGGCTCT (Reverse)
PTGS2	XM_001231378	TGACCCTGAGCTTCTGTTCAACCA (Forward)
		CGGTGCGCCAATTTCTACCATTGT (Reverse)
ACTB	NM_205518	ACACTGTGCCCATCTATGAAGGCT (Forward)
		AATTCTCTCTCGGCTGTGGTGGT (Reverse)

[1]PPAR α: Peroxisome proliferator-activated receptor alpha, PPAR γ: Peroxisome proliferator-activated receptor gamma, SREBF1: Sterol regulatory element binding transcription factor 1, PLA2G4A: Phospholipase A_2 (group IV A, cytosolic & calcium-dependent), SCD (Δ9 desaturase): Steroyl-CoA desaturase, FADS2 (Δ6 desaturase): Fatty acid desaturase 2, PTGS2 (COX-2): Prostaglandin-endoperoxide synthase 2, ACTB (β-actin): Actin, beta.

Table 21. Characteristics of genes

Gene[1]	Characteristic
PPAR α	Regulation of fatty acid oxidation
PPAR γ	Regulation of fatty acid synthesis
SREBF1	Regulation of fatty acid synthesis
PLA2G4A	Recognize and release an arachidonic acid from the sn-2 of phospholipids
SCD	Creates a double bond at the 9th carbon position from the carboxyl group
FADS2	Creates a double bond at the 6th carbon position from the carboxyl group
PTGS2	Converts an arachidonic acid to a prostaglandin H_2 (PGH$_2$)

[1]PPAR α: Peroxisome proliferator-activated receptor alpha, PPAR γ: Peroxisome proliferator-activated receptor gamma, SREBF1: Sterol regulatory element binding transcription factor 1, PLA2G4A: Phospholipase A_2 (group IV A, cytosolic & calcium-dependent), SCD (Δ9 desaturase): Steroyl-CoA desaturase, FADS2 (Δ6 desaturase): Fatty acid desaturase 2, PTGS2 (COX-2): Prostaglandin-endoperoxide synthase 2.

4. RESULTS

Live Weight, Liver Weight and Their Ratio in Broiler Chickens

Live weight, liver weight and the liver weight to live weight (liver wt./live wt.) ratio of broiler chickens fed with six different diets for 6 and 9 weeks of age were studied and summarized in Table 22.   Two way interaction due to treatment by age, did not significantly differ in live weight, liver weight and their ratio ($P > 0.05$), however, significant influence of main effect including treatment and/or age was determined ($P < 0.05$).   Six different treatments significantly influenced live weight.   Live weight was higher for broiler chickens raised on animal and vegetable oil (AVO) and soybean and olive oil (SYO) diet when compared to that of flaxseed and olive oil (FXO) and fish and olive oil (FHO) diets ($P < 0.05$).   The live weight of broiler chickens from AVO and SYO treatments were not significant ($P > 0.05$).   Moreover, neither broiler chickens from FXO nor from FHO significantly differed ($P > 0.05$).   The live weight of broiler chickens from flaxseed, eicosapentaenoic acid and olive oil (FEO) and flaxseed, docosahexaenoic acid and olive oil (FDO) treatments was similar to that of other four treatments including AVO, SYO, FXO and FHO ($P > 0.05$).

Significant differences were not determined in liver weight and liver weight to live weight ratio ($P > 0.05$) even though live weight of broiler chickens was influenced by six different dietary fats ($P < 0.05$).   It is important to indicate that both live and liver weights were increased consistently as broiler chickens were raised up to 9 weeks, and dietary fats and their combinations did not influence the morphology of liver during growth.   Liver weight and liver weight to live weight ratio of broiler chickens were ranged from 59.40 and 1.62 to 70.54 and 1.80, respectively.   Six and nine weeks of feeding significantly influenced the live weight, liver weight and liver weight to live weight ratio of broiler chickens when they were raised on six different dietary fats ($P < 0.05$).   As expected, both live weight and liver weight of 9th week broiler chickens was higher when compared to that of broiler chickens at 6 weeks of age ($P < 0.05$). However, liver weight to live weight ratio of broiler chickens at 6 week was significantly higher than that of 9th week broiler chickens, and those are 1.90 and 1.52, respectively (P

Table 22. Live weight, liver weight and their ratio of broiler chickens fed with different fat diets and processed at 6 and 9 weeks of growth (g)

Effect	LIVE WEIGHT	LIVER WEIGHT	LIVER/LIVE
TRT*WKS[1]			
P-value	0.457	0.198	0.285
TREAT[2]			
P-value	0.014	0.354	0.808
AVO	4126.8^a	70.54	1.73
SYO	4169.5^a	65.41	1.62
FXO	3682.3^b	59.71	1.69
FEO	3878.6^{ab}	67.30	1.75
FDO	3961.9^{ab}	69.36	1.80
FHO	3712.8^b	59.40	1.65
WEEK[3]			
P-value	0.001	0.001	0.001
6	3051.63^b	57.97^b	1.90^a
9	4792.29^a	72.60^a	1.52^b
ROOT MSE[4]	314.942	12.615	0.282

[1]TRT*WKS = treatment by age interaction; [2]Treatment: AVO = 5% animal fat & vegetable oil, SYO = 2.5% soybean oil + 2.5% olive oil combination, FXO =2.5% flaxseed oil + 2.5% olive oil combination, FEO = 2.45% flaxseed oil + 0.05% eicosapentaenoic acid + 2.5% olive oil combination, FDO = 2.45% flaxseed oil + 0.05% docosahexaenoic acid + 2.5% olive oil combination, FHO = 2.5% fish oil + 2.5% olive oil combination; [3]WEEK = age; [4]ROOT MSE = Root Mean Square Error; [a,b]Mean values within a row followed by the same letter are not significantly different ($P > 0.05$).

< 0.05).   As mentioned above, both live and liver weights increased consistently, but it is assumed that body weight gained rapidly when compared to liver weight.

Fatty Acid Profiles of Broiler Chicken Liver

Omega-3 and -6 fatty acids and total fatty acid profiles of broiler chicken liver fed with six different fat diets and processed at 6 and 9 weeks of growth are presented in Table 23 and 24. Two way interaction which is treatment by age for the deposition of C20:4, C22:5 and C22:6, was determined ($P < 0.05$), and C18:3, SFA and PUFA were found to be significantly affected by a factors treatment or age ($P < 0.05$).   However, the deposition of C20:3, C20:5, MUFA and the ratio of n-3 FAs to n-6 FAs did not significantly differ in two way interaction and main effects when six different fat diets were supplied to broiler chickens for 6 and 9 weeks ($P > 0.05$).   Broiler chicken liver fed with six different diets exhibited significant differences on C18:3 and did not give any significant difference in SFA and PUFA.   AVO diet which is a combination of animal and vegetable oil had similar deposition of C18:3 when compared to that of SBO, FXO and FHO diets ($P > 0.05$).   However, C18:3 of liver from FXO and FHO did not significantly differ to that of FDO ($P > 0.05$).   The broiler chicken liver sampled from FEO treatment had higher deposition of C18:3 and was insignificant to FDO ($P > 0.05$).

Age, another main effect, did not significantly influence the deposition of C18:3, C20:3, C20:5, MUFA and n-3 fatty acids to n-6 fatty acids ratio ($P > 0.05$) but had an effect on the overall content of C18:2, SFA and PUFA when broiler chickens were raised on six different fat diets and processed at 6 and 9 weeks, respectively ($P < 0.05$).   More C18:2, SFA and PUFA were deposited in liver when broiler chickens were processed and sampled at 9th week of age as compared to that of 6th week, and those are $P = 0.001$, $P = 0.029$ and $P = 0.001$.

