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Omega-3 Enrichment and Oxidative Stability of Broiler Chicken Meat

by

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ABSTRACT

Omega-3 polyunsaturated fatty acids can reduce the risk of cardiovascular disease and cancers. Enriching broiler meat opportunities lack research on product quality. The fatty acid (**FA**) profile of birds fed flaxseed for various periods was analyzed. Another experiment assessed FA profile and oxidation products in frozen-raw and cooked thigh meat in birds fed 20% linPRO (50% extruded flaxseed) with antioxidant combinations. Males deposited more omega-3 in breast meat than females. It required 26.2 d (10%flax) or 11.3 d (17%flax) feeding to achieve the 300 mg ω-3/100g of breast. Eicosapentaenoic and docosahexaenoic acids were deposited in the phospholipids whereas a-linolenic acid associated with triacylglycerols. Oxysterol appearance was reduced in thighs of high vitamin E birds while the high selenium treatment had no effect or even raised oxysterols during roasting. Antioxidants inhibited thiobarbuturic reactive acid substances in stored frozen-raw meat. Stability of ω-3 broiler meat was improved with increased dietary antioxidant levels.

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LIST OF TABLES

LIST OF FIGURES

LIST OF ABREVIATIONS

PUFA: Polyunsaturated fatty acids

EFA: Essential fatty acids

LA: Linoleic acid

LNA: alpha-linolenic acid

AA: Arachiodonic acid

EPA: Eicosapentaenoic acid

DHA: Docosahexaenoic acid

LC ω-3 PUFA: Long chain omega-3 polyunsaturated fatty acids

SFA: Saturated fatty acids

MUFA: Monounsaturated fatty acids

VLDL: Very low density lipoproteins

NAD(P)H: Cytosolic reducing equivalents

CHO: Carbohydrates

PL: Phospholipids

TG: Triacylglycerols

TBA: Thiobarbituric acid

TBARs: Thiobarbituric reactive substances

MDA: Malonaldehydes

HPLC: High performance liquid chromatography

GC: Gas chromatography

GLC: gas liquid chromatography

LDL: Low-density lipoproteins

PE: Phosphatidyl ethanolamine

PC: Phosphatidyl choline

Gpx: Glutathione peroxidase

FA: Fatty acids

FAME: Fatty acid methyl esters

SeMet: Selenomethionine

19-HC: 19-hydroxycholesterol

7α-HC: 7-alpha-hydroxycholesterol

7β-HC: 7-beta-hydroxycholesterol

25-HC: 25-hydroxycholesterol

β-CE: beta- cholestanetriol

α-CE: alpha- cholestanetriol

7-KC: 7-ketocholesterol

GC-MS: Gas Chromatography-Mass Spectrometry

ICP-MS: Inductively coupled plasma mass spectrometry

1 LITERATURE REVIEW

1.1 Consumer Trends

Meat has been categorized as a source of protein, minerals and vitamins. Today, consumers are no longer satisfied with this concept; nutritional value, quality, extended shelf life and convenience are expected in meat products. Safety and healthiness, on the other hand, are among the most important issues challenging the meat industry, in times when meat consumption is expected to decrease by 2020 (Food and Agricultural Commodity Consumption in the United States: Looking Ahead to 2020, ERS, USDA) (Table 1.1).

Recently, consumers have paid much attention to the tertiary function of foods (Dentali, 2002) which brings up a new era of functional foods. Tertiary function of foods differs from primary and secondary functions related to nutrition and preference, respectively (Arai, 1996); it is involved in the roles of food components in preventing diseases by modulating physiological systems (Dentali, 2002). The meat processing sector, particularly the value-added segments, is trying to open up a new market to fulfill consumer's demands. Possible alternatives to develop healthier meat include the following: reduction of fat content, modification of fatty acid composition, incorporation of functional ingredients, reduction of calories, nitrites and cholesterol content, among others (Jimenez-Colmenero et al., 2001).

Modification of the fatty acid composition is mainly based on the incorporation of omega-3 polyunsaturated fatty acids (**PUFA**) and conjugated linoleic acid (Hocquette et al., 2005). Furthermore, conventional processed meat products have an omega-6:omega-3 ratio higher than 15, and the healthy effects are associated with ratios lower than 4 (Simopoulos, 2002a). As a result, the best strategy to improve the ratio is by increasing the levels of ω-3 PUFA in the meat. However, ω-3 enriched chicken meat contains more double bonds, which leads to a higher lipid oxidation rate (Barroeta, 2007). In this regard, the meat industry is looking forward to develop new strategies in terms of antioxidant supply required to limit lipid oxidation, which adversely affects meat nutritional value and sensory attributes.

1.2 Functional Foods

Functional Foods are defined by Health Canada as "similar in appearance to a conventional food, consumed as part of the usual diet, with demonstrated physiological benefits, and/or to reduce the risk of chronic disease beyond basic nutritional functions" (Wildman, 2006). This definition means fruits and vegetables, whole grains, beverages, and some fortified or omega-3 enriched products can also be considered as functional foods. In fact, consumption of omega-3 is associated with reducing the risk of cardiovascular diseases, cancers, Alzheimer's disease, among others (Mozaffarian et al., 2005; Theodoratou et al., 2007). The food industry's actions are driven by health conscious consumers, a potential niche market for functional foods (Cash et al., 2006). However, the success of functional foods depends on their effectiveness in reducing the risk of a disease, as well as on their safety. Labeling is an effective tool to deliver information to consumers on nutritious attributes and health-enhancing properties of functional foods. Countries such as Canada, Sweden, and the United States, have specific laws concerning the labeling of such products. For example, in Canada a minimum level of 300 mg of ω-3 PUFA per 100 g of meat is required to label the product as a source of omega-3 (Government of Canada, 2003). However, regulations in Canada are still developing; the actual regulation govermental institutions considered ALA, EPA, DHA and DPA. It is important to highlight that the U.S. Food and Drug Administration (2004) gave qualified health claim status specifically to EPA and DHA.

1.2.1 Omega-3 Enriched Products

Dietary essential fatty acids (**EFA**) are subclassified as omega-6 and omega-3 fatty acids, indicating the location of the carbon from the omega end where the first double bond is located (Schaefer, 2002). Important nutritionally desirable EFA include linoleic (18:2 ω-6; **LA**) and α-linolenic acid (18:3 ω-3; **LNA**) which are the precursors of ω-6 and ω-3 PUFA respectively. LA and LNA

can be converted in our bodies into Arachidonic (**AA**; 20:4 ω-6), eicosapentaenoic acid (**EPA**; 20:5 ω-3) and docosahexaenoic acid (**DHA**; 22:6 ω-3) (Komprda et al., 2005) (Figure 1.1). The level of ω-3 PUFA consumed by the population is considered insufficient; most American diets provide at least 10 times more omega-6 than omega-3 fatty acids (ODS, 2005). As a result, the market of omega-3 enriched products has grown in the last years, not only because of the inadequate ω–6: ω–3 ratio in the diet but also, because of their health benefits. There were 723 omega-3 fortified foods launched in Europe and 541 in the United States in 2008 (Daniells, 2008). These products ranged from breads to milks, juices, tortillas, meal bars, margarines, chocolates, yoghurt drinks, spreads, peanut butter and eggs. Omega-3 supplements are also widely available, the most popular being fish and linseed/flaxseed oils.

1.2.2 Effects of Omega-3 on Human Health

The potential benefits of omega-3 fatty acids have been widely reported in several areas, including cardiovascular diseases, atherosclerosis, hypertension, brain development, cancer, inflammatory, autoimmune and neurological disorders.

Long Chain ω-3 PUFA (**LC ω-3 PUFA**), specifically, EPA and DHA have anti-inflammatory, antithrombotic and antiarrhythmic properties which can be helpful to prevent atherosclerosis (Moreno et al., 2003) and coronary hearth diseases (Mozaffarian et al., 2005). Studies showed that fish-oil supplements, rich in LC ω-3 PUFA, had a substantial decrease of triglycerides ranging from 10 to 33% (Balk et al., 2004). One possible mechanism to explain how LC ω-3 PUFA reduce triglycerides is because they decrease hepatic synthesis and the secretion of triglyceride-rich lipoproteins (Very Low Density Lipoproteins) by inhibiting various enzymes (Chan and Cho, 2009).

Omega-3 PUFA also act on plasma lipids, reducing blood pressure thus atherosclerosis (Esposito et al., 2004). Besides that, dietary ω-3 PUFA are critical during pregnancy since they are essential for the proper development of eye and

brain in infants (Lee et al., 2006a). In fact, DHA and AA are the major PUFA in the membranes of brain and retinal cells and they have an impact on neuronal functions (Alessandri et al., 2004). Moreover, the level of DHA in the brain is essential for memory and learning ability; DHA consumption is associated with a lower Alzheimer's disease risk (Engelhart et al., 2002).

Several studies on mice had suggested a protective effect of ω-3 PUFA and a promoting effect of ω-6 PUFA on cancer (Berquin et al., 2007). In a Scottish study, significant reductions in colorectal cancer risk were associated with higher consumption of ω-3 PUFA, also when EPA or DHA were taken individually (Theodoratou et al., 2007). Inflammatory diseases (i.e. asthma) may be possibly reduced by the consumption of EPA. In the early phase of an inflammation disease, pro-inflammatory eicosanoids, from AA metabolism, are released from the phospholipids membranes. At this phase, EPA competes with AA for enzymatic metabolism; this phenomenon induces the production of less inflammatory derivatives (Simopoulos, 2002b). The consumption of omega-3 fatty acids was also related to neurological disorders, particularly dementias. A Rotterdam Study reported that the risk of vascular dementia was negatively correlated with the consumption of omega-3 fatty acid-rich fish (Tiemeier et al., 2003).

Health authorities have determined the minimum intake of LC ω-3 PUFA in human's diet. One of the highest requirements was established in 2004 by the United Kingdom Scientific Advisory Committee on Nutrition where a minimum of 450 mg EPA + DHA is required as ω-3 EFA intake which is equivalent to 2 portions of fish/week. Other institutions like the International Society for the Study of Fatty Acids and Lipids (2006) recommended 500 mg of EPA and DHA per day. Nevertheless, the International Society for the Study of Fatty Acids and Lipids recommended a daily intake of 2,220 mg of LNA and 650 mg of EPA + DHA, with a minimum of 220 mg of both EPA and DHA (Simopoulos et al., 1999).

1.3 Lipid Metabolism

In avian species, the net deposition of lipids is coming from two sources: *de novo* lipogenesis (lipid synthesis) and direct deposition from the diet (Villaverde et al., 2006). Saturated (**SFA**) and monounsaturated fatty acids (**MUFA**) come from these two sources whereas PUFA depend almost exclusively on dietary intake. *De novo* lipogenesis is performed from dietary carbohydrates, beginning in the cytosol with the acetyl-coA molecules which are derived from the glucose through glycolisis (Drackley, 2000). Lipogenesis is a metabolic process that converts simple sugars to fatty acids and synthesizes triacylglycerols through the reaction of fatty acids with glycerol. In birds, the liver is responsible for the synthesis of fatty acids and it is also the major site of cholesterol and phospholipid synthesis (Sturkie, 1976). The main fatty acids resulting from hepatic lipogenesis are 16:0, 18:0, 18:1 ω-9 and 16:1 ω-7 (Crespo and Esteve-Garcia, 2002). After lipogenesis, triacylglycerols are packaged into very low-density lipoproteins (**VLDL**) which transport lipids and cholesterol from the site of synthesis (liver) to the deposition site (adipose tissue) (Hermier et al., 1997). However, only 70 to 80% of VLDL triglyceride secreted into the circulation is taken up by adipose tissue; 20% of the VLDL was oxidized to carbon dioxide and 10% of VLDL was taken up by the liver (Griffin et al., 1992). The synthesis of long chain ω-3 PUFA occurs in the liver and it is based on elongation/desaturation processes using LNA (Brenna, 2002) (Figure 1.1). However, it is not clear how EPA and DHA are transported within the bird. What is clear is that ω-6 enriched diets can reduce the conversion of ω-3 fatty acids by as much as 40% because of the competition for Δ6-desaturase in the conversion of LNA to 18:4 ω-3 and LA to 18:3 ω-6 (Emken et al., 1994). Most of the poultry diets include high level of ω-6 fatty acids in their fat sources, which directly affects the ω6:ω3 ratio in muscle tissues (Table 1.2.).

1.4 Factors affecting Omega-3 Enriched Chicken Meat

1.4.1 Nutrition

Exogenous factors such as nutrition interact strongly with the regulation of the energy flow in the bird. In fact, dietary protein, carbohydrate, some metal ions and dietary fatty acid composition play an important role in the synthesis of fatty acids in chickens.

Swennen et al. (2007) concluded that increasing dietary protein levels from 15 to 35% depressed *in vitro* fatty acid synthesis by reducing malic enzyme activity. The study also suggested that the reduction in hepatic fatty acid synthesis might be initiated by a limitation in the availability of cytosolic reducing equivalents [NAD(P)H] which support lipogenesis (Raheja et al., 1971). Similarly, Tanaka et al. (1983) fount that dietary carbohydrates (**CHO**) are positively correlated with *in vitro* hepatic lipogenesis and the activity of lipogenic enzymes. Their results indicated that the consumption of CHO stimulates glycolysis in birds resulting in the production of NAD(P)H which could be used in hepatic fatty acid synthesis. It is important to consider that in some cases low rates of lipogenesis in birds may be due in part to the inhibitory effect of a continuous supply of fatty acids from the diet or liver (Griffin et al., 1992). Some metal ions could also affect the synthesis of fatty acids. For example, Mg^{2+}, Fe^{2+}, Cu^{2+} did not show a significant effect on fatty acid synthesis activity but increasing Zn^{2+} concentration decreased the rate of fatty acid synthesis (Wang et al., 2003).

Dietary fat intake and composition, thus the total energy intake, are the major factors to manipulate the fatty acid profile in meat (Nuernberg et al., 2006). For the ω-3 enrichment, the incorporation of either fish or flaxseed products could be a key to the poultry industry (Gonzalez-Esquerra and Lesson, 2001). In fact, PUFA tissue deposition increases as their dietary intake increases whereas SFA and MUFA have lower concentrations with higher dietary PUFA intake (Villaverde et al., 2006). Villaverde et al. (2006) showed that when dietary fat content increased from 0 to 10%, the deposition of fatty acids from de *novo*

synthesis was reduced, 50% in SFA and 87% in MUFA. The higher reduction in MUFA could be explained by the existence of a mechanism to keep the SFA: MUFA + PUFA ratio in the biological membranes.

Dietary omega-3 sources include fish such as salmon, trout, and tuna and plant sources such as flaxseed (Schmitz and Ecker, 2008). Flaxseed oil contains 50 to 60% LNA, which is the precursor of the LC ω-3 PUFA. For these reasons, it is used widely in omega-3 PUFA muscle enrichment (Gonzalez-Esquerra and Leeson, 2000). Fishmeal, on the other hand, offers a cheaper and more plentiful source of LC ω-3 PUFA than fishoil for direct enrichment of pork and poultry (Pike and Barlow, 1999). However, the potential for fishmeal to taint meat and eggs has been widely recognized (Howe et al., 2002). The maximum degree of DHA enrichment was obtained when high fish diet was supplemented (113 and 189 mg DHA/100g meat for white and dark meat respectively) (Rymer and Givens, 2006).

1.4.2 Genetics / Animal Age / Sex

On average, the fatty acid composition of chicken dark meat is approximately as follows: 31% SFA, 48% MUFA and 21% PUFA (Sutherland and Varnam, 1995) (Table 1.3). However, this composition varies according to several factors including, diet, breed, sex, age and physiological state. The dietary response of some fatty acids could be influenced by the broiler genotypes. The LNA dietary response in dark meat is affected by genotypes with Ross 308 being more responsive than Cobb 500; however, LC ω-3 PUFA deposition was not affected by genotypes (Rymer and Givens, 2006). Lipogenesis is also affected by genetics; it is higher in fat lines compared to lean lines (Buyse et al. 1999). Similarly, Cameron et al. (2000) found that the selection for high lean growth in pigs reduced intramuscular fat. Nevertheless, nutritional dietary effects on fat characteristics are substantially greater than genetic effects. Sex, on the other hand, has some influence on fatty acid profile especially in adult birds (Rymer and Givens, 2005); females had a 10% increase of in ω-3 fatty acid deposition compared with males fed redfish meal (Hulan et al., 1989). Furthermore, Hulbert

et al. (2002) found that the concentration of DHA in phospholipids was negatively correlated to body size; small birds, with higher metabolic rates, require more DHA to help maintain sodium and calcium pumps. As a consequence, the enhancement of ω-3 fatty acids may be more efficient in bigger animals compared to smaller animals.

1.4.3 Tissue Characteristics

Lipids in meat are located in three sites: subcutaneous, intramuscular and intermuscular (Hui, 2006). In poultry, the influence of dietary lipids is especially important in the intramuscular fatty acid composition. Meat can be efficiently enriched with ω-3 PUFA because of the abundance of phospholipids (structural lipids) and triacylcycerols in muscle tissue (Ayudah et al., 1991). In this regard, the proportion of neutral lipids and structure lipids varies according to the muscle (Mourot and Hermier, 2001). For instance, as a percentage of total lipids breast meat contains more lipids as phospholipids (**PL**) while triglycerides (**TG**) predominate in thigh meat (Gonzalez-Esquerra and Lesson, 2001). Total lipids from breast meat contained 70.1% phospholipids, 22.2% triacylglycerols, 4.2% cholesterol, and 1.2% cholesterol ester; whereas total lipids from leg meat contained 42.9% phospholipids, 5 1.4% triacylglycerols, 3.7% cholesterol and 0.8% cholesterol ester (Pikul et al., 1984). Fatty acid composition of dark meat can be modified easier than in white meat because of the total lipid content of the tissue (1% and 2% for white and dark meat respectively) (Leskanich and Noble, 1997).

1.5 Potential Problems in Omega-3 Enriched Products

The sensory quality, shelf life and consumer acceptability of omega-3 enriched products are somehow compromised due to a higher number of double bonds which enhances lipid oxidation. During the oxidation, a conversion of the red muscle pigment myoglobin to brown metmyoglobin occurs and at the same time PUFA degrade leading to the development of potentially toxic compounds

(Cortinas et al., 2005), rancid odors and flavors (Wood and Enser, 1997). The peroxidation of PUFA specifically contributes to the taste of fish oil (Crawford et al., 1975) and it is responsible for excessive dripping losses of unfrozen products (O'Neil et al., 1998). The oxidation of membranal phospholipids leads to a decease in fluidity of the biomembranes (Dobretsov et al., 1977) and disruption of membrane structure and function (Slater et al., 1987). Thus, the ability of the membranes to act as a semi permeable barrier is affected which contributes to exudative loss from meat (Asghar et al., 1991).