Least squares means for treatment by age interaction of C20:4 ($P = 0.007$), C22:5 ($P = 0.004$) and C22:6 ($P = 0.022$) were analyzed and reported in Figure 7. Arachidonic acid from all six treatments was insignificant at 4 weeks of age ($P > 0.05$), however, neither C20:4 of broiler chicken liver from FHO (13.37 mg) and SYO (13.10

Table 23. Omega-3 and -6 fatty acid profiles of broiler chicken livers fed with different fat source diets and processed at 6 and 9 weeks of growth (mg/100g of fresh tissue)

Effect	C18:2	C18:3	C20:3	C20:4	C20:5	C22:5
TRT*WKS[1]						
P-value	0.278	0.131	0.410	**0.007**	0.525	**0.004**
TREAT[2]						
P-value	0.790	0.006	0.430	0.017	0.853	0.001
AVO	558.54	32.28^c	15.52	19.44	242.20	32.51
SYO	613.42	60.37^c	19.38	12.22	281.50	48.14
FXO	608.54	67.07^{bc}	16.75	17.52	280.00	40.35
FEO	643.18	143.29^a	17.67	16.48	248.50	70.31
FDO	640.88	132.29^{ab}	19.93	18.17	217.10	60.09
FHO	288.87	71.79^{bc}	15.95	11.91	400.30	88.30
WKS[3]						
P-value	0.001	0.208	0.201	0.001	0.203	0.004
6	528.91^b	73.05	15.18	11.80	223.49	57.41
9	688.90^a	95.98	19.89	20.11	333.02	55.82
ROOT MSE[5]	131.291	61.991	5.112	5.013	292.503	14.441

[1]SFA = saturated fatty acid (C14:0 + C16:0 + C18:0), MUFA = monounsaturated fatty acid (C16:1 + C18:1c9), PUFA = polyunsaturated fatty acid (C18:2 + C18:3 + c9t11 + t10c12 + C20:3 + C20:4 + C20:5 + C22:5 + C22:6), n3/n6 = ratio of omega-3 fatty acids (C18:3 + C20:5 + C22:5 + C22:6) and omega-6 fatty acid (C18:2 + C20:3 + C20:4); [2]TRT*WKS = treatment by age interaction; [3]Treatment: AVO = 5% animal fat & vegetable oil, SYO = 2.5% soybean oil + 2.5% olive oil combination, FXO =2.5% flaxseed oil + 2.5% olive oil combination, FEO = 2.45% flaxseed oil + 0.05% eicosapentaenoic acid + 2.5% olive oil combination, FDO = 2.45% flaxseed oil + 0.05% docosahexaenoic acid + 2.5% olive oil combination, FHO = 2.5% fish oil + 2.5% olive oil combination; [4]WEEK = age; [5]ROOT MSE = Root Mean Square Error; [a,b,c]Mean values within a row followed by the same letter are not significantly different (P > 0.05).

Table 24. Omega-3 and total fatty acid profiles[1] of broiler chicken livers fed with different fat source diets and processed at 6 and 9 weeks of growth (mg/100g of fresh tissue)
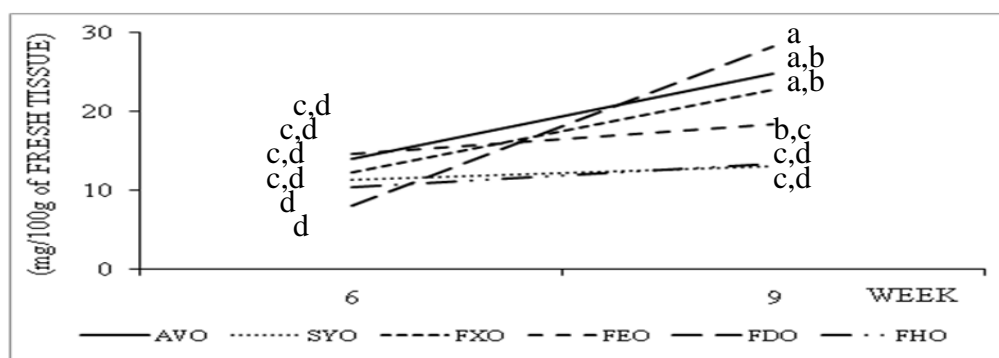
Effect	C22:6	SFA	MUFA	PUFA	n3/n6
TRT*WKS[2]					
P-value	**0.022**	0.749	0.959	0.281	0.501
TREAT[3]					
P-value	0.001	0.126	0.221	0.070	0.230
AVO	140.87	2321.6	1834.4	1160.5	0.73
SYO	160.92	1934.5	1352.5	1301.5	0.84
FXO	120.08	2073.7	1690.5	1272.0	0.76
FEO	151.92	1867.4	1439.7	1494.5	0.83
FDO	165.94	2585.7	2360.1	1418.4	0.81
FHO	300.37	2823.3	2366.6	1680.8	1.26
WEEK[4]					
P-value	0.005	0.029	0.167	0.001	0.821
6	156.62	2008.4[b]	1632.2	1199.8[b]	0.86
9	190.08	2526.9[a]	2049.1	1576.1[a]	0.89
ROOT MSE[5]	38.362	788.416	1023.036	348.219	0.452

[1]SFA = saturated fatty acid (C14:0 + C16:0 + C18:0), MUFA = monounsaturated fatty acid (C16:1 + C18:1c9), PUFA = polyunsaturated fatty acid (C18:2 + C18:3 + c9t11 + t10c12 + C20:3 + C20:4 + C20:5 + C22:5 + C22:6), n3/n6 = ratio of omega-3 fatty acids (C18:3 + C20:5 + C22:5 + C22:6) and omega-6 fatty acid (C18:2 + C20:3 + C20:4); [2]TRT*WKS = treatment by age interaction; [3]Treatment: AVO = 5% animal fat & vegetable oil, SYO = 2.5% soybean oil + 2.5% olive oil combination, FXO =2.5% flaxseed oil + 2.5% olive oil combination, FEO = 2.45% flaxseed oil + 0.05% eicosapentaenoic acid + 2.5% olive oil combination, FDO = 2.45% flaxseed oil + 0.05% docosahexaenoic acid + 2.5% olive oil combination, FHO = 2.5% fish oil + 2.5% olive oil combination;[4]WEEK = age; [5]ROOT MSE = Root Mean Square Error; [a,b,c]Mean values within a row followed by the same letter are not significantly different (*P* > 0.05).

Arachidonic Acid (C20:4, AA, n-6; *P*=0.007)
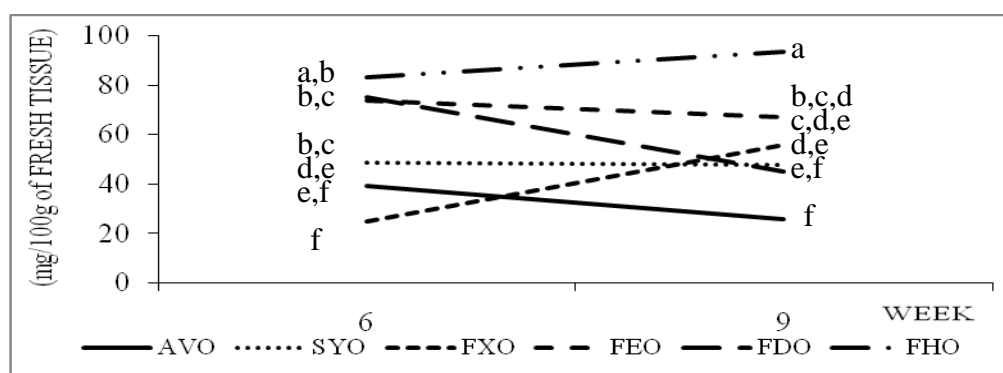
Docosapentaenoic Acid (C22:5, DPA, n-3; *P*=0.004)
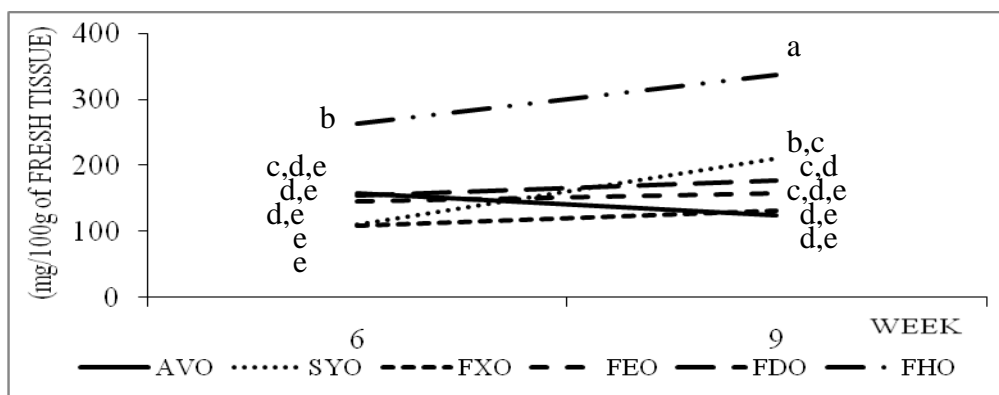
Docosahexaenoic Acid (C22:6, DHA, n-3; *P*=0.022)

Figure 7. Least squares means for treatment by age interaction for arachidonic acid, docosapentaenoic acid and docosahexaenoic acid of broiler chicken liver fed with different fat source diets.

AVO = 5% animal fat & vegetable oil, SYO = 2.5% soybean oil + 2.5% olive oil combination, FXO =2.5% flaxseed oil + 2.5% olive oil combination, FEO = 2.45% flaxseed oil + 0.05% eicosapentaenoic acid + 2.5% olive oil combination, FDO = 2.45% flaxseed oil + 0.05% docosahexaenoic acid + 2.5% olive oil combination, FHO = 2.5% fish oil + 2.5% olive oil combination.

[a-f]Mean values within a row followed by the same letter are not significantly different (*P* > 0.05).

mg) treatments nor from FEO (18.36 mg) treatment significantly differed at 9 weeks ($P > 0.05$).   The FEO, FXO and AVO had a similar deposition of C20:4, but only FXO and AVO had significantly higher overall content of C20:4 when compared to that of SYO and FHO ($P < 0.05$).   As FDO, AVO and FXO diets were fed to broiler chickens for 9 weeks, 28.25, 24.80 and 22.80 mg of C20:4 per 100 g of fresh liver tissue were determined, respectively.   They were not significantly different ($P > 0.05$).   On the other hand, C20:4 of liver samples from FDO treatment significantly differed when compared to that of FEO, FHO and SYO treatments ($P < 0.05$).   Overall C20:4 of three different treatments including SYO, FEO and FHO were similar when each diet was fed to broilers from 6 to 9 weeks.   More C20:4 was deposited when broiler chickens were raised on AVO, FXO and FDO diets for 9 weeks ($P < 0.05$), and 10.72, 10.56 and 20.15 mg of additional C20:4 per 100 g of fresh liver tissue was deposited.

Within 6 weeks of feeding to broiler chickens, overall C22:5 of broiler chicken liver from FXO treatment (24.82 mg) was neither more nor less than that of AVO treatment (39.24 mg) ($P > 0.05$).   The amount of C22:5 from AVO was not significantly different from broiler chickens raised on SYO diet (48.54 mg) ($P > 0.05$).   The AVO had a higher C22:5 content when compared to that of FXO treatment ($P < 0.05$).   The highest overall content of C22:5 was obtained when FEO, FDO and FHO diets (73.63, 75.08 and 83.16, respectively) were supplied to broiler chickens for 6 weeks.   They significantly differed to that of FXO, AVO and SYO ($P < 0.05$).   Additionally, when broiler chickens were raised on six different diets for 9 weeks, AVO and FDO had a similar overall content of C22:5, but only FDO was insignificant to that of SYO and FXO ($P > 0.05$).   Those were 25.78, 45.10, 47.73 and 55.88 mg/100 g of fresh liver tissue, respectively.

The FHO treatment (93.44 mg) showed the highest overall content of C22:5 and this treatment significantly differed to that of other treatments ($P < 0.05$).   The FEO diet (67.01 mg) induced higher C22:5 deposition in broiler chicken liver than AVO and FDO diets.   The C22:5 deposition of FEO treatment did not differ as compared to that of broiler chicken liver from SYO and FXO treatment ($P > 0.05$).   Most treatments inc-

-luding AVO, SYO, FEO and FHO maintained the deposition of C22:5 during 6 to 9 weeks of feeding. Broiler chicken livers of FXO treatment acquired more C22:5 rapidly and finally reached to 55.88 mg, which is 31.06 mg higher than C22:5 in FXO at 6 week. In contrast to the deposition of C22:5 in FXO treatment, C22:5 of liver samples collected from FDO treatment declined to 45.10 mg at 9 weeks. It is 29.98 mg lower than samples of 6 weeks.

Overall C22:6 of broiler chicken livers from FHO treatment (262.88 mg) were significantly different when compared to other treatments ($P < 0.05$). The rest of the treatments were insignificant at 6 weeks ($P > 0.05$). Treatments including AVO, FXO, FEO and FDO had an insignificant amount of C22:6 at 9 weeks. However, similar deposition of C22:6 was determined when FEO, FDO and SYO diets were supplied to broiler chickens for 9 weeks, and those were 157.79, 177.93 and 210.65 mg, respectively. Additional C22:6 was deposited in the liver of SYO when compared to that of AVO and FXO treatments (124.22 and 132.04 mg, respectively) ($P < 0.05$). The highest deposition of C22:6 was obtained when FHO diet was fed to broiler chickens for 9 weeks and it was significant when compared to other five treatments ($P < 0.05$). Two treatments, SYO and FHO, increased the overall content of C22:6, and 99.45 and 77.98 mg more C22:6 was deposited when SYO and FHO diet was supplied to broiler chickens during 6 to 9 weeks.

Nutritional Regulation of Delta-6 and -9 Desaturase

The regulation of hepatic delta-6 and -9 desaturase mRNA expression levels by omega-3 and -9 fatty acids was determined in broiler chickens raised on six different diets for 9 weeks (Figure 8). Hepatic mRNA expression levels of delta-6 desaturase varied. The only statistically significant difference detected was an increase in broiler chickens fed FXO diet compared to AVO, FEO and FHO diets, although there was no statistically significant difference between FXO diet and SYO and FDO.

Within 9 weeks of feeding broiler chickens six different diets, FXO diet (3.02) which contains 2.5% each of flaxseed and olive oils had a marked increase, statistically
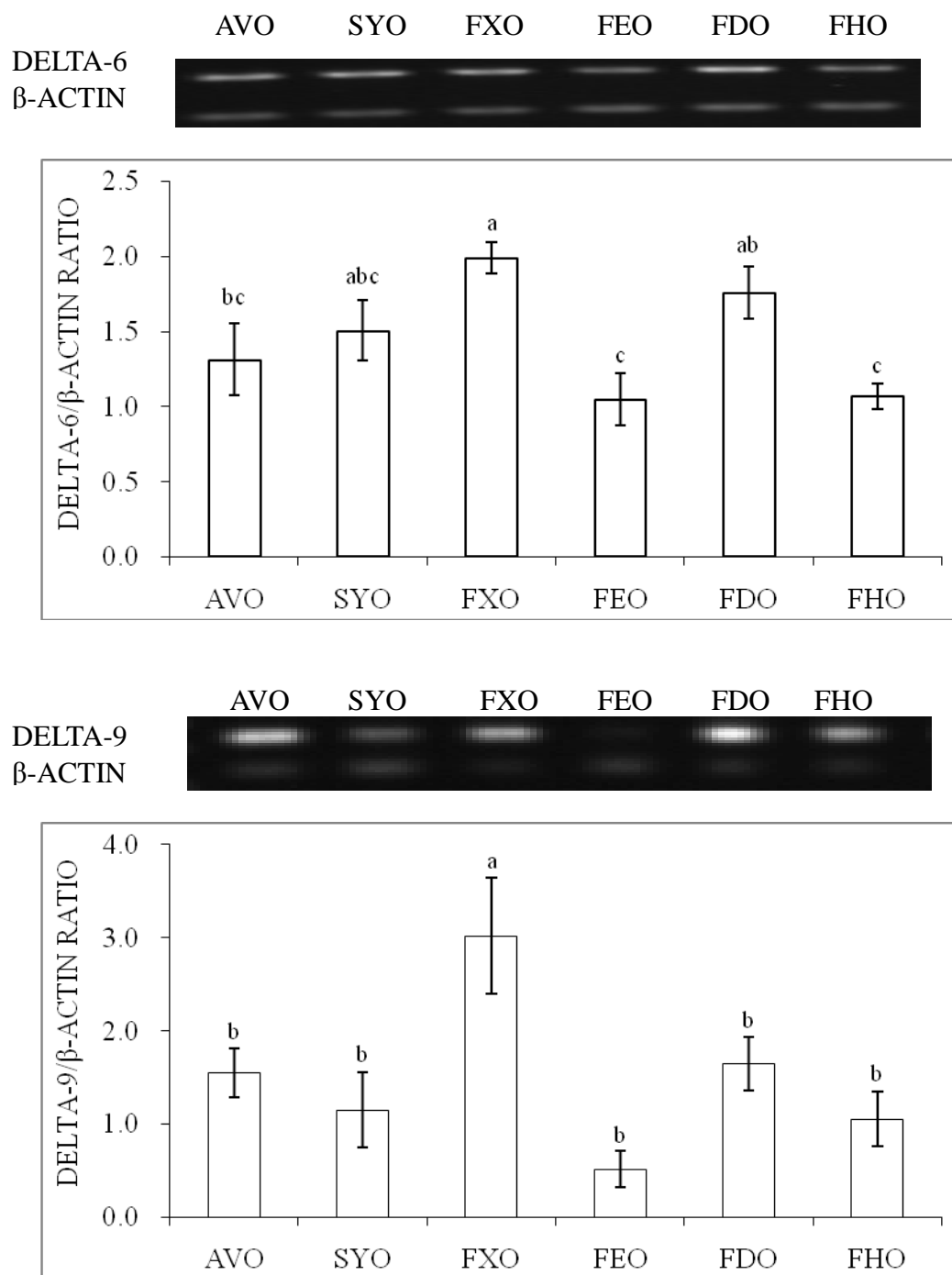
Figure 8. mRNA expression of delta-6 and delta-9 desaturase of broiler chicken livers fed with different fat source diets.