The oxidative limitation of ω-3 enriched chicken meat also varies according to the ω-3 source and its concentration in livestock feed. For example, the sensory quality of thigh meat from birds fed 10% flaxseed did not change compared to controls whereas feeding birds with 0.75% menhaden oil resulted in thigh meat with lower quality (Gonzalez-Esquerra and Lesson, 2000). Similarly, off-flavors became noticeable when dietary fish oils ranged from 0.75 to 1.5% (Miller and Robish, 1969). Consumer acceptability of meats produced from birds fed linseed oil was better than that for meats from birds fed fish oil (Lopez-Ferrer, 1999). Overall, ω-3 fatty acid dietary sources and feeding period affect sensory quality of meat parts differently which complicates meat enrichment and marketing.

1.6 Oxidation in Foods

Oxidation is one of the most important reactions in lipid chemistry since it is the responsible for the development of rancid odors and flavors. Tichivangana and Morrissey (1985) stated that oxidation of muscle foods occurs in the following order: fish > poultry > pork > beef > lamb. Oxidation occurs by following one of these three mechanisms: free radical or auto-oxidation mechanism which can act in the dark, photo-oxidation, and through metals and lipoxygenase with the latter being not fully studied (Guillen-Sans et al., 1998). Oxidation in foods occurs in two different fractions: triacylglycerols and phospholipids; in meats, the oxidation seems to be initiated in the phospholipid

membranes (Buckley et al., 1989). Auto-oxidation, the most common pathway, is the mechanism by which most of the PUFA and cholesterol oxidizes.

1.6.1 Auto-oxidation

This section refers to Pokorny et al.*, 2001*.

The auto-oxidation reaction proceeds through a free radical mechanism involving three phases: initiation, propagation and termination. The reaction is initiated with the extraction of hydrogen from a site of the lipids (LH) by a reactive species ($R^{\bullet}$) (i.e. reactive oxygen species, hydroxyl radicals). In the first phase, initiation, lipid radicals ($L^{\bullet}$) are formed as shown in equation (1). After initiation, propagation occurs in the presence of oxygen yielding peroxyl radicals ($ROO^{\bullet}$) (equation 2). This reaction is followed by an extraction of another hydrogen as shown in equation 3. Since another radical is formed from the last step of the propagation phase, the reaction keeps taking place until stable compounds are formed at the termination phase (equation 4). Chain-breaking antioxidants such as vitamin E donate a hydrogen to free radicals (i.e. $LOO^{\bullet}$) to form less reactive products (Decker et al., 2000).

Initiation $LH + R^{\bullet} \longrightarrow L^{\bullet} + RH$ (1)

Propagation $L^{\bullet} + O_2 \longrightarrow LOO^{\bullet}$ (2)

$LOO^{\bullet} + L'H \longrightarrow LOOH + L^{\bullet}$ (3)

Termination $LOO^{\bullet} + LOO^{\bullet} \longrightarrow LOOL + O_2$ (4)

1.6.2 Photo-oxidation

Photoxidation includes two different mechanisms: excitation of lipids (Type I) or excitation of oxygen (Type II) by the presence of an initiator (Pokorny et al., 2001). Type I photo-autoxidation occurs in the presence of sensitizers such as riboflavin. It is mainly a reaction between an excited triplet sensitizer and a substrate (i.e. PUFA) producing free radicals which will probably oxidize by autoxidation (Pokorny et al., 2001). Type II photo-autoxidation, on the other hand, requires the presence of either singlet or triplet oxygen (most common)

which only differ in their chemical properties (Table 1.4) (Akoh and Min, 2008). However, triplet oxygen goes to the singlet state by the uptake of energy (Decker et al., 2000) which makes singlet oxygen able to react with lipids and hydroperoxides which both exist in the singlet state. Singlet oxygen ($^1O_2$) oxidation occurs as described in equation 5 (Sutherland et al., 1995).

$$^1O_2 + \text{Lipid molecule (L-H)} \longrightarrow \text{L- OOH} \quad (5)$$

$$\text{L- OOH} \longrightarrow \text{Free radicals or autoxidation mechanism}$$

In meats, singlet oxygen can also be formed using photosensitizers, components containing hematoporphyrins (i.e. myoglobin) (Bradley and Min, 1992) and from linoleic acid peroxy radicals (Nakano et al., 1975). Singlet oxygen can react much faster than triplet oxygen. In fact, singlet oxygen reacts with linoleic acid at least 1450 times faster than triplet oxygen (Rawls and Van Santen, 1970).

1.6.3 Metal-catalysed oxidation

Many transition metals and heme protein compounds, myoglobin and hemoglobin, are associated with lipid oxidation. These include iron, copper and ferryl radicals ($FeOH^{3+}$ or FeO^{2+}). In the non-heme form, iron reacts with hydrogen-peroxide (H_2O_2) to produce reactive oxygen species which initiate lipid oxidation (Akoh and Min, 2008). The reaction can be described as: $Fe^{2+} + H_2O_2 = Fe^{3+} + OH^{\bullet} + OH^-$; Koppenol and Liebman (1984) proposed that ferryl radicals could also be products of this reaction. The level of non-heme iron in raw meat is low (2.4% to 3.9% of total chicken muscle iron) (Hazell, 1982); however, in fish muscles iron was released from the proteins during storage and cooking (Gomez-Basuri and Regenstein, 1992). Some metal ions are more effective in stimulating lipid oxidation than others. For example, Fe^{2+} was more effective to promote lipid oxidation in fish than Cu^{2+} or Cd^{2+} (Thanonkaew et al., 2006). Overall, the metal-catalyzed initiation can be summarized as follows (Pokorny et al., 2001):

$$M^{n+} + ROOH \longrightarrow RO^{\bullet} + OH^{-} + M^{(n+1)+} \quad (6)$$
$$M^{(n+1)+} + ROOH \longrightarrow ROO^{\bullet} + H^{+} + M^{(n)+}$$

1.7 Lipid Oxidation in Meat Products

1.7.1 Factors affecting Meat oxidation

Lipid oxidation is affected by both internal and external factors. In raw meat products, the primary factors that influence lipid oxidation include fatty acid composition, dietary fat quality (Engberg et al., 1996), endogenous prooxidative and antioxidative constituents, water activity and nonmeat additives (prooxidative and antioxidative) (Rojas and Brewer, 2007). Among these factors, the foremost is the level of PUFA in meat which oxidizes through a free-radical chain mechanism (Li et al., 1996). Engberg et al. (1996) found that α-tocopherol retention in broilers was significantly reduced when oxidized oil was incorporated in the diet. As a consequence, using oxidized oils in the diet requires a higher level of dietary vitamin E to maintain the antioxidant/prooxidant balance in muscle membranes. Dietary vitamin E supplementation period definitely influences the oxidative stability in meat. Morrissey et al. (1997) recommended at least 4 weeks of supplementation to guarantee oxidative protection in raw meat whereas 8 weeks were necessary to show a protective effect in cooked meat.

The presence of non-meat additives also influences the meat oxidation susceptibility, since they can be either antioxidants or prooxidants. Addition of ingredients that facilitate the formation of maillard browing reaction products (i.e. soy sauce) decrease the oxidation rate because of their antioxidant activity (Morales et al., 2001). Similarly, nitrite and phosphates exert an antioxidant effect by forming stable complexes (between heme pigments and nitrite) and metal chelation respectively (Freybler et al., 1993). Contrary to this, sodium chloride accelerates lipid oxidation because of iron displacement from heme proteins (Wettasinghe and Shahidi, 1996). Some additives such as ascorbate could have a pro- or antioxidative effect depending on their concentration. For instance,

ascorbate concentrations of 0.02% to 0.03% reported a pro-oxidative effect in beef while a concentration of 0.05% exhibited an antioxidant activity (St. Angelo et al., 1988).

Lipid oxidation in meats is also affected by external factors such as irradiation, temperature (i.e. during cooking), long-term storage and surface area in contact with oxygen. In the presence of oxygen, light and heat cholesterol oxidizes forming oxysterols (Chien et al., 2006) specifically, during cooking the rate of cholesterol oxidation varies according to time and temperature (Rao et al., 1996). In general, the level of most oxysterols increases with increasing heating time (Lee et al., 2006b). The exposure of the meat surface to air favors oxysterol formation. For example, oxysterol levels in ground pork meat proceed faster than in eggs since the yolk is protected by the albumen limiting its exposure to air (Lee et al., 2006b). Besides these external factors, biochemical changes in muscle/meat occurring in pre- and post-slaughter phases also stimulate oxidation processes. In this context, the degree of damage depends on the drop of pH during early post-mortem, carcass temperature and tenderizing techniques that disrupt cellular compartmentalization and release metal ions (Morrisey et al., 1994).

1.7.2 Primary and Secondary Oxidation Products

Lipid oxidation may occur in different stages; El-Gharbawi and Dugan (1965) found that in dried beef tissue the phospholipids oxidized first and the neutral fat oxidized later. The first product formed during the oxidation is an odorless intermediary product called hydroperoxide (Guillen-Sans and Guzman-Chozas, 1998). Latter, primary oxidation products are formed which are mainly lipid hydroperoxides and also secondary oxidation products: aldehydes (off-flavors), volatile compounds and cholesterol oxidation products or oxysterols.

Chien et al. (1998) defined that the major cholesterol oxidation pathway involves two steps: free radical formation (formation of 7α- and 7β-OH) and epoxidation (Figure 1.2). Furthermore, the formation of oxysterols is influenced by some fatty acids, for example, the presence of DHA influences the formation

of α- and β-epoxides (Hu and Chen, 2002). The oxidation of cholesterol brings up approximately 75 oxysterols (Smith, 1996); however, in food the most common oxysterols are the following: 7α-hydroxycholesterol, 7β-hydroxycholesterol, 25-hydroxycholestrol, 7-ketocholesterol and cholestanetriol (Morrissey and Kiely, 2006).

1.7.3 Test to Determine Lipid oxidation in Meat

The methods to assess the oxidative status of biological systems can be classified as follows: detection of primary or secondary (i.e. malonaldehyde, oxysterols) and fluorescent products; conjugated dienes assay; estimation of hydrocarbon gases; and loss of PUFA (Logani and Davies, 1979). However, sensory analysis is often used to detect oxidative off-flavors by taste or smell in order to decide if a lipid-containing food is suitable for consumption (Pokorny et al., 2001). Primary products of lipid oxidation include lipid hydroperoxides which can be quantified using the ferrous oxidation-xylenol orange test. This method is based on the reaction between the dye xylenol orange and ferric ions which produces a blue-purple complex with a maximum of absorbance between 550 and 600 nm (Grau et al., 2000).

The measurement of secondary oxidation products can be conducted by quantification of cholesterol oxidation products (oxysterols) or by the determination of thiobarbituric acid (**TBA**) value. The quantification of oxysterols is still controversial; in most of the cases it involves lipid extraction, purification: saponofication and column or thin layer chromatography fractionation (Guardiola et al., 1995). Since many compounds can elute at or around the retention site of the sterol of interest, mis-identification can be easily done; therefore, confirmation of the sterols by other techniques (i.e. mass-spectrometry) is necessary. A lot of difficulties are present in oxysterol determination mainly because of their trace levels and similar structures (Table 1.5).

In contrast, thiobarbituric acid test is a straight forward procedure, proposed over 40 years ago, that measures the quantity of TBA-reactive

substances (**TBARs**), such as malondialdehyde (**MDA**), a breakdown product formed mainly from oxidized PUFA (Veberg et al., 2006). TBA also reacts with other compounds rather than MDA (aldehydes, ketones, organic acids, esters, amides, amino acids, oxidized proteins, pyridines, pyrimidines, and vitamins) (Guillen-Sanz et al., 1998). Thiobarbituric acid value assay is based on MDA's ability to react with TBA to give a pink complex absorbing at 530 to 537 nm (Raharjo and Sofos, 1993). However, fluorescence is a more sensitive test compared to TBA test (10 to 100 times); therefore, it is an alternative method to measure lipid oxidation in biological tissues (Dillard and Tappel, 1971). The fluorescence procedure is based on the reaction between MDA with proteins and other cellular compounds which results in the formation of fluorescent products (Logani and Davies, 1979). Csallany et al. (1984) published another method for the direct quantification of MDA using high-performance liquid chromatography (**HPLC**), concluding that TBA test overestimate the MDA formation.

A rapid method to monitor oxidative deterioration of oils is the determination of conjugated dienes (C=C-C=C-C). The formation of hydroperoxides from PUFA oxidation leads to conjugation of the pentadiene structure (Pokorny et al., 2001). Conjugated dienes can be measured because they show an intense absorption at 233 nm (Logani and Davies, 1979). Another technique is based on the quantification of volatile compounds including pentane and ethane which are formed from ω-3 and ω-6 fatty acids respectively during oxidation (Dumelin and Tappel, 1977). This quantification is done using gas chromatography (**GC**) and it is normally accompanied with a sensory assessment of oil deterioration (Pokorny et al., 2001). The loss of PUFA is also a method to monitor changes in oxidation. In this procedure, total fatty acid composition is measured by either GC or gas liquid chromatography (**GLC**) (Logani and Davies, 1979). The loss of PUFA is one of the most direct methods because it is done on the lipids themselves.

1.7.4 Implications of Lipid Oxidation Products on Human Health

The effect of oxidation products on human health has been studied in three main areas: lipid peroxides, malonaldehyde and oxysterols. In general oxidation products are considered as inducers of cardiovascular and atherogenesis problems. The observation that oxidized low-density lipoproteins (**LDL**) triggers early steps in atherogenesis (Ross et al., 1993) has brought attention to oxidation products since they seems to influence the progression of this disease. When LDL are oxidized, the associated PUFA content is reduced, and the levels of lipid peroxides, aldehydes and oxysterols increase (Chang et al., 1997). There is supportive evidence that lipid peroxides in serum play a role in coronary artery disease (Addis et al., 1986). Furthermore, Cornwall and Morisaki (1984) suggested that lipid peroxides inhibit cell proliferation, and then antioxidants would therefore support tumor growth by reducing the levels of peroxides.

In recent years the control of the occurrence of oxysterols has increased due to their potentially damaging effects on human health. Oxysterols may be formed in the human body endogenously (free-radical reaction with cholesterol) or exogenously by autoxidation of cholesterol in foods (Morrissey and Kiely, 2006). The ingestion of oxysterols may have cytotoxic, atherogenic, mutagenic and carcinogenic effects (Bosinger, 1992). Also, oxidized cholesterols at a concentration of 10^{-9} and 10^{-6} M inhibit cholesterol synthesis *in vitro* and *in vivo* (Kandutsch et al., 1978), affecting cholesterol concentration in cells and their membranes, thus cellular functions. Among all oxysterols, Yin et al. (2000) found that 7-ketocholesterol was the strongest inhibitor of cell proliferation and 25-hydroxycholesterol was the most effective inducer of cell death or apoptosis. The absorption of oxysterols in rats, rabbits and human varies from 93% (Bascoul et al., 1986) to 6% (Vine et al., 1997). Emmanuel et al. (1991) suggested that some oxysterols are preferentially absorbed in plasma; this study found that 7-ketocholesterol was the major oxysterol in plasma even though its concentration was not the highest one in spray-dried eggs. As a result, the toxicity of ingested oxysterols is not clear due to the differences in their behavior among individuals.

1.8 Impact of Cooking on Meat

Cooking meat leads to structural changes which mainly affect its oxidative status. Some of these changes include: protein denaturion which leads to a loss of antioxidant enzyme activity (i.e. gluthathione peroxidase and catalase), whereas the decomposition of myoglobin and hydroperoxides formed prooxidant species (alkoxyl and hydroxyl radicals). Moreover, during cooking endogenous antioxidants such as α-tocopherol (Grau et al., 2001; Decker and Xu, 1998) are lost, while there is little or no loss of selenium (Higgs et al., 1972). However, the forms may differ in raw and cooked meat. Tanticharoenkiat et al. (1988) found that the percent of dialyzable selenium or low molecular weight (i.e. selenomethionine and selenocystine) was higher in cooked chicken than in raw chicken. Besides this, maillard reaction products are formed during cooking at high temperatures which may potentially inhibit lipid oxidation (Gray and Pearson, 1987).

Fatty acids, on the other hand, can also be modified during cooking as a result of some chemical factors such as oxidation, hydrolysis and polymerization (Lee et al., 1973). Small increases in the amount of 16:0 and 18:0 in broiled steaks were reported by Terrel et al. (1967). Similarly, Rao et al. (1996) reported significant increases in the contents of lipids, phospholipids, free fatty acids, glycolipids and glycerides in cooked buffalo meat. Additionally, Howe et al. (2002) stated that cooking can more than double the FA content in the tissue, probably because of moisture loss. In fact, cooking of meat does not liberate any appreciable quantity of the more ‘tightly bound lipids’ from the phospholipids (Giam and Dugan, 1965). In constrast, saturated fats in ground beef present greater losses during cooking (Janicki and Appledorf, 1974).

Regarding oxidation, Mohamed et al. (1990) found an increase in TBA values after cooking, with the increase influenced by cooking conditions. Boiling increased TBA value in breast meat 3-fold while roasting increased TBA value 4-fold. Warmed-over flavors are also developed from oxidation in cooked meat which was influenced by changes in the phospholipids during cooking (Igene and Pearson, 1979). The stability of the phospholipid components, phosphatidyl

ethanolamine (**PE**) and phosphatidyl choline (**PC**), during cooking differs; Tgene et al. (1981) demonstrated that PE was completely absent in the drippings showing that it is more bound to the membranes than PC. As a result, the PE proportion in cooked meat is fundamental to determine the oxidative status because it reacts easily with oxygen (Tsai and Smith, 1971) and it also produces 70-77% of the total MDA among all the phospholipids (Pikul and Kummerow, 1965).

1.9 Antioxidants

Antioxidants have been successfully added to livestock feeds in order to increase meat oxidative stability (Barroeta, 2007). Calvert and Decker (1992) suggested that a combination of different antioxidants may be more effective in retarding lipid oxidation rather than the use of a single antioxidant. Both, natural and synthetic antioxidants (i.e. butylated hydroxyanisole and butylated hydroxytoluene) have been tested in meat; however, natural antioxidants such as vitamin C and E are strongly preferred by consumers.

1.9.1 Type I Antioxidants

The most common type of antioxidants are called chain-breaking antioxidants which include vitamin E, C, β-carotene, lutein, carotenoids, among others. Vitamin E is the most important of these antioxidants in plasma lipids since its concentration is 15 times higher than any of the others (Burton et al., 1993).

1.9.1.1 Vitamin E

Tocopherols are a family of major lipid-soluble antioxidants but they cannot be synthesized by animals; therefore, they must be included in the animal's diet. There are four different tocopherols named as α-, β-, γ- and δ-tocopherols which only differ in the number and position of methyl groups (Figure 1.3) (Saladino et al., 2008). α-tocopherol, known as vitamin E, is

deposited in membranes in positions adjacent to the PUFA as part of the phospholipids (Fukuzawa and Fujii, 1992).

The level of vitamin E in the muscle depends on its dietary level, the duration of the supplementing period, the fiber type distribution and on metabolic characteristics (Jensen, 1998). For instance, the absorption of vitamin E in broilers is variable with an average of 42% (Barroeta, 2007) and it is strongly affected by dietary PUFA content (Barroeta, 2007; Cortinas et al., 2006). Furthermore, vitamin E is influenced by the type of the muscle fibers; vitamin E level in thigh meat is 1.8 to 2 fold higher than in breast meat (Cortinas et al., 2006). A decrease in the oxidation rate is usually observed at all muscle vitamin E levels. However, when vitamin E muscle levels in porcine were above critical levels (3.5 μg/g) no beneficial effect on color was observed (Jensen et al., 1997).

Vitamin E asserts its antioxidant function in membrane lipids; it donates a hydrogen to form a hydroperoxide and a tocopheroxyl radical, and thus preventing propagation of the chain reaction as described in equation 7 (Morrissey et al. 1998). However, high concentrations of vitamin E exert a pro-oxidative effect on LA oxidation (Mahoney and Graf, 1986).

$$TOH + ROO^{\cdot} \longrightarrow ROOH + TO^{\cdot} \qquad (7)$$

1.9.1.2 Stability of Vitamin E

The stability of vitamin E has been studied in the following areas: heating processes, exposure to light (sunlight and artificial) and processing operations. The heating processes that occur during cooking affect the vitamin E content of foods. Murcia et al. (1999) reported decreases in the vitamin E level of eggs after boiling and heating in a microwave, with a higher degradation of vitamin E when microwave heating was applied. Similar decreases have also been observed in olive oil (Nissiotis and Tasioula-Margari, 2002) and tofu (Guzman and Murphy, 1986) after heating them at 100°C and 85°C respectively. In most of these studies, vitamin E losses increase as the heating/cooking time increases. Steaming salmon

fillets at 66°C for 12 min, on the other hand, cause no significant vitamin E losses (Al-Saghir et al., 2004). Similarly, boiling broccoli and sweet pepper in the presence of water did not reduce vitamin E levels (Bernhardt and Schlich, 2005).

Tocopherols are generally sensitive to both sunlight and artificial light. Studies have reported loss of vitamin E in the presence of radiation and its decrease rate has been positively correlated to the irradiation level. Lakritz et al. (1995) reported vitamin E losses in red meat (beef, lamb, pork) and turkey meat (breast, leg) that were related to the irradiation dose. Correspondingly, vitamin E losses were also reported in pork due to radiation; however, the rate of α-tocopherol loss decreased with increasing water content (Fox et al., 1997). Besides radiation, vitamin E level is affected by some processing operations (i.e. grinding, sterilization and extrusion) as they promote lipid oxidation in food products. Among all the processing operations, freezing did not affect the vitamin E content of the foods but long-term frozen storage may decrease vitamin E levels (Braunstein, 2006).

1.9.2 Type II Antioxidants

An important antioxidant mechanism involves enzymes (i.e. catalase and glutathionine peroxidase) that work together to convert O^{-}_{2} through H_2O_2 to H_2O, thereby minimizing the production of HO^{-} (Morrissey et al., 1998). Nevertheless, reactions between peroxidized lipids and proteins have been shown to cause loss of enzyme activities (Chio and Tappel, 1969). Glutathione peroxidase (**GPx**) is an enzyme that plays a role in nutrient metabolism and regulation of cellular events (including protein synthesis, cell growth, and immune response) and it contains selenium (Chan and Decker, 1994). As a result, the incorporation of selenium in broiler diets is used to improve oxidative stability in tissues. Gpx catalyzes hydrogen or lipid peroxides (**LOOH**) using reduced glutathionine which inhibits lipid oxidation directly (non-enzymically) (**GSH**) as described in equation 8 (Decker and Mei, 1996).

$$H_2O_2 + 2\ GSH \longrightarrow 2\ H_2O + \text{Oxidized gluthathione} \quad (8)$$

Or

$$LOOH + 2\ GSH \longrightarrow LOH + H_2O + \text{Oxidized gluthathione}$$

Selenium exists in two forms: organic and inorganic. Organic selenium occurs in plants while inorganic selenium occurs in +6, +4, 0 or -2 oxidation states (Ali, 2000). Organic selenium includes selenomethionine and sodium selenite and its absorption is higher than that of the inorganic form (Mahan and Kim, 1996). Selenomethionine is absorbed as amino acids in the intestine, and in the tissues it is competing with methionine in building into proteins (Surai, 2000). However, it can also be converted to selenocysteine and incorporated into GPx (Schrauzer, 2000). Selenium plays an important role in physiologic effects including functioning of proteins, immune system and reduction of cancer risk (Behne and Kyriakopoulos, 2001; Lu and Jiang, 2005). Consequently, the enrichment of meat with selenium is nutritionally desirable.

1.10 Research Objectives

1.10.1 Main Objective

The objective of this thesis project was to evaluate the viability of nutritional strategies to enrich broiler white and dark meat with omega-3 fatty acids. Understanding the omega-3 fatty acid distribution among lipid fractions, their potential adverse effects on muscle lipid oxidation and the influence of sex on ω-3 PUFA enrichment are key factors being explored. This will improve our understanding of these factors as more of the value-added products are marketed.

1.10.2 Specific Objectives

- Determine the optimum broiler feeding period to achieve the minimum levels of α-linolenic acid in breast and thigh meat to label a product as a source of omega-3 in Canada.

- Establish the distribution of omega-3 fatty acids in between phospholipids and triacylglyerols of breast and thigh meat.
- Relate broiler sex and fatty acid deposition in breast meat.
- Evaluate the effect of vitamin E and selenomethionine on breast drip loss.
- Determine appearance of oxysterols (cholesterol oxidation products) in ω-3 enriched dark chicken meat.
- Establish the influence of different cooking methods (i.e. roasting, pan-frying and boiling) on the formation of oxidation products (TBARs and oxysterols) in ω-3 enriched dark chicken meat.
- Verify the antioxidative properties of vitamin E and selenomethionine in preventing the formation of TBARs and oxysterols in frozen-raw and cooked dark chicken meat.

1.11 Approach

The objectives of this thesis were assessed in two broiler experiments followed by laboratory analysis.

Experiment 1. A factorial design (2 x 8) was used to study the effects of two levels of dietary flaxseed (Low 10%, High 17%) on fatty acid composition in breast and thigh meat, as well as fatty acid deposition in the phospholipid membranes of the cells. Fatty acid profiles after different feeding periods (0 [Control], 4, 8, 12, 16, 20, 24, and 35 d) prior to processing were analyzed to determine fatty acids were distribution between TG and PL, and also when α-linolenic acid reached the level of 300 mg/100 g meat, required to label products as a source of ω-3 fatty acids.

Experiment 2. Four broiler finisher diets (21 – 61d) were fortified with linPRO (50% flaxseed and legumes) and antioxidants (vitamin E and selenomethionine)

to mitigate oxidation in ω-3 enriched chicken meat**.** Broiler carcass yield traits were determined after processing. Equal number of female and male thigh meat samples were pooled prior lo laboratory analysis. Lipid profile, TBARs and oxysterols were studied in frozen-raw and cooked (boiled, pan-fried and roasted) thigh chicken meat to establish the oxidative status of omega-3 enriched dark chicken meat after using antioxidant supplementation.

***Experiment 3*.** The effect of broiler sex on lipid content, fatty acid composition and meat quality parameters was determined. Individual breast samples collected from experiment # 2 were used to run the analyses.

1.12 TABLES

Table 1.1. Prediction of change in meat consumption from 2000 to 2020.

Type of Meat	Prediction of Change in Consumption from 2000 to 2020
Beef	-1.36%
Pork	0.09%
Poultry	-1.26%
Fish	1.76%

Modified from: Food and Agricultural Commodity Consumption in the United States: Looking Ahead to 2020, ERS, USDA.

Table 1.2. Omega 6:Omega 3 Ration of Poultry Dietary Fat Sources.

Dietary Fat Source	Omega 6: Omega3 Ratio
Corn oil	57
Sunflower oil	71
Safflower	76
Soybean oil	6.75
Cottonseed oil	54
Canola oil	2.2
Flaxseed oil	0.3

Modified from: Cherian, 2007.

Table 1.3. Fatty acid composition of fats of the major meat animals (m/m %).

Fatty acid	Common Name	Beef	Pork	Chicken
$C_{14:0}$	Myristic acid	2.5	1.5	1.3
$C_{14:1}$	Myristoleic acid	0.5	0.5	0.2
$C_{15:0}$		0.5		
$C_{16:0}$	Palmitic acid	24.5	24.0	23.2
$C_{16:1}$	Palmitoloic acid	3.1	3.5	6.5
$C_{17:0}$		1.0	0.5	0.3
$C_{18:0}$	Stearic acid	18.5	14.0	6.4
$C_{18:1}$	Oleic acid	40.0	43.0	41.6
$C_{18:2}$	Linoleic acid	5.0	9.5	18.9
$C_{18:3}$	Linolenic acid	0.5	1.0	1.3
$C_{20:0}$	Arachidic acid	0.5	0.5	
$C_{20:1}$		0.5	1.0	
Others		2.5	1.5	0.3
PUFA/SFA[1] ratio		0.11	0.25	0.64

[1]PUFA = Polyunsaturated fatty acids; SFA = saturated fatty acids.
Modified from: Varnam and Sutherland, 1995.

Table 1.4. Triplet and singlet oxygen properties.

Characteristics	Triplet Oxygen	Singlet Oxygen
Energy level	0 kcal/mol	22.4 kcal/mol
Temperature	Significant effect on oxidation	Little effect on oxidation
Nature	Diradical	Electrophilic, non-radical (unpaired electrons)
Reaction	Radical compound	Electron-rich compounds (Reacts with PUFA[1])

[1]PUFA = polyunsaturated fatty acids.
Modified from: Min and Boff, 2002.

Table 1.5. Some difficulties in the quantification of oxysterols in foods.

Problem	Example
1. Loss of oxysterols	Hot saponification (α-epoxide)
2. Production of artefacts	7-ketocholesterol to 3,5-cholestadiene-7-one
3. Loss of 7-ketocholesterol with purification	Same as (2)
4. Mis-identification	No use of MS[3], IR[3] or NMR[3]
5. Insolubility of oxysterol in non-polar solvent	Cholestanetriol insoluble in petroleum ether
6. Poor resolution	TLC[1]
7. Instability during gas chromatography	Production of dehydration products of oxysterols at high column temperatures

[1]TLC = Thin Layer Chromatography.
[2]HPLC = High Performance Liquid Chromatography.
[3]MS = mass spectrometry; IR = infra red; NMR = nuclear magnetic resonance.
Sources: Tsai (1984) and Park (1985)
Modified from: Addis (1986).

1.13 FIGURES

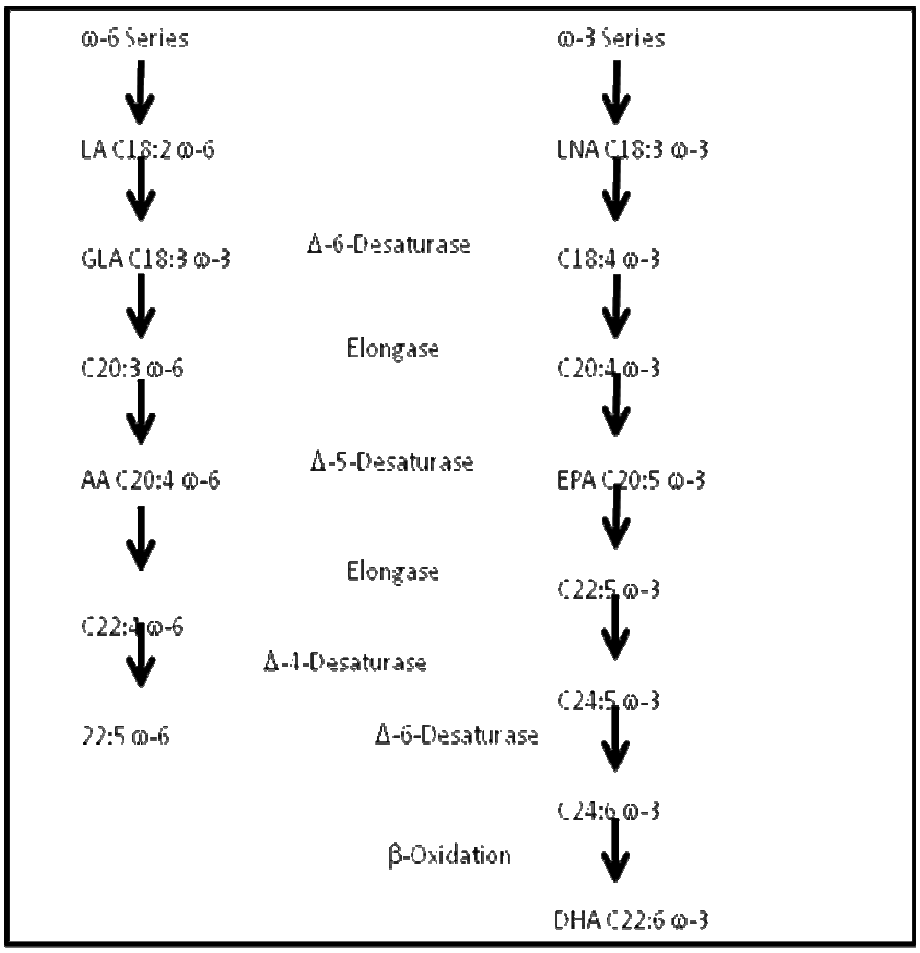


Figure 1.1. Synthesis of essential fatty acids. LA, linoleic acid; LNA, linolenic acid; GLA, γ-linolenic acid; AA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

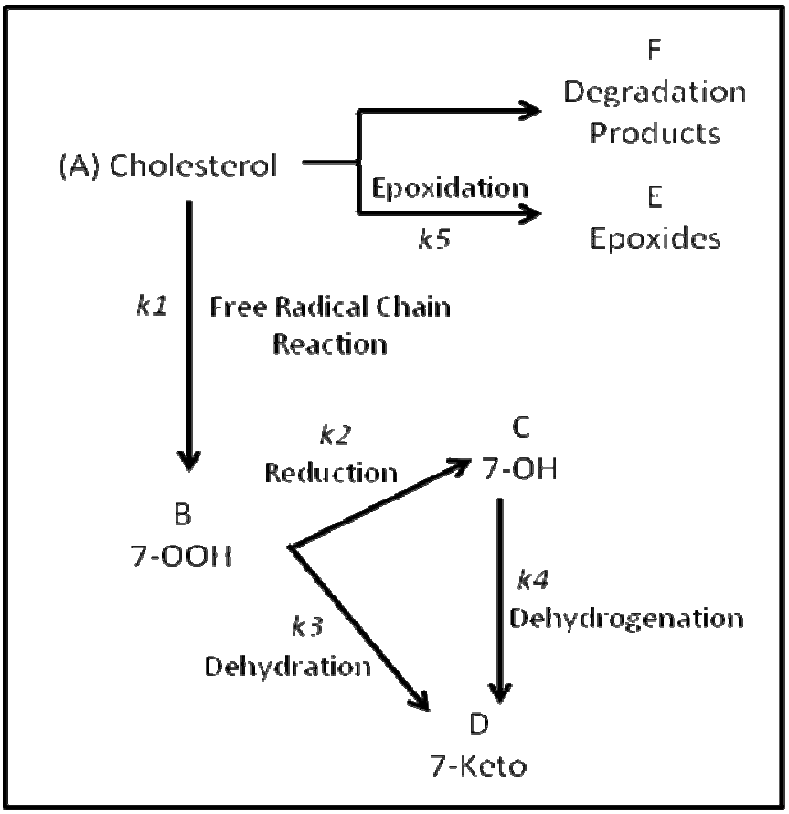
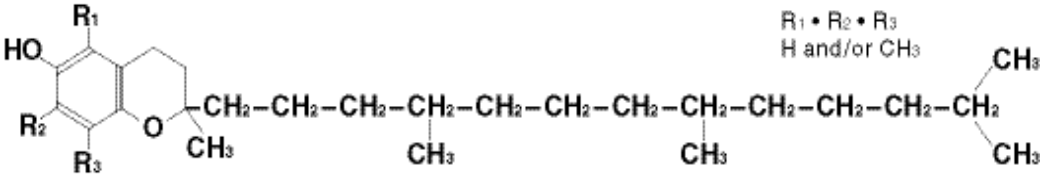


Figure 1.2. Major pathways of cholesterol oxidation: A, cholesterol; B, 7-OOH (7α- and 7β-hydroperoxycholesterol); C, 7-OH (7α- and 7β-hydroxycholesterol); D, 7-keto (7-ketocholesterol); E, epoxides (α- and β-epoxides). *k1*, *k2*, *k3* and *k4* rate constants of the reaction (*k1* > *k5* > *k3* > *k2* > *k4*).

Modified from: Morrissey and Kiely, 2006.



http://www.rikenvitamin.jp/int/tocopherol/toc1.html

α-tocopherol: $R_1 = R_2 = R_3 = CH_3$

β-tocopherol: $R_1 = R_3 = CH_3$; $R_2 = H$

γ-tocopherol: $R_1 = R_2 = CH_3$; $R_3 = H$

δ-tocopherol: $R_1 = CH_3$; $R_2 = R_3 = H$

Figure 1.3. Structure of individual tocopherols in vitamin E.

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2 OMEGA-3 ENRICHED BROILER MEAT: FATTY ACID DISTRIBUTION BETWEEN TRIACYLGLYCEROL AND PHOSPHOLIPID CLASSES[1]

2.1 INTRODUCTION

Coronary Heart Disease (**CHD**) is the most common cause of death in the Western society. It can be hereditary or developed from human dietary sources. For instance, saturated dietary fats and omega-6 polyunsaturated fatty acids (**ω-6 PUFA**) are related to CHD. The consumption of omega-3 polyunsaturated fatty acids **(ω-3 PUFA)**, on the other hand, may reduce the incidence of CHD. Eicosapentaenoic acid (**EPA**) (20:5 ω-3) and docosa hexaenoic acid (**DHA**) (22:6 ω-3) seem to be the most effective in this regard (Simopoulos, 1991). The ω-6:ω-3 ratio of the human diet has evolved from a healthier ratio of 1:1-4:1 (Eaton et al., 1996; Simopoulus, 1999) to a ratio of 10:1 or higher (Azain, 2004). Reducing both dietary saturated fats and ω-6 PUFA contribute to a more appropriate balance of ω-6 to ω-3 PUFA, thus reducing the risk of CHD (Woodside and Kromhout, 2005). Major sources of ω-6 PUFA include vegetable oils such as corn, safflower and soybean oil, whereas major ω-3 PUFA sources include fish (i.e. salmon, trout and tuna) and flaxseed (Schimtz et al., 2008).