AVO = 5% animal fat & vegetable oil, SYO = 2.5% soybean oil + 2.5% olive oil combination, FXO =2.5% flaxseed oil + 2.5% olive oil combination, FEO = 2.45% flaxseed oil + 0.05% eicosapentaenoic acid + 2.5% olive oil combination, FDO = 2.45% flaxseed oil + 0.05% docosahexaenoic acid + 2.5% olive oil combination, FHO = 2.5% fish oil + 2.5% olive oil combination.
[a,b,c]Mean values within a row followed by the same letter are not significantly different ($P > 0.05$).

significant in delta-9 desaturase mRNA expression level over AVO, SYO, FEO, FDO and FHO diets ($P < 0.05$).   Interestingly, despite different fatty acid compositions, hepatic mRNA levels of delta-9 desaturase were not statistically significant when AVO, SYO, FEO, FDO and FHO diets were fed to broiler chickens for 9 weeks.

Fatty Acid Profiles of Broiler Chicken Breast Muscle

Two way interaction due to treatment by age for n-3/n-6 ratio of breast samples was observed when broiler chickens were raised on six different diets for 6 and 9 weeks ($P < 0.05$) (Tables 25 and 26).   A main effect of treatment and/or age was significantly influenced C18:2, C18:3, C20:3, C20:4, C22:5, C22:6 and SFA ($P < 0.05$), however, C20:5, MUFA and PUFA were not influenced neither by treatment nor by age ($P > 0.05$). Breast fatty acids profiles of broiler chickens fed with AVO diet had significantly higher amounts of C18:2, C20:3, C20:4 and SFA as compared to broiler chickens fed FXO, FDO and FHO diets ($P < 0.05$).   Interestingly, AVO diet provided low deposition of C18:3, C22:5 and C22:6 in broiler chicken breasts and had similar C18:3, C22:5 and C22:6 depositions when compared to that of SYO ($P > 0.05$).

Soybean and olive oil combination diet (SYO) showed similar fatty acid profiles for breast muscles as that of AVO diet for breast samples ($P > 0.05$), and this was expected.   However, the amount of SFA was significantly different between broiler chicken breast samples from SYO and AVO ($P < 0.05$).   Ideally, flaxseed and olive oil combination diet (FXO) should contain a high level of C18:3, but the current study indicated insignificant amount of C18:3 for FXO treatment when compared to that of other four treatments including AVO, SYO, FDO and FHO ($P > 0.05$).   The accumulation of C20:3 and C20:4 which are long-chain-fatty acids of n-6 fatty acids, was lower in FXO than in SYO ($P < 0.05$) even though C18:2 deposition of breast samples from FXO and SYO treatment was insignificant.   On the other hand, overall C22:5 of broiler samples from FXO treatment was similar to the case of AVO and SYO ($P < 0.05$), whereas C22:6 observed in FXO was neither more nor less when compared to that of FEO and FDO ($P > 0.05$).

Table 25. Omega-3 and -6 fatty acid profile of broiler chicken breast fed with different fat source diets and processed at 6 and 9 weeks of growth (mg/100g of fresh tissue)

Effect	C18:2	C18:3	C20:3	C20:4	C20:5	C22:5
TRT*WKS[1]						
P-value	0.977	0.822	0.002	0.036	0.328	0.001
TREAT[2]						
P-value	0.045	0.001	0.001	0.001	0.507	0.001
AVO	347.16^a	28.51^b	13.23^a	69.20^{ab}	30.82	24.71^{bc}
SYO	243.20^{ab}	15.75^b	13.55^a	79.05^a	41.19	26.80^b
FXO	208.55^b	52.35^b	7.01^b	46.18^{cd}	36.08	29.57^b
FEO	269.75^{ab}	111.35^a	12.15^a	58.75^{bc}	18.31	40.46^a
FDO	170.18^b	49.94^b	4.89^b	36.65^d	24.37	16.03^c
FHO	188.63^b	31.86^b	5.60^b	36.07^d	17.16	42.95^a
WKS[3]						
P-value	0.642	0.337	0.285	0.354	0.120	0.326
6	245.65	42.61	10.04	52.43	21.23	28.85
9	230.18	53.98	8.77	56.21	34.74	31.32
ROOT MSE[5]	114.410	40.487	4.057	13.939	29.354	8.581

[1]SFA = saturated fatty acid (C14:0 + C16:0 + C18:0), MUFA = monounsaturated fatty acid (C16:1 + C18:1c9), PUFA = polyunsaturated fatty acid (C18:2 + C18:3 + c9t11 + t10c12 + C20:3 + C20:4 + C20:5 + C22:5 + C22:6), n3/n6 = ratio of omega-3 fatty acids (C18:3 + C20:5 + C22:5 + C22:6) and omega-6 fatty acid (C18:2 + C20:3 + C20:4); [2]TRT*WKS = treatment by age interaction; [3]Treatment: AVO = 5% animal fat & vegetable oil, SYO = 2.5% soybean oil + 2.5% olive oil combination, FXO =2.5% flaxseed oil + 2.5% olive oil combination, FEO = 2.45% flaxseed oil + 0.05% eicosapentaenoic acid + 2.5% olive oil combination, FDO = 2.45% flaxseed oil + 0.05% docosahexaenoic acid + 2.5% olive oil combination, FHO = 2.5% fish oil + 2.5% olive oil combination; [4]WEEK = age; [5]ROOT MSE = Root Mean Square Error; [a,b,c]Mean values within a row followed by the same letter are not significantly different ($P > 0.05$).

Table 26. Omega-3 and total fatty acid[1] profiles of broiler chicken breast fed with different fat source diets and processed at 6 and 9 weeks of growth (mg/100g of fresh tissue)

Effect	C22:6	SFA	MUFA	PUFA	n3/n6
TRT*WKS[2]					
P-value	0.001	0.948	0.980	0.734	**0.042**
TREAT[3]					
P-value	0.001	0.042	0.066	0.069	0.001
AVO	36.80^{bc}	612.82^a	851.10	550.44	0.30
SYO	38.94^b	385.58^b	447.50	458.47	0.37
FXO	20.39^d	378.54^b	474.50	400.11	0.51
FEO	27.87^{cd}	436.68^b	620.70	538.62	0.59
FDO	19.59^d	350.49^b	418.70	321.64	0.51
FHO	80.20^a	393.92^b	479.70	402.46	0.82
WEEK[4]					
P-value	0.747	0.635	0.964	0.752	0.001
6	36.84	437.93	550.68	437.64	0.44
9	37.76	414.74	546.68	452.94	0.59
ROOT MSE[5]	9.748	167.843	305.887	166.334	0.140

[1]SFA = saturated fatty acid (C14:0 + C16:0 + C18:0), MUFA = monounsaturated fatty acid (C16:1 + C18:1c9), PUFA = polyunsaturated fatty acid (C18:2 + C18:3 + c9t11 + t10c12 + C20:3 + C20:4 + C20:5 + C22:5 + C22:6), n3/n6 = ratio of omega-3 fatty acids (C18:3 + C20:5 + C22:5 + C22:6) and omega-6 fatty acid (C18:2 + C20:3 + C20:4); [2]TRT*WKS = treatment by age interaction; [3]Treatment: AVO = 5% animal fat & vegetable oil, SYO = 2.5% soybean oil + 2.5% olive oil combination, FXO =2.5% flaxseed oil + 2.5% olive oil combination, FEO = 2.45% flaxseed oil + 0.05% eicosapentaenoic acid + 2.5% olive oil combination, FDO = 2.45% flaxseed oil + 0.05% docosahexaenoic acid + 2.5% olive oil combination, FHO = 2.5% fish oil + 2.5% olive oil combination;[4]WEEK = age; [5]ROOT MSE = Root Mean Square Error; [a,b,c]Mean values within a row followed by the same letter are not significantly different (*P* > 0.05).