Humans cannot synthesize either alpha-linolenic acid (**LNA**) or linoleic acid (**LA**); therefore, LNA and LA belong to the essential fatty acid family. Once consumed, these fatty acids are further metabolized in the cells to long chain polyunsaturated fatty acids (**LC-PUFA**). From LA convertion, γ-linolenic acid (18:3 ω-6) and dihomo-γ-linolenic acid (20:3 ω-6) are produced, which are intermediates to form arachidonic acid (**AA**) (20:4 ω-6) by the action of desaturase- and elongase-enzymes. Similarly, from LNA (18:3 ω-3), stearodinic acid (18:4 ω-3) and eicosatetraenoic acid (20:4 ω-3) are formed, which will synthesize eicosapentaenoic acid (EPA) (20:5 ω-3) using the same series of enzymes as those used to elongate and desaturase LA. EPA is further metabolized

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to DHA or eicosanoids (Schimtz and Ecker, 2008). The production of eicosonoids from LA is associated with CHD, inflammatory disorder and cancer (Emken et al., 1994; Siddiqui et al., 2005).

The U.S. Food and Drug Administration (2004) gave qualified health claim status to ω-3 PUFA - specifically to EPA and DHA. It was stated that their consumption may reduce the risk of coronary heart disease. As a result of these benefits, ω-3 PUFA enriched products have the potential to help consumers increase the ω-3 PUFA intake. To label a product as a source of ω-3 in Canada, a minimum level of 300 mg of ω-3 PUFA per 100 g meat is necessary (Government of Canada, 2003).

The concentration of LNA in the tissues is related to LNA concentration in the broiler diet (Rymer and Givens, 2005). Flaxseed oil is used widely in ω-3 fatty acid meat enrichment since it contains 50-60% LNA, which is the precursor of the ω-3 family (Plourde and Cunnane, 2007; Villaverde et al., 2006; Dublecz et al., 2004; Cortinas et al., 2004).

Fatty acids in muscles are primarly distributed between the neutral lipids and the phospholipids (**PL**). As an essential component of cell membranes, PL amounts remain fairly constant, or increase slightly, as the animal increases in fatness (Wood et al., 2008). Neutral lipids, or triglycerides, rich in saturated and monounsaturated fatty acids, are located in the perimysium (intramuscular adipocytes) (Sanosaka et al., 2008). Adipocyte number and size increases with the total lipid content of the muscle (Mourot and Hermier, 2001; Wood et al., 2008). Breast muscle contains more lipids as phospholipids while in thigh muscle predominant lipids are triglycerides (**TG**). As a consequence, omega-3 enrichment of breast meat may be more difficult since this lipid depot is smaller.

In order to increase ω-3 PUFA concentration in the chicken tissues to adequate enrichement levels, moderate or high levels of flaxseed were fed for different periods prior to broiler processing. The present study characterizes the fatty acid profile and distribution in the triacylglycerol and phospholipid fraction

of breast and thigh meat. Additionally, in this study the feeding period necessary to achieve 300 mg of LNA / 100 g meat was determined.

2.2 MATERIALS AND METHODS

2.2.1 Study Design

Experimental protocols were approved by an Institutional Animal Policy and Welfare Committee. Two levels (10% and 17%) of ground whole flaxseed meal (Omegaflax®, Archer Daniels Midland, AB, Canada) were fed for 8 lengths of time prior to processing at 35 d of age. A starter (ME=3086 kcal/kg; 22% CP), grower (ME=3110 kcal/kg; 18% CP) and finisher (ME=3196 kcal/kg; 18% CP) nutritional phases were implemented (Table 2.1.). Due to possible performance issues derived from feeding high-flax diets from a young age, only two diets (Control and 10%) were provided to low- and high-enrichment x 35 d birds during the 0 to 11 d- starter phase. From 11 d, birds in the high enrichment treatment received 17% ground flaxseed. Thus, birds received diets enriched with flaxseed meal for 0 (Control), 4, 8, 12, 16, 20, 24, or 35 d prior to processing.

2.2.2 Stocks, Management, and Sampling

One hundred and thirty-two Ross x Ross 308 mixed-sex broilers were evaluated in this study. At day of hatch, 22 chicks were placed randomly into 6 pens. The birds were provided *ad libitum* access to experimental diets and to water. A lighting program of 23L:1D was used for the entire 35 d growing period. At the age of 11 d, birds were switched to an enriched diet with either 10 or 17% flaxseed. At the age of 35 d, 8 birds per treatment were processed after a 12-h feed withdrawal period. The birds were electrically stunned, and then bled for 2 minutes. After scalding (63°C) for 45 seconds, carcasses were mechanically defeathered. Carcasses were manually eviscerated, and cut up after reaching an internal carcass temperature of 4°C. The fatty acid profile of broiler tissues was determined in skinless meat. Thigh and breast tissues were analysed skinless.

Meat from eight carcasses per each dietary treatment were pooled and grounded; two replicates per treatment were used for the analysis.

2.2.3 Fatty Acid Analysis

The tissue samples (2 g) were pulverized at dry ice temperature (-78ºC) according to the procedure described by Kramer and Hulan (1978). The total lipids were extracted as described by Folch et al. (1957). Thin layer chromatography procedure was used to separate PL and TG fractions following Christie and Breckenridge (1989). The developing agent was hexane:ether:formic acid (80:20:2 v/v; Fisher scientific Ltd. Nepean, Ontario, Canada); silica G plates 20 x 20 cm were used for the procedure (Analtech Inc., Newark, DE).

After the extraction and separation of lipids, the fatty acid profile of TG and PL fraction of breast and thigh meat were analyzed by gas chromatography (GC). Derivatization was conducted using 2 mL of methanolic HCl (HCl in methanol; SUPELCO, Bellefonte, PA) which were added to 50 µL of the mixture (extracted fat and chloroform). To complete the reaction, samples were put into a water bath at 50 ºC for 60 min. The fatty acid methyl esters were determined with a GC Varian 3400 gas chromatograph (Varian Walnut Creek, California, USA) equipped with a flame ionization detector and a SUPELCO SP™-2560 capillary column (100 m x 0.25 mm x 0.2 µm film thickness; SUPELCO Bellefonte, PA, USA). The operating conditions for the GC were as described by Betti et al. (2009). Fatty acids were quantified using heptadecanoic acid (17:0) as an internal standard. The fatty acid peak integration was performed using the Galaxie Chromatography Data System (Varian).

Saturated fatty acid (**SFA**) levels were calculated as 14:0 + 16:0 + 18:0 + 20:0. Monounsaturated fatty acid (**MUFA**) levels were calculated as 16:1 ω-7 + 18:1 ω-7 + 18:1 ω-9 + 22:1 ω-9. Polyunsaturated fatty acid (**PUFA**) levels were calculated as LA + LNA + 18:3 ω-6 + 20:3 ω-6 + AA + EPA + docosapentaenoic acid (22:5 ω-3; **DPA**) + DHA. Total ω-3 fatty acid levels were calculated as LNA + EPA + DPA + DHA. Total ω-6 fatty acid levels were calculated as LA + 18:3 ω-6 + 20:2 ω-6 + 20:3 ω-6 + arachidonic acid (20:4 ω-6; **AA**) +22:4 ω-6.

2.2.4 Statistical Analysis

A 2 x 8 factorial design was analysed using ANOVA, with two dietary levels of ground flaxseed (Low 10% and high 17%), and eight feeding period durations prior to processing: 0 (control), 4, 8, 12, 16, 20, 24 and 35 d. The Mixed procedure of SAS (SAS System, 2002) was used. Least squares means were separated using Tukey's adjustment and were considered significant at the $P < 0.05$ level.

The duration of the dietary flaxseed required to reach the claim labelling level of total omega-3 was determined by a piecewise regression or broken stick analysis (Toms and Lesperance, 2003). The NLIN procedure of SAS (SAS System, 2002) was used to calculate the equation, as follows:

$$Y_i = \begin{cases} \beta_0 + \beta_1 x_i & \text{for } x_i < \alpha \\ C & \text{for } x_i > \alpha \end{cases}$$

Where y was the concentration of ω-3 PUFA in the tissues (mg/100 g of meat); x was the flaxseed feeding period and C is the maximum level of ω-3 PUFA achieved with a dietary flaxseed level. The constant "C" was calculated as follows: $C = \beta_0 + \beta_1(X)$; X is the breakpoint or the threshold duration to reach the plateau ($X = (C - \beta_0)/\beta_1$ where β_0 is the intercept and β_1 is the slope).

2.3 RESULTS AND DISCUSSION

Effects of dietary treatments on body weight, feed conversion rate, carcass yield and fatty acid profile are reported elsewhere (Zuidhof et al., 2008; Betti et al., 2009).

2.3.1 Fatty Acid Content of Diets

The addition of 10% flaxseed to the starter diet resulted in a 4-fold increase in dietary LNA whereas a 3-fold increase in LNA was observed in the grower and finisher diets (Table 2.2). As expected, the addition of 17% flaxseed meal in the grower and finisher diets resulted in higher increases of the dietary

LNA content (4- and 5-fold increase for grower and finisher diets, respectively). During the finisher phase, 25% and 33% of the total fatty acid intake was LNA when broilers where fed 10% flaxseed and 17% flaxseed respectively.

2.3.2 Fatty Acid Distribution between Lipid Classes

Results for PL and TAG fatty acids composition of both breast and thigh meat for main effects and treatment interactions (level of flaxseed and duration) are presented in Table 2.3. The means of the PL and TG fatty acid content of both breast and thigh are reported in Tables 2.4, 2.5, 2.6 and 2.7.

2.3.2.1 Phospholipid Fraction

In the phospholipid fraction, Saturated fatty acids, MUFA and PUFA did not increase after 35 d of high or low flaxseed supplementation in either breast or thigh meat compared to control groups (Tables 2.4 and 2.5). On average, PUFA content of thigh PL was higher than that of the breast PL. These results could be related by the differences in muscle fibers. Kriketos et al. (1995) reported higher content of ω-6 and ω-3 PUFA in type I (slow-twitch oxidative) and type IIa fibers (fast-twitch oxidative-glycolytic) than type IIb fibers (fast-twitch glycolytic). Thigh muscles are mainly composed by type I and type IIa fibers which may explain our findings.

In both breast tissue (Table 2.4) and thigh tissue (Table 2.5), LNA content in the phospholipid fraction did not significantly increase after feeding the birds with either 10% flaxseed or 17% flaxseed compared to control group. After 35 d of supplementation, the amount of LNA in the PL fraction of the breast was 3.07 and 3.21 mg/100 g of meat for the low and high dietary flaxseed level, respectively. The content of LNA in the thigh was higher than that of the breast PL fraction (7.20 and 11.9 mg/100g of meat for 10% and 17% flaxseed level respectively). The LNA enrichment was not observed in the phospholipid fraction, which is in agreement with Gonzalez-Esquerra and Lesson (2001).

The long chain ω 3 PUFA (**LC ω-3 PUFA**) are synthesized in the liver from LNA and LA using the Δ6 and Δ5 desaturase and elongase enzymes (Wood,

2008). The main LC ω 3 PUFA in the PL fraction of breast (Table 2.4) and thigh meat (Table 2.5) was DPA **(**22:5 ω-3). In breast muscle, low dietary flaxseed level significantly increased DPA after 24 d (12.4 *vs*. 8.72 mg/100g of meat). However, with 17% flaxseed supplementation only 8 d were needed to produce a significant increase (13.7 *vs*. 9.95 mg/100g of meat). In thigh meat, DPA did not undergo a significant change when low levels of flaxseed were supplemented. However, with high level of flaxseed DPA had a significant increase of 36% after 35 d of supplementation. In breast meat, EPA (20:5 ω-3) was increased (+ 48%) only after 35 d of supplementation with the low level of flaxseed. However, with high dietary flaxseed, a significant increase of EPA was detected after only 8 d (53%). In thigh meat, EPA significantly increased after 16 and 12 d for low and high levels of flaxseed supplementation respectively. The content of DHA (22:6 ω -3) in breast meat did not increase with either low or high levels of flaxseed. Of the ω-3 PUFA in broiler meat, EPA and DPA are of importance in the development of value-added products because they have a proven role in reducing the risk of heart disease.

Findings from this study are supported by the theory that chickens convert LNA to its ω-3 derivatives: EPA, DHA and DPA (Lopez-Ferrer et al., 2001a; 1999). Results are also in agreement with Wood's (2008) and Warnants et al. (1999), who found that ω-3 and ω-6 LC PUFA are mainly deposited in the phospholipid membranes. When higher levels of EPA and DHA are desired, sources of EPA and DHA should be fed rather than relying only on the synthesis *de novo* of EPA and DHA from LNA (Kronberg et al., 2006). Lopez-Ferrer et al. (2001b) stated that ω-3 enrichment of meat seems to be less effective when vegetable fat sources compared to marine oils. Nevertheless, fish oil concentrations higher than 1 to 2% in poultry diets brings organoleptic problems in meat (Hargis and Van Elswyk, 1993).

The total ω -3 content in the PL of breast meat was increased 1.5-fold after 35 d of flaxseed supplementation. Significant increases in the total ω-3 content of the breast meat were observed after 24 and 20 d with 10% and 17% flaxseed

respectively (Table 2.4). In thigh meat, total ω-3 increased after 20 d only when high levels of flaxseed were used in the diets (41.4 *vs*. 23.1 mg/100g of meat). In contrast, the content of total ω-6 did not increased as feeding period increased. However, in thigh meat a significant decrease of ω-6 started after 12 d of 10% flaxseed supplementation. In agreetment with Komprda et al. (2005) AA (20:4 ω-6), the most important metabolite of LA, decreased when LC ω-3 PUFA increased in thigh and breast meat (Table 2.4 and 2.5). This could be explained by the fact that LA and LNA compete for the same enzymes (desaturase and elongase), where LNA suppressed bioconversion of AA from LA (Garg et al., 1988).

In the PL fraction of breast and thigh meat, the ω-6:ω-3 ratio decreased with the inclusion of flaxseed in the broiler diet, with a decreasing tendency with longer durations of flaxseed feeding (Table 2.4 and 2.5). On day 35, ω-6:ω-3 phospholipids thigh meat ratio was 3.05 with 10% flaxseed and 2.91 with 17% flaxseed in the diet. By day 35, breast meat obtained an ω-6:ω-3 ratio in the PL fraction of 3.06 and 3.23 for low and high dietary flaxseed levels, respectively. The reduction of the ω-6:ω-3 ratio was below the recommended healthy ratio (<4.0) as a consequence of the ω-3 enrichment and total ω-6 reduction (Scollan et al., 2006).

2.3.2.2 Triacylglycerols Fraction

The major fatty acid in the triacylglycerol fraction was oleic acid (18:1 ω-9) in both breast and thigh meat (Table 2.6 and 2.7). This essential fatty acid is more predominant in neutral lipids (TG) than in polar lipids (PL) (Wood, 2008). The SFA and MUFA levels in breast meat did not change due to dietary flaxseed levels while in thigh meat SFA and MUFA levels increased by d 12 (17% flaxseed) or by d 35 (10% flaxseed).

In both tissues, LNA content significantly increased after 8 d when 17% flaxseed was fed to the birds compared with control group. With the inclusion of 10% flaxseed in the diet, a significant increase in the LNA content occurred in

breast meat after 16 d (242.8 *vs*. 70.7 mg/100g of meat) but in thigh meat only 8 d were necessary (384.9 *vs*. 195 mg/100g of meat). In both muscles, LNA accounted for 96 to 98% of the total ω-3 enrichment. The higher amount of LNA in thigh compared to breast meat is explained by the fact that thigh meat contains more lipids as triacylglycerols (Gonzalez-Esquerra and Lesson, 2001) which concur with the finding that LNA is mainly deposited in the TG fraction. Results from this study also support the theory that the fatty acid profile of PL is less affected by dietary fat profile than TG fraction. It may be because changes in the fatty acid composition of the TG fraction are only related to energy storage rather than to cell metabolism (Villaverde et al., 2006).

The level of LC ω-3 PUFA (EPA, DHA, and DPA) in the thigh and breast meat triacylglycerols was lower than in the phospholipid fraction. In breast meat, high dietary flaxseed still resulted in significant increases in EPA content by day 8 (Table 2.6) while in thigh meat a significant increase was produced by day 4 (Table 2.7). After 35 d, EPA levels in breast meat from high and low flaxseed dietary treatments increased by 584% and 514% respectively. Comparable increases were found in the TG fraction of the thigh meat with both levels of flaxseed (427% and 568% for low and high flaxseed levels respectively). In breast meat, low and high flaxseed feeding increased DPA content by day 24 and by day 8 respectively. In thigh meat, the level of DPA changed by day 8 (10% flaxseed) and by day 4 (17% flaxseed) based on dietary flaxseed levels. DHA was not detected in breast after 20 d (17% flaxseed) or 35 d (10% flaxseed) of feeding the birds. However, in thigh meat, DHA was detected after 12 d and after 8 d on the 10% and 17% flaxseed diets respectively. During the enrichment, LC ω-3 PUFA are observed in small amounts, and they appear to be preferentially incorporated into the PL fraction of the tissues rather than in the TG fraction. In fact, after 35 d of 10% flaxseed dietary supplementation LC ω-3 PUFA in the thigh meat phospholipids accounted for 61% of the total LC ω-3 PUFA.

The total ω-3 content in the TG fraction was much higher than in the PL lipid fraction because LNA is mainly deposited in the TG fraction in both breast

and thigh meat. In breast meat, total ω-3 was significantly increased by day 35 with the 10% flaxseed level and by day 8 with 17% flaxseed (Table 2.6). In thigh meat, on the other hand, total ω-3 increased by day 8 (10% flaxseed) and by day 4 (17% flaxseed) (Table 2.7). Our results concur with Leskanich et al. (1993), who reported significant effects of dietary ω-3 PUFA on fatty acid composition of TG fraction in pigs that were related to the duration of the dietary supplementation. In both, thigh and breast muscle, the ω-6:ω-3 ratio in the TG fraction decreased down to the healthy range (Table 2.6 and 2.7). In this experiment, an adequate ratio (below 4) was obtained after 4 d with either low or high levels of dietary flaxseed.

Results from this study clearly demonstrated that LNA is deposited mainly in the TG fraction whereas the major source of LC ω-3 PUFA was in the PL fraction. As a consequence, LNA is used as a source of energy in muscle metabolism instead of structural fatty acid in PL membranes (Plourde and Cunnane, 2007). These findings are similar to those reported in pigs and bovine muscle enrichment (Kronberg et al., 2006; Warnants et al., 1999).