Most n-3 and -6 fatty acid profiles of broiler chicken breast from FEO did not differ to that of AVO, a control in our experiment, but FEO showed differences in C18:3 and C22:5 ($P < 0.05$).   The amount of C18:3 was highest in breast samples (111.35 mg/100 g) from FEO treatment was determined when compared to other five treatments ($P < 0.05$).   Besides, a significant difference in C22:5 did not exist between the FEO and FHO diets ($P > 0.05$) but was determined with other treatments ($P < 0.05$).   FDO and FHO diets containing C22:6, induced similar deposition of C18:2, C18:3, C20:3, C20:4 and C20:5 in broiler chicken breast, whereas significant differences between FDO and FHO were observed in C22:5 and C22:6.   In contrast to the diet of FDO, FHO diet showed markedly higher amount of C22:5 and C22:6, and those were 42.95 and 80.20 mg, respectively ($P < 0.05$).   None of n-3, n-6, SFA, MUFA and PUFA profiles was significantly influenced by two different ages of broiler chickens ($P > 0.05$).

As shown in figure 9, the ratio of n-3/n-6 had a two-way interaction in breast samples of broiler chickens fed six different diets for two different weeks, 6 and 9 ($P < 0.05$).   For 6 weeks of feeding, the breast samples from AVO, FDO and SYO treatment (0.23, 0.29 and 0.35 mg, respectively) did not significantly differ ($P > 0.05$) but had a low n-3/n-6 ratio when compared to the case of FEO and FHO (0.59 and 0.77 mg) ($P < 0.05$).   However, n-3/n-6 ratio of breast samples in FXO (0.42 mg) was similar to that of AVO, FDO, SYO and FEO treatment.   Higher n-3/n-6 ratio of breast samples was observed in FHO diet, but the ratio was not significant as compared to the case of FEO, only.   Most diets including AVO, SYO, FXO, FEO and FHO maintained their n-3/n-6 ratio of breast samples up to 9 weeks of growth, and those were 0.38, 0.40, 0.60, 0.58 and 0.86 mg, respectively.   FDO diet increased the ratio of n-3/n-6 from 0.29 to 0.73 mg when broiler chickens were raised during 6 to 9 weeks.

n-3/n-6 Ratio (*P*=0.042)
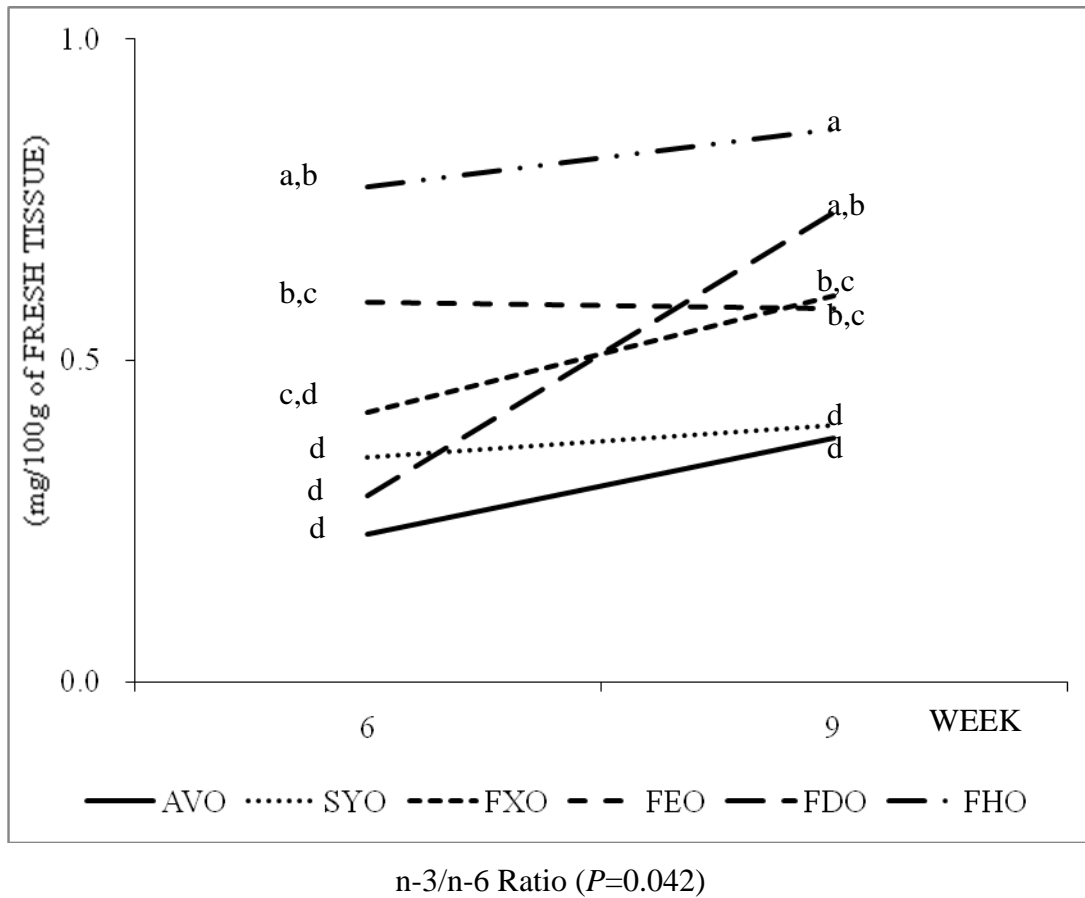
Figure 9. Least squares means for treatment by age interaction for omega-3 and -6 ratio of broiler chicken breast fed with different fat source diets.

AVO = 5% animal fat & vegetable oil, SYO = 2.5% soybean oil + 2.5% olive oil combination, FXO =2.5% flaxseed oil + 2.5% olive oil combination, FEO = 2.45% flaxseed oil + 0.05% eicosapentaenoic acid + 2.5% olive oil combination, FDO = 2.45% flaxseed oil + 0.05% docosahexaenoic acid + 2.5% olive oil combination, FHO = 2.5% fish oil + 2.5% olive oil combination.

[a,b,c,d]Mean values within a row followed by the same letter are not significantly different (*P* > 0.05).

Fatty Acid Profiles of Broiler Chicken Thigh Muscle

Omega-3 and -6 fatty acids, SFA, MUFA, PUFA and n-3/n-6 ratio profiles of thigh meats from broiler chickens fed with six different diets for 6 and 9 weeks are investigated and presented in Tables 27 and 28. Significant treatment by age interaction was not confirmed ($P > 0.05$), but a main effect due to treatment and/or age was determined in C18:3, C20:4, C20:5, C22:5, C22:6 and n-3/n-6 ratio ($P < 0.05$). Animal fat and vegetable oil (AVO) and soybean and olive oil (SYO) treatments showed an increase in C20:4 but a reduction in C22:6 depositions. C20:4 and C22:6 profiles of thigh samples from AVO and SYO treatments significantly differed to those of fish and olive oil (FHO) treatment ($P < 0.05$). Similar amount of C18:3 and C22:5 were deposited in thigh samples due to the supply of AVO and SYO diets to broiler chickens. In contrast to C18:3 of AVO treatment, SYO showed a significantly different amount of C18:3 when compared to FEO treatment ($P < 0.05$). The amount of C22:5 in AVO treatment was not significant as compared to that of other five treatments, however, n-3/n-6 ratio was significantly lowered in AVO than in the cases of the FXO, FEO, FDO and FHO treatments ($P < 0.05$).