2.3.3 Duration Required in Labelling a Product "ω-3 Enriched"

To establish the duration required to label a product "ω-3 enriched" total fatty acids of breast meat were used (Table 2.8). The broken-stick analysis resulted in the following equations:

10% or Low Flaxseed	Y= 93.01 + 7.912 x	$P < .0001$
	C = Undefined; it did not break within 35 d	
17% or High Flaxseed	Y=146.3 + 13.6 x	$P < .0001$
	C = 398.06	

The equations indicate that the increasing ratio of total ω-3 PUFA concentrations was higher when high level of flaxseed was used (7.91 *vs*. 13.6 mg/100 g per day). The maximum level of ω-3 enrichment (398.1 mg/100 g of

meat) was reached after 18.5 d with high levels of flaxseed. The broken stick analysis indicated that the threshold level of 300 mg of total ω-3 PUFA per 100 g of breast meat was reached at 11.3 or 26.2 d with the high and low flaxseed dietary treatments (Figure 2.1). These results are consistent with those reported by Zuidhof et al. (2009).

2.4 CONCLUSIONS

Flaxseed is a good source of enrich meat of poultry with ω-3 fatty acids because of its LNA content. Tissue structure, in the target muscles influenced the location of fatty acid deposition. Modifying the dietary fatty acid profile stimulates LNA enrichment in the triacylglycerol fraction of the meat, while a much smaller enrichment is observed in phospholipid membranes. In dark chicken meat, LNA enrichment was higher than in white meat due to differences in the amount of TG. In order to achieve adequate ω-3 PUFA levels (300 mg/100 g of meat) to label breast meat as a source of ω-3 fatty acids, birds need to be fed for 26.2 or 11.3 d with 10% flaxseed meal or 17% flaxseed meal respectively. An important aspect of this project was the confirmation that DPA was the most abundant LC ω-3 PUFA in the lipids, which is in agreement with other studies in pigs and ruminants (Riley et al., 2000; Kronberg et al., 2006; Scollan et al., 2006).

2.5 TABLES

Table 2.1. Diet composition and nutrient content of diets containing Control, Low, and High levels of dietary enrichment with 0, 10, and 17% dietary ground flaxseed, respectively.

Phase	Starter (0 to 11 d)		Grower (11 to 21 d)			Finisher (21 to 35 d)		
Enrichment	Control	Low	Control	Low	High	Control	Low	High
								%
Ingredient								
Corn	5.000	5.000	30.42	5.00	–	39.75	25.00	25.00
Corn gluten meal	1.00	5.00		5.00	7.27	–	–	–
Soybean meal	26.80	15.61	22.67	4.83		21.77	17.09	15.55
Wheat bran	10.69	–	21.37	6.14	4.51	18.00	5.87	–
Calcium Carbonate	1.43	1.38	1.36	1.30	1.27	1.30	1.21	1.16
Dicalcium Phosphate	1.82	1.95	1.50	1.67	1.68	1.41	1.52	1.59
Salt (NaCl)	0.47	0.42	0.49	0.45	0.39	0.50	0.47	0.42
L-Lysine	0.33	0.30	0.32	0.36	0.24	0.26	–	–
D,L-Methionine	0.29	0.01	0.37	0.01	–	0.32	0.05	–
L-Threonine	0.08	–	0.08	–	–	0.06	–	–
Wheat	43.67	55.39	10.32	59.50	61.80	5.62	30.19	31.51
Ground flaxseed meal[2]	–	10.00	–	10.00	17.00	–	10.00	17.00
Canola oil	6.97	3.48	9.66	4.30	4.39	9.56	7.15	6.33
Microingredients[1]	1.45	1.45	1.45	1.45	1.45	1.45	1.45	1.45
Calculated analysis								
ME, kcal/kg	3086	3086	3110	3110	3110	3196	3196	3196
Crude protein, %	23.0	22.0	19.0	18.0	18.0	18.0	18.0	18.0
Crude fat, %	8.69	8.47	12.00	10.00	12.69	12.00	13.00	14.75
Crude fibre %	3.30	3.15	3.70	5.46	7.08	3.38	5.18	6.48
Ca %	1.00	1.00	0.90	0.90	0.90	0.85	0.85	0.85
Available P %	0.50	0.50	0.45	0.45	0.45	0.42	0.42	0.42
Met + Cys %	0.97	1.00	0.85	0.85	1.00	0.80	0.80	0.91
Met %	0.64	0.53	0.64	0.47	0.56	0.59	0.45	0.48
Lys %	1.35	1.42	1.18	1.18	1.22	1.09	1.13	1.33
Try %	0.31	0.42	0.26	0.36	0.45	0.25	0.38	0.48
Thr %	0.87	1.00	0.76	0.82	1.00	0.72	0.91	1.10
Arg %	1.46	1.99	1.28	1.65	2.11	1.22	1.90	2.41

[1]The microingredient mix contained (per kg of diet): iron, 100 mg; zinc, 80 mg; manganese, 70 mg; copper, 8.5 mg; iodine, 0.5 mg; selenium, 0.1 mg; vitamin A, 10000 IU; vitamin D3, 2500 IU; vitamin E, 50 IU; vitamin K (menadione), 2 mg; niacin, 65 mg; D-pantothenic acid, 14 mg; riboflavin, 5 mg; pyridoxine, 4 mg; thiamine, 2 mg; folic acid, 0.8 mg; biotin, 0.18 mg; vitamin B12, 0.015 mg; choline, 0 mg; Avizyme 1300 (Danisco Animal Nutrition, Marlborough, Wiltshire, UK), 500 mg; Bacitracin methylene Disalicylate (BMD 110 G, Alpharma, Mississauga, ON, Canada), 500 mg; Choline Cl, 400 mg; Sacox 120 (Intervet Canada), 500 mg.

[2]Omegaflax ground whole flaxseed meal (ADM Animal Health & Nutrition, Lethbridge, AB, Canada).

Table 2.2. Fatty acid profile (mg / g of diet) of experimental diets[1].

Phase	Starter		Grower			Finisher			
Flax level	0% Flax	10% Flax	0% Flax	10% Flax	17% Flax	0% Flax	10% Flax	17% Flax	SEM[2]
Fatty acid	— mg/g of diet —		———— mg/g of diet ————			———— mg/g of diet ————			
14:0	0.05[a]	0.05[a]	0.06[a]	0.0491[a]	0.0475[a]	0.0614[a]	0.0513[a]	0.0565[a]	0.0050
15:0	0.0302[a]	0.0257[a]	0.0318[a]	0.0288[a]	0.0271[a]	0.0226[a]	0.0230[a]	0.0267[a]	0.0030
16:0	5.5[a]	5.7[a]	5.5[a]	5.9[a]	5.6[a]	5.18[ab]	5.20[b]	5.81[a]	0.220
16:1 ω-7	0.18[a]	0.11e[b]	0.19[a]	0.16[ab]	0.126[b]	0.240[a]	0.159[b]	0.180[b]	0.014
18:0	1.5[b]	2.1[a]	1.3[b]	1.8[a]	1.9[a]	1.2[b]	1.6[a]	1.8[a]	0.120
18:1 ω-7	1.3[a]	0.73[b]	1.540[a]	0.755[b]	ND	ND	0.976[a]	ND	0.0500
18:1 ω-9	33.1[a]	23.9[b]	38.0[a]	26.1[b]	23.3[b]	36.2[a]	26.5[c]	30.8[b]	1.80
18:2 ω-6	20.0	19.9	22.5	21.3	19.4	21.9[ab]	19.9[b]	22.2[a]	0.958
18:3 ω-3	5.30[b]	23.5[a]	6.80[c]	22.7[b]	27.4[a]	5.90[c]	18.8[b]	30.9[a]	0.80
18:3 ω-6	0.03[a]	0.13[a]	0.16[a]	0.02[b]	0.09[ab]	0.19[ab]	0.092[b]	0.189[a]	0.036
22:1 ω-9	0.19[a]	0.16[a]	0.17[a]	0.15[a]	0.14[a]	0.114[a]	0.126[a]	0.132[a]	0.012
22:4 ω-6	0.11[a]	0.11[a]	0.085[a]	0.090[a]	0.0864[a]	0.0448[b]	0.0741[ab]	0.0843[a]	0.0112
SFA[3]	7.2	78.0	7.0	8.0	7.65	6.69[b]	7.00[b]	7.90[a]	0.310
MUFA[4]	36.8[a]	26.2[b]	42.2[a]	28.5[b]	24.7[b]	38.5[a]	29.4[b]	32.5[b]	1.89
PUFA[5]	25.5[b]	43.7[a]	29.6[b]	44.2[a]	47.0[a]	28.1[c]	38.9[b]	53.4[a]	1.59
Total ω-3	5.3[b]	23.5[a]	6.8[c]	22.7[b]	27.4[a]	5.90[c]	18.8[b]	30.9[a]	0.81
Total ω-6	20.1	20.2	22.8[a]	21.4[ab]	19.5[b]	22.1[ab]	20.0[b]	22.5[a]	0.97

[1]The following fatty acids were not detected: 20:0, 20:2 ω-6, 20:3 ω-6, 20:4 ω-6, 20:5 ω-3, 22:0, 22:5ω-3, 22:6 ω-3. Means within fatty acids and within dietary phase with no common superscript are significant different (P<0.05)

[2]SEM = standard error.

[3]SFA = saturated fatty acids

[4]MUFA = monounsaturated fatty acids

[5]PUFA = polyunsaturated fatty acids

SFA levels were calculated as 14:0 + 16:0 + 18:0 + 20:0 + 22:0. MUFA levels were calculated as 16:1 ω-7 + 18:1 ω-7 + 18:1 ω-9 + 22:1 ω-9. PUFA levels were calculated as 18:2 ω-6 + 18:3 ω-3 + 18:3 ω-6 +AA + 22:4 ω-6. Total ω-3 were calculated as 18:3 ω-3 . Total ω-6 were calculated as LA +18:3 ω-6 + AA + 22:4

Table 2.3. Statistical significance of treatment effects for the fatty acid profile.

	Breast Meat						Thigh Meat					
Lipid Fraction	Phospholipids			Triacylglycerols			Phospholipids			Triacylglycerols		
Fatty Acids	Duration	Level	Duration x Level	Duration	Level	Duration x Level	Duration	Level	Duration x Level	Duration	Level	Duration x Level
14:0	0.1842	0.6264	0.2290	0.0742	0.1658	0.0422	0.1447	<.0001	0.0032	0.0001	<.0001	<.0001
16:0	0.0010	0.0017	0.0449	0.0295	0.0917	0.0148	0.4044	0.2305	0.0557	<.0001	<.0001	0.0001
16:1 ω-7	0.0002	0.0001	<.0001	0.0021	0.0011	0.0013	0.1741	0.0122	0.0398	<.0001	<.0001	<.0001
18:0	<.0001	<.0001	0.0003	0.0515	0.8313	0.0350	0.0008	<.0001	<.0001	0.0007	0.0078	0.0084
18:1 ω-7	<.0001	0.3291	0.0368	0.0751	0.5135	0.0285	<.0001	0.1049	0.0198	0.0310	0.0014	0.0030
18:1 ω-9	0.0017	0.0006	0.0066	0.0545	0.7782	0.0295	0.5550	0.1872	0.2188	0.0107	0.0081	0.0071
18:2 ω-6	<.0001	<.0001	0.0002	0.0731	0.2317	0.0593	0.0172	0.0086	0.0030	0.0777	0.3887	0.0503
18:3 ω-3	0.0111	0.0046	0.0060	<.0001	<.0001	0.0573	0.0561	0.0103	0.1717	<.0001	<.0001	0.0783
18:3 ω-6										0.4670	0.2791	0.0194
20:0										<.0001	<.0001	0.0008
20:2 ω-6	<.0001	0.0014	0.0002	0.2315	0.7532	0.0577	<.0001	0.0085	<.0001	0.0091	0.0003	0.0047
20:3 ω-6							0.3505	0.244	0.0184	<.0001	0.7144	0.0619
20:4 ω-6	<.0001	<.0001	<.0001	0.0029	0.0002	0.0011	<.0001	0.0577	0.0013	0.0342	0.0001	<.0001
20:5 ω-3	<.0001	<.0001	<.0001	<.0001	<.0001	0.2037	<.0001	<.0001	0.0078	<.0001	<.0001	<.0001
22:1				<.0001	0.0042	0.0802						
22:4 ω-6							<.0001	<.0001	0.0029			
22:5 ω-3	<.0001	<.0001	0.0006	<.0001	<.0001	0.0609	0.0008	0.0142	0.1324	<.0001	0.0001	0.0003
22:6 ω-3	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0108	<.0001	<.0001	<.0001	0.0117	0.0002
SFA[1]	0.0004	<.0001	0.0062	0.0344	0.1924	0.0189	0.0417	0.0390	0.0034	<.0001	<.0001	0.0003
MUFA[2]	0.0027	0.0041	0.0106	0.0489	0.5737	0.0250	0.2948	0.2295	0.2237	0.0046	0.0015	0.0029
PUFA[3]	<.0001	<.0001	0.0009	0.0054	0.0170	0.0893	0.0803	0.0026	0.0029	<.0001	0.0006	0.2162
Total ω-3	<.0001	<.0001	0.0003	<.0001	<.0001	0.0615	0.0006	0.0001	0.0154	<.0001	<.0001	0.0289
Total ω-6	<.0001	<.0001	0.0012	0.0720	0.2219	0.0584	0.0005	0.0146	0.0012	0.0803	0.3901	0.0512
ω -6:ω-3	<.0001	<.0001	0.0024	<.0001	<.0001	0.0002	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

[1]SFA = saturated fatty acids
[2]MUFA = monounsaturated fatty acids
[3]PUFA = polyunsaturated fatty acids

Table 2.4. Fatty acid profile (mg fatty acids/100 g of meat) in breast meat phospholipids[1].

	Low (10% Flaxseed)								High (17% Flaxseed)								
Fatty acids	0 d	4 d	8 d	12 d	16 d	20 d	24 d	35 d	0 d	4 d	8 d	12 d	16 d	20 d	24 d	35 d	SEM[2]
14:0	1.03	1.16	1.02	1.05	1.18	1.25	1.16	1.15	1.15	0.97	1.09	1.08	1.17	1.11	1.19	1.14	0.059
16:0	66.60^{abc}	62.62^{bc}	60.90^{bc}	56.52^{c}	67.04^{abc}	62.29^{bc}	63.31^{bc}	73.24^{ab}	67.93^{abc}	65.86^{abc}	65.36^{bc}	60.75^{bc}	66.58^{abc}	80.02^{a}	71.00^{ab}	72.93^{ab}	2.523
16:1 ω-7	1.85^{bcde}	1.59^{e}	1.15^{e}	1.59^{e}	2.46^{abc}	2.68^{a}	1.69^{de}	2.52^{ab}	1.41^{e}	1.12^{e}	1.86^{bcde}	2.36^{abcd}	1.30^{e}	1.48^{e}	1.58^{e}	1.70^{cde}	0.135
18:0	33.10^{cde}	31.04^{def}	31.96^{cdef}	27.11^{ef}	31.19^{def}	25.25^{f}	36.85^{abcd}	36.66^{abcd}	37.30^{abcd}	37.17^{abcd}	33.24^{cde}	32.11^{cdef}	35.64^{bcd}	43.84^{a}	43.05^{ab}	39.11^{abc}	1.338
18:1 ω-7	17.25^{ab}	14.06^{abcd}	16.23^{abc}	12.50^{d}	13.83^{bcd}	13.50^{cd}	12.50^{d}	16.25^{abc}	17.59^{a}	13.74^{bcd}	15.71^{abcd}	13.77^{bcd}	14.77^{abcd}	15.73^{abcd}	13.77^{bcd}	13.56^{cd}	0.628
18:1 ω-9	68.27^{abcd}	63.23^{bcd}	62.56^{bcd}	58.77^{d}	74.02^{ab}	66.66^{abcd}	60.60^{cd}	78.01^{a}	69.10^{abcd}	67.39^{abcd}	72.72^{abc}	72.54^{abc}	68.82^{abcd}	74.66^{ab}	70.31^{abcd}	75.11^{ab}	2.271
18:2 ω-6	68.61^{bc}	59.77^{cdef}	54.88^{def}	51.88^{f}	63.93^{bcdef}	54.28^{ef}	64.44^{bcde}	74.57^{ab}	67.18^{bcd}	63.70^{bcdef}	63.70^{bcdef}	62.37^{bcdef}	74.39^{ab}	82.49^{a}	83.93^{a}	84.84^{a}	2.188
18:3 ω-3	2.76^{abc}	2.74^{abc}	2.35^{bc}	2.09^{c}	2.51^{abc}	2.34^{bc}	3.02^{ab}	3.07^{ab}	2.45^{abc}	2.43^{abc}	2.87^{abc}	2.88^{abc}	2.87^{abc}	3.33^{a}	2.97^{abc}	3.21^{ab}	0.162
20:2 ω-6	1.19^{h}	1.69^{defgh}	1.53^{efgh}	1.36^{fgh}	1.96^{cdefgh}	3.01^{a}	1.99^{bcdefg}	2.49^{abc}	1.34^{fgh}	1.24^{gh}	2.29^{abcde}	2.47^{abcd}	2.06^{bcdef}	2.46^{abcd}	2.75^{abc}	2.78^{ab}	0.141
20:4 ω-6	20.64^{b}	17.83^{bcde}	19.56^{bcd}	14.04^{e}	15.24^{e}	9.64^{f}	17.40^{bcde}	14.04^{e}	24.89^{a}	25.75^{a}	20.27^{bc}	15.82^{de}	17.06^{bcde}	19.09^{bcd}	15.17^{e}	16.54^{cde}	0.671
20:5 ω-3	3.14^{fg}	3.18^{fg}	2.41^{g}	3.58^{efg}	4.10^{def}	4.36^{def}	4.41^{def}	6.02^{abc}	2.30^{g}	3.46^{fg}	4.91^{cde}	6.89^{a}	5.39^{bcd}	6.48^{ab}	6.4^{ab}	6.41^{ab}	0.241
22:5 ω-3	8.72^{f}	8.66^{f}	9.688^{def}	9.31^{ef}	10.68^{cdef}	9.76^{def}	12.40^{bcde}	14.37^{ab}	9.95^{def}	12.37^{bcde}	13.74^{bc}	12.46^{bcd}	13.72^{bc}	17.46^{a}	14.77^{ab}	15.44^{ab}	0.549
22:6 ω-3	5.01^{cdef}	4.76^{cdef}	6.18^{bcde}	4.44^{def}	4.13^{f}	4.29^{ef}	6.55^{bc}	6.26^{bcd}	7.65^{b}	10.29^{a}	6.18^{bcde}	5.93^{bcdef}	6.09^{bcde}	9.61^{a}	7.465^{b}	7.22^{b}	0.346
SFA[3]	100.7^{bcde}	94.83^{bcde}	93.88^{cde}	84.68^{e}	99.42^{bcde}	88.78^{de}	101.3^{bcde}	111.1^{abc}	106.4^{abcd}	104.0^{bcde}	99.70^{bcde}	93.93^{cde}	103.4^{bcde}	125.0^{a}	115.2^{ab}	113.2^{abc}	3.645
MUFA[4]	87.36^{abcd}	78.89^{bcd}	79.94^{bcd}	72.85^{d}	90.30^{abc}	82.83^{abcd}	74.79^{cd}	96.78^{a}	88.09^{abcd}	82.26^{abcd}	90.28^{abc}	88.67^{abcd}	84.90^{abcd}	91.87^{ab}	85.66^{abcd}	90.37^{abc}	2.862
PUFA[5]	110.1^{def}	98.63^{efgh}	96.60^{fgh}	86.71^{h}	102.5^{defgh}	87.68^{gh}	110.2^{def}	120.8^{abcd}	115.8^{bcdef}	119.2^{bcde}	114.0^{cdef}	108.8^{defg}	121.6^{abcd}	140.9^{a}	133.5^{abc}	136.4^{ab}	3.765
Total ω-3	19.63^{e}	19.34^{e}	20.63^{de}	19.42^{e}	21.42^{de}	20.75^{de}	26.38^{bcd}	29.73^{b}	22.35^{cde}	28.56^{b}	27.70^{bc}	28.17^{bc}	28.06^{bc}	36.87^{a}	31.61^{ab}	32.28^{ab}	2.807
Total ω-6	90.43^{abcd}	79.29^{cde}	75.97^{de}	67.29^{e}	81.13^{cde}	66.93^{e}	83.83^{cd}	91.10^{abcd}	93.40^{abc}	90.68^{abcd}	86.26^{bcd}	80.66^{cde}	93.50^{abc}	104.0^{a}	101.8^{ab}	104.2^{a}	1.076
ω -6:ω-3	4.61^{a}	4.13^{ab}	3.68^{bcd}	3.46^{cde}	3.79^{bc}	3.22^{cdef}	3.18^{def}	3.06^{ef}	4.19^{ab}	3.18^{def}	3.11^{def}	2.86^{ef}	3.33^{cdef}	2.82^{f}	3.22^{cdef}	3.23^{cdef}	0.108

[1]Means within row with no common superscript are significant different (P < 0.05)
[2]SEM = standard error
[3]SFA = saturated fatty acids
[4]MUFA = monounsaturated fatty acids
[5]PUFA = polyunsaturated fatty acids

Table 2.5. Fatty acid profile (mg fatty acids/100 g of meat) in thigh meat phospholipids[1].