Flaxseed and olive oil (FXO) induced the deposition of C22:5 as compared to that of soybean and olive oil combination (SYO) ($P < 0.05$), whereas significant difference did not exist between FXO and SYO treatments in C18:3, C20:4 and C22:6 profiles ($P > 0.05$). Generally, C20:4, C22:5, C22:6 and n-3/n-6 ratio ofthigh samples in FEO and FDO treatments were similar to those of FXO but differed for C18:3. The amount of C18:3 obtained in FXO treatment was neither more nor less when compared to that of FEO ($P > 0.05$) but significantly differed in FDO ($P < 0.05$).

Table 27. Omega-3 and -6 fatty acid profile of broiler chicken thigh fed with different fat source diets and processed at 6 and 9 weeks of growth (mg/100g of fresh tissue)

Effect	C18:2	C18:3	C20:3	C20:4	C20:5	C22:5
TRT*WKS[1]						
P-value	0.577	0.703	0.646	0.087	0.819	0.473
TREAT[2]						
P-value	0.087	0.001	0.105	0.022	0.302	0.021
AVO	373.80	25.01[d]	6.93	54.76[a]	11.38	16.66[ab]
SYO	498.20	41.15[bcd]	10.14	67.03[a]	34.39	11.76[b]
FXO	368.80	104.74[ab]	9.59	57.38[a]	38.84	25.92[a]
FEO	450.20	168.68[a]	9.78	54.85[a]	23.78	29.48[a]
FDO	271.90	97.94[bc]	8.48	53.81[a]	27.65	30.63[a]
FHO	227.30	32.81[cd]	5.95	34.52[b]	32.90	17.75[ab]
WKS[3]						
P-value	0.167	0.319	0.337	0.028	0.023	0.003
6	324.25	68.97	8.00	48.03[b]	36.60[a]	16.23[b]
9	405.80	87.81	8.96	59.42[a]	19.72[b]	27.84[a]
ROOT MSE[5]	200.059	64.546	3.418	17.197	24.587	12.438

[1]SFA = saturated fatty acid (C14:0 + C16:0 + C18:0), MUFA = monounsaturated fatty acid (C16:1 + C18:1c9), PUFA = polyunsaturated fatty acid (C18:2 + C18:3 + c9t11 + t10c12 + C20:3 + C20:4 + C20:5 + C22:5 + C22:6), n3/n6 = ratio of omega-3 fatty acids (C18:3 + C20:5 + C22:5 + C22:6) and omega-6 fatty acid (C18:2 + C20:3 + C20:4); [2]TRT*WKS = treatment by age interaction; [3]Treatment: AVO = 5% animal fat & vegetable oil, SYO = 2.5% soybean oil + 2.5% olive oil combination, FXO =2.5% flaxseed oil + 2.5% olive oil combination, FEO = 2.45% flaxseed oil + 0.05% eicosapentaenoic acid + 2.5% olive oil combination, FDO = 2.45% flaxseed oil + 0.05% docosahexaenoic acid + 2.5% olive oil combination, FHO = 2.5% fish oil + 2.5% olive oil combination; [4]WEEK = age; [5]ROOT MSE = Root Mean Square Error; [a,b,c]Mean values within a row followed by the same letter are not significantly different (*P* > 0.05).

Table 28. Omega-3 and total fatty acid[1] profiles of broiler chicken thigh fed with different fat source diets and processed at 6 and 9 weeks of growth (mg/100g of fresh tissue)

Effect	C22:6	SFA	MUFA	PUFA	n3/n6
TRT*WKS[2]					
P-value	0.627	0.569	0.281	0.536	0.744
TREAT[3]					
P-value	0.004	0.251	0.244	0.071	0.001
AVO	21.91[b]	664.0	158.07	488.5	0.18[b]
SYO	21.51[b]	690.7	125.23	662.7	0.20[b]
FXO	15.63[b]	607.3	123.58	605.2	0.43[a]
FEO	21.71[b]	779.7	126.23	736.7	0.49[a]
FDO	28.62[b]	459.2	71.41	490.4	0.56[a]
FHO	48.31[a]	470.9	84.03	351.2	0.49[a]
WEEK[4]					
P-value	0.119	0.074	0.065	0.166	0.789
6	22.62	531.17	94.02	502.07	0.40
9	29.93	692.77	135.50	609.54	0.39
ROOT MSE[5]	15.840	303.964	75.426	263.255	0.136

[1]SFA = saturated fatty acid (C14:0 + C16:0 + C18:0), MUFA = monounsaturated fatty acid (C16:1 + C18:1c9), PUFA = polyunsaturated fatty acid (C18:2 + C18:3 + c9t11 + t10c12 + C20:3 + C20:4 + C20:5 + C22:5 + C22:6), n3/n6 = ratio of omega-3 fatty acids (C18:3 + C20:5 + C22:5 + C22:6) and omega-6 fatty acid (C18:2 + C20:3 + C20:4); [2]TRT*WKS = treatment by age interaction; [3]Treatment: AVO = 5% animal fat & vegetable oil, SYO = 2.5% soybean oil + 2.5% olive oil combination, FXO =2.5% flaxseed oil + 2.5% olive oil combination, FEO = 2.45% flaxseed oil + 0.05% eicosapentaenoic acid + 2.5% olive oil combination, FDO = 2.45% flaxseed oil + 0.05% docosahexaenoic acid + 2.5% olive oil combination, FHO = 2.5% fish oil + 2.5% olive oil combination;[4]WEEK = age; [5]ROOT MSE = Root Mean Square Error; [a,b,c]Mean values within a row followed by the same letter are not significantly different (*P* > 0.05).

Insignificant difference of C18:3 for FXO and FDO was determined. As mentioned above, thigh samples derived from FHO treatment had a similar amount of C22:5 and ratio of n-3/n-6 as compared to those from FXO, FEO and FDO treatments ($P > 0.05$). The lowest C20:4 but highest C22:6 with values of 34.52 and 48.31 mg, respectively, were observed in FHO treatment. The two fatty acids were found to be significant for FHO when compared to other five treatments ($P < 0.05$). Also, the amount of C18:3 in FHO treatment differed to that of FXO and FEO.

Age, one of main effects, did not influence the amount of C18:2, C18:3, C20:3, C22:6, SFA, MUFA and PUFA deposited and also had not an effect on the ratio of n-3/n-6. In contrast, the amount of C20:4, C20:5 and C22:5 were significantly affected. Feeding time had promoted the amount of C20:4 and C22:6, but amount of C20:5 was reduced ($P < 0.05$).

No statistically significant differences in hepatic mRNA levels of phospholipase A_2 (PLA2G4A) and cyclooxygenase2 (COX2) were observed between broiler chickens fed six different diets for 9 weeks (Figure 10). No statistically significant difference in plasma prostaglandin E_2 (PGE$_2$) concentration was detected between broiler chickens fed six different diets for 9 weeks ($P > 0.05$). The concentration of PGE$_2$ ranged from 0.41 to 0.50 pg/ml depending on the diet (Table 29).
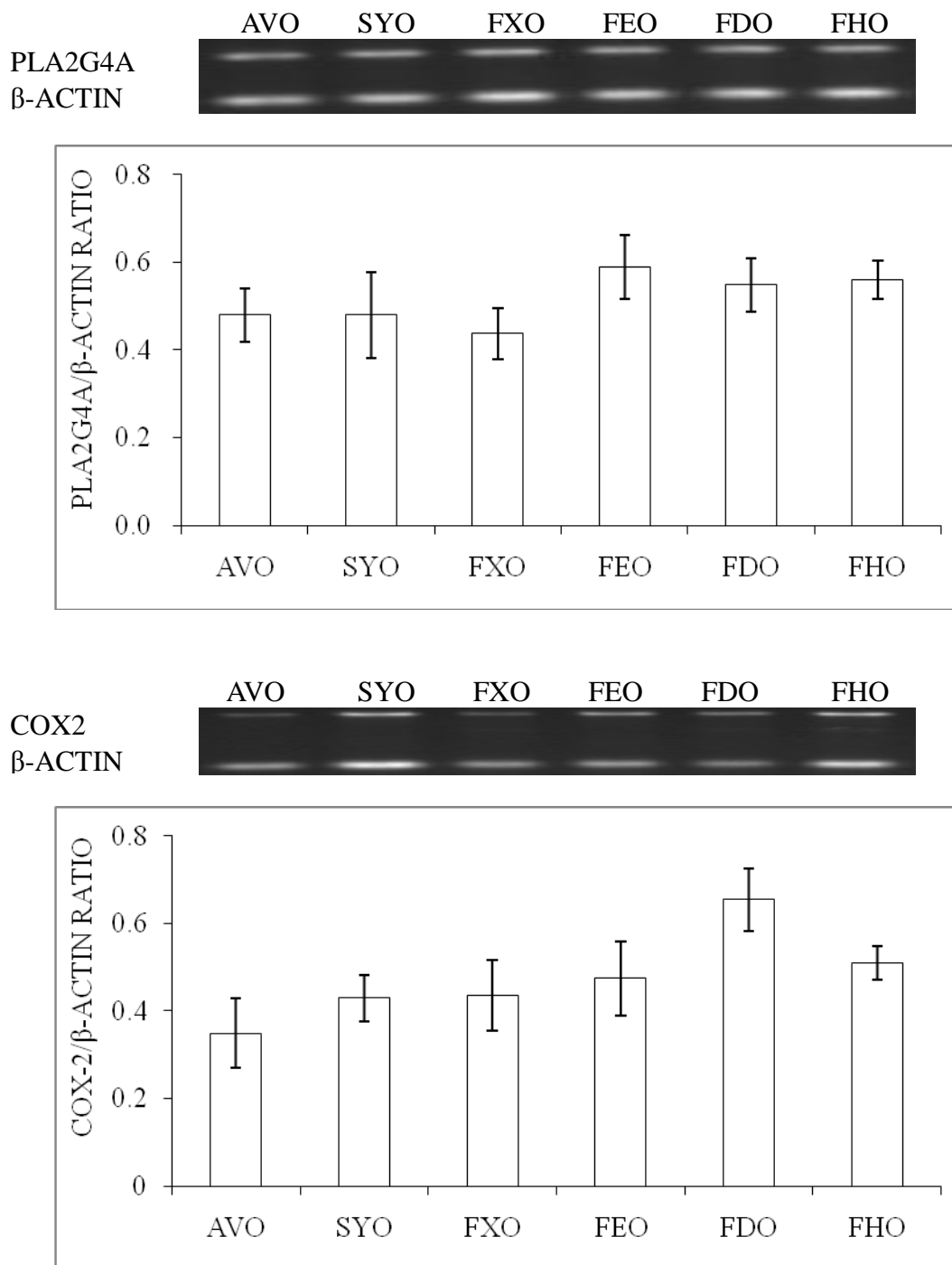
Figure 10. mRNA expression of phospholipase A_2(PLA2G4A) and cyclooxygenase2 (COX2) of broiler chicken liver fed with different fat source diets.

AVO = 5% animal fat & vegetable oil, SYO = 2.5% soybean oil + 2.5% olive oil combination, FXO = 2.5% flaxseed oil + 2.5% olive oil combination, FEO = 2.45% flaxseed oil + 0.05% eicosapentaenoic acid + 2.5% olive oil combination, FDO = 2.45% flaxseed oil + 0.05% docosahexaenoic acid + 2.5% olive oil combination, FHO = 2.5% fish oil + 2.5% olive oil combination.

Table 29. Prostaglandin E_2 (PGE$_2$) of broiler chickens fed with six different fat source diets and processed at 6 and 9 weeks of growth (pg/mL)

Effect	Prostaglandin E_2
TRT*WKS[1]	
P-value	0.2917
TRT[2]	
P-value	0.2680
AVO	0.49
SYO	0.50
FXO	0.41
FEO	0.47
FDO	0.47
FHO	0.42
WKS[3]	
P-value	0.7383
6	0.46
9	0.45
ROOT MSE[4]	0.085169

[1]TRT*WKS = treatment by age interaction; [2]Treatment: AVO = 5% animal fat & vegetable oil, SYO = 2.5% soybean oil + 2.5% olive oil combination, FXO =2.5% flaxseed oil + 2.5% olive oil combination, FEO = 2.45% flaxseed oil + 0.05% eicosapentaenoic acid + 2.5% olive oil combination, FDO = 2.45% flaxseed oil + 0.05% docosahexaenoic acid + 2.5% olive oil combination, FHO = 2.5% fish oil + 2.5% olive oil combination;[3]WKS = age; [4]ROOT MSE = Root Mean Square Error; [a,b,c]Mean values within a row followed by the same letter are not significantly different (*P* > 0.05).

5. DISCUSSION

This study was undertaken to evaluate the effectiveness of the combination of n-3 and n-9 fatty acids in poultry diet to reduce the deposition of n-6 fatty acids which are linoleic acid (C18:2, LA, n-6) and arachidonic acid (C20:4, AA, n-6) in poultry chicken meats. As the palmitic acid (C16:0, PA, SFA), a predominant saturated fatty acid (SFA) in the U.S. diet, was known as a fatty acid leading inflammatory responses and impaired insulin sensitivities (Valsta et al., 2005; Kennedy et al., 2009), unsaturated fatty acids (UFA) mainly from seed oils was added to poultry diets providing balanced UFA to SFA (U/S) poultry meat to consumers. On the other hand, the addition of seed oils in poultry diets markedly increases the deposition of n-6 fatty acid and finally reduces a n-3 to n-6 fatty acids ratio of poultry meats. Due to over intake of n-6 fatty acids *via* rich in C18:2 and/or C20:4 of diets, 1.6 billion people around the world were overweight (Kennedy et al., 2009).

For human adults, 2700 Kcal of energy per day is required, and only 9000 mg of fatty acids must be ingested from n-6 fatty acids (Costa, et al., 2008). The ratio of linoleic acid (C18:2, LA, n-6), a major feed ingredient, is ranged from 0.5 to 7% of PUFA in meats, while linolenic acid (C18:3, LNA, n-3) of meat is only 0.5% (Valsta et al., 2005). Most C18:2 derived from the meats can be absorbed and incorporated to human tissue. Thereby, nutritionally imbalanced n-3 to n-6 ratio may be accomplished to consumers. Modification of poultry diet is necessary to provide nutritionally balanced poultry chicken meat for consumers in commercial market. To reduce n-6 fatty acids in poultry meat should be emphasized. Surprisingly, poultry meat contains 10 ~ 12g of fat per 100g of chicken meat with skin (Valsta et al., 2005), and poultry chicken meat is a good dietary fat source to consumers.

Olive oil contains sufficient C18:2 (3.3 mol%), and it may help preventing the essential fatty acid deficiency (EFAD) of broiler chickens (Wang et al., 2005). Besides, additional C18:2 was supplied to broiler chickens *via* basal diet including corn and soybean mill as shown in Table 12. Under 3800g of broiler chicken weight from FXO and FHO treatments of our experiment may not be due to deficient of C18:2 but may be

due to increased mitochondrial oxidation that burnt fat for energy (Flachs et al., 2005; Vijaimohan et al., 2006).   Omega-3 PUFAs are preferred to be used for generating energy when compared to n-6 PUFAs, and it is influenced due to the chain length and degree of saturation (Newman et al., 2002).   Interestingly, our FEO and FDO treatment did not show significantly different weight gain as compared to that of AVO, SYO, FXO and FHO, and it was similar to study conducted by Willumsen et al. (1993).

Hepatic fatty acid profiles of broiler chickens from six different treatments were not strongly influenced by dietary fatty acids even though broiler chicken has single stomach.   As we provided specifically compromised fatty acids diets for broiler chickens, fatty acids which are deficient, may be replenished due to *de novo* lipogenesis of broiler chicken liver.   The liver of broiler chickens is a major organ generating very-long-chain fatty acids (carbon $\geq$ 20) including C20:4, C20:5, C22:5 and C22:6 because of a great number of delta-5 and delta-6 desaturases (Viveros et al., 2009).   Delta-5 and -6 desaturases are greatly involved to feedback regulation of C18:2 and C18:3 generating C20:4, C20:5 and C22:6.   Tang and his/her colleagues (2003) demonstrated that the delta-6 desaturase gene transcription of rodent liver was inhibited when n-3 (menhaden fish oil) or n-6 (safflower oil) fatty acids was supplied to male rats for 5 days. However, to reach the end-point fatty acids (C20:4 and C20:5, respectively) of C18:2 and C18:3, the competition for delta-5 and -6 desaturases is necessary.