	Low (10% Flaxseed)								High (17% Flaxseed)								
Fatty acids	0 d	4 d	8 d	12 d	16 d	20 d	24 d	35 d	0 d	4 d	8 d	12 d	16 d	20 d	24 d	35 d	SEM[2]
14:0	ND	ND	ND	ND	ND	0.51^{bc}	0.35^{bc}	0.20^{bc}	1.08^{ab}	1.13^{ab}	1.13^{ab}	1.05^{abc}	2.09^{a}	1.13^{ab}	1.01^{bc}	0.92^{bc}	0.190
16:0	69.25	59.12	66.65	61.13	64.19	60.55	57.97	53.75	63.65	61.91	51.13	57.31	52.49	62.73	63.05	61.98	3.680
16:1 ω-7	2.81	2.89	2.08	2.11	2.68	2.32	2.80	2.08	2.68	1.44	2.29	2.21	1.17	3.05	1.30	1.46	0.370
18:0	67.87^{ab}	47.44^{cd}	55.52^{abcd}	44.29^{d}	45.86^{d}	47.57^{cd}	45.61^{d}	43.54^{d}	60.33^{abcd}	53.54^{bcd}	44.62^{d}	53.34^{bcd}	65.93^{ab}	48.86^{cd}	63.83^{abc}	71.84^{a}	2.970
18:1 ω-7	16.19^{ab}	12.44^{bcd}	13.47^{abc}	10.94^{cd}	10.86^{cd}	10.24^{cd}	9.53^{d}	10.33^{cd}	16.48^{a}	13.49^{abc}	10.03^{cd}	12.09^{cd}	12.25^{cd}	10.62^{cd}	12.02^{cd}	11.67^{cd}	0.674
18:1 ω-9	69.44	61.98	75.00	60.96	64.20	61.64	59.12	55.75	76.80	67.40	58.62	65.63	58.06	70.72	79.42	66.12	6.293
18:2 ω-6	99.47^{ab}	77.34^{abc}	95.97^{abc}	74.65^{abc}	78.87^{abc}	81.54^{abc}	69.26^{c}	71.47^{bc}	101.20^{a}	81.76^{abc}	71.03^{bc}	82.24^{abc}	97.49^{abc}	84.05^{abc}	99.68^{ab}	93.11^{abc}	5.184
18:3 ω-3	4.68^{b}	5.49^{ab}	11.11^{ab}	7.67^{ab}	8.25^{ab}	8.30^{ab}	7.21^{ab}	7.20^{ab}	5.56^{ab}	6.08^{ab}	8.60^{ab}	12.12^{ab}	9.19^{ab}	15.35^{ab}	18.67^{a}	11.92^{ab}	2.370
20:2 ω-6	2.35^{a}	ND	2.09^{ab}	1.50^{abc}	1.53^{abc}	1.12^{bc}	0.45^{cd}	ND	1.63^{ab}	1.79^{ab}	1.36^{abc}	1.15^{bc}	1.37^{abc}	1.29^{abc}	1.40^{abc}	1.41^{abc}	0.198
20:3 ω-6	2.74^{a}	2.42^{ab}	2.59^{ab}	2.35^{ab}	2.56^{ab}	2.01^{ab}	2.27^{ab}	1.73^{b}	2.02	2.05	2.14	2.37	2.24	2.49	2.29	2.26	0.169
20:4 ω-6	33.78^{a}	24.00^{bcd}	26.48^{bc}	20.55^{cde}	20.07^{cde}	19.15^{de}	18.38^{de}	15.48^{e}	30.58^{ab}	26.59^{abc}	20.35^{cde}	22.88^{cd}	24.58^{bcd}	18.73^{de}	20.85^{cde}	23.79^{bcd}	1.280
20:5 ω-3	2.35^{gh}	2.89^{fgh}	3.66^{efgh}	3.86^{defgh}	4.95^{cdef}	4.45^{cdefg}	5.24^{bcde}	4.89^{cdef}	2.20^{h}	2.33^{gh}	4.01^{cdefgh}	5.59^{abcde}	6.14^{abc}	5.88^{abcd}	7.37^{ab}	7.62^{a}	0.391
22:4 ω-6	5.51^{a}	3.72^{b}	3.41^{bc}	2.96^{bcd}	2.51^{cde}	1.47^{ef}	1.98^{def}	1.38^{ef}	3.59^{bc}	3.26^{bc}	1.97^{def}	2.11^{def}	1.47^{ef}	1.34^{f}	1.29^{f}	1.38^{ef}	0.200
22:5 ω-3	10.56^{abcd}	8.52^{d}	10.44^{abcd}	10.72^{abcd}	12.51^{abcd}	10.91^{abcd}	11.09^{abcd}	11.59^{abcd}	9.02^{cd}	9.53^{bcd}	10.10^{abcd}	12.52^{abcd}	12.68^{abcd}	13.69^{ab}	13.33^{abc}	14.05^{a}	0.781
22:6 ω-3	7.82^{abcd}	4.61^{f}	6.03^{cdef}	5.76^{def}	4.41^{f}	5.63^{def}	5.00^{ef}	5.85^{cdef}	6.31^{bcdef}	7.48^{abcde}	4.75^{f}	6.43^{bcdef}	9.31^{a}	6.50^{bcdef}	8.71^{ab}	8.33^{abc}	0.439
SFA[3]	137.1^{a}	106.6^{ab}	122.2^{ab}	105.4^{ab}	110.1^{ab}	108.6^{ab}	103.9^{ab}	97.49^{b}	125.1^{ab}	116.6^{ab}	96.87^{b}	111.7^{ab}	120.5^{ab}	112.7^{ab}	127.9^{ab}	134.7^{a}	6.083
MUFA[4]	88.44	77.31	90.55	74.00	77.74	74.21	71.44	68.16	95.95	82.33	70.94	79.93	71.48	84.39	92.74	79.25	7.031
PUFA[5]	169.3^{ab}	129.0^{ab}	161.8^{ab}	130.0^{ab}	135.7^{ab}	134.6^{ab}	120.9^{b}	119.6^{b}	162.1^{ab}	140.9^{ab}	124.3^{ab}	147.4^{ab}	164.5^{ab}	149.3^{ab}	173.6^{a}	163.9^{ab}	8.808
Total ω-3	25.41^{cd}	21.51^{d}	31.23^{bcd}	28.00^{bcd}	30.13^{bcd}	29.30^{bcd}	28.55^{bcd}	29.53^{bcd}	23.09^{d}	25.42^{cd}	27.47^{bcd}	36.65^{abcd}	37.32^{abcd}	41.43^{abc}	48.08^{a}	41.92^{ab}	2.896
Total ω-6	143.8^{a}	107.5^{bcde}	130.5^{abc}	102.0^{cde}	105.5^{bcde}	105.3^{bcde}	92.34^{de}	90.06^{e}	139.1^{ab}	115.4^{abcde}	96.86^{cde}	110.8^{abcde}	127.2^{abcd}	107.9^{bcde}	125.5^{abcd}	122.0^{abcde}	6.166
ω -6:ω-3	5.66^{a}	4.99^{b}	4.18^{c}	3.65^{d}	3.50^{de}	3.60^{d}	3.24^{def}	3.05^{efg}	6.02^{a}	4.54^{bc}	3.53^{de}	3.02^{efg}	3.40^{def}	2.64^{g}	2.64^{g}	2.91^{fg}	0.092

[1]Means within row with no common superscript are significant different (P < 0.05)./ ND: not detected
[2]SEM = standard error
[3]SFA = saturated fatty acids
[4]MUFA = monounsaturated fatty acids
[5]PUFA = polyunsaturated fatty acids

Table 2.6. Fatty acid profile (mg fatty acids/100 g of meat) in breast meat triacylglycerols[1].

	Low (10% Flaxseed)								High (17% Flaxseed)								
Fatty acids	0 d	4 d	8 d	12 d	16 d	20 d	24 d	35 d	0 d	4 d	8 d	12 d	16 d	20 d	24 d	35 d	SEM[2]
14:0	5.47^{ab}	4.93^{ab}	3.70^{b}	4.12^{b}	7.42^{ab}	5.61^{ab}	6.16^{ab}	9.17^{a}	4.69^{ab}	5.72^{ab}	6.23^{ab}	5.12^{ab}	5.11^{ab}	4.83^{ab}	4.39^{b}	5.68^{ab}	0.830
16:0	239.4^{ab}	183.8^{b}	148.4^{b}	167.8^{b}	296.4^{ab}	218.3^{ab}	239.1^{ab}	403.5^{a}	184.3^{b}	250.3^{ab}	259.5^{ab}	192.0^{b}	185.5^{b}	181.9^{b}	161.9^{b}	233.6^{ab}	34.51
16:1 ω-7	45.27^{b}	30.41^{b}	26.54^{b}	30.61^{b}	59.28^{b}	58.54^{b}	47.64^{b}	101.0^{a}	28.74	41.92	46.47	34.92	28.65	32.02	27.93	41.76	7.372
18:0	62.12^{ab}	51.51^{ab}	41.32^{b}	45.51^{ab}	80.71^{ab}	56.59^{ab}	70.71^{ab}	93.92^{a}	56.67	81.54	73.95	56.77	60.47	56.19	50.38	74.19	8.988
18:1 ω-7	44.59	37.26	30.67	32.54	57.28	36.81	39.29	63.78	41.59	53.50	49.82	32.57	36.7	40.15	30.18	41.37	6.112
18:1 ω-9	617.5^{ab}	495.5^{ab}	419.0^{b}	437.4^{ab}	797.5^{ab}	526.3^{ab}	566.5^{ab}	920.5^{a}	551.8	757.6	707.0	477.8	531.9	575.1	446.3	632.9	87.00
18:2 ω-6	361.8	337.5	268.2	273.0	499.5	270.9	354.5	508.0	374.2	478.9	449.7	306.6	378.1	418.6	316.8	407.2	51.61
18:3 ω-3	70.68^{f}	97.07^{ef}	91.93^{ef}	119.4^{def}	242.8^{abcd}	148.5^{cdef}	175.1^{bcdef}	295.4^{ab}	76.86^{f}	140.6^{cdef}	216.4^{abcde}	199.7^{abcdef}	241.6^{abcd}	302.2^{ab}	258.0^{abc}	334.4^{a}	24.20
18:3 ω-6	1.85	1.50	1.13	1.32	2.24	1.40	1.72	2.28	1.78	2.55	2.12	1.52	1.66	1.69	1.43	1.84	0.260
20:0	1.29^{abcd}	1.12^{bcd}	0.97^{d}	1.05^{bcd}	ND	ND	1.42^{abcd}	1.68^{ab}	1.37^{abcd}	1.86^{a}	1.60^{abc}	0.98^{cd}	1.33^{abcd}	1.26^{abcd}	1.12^{bcd}	1.46^{abcd}	0.111
20:2 ω-6	2.40	2.37	1.82	1.86	2.65	2.01	2.37	3.11	2.46	2.74	2.75	1.99	2.06	2.37	1.71	2.16	0.281
20:3 ω-6	1.27	ND	ND	ND	1.67	1.30	1.40	1.92	1.28	1.62	1.66	1.29	1.47	1.37	1.22	1.90	0.145
20:4 ω-6	2.18	2.01	2.19	2.01	2.94	2.10	2.79	2.91	3.34^{abc}	4.49^{a}	3.33^{abc}	2.03^{c}	2.80^{bc}	2.57^{bc}	2.22^{bc}	3.71^{ab}	0.267
20:5 ω-3	0.44^{f}	0.68^{ef}	1.04^{def}	1.27^{cdef}	2.17^{bcd}	1.90^{bcde}	2.02^{bcde}	3.01^{b}	0.75^{ef}	1.40^{cdef}	2.51^{bc}	2.15^{bcd}	2.78^{b}	2.88^{b}	2.91^{b}	4.61^{a}	0.238
22:1	0.99^{d}	1.02^{d}	1.00^{d}	1.21^{cd}	2.12^{abc}	1.63^{cd}	1.97^{abcd}	2.74^{a}	1.16^{ef}	1.47^{def}	1.93^{cd}	1.75^{cd}	1.96^{cd}	2.05^{c}	2.05^{c}	2.61^{ab}	0.173
22:5 ω-3	1.12^{e}	1.14^{e}	1.27^{de}	1.65^{cde}	2.74^{bcde}	2.52^{bcde}	2.89^{bcd}	3.79^{ab}	1.40^{de}	2.29^{bcde}	3.30^{bc}	2.59^{bcde}	3.38^{b}	3.22^{bc}	2.86^{bcd}	5.37^{a}	0.300
22:6 ω-3	ND	ND	ND	ND	ND	ND	ND	1.05^{b}	ND	1.05^{b}	ND	ND	ND	1.00^{b}	0.96^{b}	2.07^{a}	0.048
SFA3	308.3^{ab}	241.4^{b}	194.4^{b}	218.5^{b}	384.5^{ab}	280.5^{ab}	317.4^{ab}	508.3^{a}	247.1	339.4	341.3	254.9	252.4	244.2	217.8	314.9	44.33
MUFA4	708.3^{ab}	564.2^{ab}	477.2^{b}	501.8^{b}	916.2^{ab}	623.3^{ab}	655.4^{ab}	1088^{a}	623.3	854.5	805.3	547.1	599.2	649.3	506.4	718.7	100.4
PUFA5	441.7^{ab}	442.3^{ab}	367.5^{b}	400.6^{ab}	756.7^{ab}	430.6^{ab}	542.8^{ab}	821.5^{a}	462.0	635.7	681.7	517.9	633.8	735.9	588.0	763.2	76.46
Total ω-3	72.24^{f}	98.89^{ef}	94.24^{ef}	122.3^{def}	247.7^{abcd}	153.0^{cdef}	180.0^{bcdef}	303.3^{ab}	79.00^{d}	145.4^{cd}	222.2^{abc}	204.5^{bcd}	247.8^{abc}	309.3^{ab}	264.7^{abc}	346.4^{a}	24.70
Total ω-6	369.5	343.4	273.3	278.3	509.0	277.7	362.7	518.3	383.0	490.3	459.5	313.4	386.1	426.6	323.3	416.8	52.51
ω -6:ω-3	5.12^{a}	3.48^{b}	2.92^{c}	2.28^{d}	2.06^{def}	1.81^{efg}	2.02^{def}	1.71^{fgh}	4.83^{a}	3.37^{b}	2.07^{de}	1.53^{ghi}	1.56^{ghi}	1.38^{hi}	1.22^{i}	1.20^{i}	0.063

[1]Means within row with no common superscript are significant different (P < 0.05). ND: not detected
[2]SEM = standard error
[3]SFA = saturated fatty acids
[4]MUFA = monounsaturated fatty acids
[5]PUFA = polyunsaturated fatty acids

Table 2.7. Fatty acid profile (mg fatty acids/100 g of meat) in thigh meat triacylglycerols[1].