Due to the competition of C18:2 for the delta-6 desaturase against C18:3, dietary composition of n-3 and n-6 fatty acids is important.   The abundance of hepatic delta-6 desaturase mRNA was markedly reduced while C20:4, C20:5 and/or C22:6 were supplied to mice and human, whereas triolein had no effect (Raz et al., 1998; Emken et al., 1999; Matsuzaka et al., 2002; Tang et al., 2003).   Therefore, dietary fatty acids containing $\geq$ 20 carbons may be a control point generating very-long-chain fatty acids of n-3 and reducing n-6 fatty acids in poultry chicken meats.   In contrast to studies conducted by Emken et al. (1999) and Portolesi et al. (2008), the amount of delta-6 desaturase mRNA was ameliorated when FXO and FDO diets of our study were fed to broiler chickens for 9 weeks as compared to that of FEO and FHO diets.   As a result,

insignificant amount of C20:5 was deposited in broiler chicken breast and thigh meats of six different diet treatments (Tables 20 and 21).

Generally, it takes 6 weeks for eicosapentaenoic acid (C20:5, EPA, n-3) to reach maximum accumulation in monocytes, but it quickly returns to a normal stage (Simopoulos, 2002). Due to deposition of EPA, expression of delta-6 desaturase mRNA may be declined, thereby, the desaturation of C18:2 to C20:4 and C18:3 to C20:5 could be decreased, as well. Moreover, as EPA was returning to a normal dose from 6 to 9 weeks, liver accumulated C20:4 rapidly as shown in figure 6. The abundance of delta-6 desaturase mRNA from FEO was significantly low as compared to that of FXO and FDO treatments. Similar mechanism was not observed when C22:6 (DHA, n-3) was supplied to broiler chickens for 6 and 9 weeks (FDO treatment), and it is because DHA needs more weeks to reach a maximum deposition when compared to deposition of EPA (Simopoulos, 2002).

Around 19.59 mg of C22:6 in 100 g of fresh breast tissue, however, 28.62 mg of C22:6 in fresh thigh tissue were deposited when FDO diet was supplied to broiler chickens for 9 weeks. The highest accumulation of C22:6 in breast and thigh meat sampled from FDO treatment was not accomplished even if 0.05% of C22:6 was blended to a diet. It is an unexpected result. Normally, broiler chicken breast and thigh muscles are composed of two different fiber types, and these are type I and type IIB. The type IIB fiber is a major component of broiler breast muscle; in contrast, broiler thigh muscle is mostly composed of type I fiber. In spite of higher composition of phospholipid due to a great number of mitochondria in type I fiber, very-long-chain fatty acids of n-3 are favorably absorbed and immediately burnt to energy (Newman et al., 2002). Thereby, more mg of C22:6 was deposited in breast muscle, but similar effect was not observed in breast muscle of FDO treatment.

Arachidonic acid (C20:4, AA, n-6) is a precursor of prostaglandin E_2 (PGE_2), and it induces chronic diseases including obesity, type-2 diabetes and cancers when disordered. General dietary lipids of U.S. for farm animals are from restaurant grease or hydrogenated oil of food industry; thereby, dietary lipids contain a large amount of

saturated fatty acid, *trans* fatty acid and n-6 fatty acid but have low in n-3 fatty acids (Cherian, 2007). Chances of C20:4 to be incorporated in cell membrane of broiler chickens are increased. Now, C20:4 of cell membrane is able to be released by cPhospholipase A2 (cPLA2) (Moreira et al., 2009; Rosa and Rapoport, 2009) and then converted to PGE_2 by cyclooxygenase2 (COX2). Therefore, the amount of C20:4 incorporated in cell membrane is important, and effort to minimize C20:4 in cell membrane is necessary.

In our experiment, reduction of C20:4 by FDO diet was observed depending on fiber types, and significantly decreased amount of C20:4 from both breast and thigh muscle was determined when FHO diet was supplied to broiler chickens. However, as C18:2 increased in broiler chicken breast, significantly higher deposition of C20:4 in FEO was obtained as compared to that of FDO and FHO. Delta-6 desaturase is competitively functions due to the demand of C20:4 and C20:5. As mentioned above, opportunities to use delta-6 desaturase may be increased to C18:2 if more C18:2 is available than C18:3. Besides, C20:5 of breast samples from FEO was converted to C22:6 because of insufficient C22:6 supply *via* FEO diet. It happened to FDO treatment as well. Sufficient C20:5 was deposited due to retroconversion of C22:6 to C20:5, thereby, C20:5 is able to depress the expression of delta-6 desaturase to C18:2.

Surprisingly, in spite of different deposition of C20:4, expression of cPLA2 and COX2 mRNA did not change, and as a result, they generated 0.41 ~ 0.50 pg/mL of PGE_2 content in six treatments. It is not expected. However, it may have happened because our experiment was designed to provide a normal environmental condition to broiler chickens, and cPLA2 released C20:4 as needed. Phospholipase A2 (cPLA2) is a Ca^{2+} dependent cytosolic enzyme, and cPLA2 is activated when it is translocated to a selective phospholipid containing C20:4 (Moreira et al., 2009; Rosa and Rapoport, 2009; Chen et al., 2010). Calcium ion (Ca^{2+}) was generally released from an endoplasmic reticulum (ER), and as confirmed by expression of SREBP1 mRNA (data not shown), over release of Ca^{2+} due to disruption of ER did not occurred. Therefore, only C20:4 targeted to cPLA2 was able to be generated.

In conclusion, although the generation of PGE_2 was not affected due to combination of n-3 and n-9 fatty acids in our diets, the deposition of n-6 fatty acids including C18:2 and C20:4 was decreased in broiler chicken breast and/or thigh muscles as n-3 fatty acids were supplied to broiler chickens for 9 weeks. Eicosapentaenoic acid (C20:5, EPA, n-3) addition to poultry diet did not reduce the deposition of C18:2 and/or C20:4 as much as C22:6 did. However, C22:6 of FDO diet significantly reduced the overall content of C20:4 in broiler chicken breast muscle, thereby, increased the n-3 to n-6 ratio at 9 weeks. When C20:5 and C22:6 were blended to poultry diet and fed to broiler chickens for 9 weeks, synergistic effects were observed. Reduction of C20:4 was obtained when FHO diet was fed to broiler chickens, and addition of C20:5 and C22:6 as a mixed form to poultry diet may be recommendable to reduce C20:4 accumulation in both broiler chicken breast and thigh meats.

CHAPTER V

OVERALL CONCLUSION

1. EXPERIMENT I

The conjugated linoleic acid and flaxseed oil combination diet (CXO) had a higher deposition of C18:2 and C20:4, in contrast, had low percent of C20:5, C22:5 and C22:6 when compared to those of CHO which is a conjugated linoleic acid and fish oil combination diet. The CXO provided only 0.41 of n-3/n-6 ratio in breast and thigh meat. It was lower than that of flaxseed oil diet (FXO), however, it may not be acceptable in commercial market if CXO has similar problems as that of FXO. The CHO which decreased the deposition of C18:2 and C20:4, decreased the SFA, and increased the PUFA in breast and thigh muscles, is recommendable, and it may provide 'functional' broiler chicken meats to consumers.

2. EXPERIMENT II

Although the generation of prostaglandin E_2 (PGE_2) was not affected due to combination of n-3 and n-9 fatty acids in our diets, the deposition of n-6 fatty acids including C18:2 and C20:4 was decreased in broiler chicken breast and/or thigh muscles as n-3 fatty acids were supplied to broiler chickens for 9 weeks. Eicosapentaenoic acid (C20:5, EPA, n-3) addition to poultry diet did not reduce the deposition of C18:2 and/or C20:4 as much as C22:6 did. However, C22:6 of flaxseed oil and docosahexaenoic aicd combination diet (FDO) significantly reduced the overall content of C20:4 in broiler chicken breast muscle, thereby, increased the n-3 to n-6 ratio at 9 weeks. When C20:5 and C22:6 were blended to poultry diet and fed to broiler chickens for 9 weeks, synergistic effects were observed. Reduction of C20:4 was obtained when fish oil diet (FHO) was fed to broiler chickens, and addition of C20:5 and C22:6 as a mixed form to poultry diet may be recommendable to reduce C20:4 accumulation in both broiler chicken breast and thigh meats.

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