	Low (10% Flaxseed)								High (17% Flaxseed)								
Fatty acids	0 d	4 d	8 d	12 d	16 d	20 d	24 d	35 d	0 d	4 d	8 d	12 d	16 d	20 d	24 d	35 d	SEM[2]
14:0	14.49^{bcde}	15.49^{bc}	11.72^{cde}	13.52^{bcde}	15.06^{bcd}	17.92^{ab}	20.56^{a}	21.66^{a}	13.50^{bcde}	11.21^{cde}	15.61^{bc}	13.25^{bcde}	9.678^{e}	10.31^{de}	13.18^{bcde}	14.00^{bcde}	0.863
16:0	588.7^{cd}	604.7^{bcd}	441.2^{d}	531.8^{cd}	594.5^{cd}	749.2^{abc}	818.2^{ab}	932.9^{a}	518.8^{d}	428.6^{d}	601.7^{bcd}	501.8^{d}	387.3^{d}	459.0^{d}	535.5^{cd}	567.4^{cd}	39.15
16:1 ω-7	165.3^{bc}	149.2^{bcd}	75.37^{fg}	105.3^{cdefg}	129.9^{bcdef}	176.9^{b}	237.8^{a}	260.9^{a}	114.4^{cdefg}	67.05^{g}	137.1^{bcde}	109.4^{cdefg}	79.83^{efg}	103.2^{defg}	131.7^{bcdef}	117.9^{bcdefg}	10.64
18:0	91.30^{d}	110.3^{cd}	123.4^{abcd}	130.7^{abcd}	137.8^{abcd}	174.0^{ab}	166.1^{abc}	180.6^{a}	119.1^{bcd}	117.2^{bcd}	136.1^{abcd}	121.2^{abcd}	101.0^{d}	110.0^{cd}	128.4^{abcd}	151.2^{abcd}	10.68
18:1 ω-7	111.7^{abc}	105.7^{bc}	99.94^{bc}	108.3^{bc}	99.33^{bc}	123.8^{ab}	117.1^{abc}	145.7^{a}	112.6^{abc}	109.5^{abc}	113.9^{abc}	108.1^{bc}	85.81^{c}	89.80^{bc}	92.78^{bc}	98.02^{bc}	6.565
18:1 ω-9	1548^{ab}	1440^{b}	1394^{b}	1498^{b}	1405^{b}	1778^{ab}	1748^{ab}	2078^{a}	1486^{b}	1463^{b}	1666^{ab}	1566^{ab}	1258^{b}	1331^{b}	1461^{b}	1518^{b}	94.06
18:2 ω-6	977.5	956.6	1027	1083	995.3	1129	947.8	1198	1064	1153	1154	1194	990.0	938.5	973.6	1054	58.37
18:3 ω-3	195.0^{h}	274.1^{gh}	384.9^{fg}	480.7^{ef}	533.8^{ef}	631.1^{cde}	552.7^{def}	776.6^{abc}	255.0^{gh}	402.8^{fg}	561.7^{def}	733.7^{bcd}	725.5^{bcd}	730.9^{bcd}	819.6^{ab}	935.0^{a}	33.17
18:3 ω-6	5.01^{ab}	4.56^{ab}	4.38^{ab}	4.49^{ab}	4.10^{ab}	5.43^{a}	5.04^{ab}	4.92^{ab}	4.72^{ab}	4.92^{ab}	5.084^{ab}	5.35^{ab}	4.27^{ab}	4.77^{ab}	3.82^{b}	4.63^{ab}	0.281
20:0	2.54^{abc}	2.21^{abc}	2.47^{abc}	2.48^{abc}	2.23^{abc}	3.08^{a}	2.38^{abc}	2.89^{ab}	ND	ND	ND	ND	ND	1.46^{c}	1.10^{abc}	1.86^{bc}	0.215
20:2 ω-6	5.39^{abc}	4.98^{abc}	5.63^{ab}	5.25^{abc}	4.94^{abc}	6.42^{a}	4.23^{abc}	5.46^{abc}	5.50^{ab}	5.87^{ab}	4.39^{abc}	5.08^{abc}	3.89^{bc}	3.20^{c}	3.18^{c}	3.76^{bc}	0.406
20:3 ω-6	2.44^{c}	2.47^{c}	2.95^{abc}	3.14^{abc}	3.48^{abc}	3.32^{abc}	3.15^{abc}	3.70^{ab}	2.47^{c}	2.64^{bc}	2.81^{abc}	3.99^{a}	2.95^{abc}	2.71^{bc}	3.44^{abc}	3.95^{a}	0.211
20:4 ω-6	4.81^{de}	4.50^{e}	7.01^{abcde}	7.16^{abcde}	5.76^{cde}	6.89^{abcde}	5.66^{cde}	5.50^{cde}	6.33^{bcde}	9.52^{a}	5.23^{cde}	7.43^{abcd}	7.87^{abc}	5.02^{de}	7.33^{abcde}	8.62^{ab}	0.502
20:5 ω-3	1.17^{h}	2.11^{gh}	3.66^{fg}	4.77^{ef}	5.31^{ef}	5.34^{ef}	5.84^{de}	6.17^{cde}	1.67^{h}	3.57^{fg}	4.52^{ef}	7.64^{bcd}	8.02^{bc}	6.08^{de}	9.27^{b}	11.16^{a}	0.331
22:5 ω-3	2.11^{ef}	2.23^{ef}	4.38^{cd}	5.12^{bcd}	6.24^{bc}	5.92^{bcd}	5.35^{bcd}	6.12^{bc}	1.91^{f}	3.99^{de}	4.06^{de}	6.64^{b}	6.47^{b}	5.07^{bcd}	6.85^{b}	9.47^{a}	0.352
22:6 ω-3	ND	ND	1.50^{cde}	1.65^{bcd}	1.10^{cde}	2.34^{abcd}	1.65^{bcd}	2.08^{abcd}	ND	1.29^{cde}	ND	0.93^{de}	2.48^{abc}	2.34^{abcd}	3.01^{ab}	3.29^{a}	0.266
SFA[3]	697.0^{cd}	732.6^{bcd}	578.8^{d}	678.4^{cd}	749.6^{bcd}	944.1^{abc}	1007^{ab}	1138^{a}	651.4^{d}	557.0^{d}	753.4^{bcd}	636.3^{d}	498.0^{d}	580.8^{d}	679.1^{cd}	734.4^{bcd}	49.68
MUFA[4]	1825^{bc}	1695^{bc}	1569^{bc}	1711^{bc}	1634^{bc}	2078^{ab}	2103^{ab}	2485^{a}	1714^{bc}	1640^{bc}	1917^{abc}	1783^{bc}	1424^{c}	1524^{bc}	1685^{bc}	1734^{bc}	109.5
PUFA[5]	1193^{e}	1252de	1442^{bcde}	1595^{abcde}	1561^{abcde}	1796^{abc}	1531^{abcde}	2008^{a}	1341^{cde}	1587^{abcde}	1742^{abcd}	1965^{ab}	1752^{abcd}	1699^{abcde}	1830^{abc}	2034^{a}	92.70
Total ω-3	198.3^{i}	278.4^{ghi}	394.5^{fgh}	492.2^{ef}	546.4^{ef}	644.7^{cde}	565.5^{def}	791.0^{abc}	258.6^{hi}	411.6^{fg}	570.3^{def}	748.9^{bcd}	742.5^{bcd}	744.4^{bcd}	838.7^{ab}	959.0^{a}	34.01
Total ω-6	990.1	968.6	1043	1098	1009	1146	960.8	1212	1078	1171	1166	1210	1005	949.4	987.5	1071	59.38
ω -6:ω-3	4.99^{a}	3.48^{b}	2.64^{c}	2.23^{d}	1.85^{e}	1.78^{ef}	1.70^{fg}	1.53^{g}	4.17^{b}	2.84^{d}	2.05^{g}	1.62^{jk}	1.35^{l}	1.28^{lm}	1.18^{mn}	1.12^{n}	0.021

[1]Means within row with no common superscript are significant different (P < 0.05). ND: not detected
[2]SEM = standard error
[3]SFA = saturated fatty acids
[4]MUFA = monounsaturated fatty acids
[5]PUFA = polyunsaturated fatty acids

Table 2.8. ω-3 PUFA detected (mg fatty acids/100 g of meat) in total lipid tissue of breast meat[1].

Level	Duration	LNA (18:3 ω-3)	EPA (20:5 ω-3)	DPA (22:5 ω-3)	DHA (22:6 ω-3)	Total ω-3
Low (10% Flaxseed)		178.9^{b}	7.37^{b}	16.31^{b}	8.12^{b}	210.7^{b}
High (17% Flaxseed)		268.5^{a}	9.83^{a}	19.90^{a}	10.68^{a}	308.9^{a}
SEM[2]		5.16	0.16	0.35	0.25	5.20
	0d	96.23^{f}	5.00^{e}	14.21^{d}	9.13^{abc}	124.6^{f}
	4d	141.8^{ef}	5.82^{de}	15.24^{d}	10.49^{ab}	173.3^{ef}
	8d	175.1^{de}	6.78^{d}	16.74^{cd}	8.82^{abc}	207.4^{de}
	12d	208.2^{cd}	9.54^{bc}	17.11^{cd}	8.27^{bc}	243.2^{cd}
	16d	253.2^{bc}	8.56^{c}	17.24^{bcd}	7.57^{c}	286.6^{bc}
	20d	262.0^{b}	9.80^{bc}	19.49^{bc}	9.35^{abc}	300.7^{b}
	24d	256.9^{bc}	10.16^{b}	20.62^{b}	10.52^{ab}	298.2^{b}
	35d	395.9^{a}	13.14^{a}	24.19^{a}	11.05^{a}	444.3^{a}
SEM[2]		10.31	0.31	0.70	0.51	10.39
Low (10% Flaxseed)	0d	95.76^{h}	5.52^{g}	14.03^{ef}	7.58^{de}	122.9^{g}
	4d	111.6^{gh}	5.35^{g}	13.17^{f}	6.92^{de}	137.1^{fg}
	8d	109.2^{gh}	4.75^{g}	14.80^{def}	9.56^{bcd}	138.3^{fg}
	12d	138.3^{fgh}	6.56^{efg}	14.31^{def}	6.82^{de}	166.0^{efg}
	16d	205.1^{def}	6.25^{fg}	13.24^{f}	5.39^{e}	230.0^{de}
	20d	181.7^{efg}	8.74^{def}	16.19^{cdef}	6.95^{de}	213.6^{def}
	24d	232.6^{cde}	9.70^{cd}	22.31^{ab}	11.86^{abc}	276.4^{cd}
	35d	356.9^{ab}	12.15^{abc}	22.42^{ab}	9.90^{bcd}	401.4^{b}
High (17% Flaxseed)	0d	96.70^{h}	4.50^{g}	14..39^{def}	10.69^{abcd}	126.3^{g}
	4d	171.9^{efgh}	6.29^{fg}	17.31^{bcdef}	14.05^{a}	209.6^{def}
	8d	241.0^{cde}	8.81^{de}	18.69^{bcdef}	8.09^{cde}	276.6^{cd}
	12d	278.2^{bcd}	12.53^{ab}	19.90^{bcd}	9.71^{bcd}	320.4^{bc}
	16d	301.2^{bc}	10.86^{bcd}	21.24^{abc}	9.76^{bcd}	343.1^{bc}
	20d	342.4^{b}	10.86^{bcd}	22.80^{ab}	11.74^{abc}	387.8^{b}
	24d	281.3^{bcd}	10.63^{bcd}	18.92^{bcde}	9.18^{bcde}	320.0^{bc}
	35d	434.9^{a}	14.13^{a}	25.95^{a}	12.20^{ab}	487.2^{a}
SEM[2]		14.59	0.44	0.99	0.72	14.70

Source of Variation	---------- Probability----------				
Level	<.0001	<.0001	<.0001	<.0001	<.0001
Duration	<.0001	<.0001	<.0001	0.0020	<.0001
Level x Duration	0.0008	<.0001	0.0008	<.0001	0.0004

[1] Means within row with no common superscript are significant different (P < 0.05)
[2] SEM = standard error

2.6 FIGURES

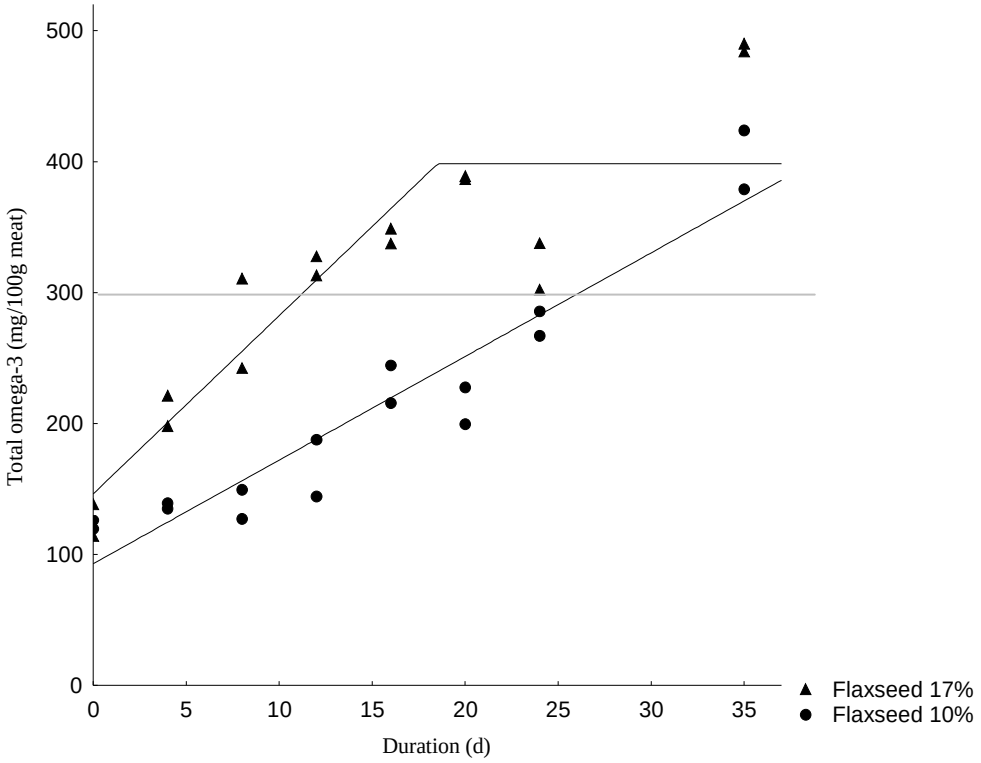

Figure 2.1. Optimization of duration of feeding a Low (10%) or High (17%) level of dietary flaxseed for omega-3 enrichment of broiler meat to a level of 300 mg/100 g of broiler chicken breast meat.

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3 EFFECTS OF VITAMIN E AND ORGANIC SELENIUM ON OXIDATIVE STABILITY OF OMEGA-3 ENRICHED DARK CHICKEN MEAT DURING COOKING

3.1 INTRODUCTION

Omega-3 polyunsaturated fatty acids (**ω-3 PUFA**) play an important role in the prevention and treatment of cardiovascular diseases, diabetes, inflammatory diseases and some types of cancer (Yashodhara et al., 2009). As a consequence, health authorities in Western countries are advising people to consume ω-3 fatty acids, specifically the long chain fatty acids, eicosapentaenoic (**EPA**) and docosahexaenoic (**DHA)** acids (Riley et al., 2000). An effective approach for raising ω-3 PUFA consumption is by increasing ω-3 PUFA levels in edible muscle tissues (Rymer and Givens, 2005).

Flaxseed oil contains 50-60% of α–linolenic acid (**LNA**), which is a precursor of EPA and DHA. For this reason, it is widely used in ω-3 PUFA muscle enrichment (Cortinas et al., 2005; Betti et al., 2009). However, by using high levels of unsaturated fatty acids in broiler diet, the degree of unsaturation of muscle membrane lipids increases, leading to a reduced oxidative stability of the meat (Morrissey and Kiely, 2006). Additionally, post slaughter biochemical changes are accompanied by a loss of cellular antioxidant defences, increasing the propensity of meat lipids to undergo oxidation (O'Neill et al., 1998). Lipid oxidation contributes to undesirable changes in a number of meat quality parameters, including loss of texture, flavour and water-holding capacity (Morrissey and Kiely, 2006).

In meats, in addition to PUFA oxidation cholesterol oxidation occur resulting in a formation of oxysterols, especially when meat is exposed to light, radiation, long-term storage and elevated temperatures during cooking. Oxysterols are absorbed from the diet and are transferred into cholesterol-rich lipoproteins such as very low density lipoproteins, low density lipoproteins (**LDL**), and chylomicrons (Colles et al., 2001). Oxysterols can modify cell membrane fluidity and permeability and inhibit cholesterol biosynthesis by suppressing hydroxy methyl glutaryl-CoA reductase activity. Oxysterols are cytotoxic, immunosuppressant and affect the production of prostacyclin by endothelial cells and cellular cholesterol efflux (Guardiola et al., 1996). All these deleterious effects support the hypothesis that cholesterol oxidation products (oxysterols) are involved in atherosclerosis.

Ohshima et al. (1993) have reported that cholesterol oxidation in fish products proceeds in conjunction with oxidative decomposition of the coexisting fish oils PUFA. Cholesterol oxidation might also be accelerated in the presence of ω-3 PUFA in poultry meat.

Dietary supplementation with antioxidants is an effective way to maintain lipid stability in meat (Grau et al., 2001). Vitamin E is a major lipid-soluble antioxidant able to stabilize the membrane-bound lipid against metmyoglobin/H_2O_2-initiated oxidation (Buckley et al., 1989). Vitamin E is thought to reduce fatty acyl hydroxyperoxy radicals (ROO˚) to yield less reactive hydroperoxides (ROOH). Selenium is a constituent of cellular and plasma glutathione peroxidase, an essential enzyme in nutrient metabolism and cellular function including protein synthesis, cell growth, and immune response. Glutathione peroxidise acts as an antioxidant by catalyzing the reduction of hydrogen peroxide and many lipid peroxides (Hoac et al., 2006).

The presence of oxysterols and thiobarbituric acid reactive substances (**TBARs**) in the diet needs to be reduced since they seem to have potential damaging health effects. There are many studies on lipid oxidation; however, little research has been done to study the effect of cooking on cholesterol oxidation in ω-3 enriched products. The aim of this study was to investigate the individual and combined effect of different types of dietary antioxidants, vitamin E and selenomethionine (**SeMet**) on lipid and cholesterol oxidation of ω-3 enriched broiler dark meat during cooking and storage.

3.2 MATERIALS AND METHODS

3.2.1 Study Design

The University of Alberta's Faculty of Agricultural, Life and Environmetal Sciences Economics Animal Policy and Welfare Committee approved the experimental protocol used in this experiment. Animals used in this project were managed according to the Guide to the Care and Use of Experimental Animals (Canadian Council on Animal Care. Guide to the Care and Use of Experimental Animals. 1984, Vol. 2. Can. Counc. Anim. Care, Ottawa, Ontario, Canada). From 21 to 61 d of age, broilers were fed four dietary treatments arranged as a 2 x 2 factorial, with low and high levels of vitamin E (LE and HE, respectively), and low and high levels of SeMet (LSe and HSe, respectively). The four diets were: low level of antioxidants (**LE x LSe;** 50 IU of vitamin E and 0.1 mg of sodium selenite per kg of feed), high level of vitamin E with low levels of selenium (**HE x LSe;**

250 IU of vitamin E and 0.1 mg of sodium selenite per kg of feed), low level of vitamin E with high level of SeMet (**LE x HSe;** 50 IU of vitamin E, 0.3 mg of SeMet and 0.1 mg of sodium selenite per kg of feed) and high levels of vitamin E and SeMet (**HE x HSe,** 250 IU of vitamin E, 0.3 mg of SeMet and 0.1 mg of sodium selenite per kg of feed). Fatty acid composition and oxidative status were analyzed in frozen-raw, boiled, pan-fried and roasted dark chicken meat. Experimental data was analysed as a 2 x 2 x 4 factorial, with two levels of vitamin E, SeMet and four cooking methods: frozen-raw, boiling, pan-frying and roasting.

3.2.2 Stocks, Management, and Sampling

A commercial hatchery provided one-hundred twenty-eight mixed-sex Ross 308 broilers. Three nutritional phases (starter, grower and finisher) were administered to the broilers (Table 3.1.). Birds began on a starter diet from 0 to 11 d, a grower diet to 21 d and a finisher diet to 61 d. The birds were provided *ad libitum* access to feed and water. A lighting program of 23 h of light and 1 h of darkness was used for the entire 61 d growing period. In the finisher phase (21 to 61 d of age) animals were randomly distributed into four isocaloric experimental dietary treatments of 32 birds each (Table 3.1). All the experimental diets contained 20% of LinPRO (flaxseed) as α-linolenic acid (**LNA**) source. LinPRO is an extruded product of linseed and legumes. The fatty acid composition of the dietary treatments is described in Table 3.2.

On day 61, birds were processed according to standard commercial procedures. Carcasses were cut up after reaching an internal temperature of 4 ºC. Thirty-two carcasses from each dietary treatment were used for analysis. Meat samples from each dietary treatment were pooled and ground (175 Mixer Grinder, Hollymatic, 7.5 HP, 200 V, 25 A, 60 Hz). Ground meat was used to produce thigh meat patties with similar weight (12 to 14 g) and size (d = 3 cm). The patties were vacuum-packaged in high-layer and multi-barrier bags, and stored at 4 ºC in a refrigerator for 24 h prior to cooking. Sixteen patties from each dietary treatment were produced from the ground meat. Patties were used as frozen-raw (n=4), boiled (n=4), pan-fried (n=4) and roasted (n=4) samples for subsequent analysis. Fatty acid profile and oxidative parameters were determined in skin-less thigh meat.

3.2.3 Cooking Conditions

Patties were divided into two portions: the first portion served as frozen-raw samples and the second portion as cooked samples. Meat patties were stored at -30 ºC until analyses were conducted. Meat temperature during cooking was monitored by using thermocouple wires inserted into the core of the patties during each of the cooking procedures. Pan-frying was done in an electrical frying pan (Multi-cuisine SK200 non-stick frying pan, Black&Decker, Towson, Maryland, USA) which was preheated for 30 min at 177 ºC before samples were placed. Meat patties were pan-fried for 10 min, without oil, until reaching an internal temperature of 95 to 98 ºC. Roasting was done in a convection oven (9900 W, 60 Hz, 3 phases, 208 V, Bakers Pride, New Rochelle, NY, USA) which was preheated for 60 min at 177 ºC. Patties were roasted for approximately 10 min to an internal temperature of 95 to 98 ºC. Boiling was done in 50 mL beakers in a water bath (Labline, Inc- Chicago, IL 1500W, 115V) which was preheated for 60 min. Meat patties were cooked in a water bath for approximately 12 min, until they reached an internal temperature of 92-95 ºC. This cooking method was named as boiling which was defined as cooking meat kept in beakers that were depth in boiling water. All cooked samples were vacuum packaged (FoodSaver® Vac 1200) with 5 layers of polyethylene and an outer layer of nylon to prevent air and moisture penetration, and stored at -30 ºC until analysis was performed.

3.2.4 Fat Extraction and Fatty Acid Profile

Fatty acid analysis was conducted after two months of storage at -30 ºC. The tissue samples (2 g) were freeze-dried at -78 ºC according to the procedure described by Kramer and Hulan (1978). The total lipids were extracted from the fine powder of frozen-raw, boiled, pan-fried and roasted meat samples in chloroform by direct extraction, with the total lipid content determined gravimetrically. Derivertisation to fatty acid methyl esters (**FAME**) was achieved using methanolic HCl (hydrochloric acid in methanol) in a water bath at 55 °C for 1 h. After the mixture cooled, water, hexane and internal standard were added. FAME were quantified using heptadecanoic acid (17:0) as an internal standard (Nu-chek prep Inc. P.O. Box 295 Elysian, MN 56028 USA). The FAME profile was analyzed using a Varian 3400 gas chromatograph (Varian Walnut Creek, CA 94598-1675 USA) equipped with a flame ionization detector and a SP™-2560 capillary column (100 m x 0.25 mm x 0.2 µm film thickness; SUPELCO 595 North Harrison Road Bellefonte, PA 16823-

0048 USA.). Operating conditions were as follows: initial temperature of 45°C (4 min); increased 13°C/min to 175°C, which was held for 27 min. A temperature of 215°C was reached, at a rate of 4°C/min, which was maintained for 29 min. Fatty acid identification was done by comparing retention time of the peaks with those known for the standard (Nu-chek prep Inc. P.O. Box 295 Elysian, MN 56028 USA). Peak identification was performed using the Galaxie Chromatography Data System (Varian 3120 Hansen Way, Palo Alto, CA 94304-1030, USA). Duplicate derivertisations were done for each sample.

Total ω-3 fatty acid levels were calculated as LNA + eicosapentaenoic (20:5 ω-3; **EPA**) + docosapentaenoic acid (22:5 ω-3; **DPA**) + docosahexaenoic acid (22:6 ω-3; **DHA**) + 20:3 ω-3. Total ω-6 fatty acid levels were calculated as linoleic acid (18:2 ω-6; **LA**) + 20:3 ω-6 + arachidonic acid (20:4 ω-6; **AA**) + 18:3 ω-6. Saturated fatty acid (**SFA**) levels were calculated as 14:0 + 16:0 + 18:0. Monounsaturated fatty acid (**MUFA**) levels were calculated as 14:1 ω-7 + 16:1 ω-7 + 18:1 ω-7 + 18:1 ω-9. Polyunsaturated fatty acid (**PUFA**) levels were calculated as LA + LNA + 18:3 ω-6 + 20:3 ω-6 + AA + EPA + DPA + DHA.

3.2.5 Determination of Oxysterols

Total oxysterols determination was conducted after 4 mo of storage at -30 ºC. The procedure is made up of the following stages: lipid extraction, cold saponification, cartridge purification and silanization. Total lipids from frozen-raw and cooked thigh meat were extracted by following the procedure described by Folch et al. (1957). One gram of dried meat was left under Folch solution (chloroform: methanol; 2:1, v/v) overnight at room temperature (22 °C). The liquid phase was decanted through filter paper, and the residue was rewashed two times with 8 mL of Folch solution. Sodium chloride (0.88%, w/w) was added and the mixture was centrifuged at 1100*g* for 15 min. The supernatant was transferred into a scintillation vial to evaporate the solvent under nitrogen. 19-Hydroxycholesterol (50 µg) was added as an internal standard (Steraloids, Inc. Newport, RI, USA). Cold saponification was performed following the procedure described by Guardiola et al. (1995). The non-saponificable obtained was re-dissolved in 5 mL of hexane and applied to a silica cartridge (Sep-pack, Vac 6cc (1g) SPE, Waters, Millpore, Bedford MA, USA). The cartridge purification followed the method of Guardiola et al. (1995). Briefly, solvent mixtures (hexane/ether) of increasing polarity were applied to the cartridge in order to remove the interferencing compounds and then 10 mL of acetone/methanol (75:25, v/v) were used to isolate the oxysterols portion. The final residue was dried under

nitrogen and re-dissolved in 50 µL of anhydrous pyridine. To complete the silanization reaction, 50 µL of Sylon BTZ (Supelco Inc., Supelco Park, Bellefonte, PA 16823, USA) were added; the mixture was left for 20 min at room temperature (22 °C). Oxysterols were separated and quantified by gas chromatography (**GC**) (Model 3400, Varian, Palo Alto, CA, USA) with a J&W DB5-column (Agilent J&W GC Columns, Brockville, Ontario, Canada) and a flame ionization detector. A cool-on-column injector was used with Helium as a carrier gas. The GC conditions were set as follows: initial column temperature was 70 °C with an initial hold of 0.20 min. Two GC programs were used which only differed in the final conditions. Program I ramped from 70 °C to 250 °C at a rate of 20 °C/min with a final hold of 8 min while program II ramped from 70 °C to 280 °C at a rate of 15 °C/min with a final hold of 17 min. The temperature of the injector and detector was 90 °C and 300 °C, respectively. The head pressure of the column was set at 22-23 psi. A mixed external standard was prepared with pure substances (Sigma Chemical Co., St Louis, MO, USA): 50 µg of cholesterol, 50 µg of 19-hydroxycholesterol (**19-HC**), 50 µg of 7-α-hydroxycholesterol (**7α-HC**), 50 µg of 7-β-hydroxycholesterol (**7β-HC**), 25 µg of 25-hydroxycholesterol (**25-HC**), 25 µg of β-cholestanetriol (**β-CE**), 25 µg of α-cholestanetriol (**α-CE**) and 25 µg of 7-keto cholesterol (**7-KC**). The mixed external standard was derivatized in the same manner as the samples. Detected peaks were identified by comparison of retention times of pure substances with those found in meat samples. Different concentrations of the mixed standard were analysed to confirm the linearity and the relatively response factors. To verify the identity of detected peaks, Gas Chromatography-Mass Spectrometry (**GC-MS**) was used. Pure standards, as described above, were run in GC-MS in order to build their mass spectometrum.

3.2.6 Thiobarbituric Acid Reactive Substances

The thiobarbituric acid reactive substances (**TBARs**) test was done using the extraction method described by Raharjo et al. (1992). TCA-TBA-HCl (15%, w/v trichloroacetic acid; 0.375%, w/v thiobarbituric acid; 0.25 N hydrochloric acid) was used to determine TBARs. Samples were weighed in a tube and 10 mL of potassium chloride (KCl), 4 mL of TCA-TBA-HCl (Fisher Scientific, Ottawa, ON, Canada; Sigma-Aldrich, Oakvilly, ON, Canada) and a 1 mL of buffer solution were immediately added to the samples. The mixture was incubated in a water bath for up to 15 min. After cooling down, the upper layer was removed by centrifugation at 1000*g* for 5 min. The final solution and

blank (distilled water) were measured spectrophotometrically (UV/VIS Spectophotometer) at 532 nm. The absorbance of blanks was subtracted from that of the samples. Absorbance values and an extinction coefficient of 1.56 x 10^5 M^{-1} cm^{-1} (Slater and Sawyer 1971) were used to estimate the malonaldehyde (**MDA**) values. TBARs were measured after 6 mo and at 12 mo of storage at -30 ºC. Four replicates per treatment were conducted in each analysis.

3.2.7 Vitamin E Levels

Vitamin E levels in frozen-raw chicken meat were measured after 12 mo of storage according to Abdollahi et al. (1993) and Drotleff and Ternes (1999) with modifications. 2 g of fresh meat was homogenized with 6 mL of water; subsequently, 4 mL of methanol were added and mixed. Saturated methanolic potassium hydroxide (MeOH-KOH) was added (4 mL; 33%, w/w) and the homogenate was vortexed for 20 s. Saponification was carried out at 70 °C for 16 h. The sample was then cooled to room temperature (22 °C) and 10 mL of acetone:chloroform (3:7, v/v) were added. Samples were centrifuged at 2000*g* for 5 min and the supernatant containing the organic layer was evaporated to dryness in a nitrogen stream. The residue was redissolved in 1 mL of methanol and then centrifuged at 7800*g* for 5 min. The clear supernatant was injected on a Varian 9010 high-performance liquid chromatography (**HPLC**) system, equipped with a SUPELCOSIL^{TM} LC-18 (Supelcosil, St. Louis, MO, USA) column (15 cm x 4.6 mm, 5µm). Detection and quantification was performed by using a fluorescence detector (EX 295 nm; Shimadze Corparation, RF-335, Japan). Samples were eluted with three solvents: acetonitrile, methanol and iso-propanol, at a flow rate of 1 mL/min. The total running time was 23 min. Quantification of vitamin E was performed using an external calibration method. Six concentrations for alpha-tocopherol were injected and the calibration curve was obtained by plotting the concentration against area. Duplicate analysis was conducted on freeze-dried frozen-raw meat samples.

3.2.8 Selenium Levels

After 12 mo of storage at -30 °C, selenium levels were determined using inductively coupled plasma mass spectrometry (**ICP-MS**) on a Perkin Elmer Elan 6000 instrument (PerkinElmer Life And Analytical Sciences, Inc., 940 Winter Street, Waltham, Massachusetts 02451 USA). Four concentrations were used for the standard calibration

curve (0.005, 0.010, 0.020 ppm). Triplicate analysis was conducted on freeze-dried frozen-raw meat samples.

3.2.9 Statistical Analysis

This experiment was conducted as a 2 x 2 x 4 factorial arrangement with two levels of vitamin E (high and low), two levels of SeMet (high and low) and four cooking methods (frozen-raw, pan-frying, boiling and roasting). A multi-factor ANOVA was done by using the mixed procedure of SAS System (SAS 9.1. Copyright (C) 2002-2003. SAS Institute Inc., 2002**,** Cary, NC, USA). Differences between least square means were determined using Tukey's test. Differences were considered to be significant based on a 0.05 level of probability.

3.3 RESULTS

3.3.1 Lipid Content

Total lipid content in raw dark chicken meat was in the range of 6.5 to 9.1% (Table 3.3). Cooking tended to reduce the percentage of fat in most of the samples compared to raw meat with the exception of roasted samples ($P < .0001$). In most cases, roasted meat showed the lowest lipid loss compared to boiled and pan-fried meat. For instance, in roasted low antioxidants-enriched meat the lipid loss was 3.0% while pan-fried and boiled meat presented a lipid loss of 13% and 23% respectively. Significant differences in the lipid content of raw meat were observed due to high vitamin E supplementation (7.6% *vs*. 8.1% for low and high vitamin E). Whereas, incorporation of high levels of selenium did not significantly affect lipid content. The interaction of vitamin E and selenium had a significant effect on the lipid content ($P = 0.0493$). Meat enriched with high levels of both antioxidants had higher lipid content than meat from both LE x LSe and LE x HSe dietary treatments (8.3% *vs*. 7.6%).

3.3.2 Fatty Acid Composition

In this experiment, fatty acid (**FA**) content was expressed as mg FA/100 g of meat. The FA profile of frozen-raw and cooked thigh meat samples is given in Table 3.4.

3.3.2.1 Fatty Acid Composition in Frozen-Raw Meat

The amount of SFA and MUFA was significantly higher in meat obtained from HE x HSe dietary treatment compared to the control (LE x LSe). For example, a 1.3-fold

increase of palmitic acid (16:0) was observed with HE x HSe diet compared to meat samples from the control group (LE x LSe) (1425 *vs*. 1102 mg/100 g of meat). The level of ω-3 PUFA found in frozen-raw meat was not significantly affected by antioxidants.

3.3.2.2 Fatty Acid Composition in Cooked Meat

The content of SFA, MUFA and PUFA was significantly decreased during cooking, especially during boiling. The cooking effect indicated that the loss of fatty acid in roasted meat was the lowest one among cooking methods ($P < .0001$ for SFA, MUFA and PUFA). By looking at the interaction between vitamin E, selenium and cooking method, the highest FA loss was observed in boiled meat samples (30%, 33% and 31% in the LE x LSe diet for SFA, MUFA and PUFA respectively) (Table 3.4). A strong decrease in LA (31%) and LNA (32%) was also observed by using this cooking treatment, especially in meat samples from the LE x LSe diet. However, the percentage of LA and LNA loss was lower when the HE x LSe (24% and 22% for LA and LNA respectively) and LE x HSe (26% and 25% for LA and LNA respectively) diets were fed compared to losses in meat from the HE x HSe dietary treatment. The content of EPA, DPA and DHA did not significantly decrease in cooked samples compared to frozen-raw samples, with the exception of DHA in boiled meat from the HE x HSe group.

In this study, it appears to be that cooking has a greater impact on FA concentrations than antioxidant supplementation (Table 3.4). This effect is probably a consequence of the meat lipid release during thermal processing; in this regard, roasted meat results in the least change. For instance, Duckett and Wagner (1998) reported a substantial loss of LNA and PUFA in *longissimus* muscle after cooking to an internal temperature of 70 ˚C whereas Bonoli et al. (2007) found that oven-cooking did not lead to significant differences of the meat FA composition compared to raw meat. These studies are thus consistent with results presented here that indicate a milder effect of oven cooking (roasting) on the FA composition compared to boiling and pan-frying.

The retention of long chain ω-3 PUFA (**LC ω-3 PUFA**) during cooking may be explained by the fact that during cooking the majority of lipids that are released originate from the triacylglycerols (**TG**) fraction. The TG are normally found between muscle bundles and they include very limited amount of LC ω-3 PUFA (Betti et al., 2009). In contrast, phospholipids (**PL**), found in muscle cell membranes, contain a relatively high

amounts of LC ω-3 PUFA. For example, Betti et al., (2009) found that LC ω-3 PUFA accounted for 71 to 89% of total ω-3 PUFA content in the PL fraction of breast and thigh meat respectively. The release of TG and water during heat treatment likely resulted in a concentration of PL, enhancing the relative level of EPA, DPA and DHA. This is supported by Igene et al. (1981), who found an increased proportion of phosphatidyl ethanolamine in cooked meat compared to raw, following the observation that FA found in PL could be concentrated during cooking. In fact, results for the main effect of cooking indicated that DHA concentrates during cooking (5.1 *vs*. 6.5, 5.9 and 5.8 mg/100g of meat for boiled, pan-fried and roasted meat respectively).

3.3.3 Oxysterols

Oxysterols were measured in frozen-raw and cooked meat after 4 mo of frozen storage at -30 °C. The most common oxysterols in foods were tested; however, only the following oxysterols were consistently identified in all the samples: 7α-HC, 7β-HC, β-CE, α-CE and 7-KC (Table 3.5).

3.3.3.1 Oxysterols in Frozen-Raw Meat

7α-HC, 7-KC and 7β-HC were the most abundant oxysterols found in frozen-raw meat after 4 mo of storage, which is in agreement with Conchillo et al. (2003), Al-Saghir et al. (2004), who studied levels of oxysterols in salmon, and Hsien-Wei et al. (2006), who reported oxysterols in marinated ground pork. In the current study, 25-hydroxycholesterol, which is considered most cytotoxic (Monahan et al., 1992), was not detected in any of the samples. The individual effect of vitamin E indicated that high level of vitamin E significantly reduced all the detected oxysterols (Table 3.5). In contrast, selenium did not significantly reduce oxysterols formation. The interaction between vitamin E and selenium reflected that antioxidants reduced oxysterols, excluding 7-KC, 7b-HC and a-CE in LE x HSe-enriched meat (i.e. 8.1 *vs*. 6.8 μg of 7-KC/g of fat for LE x LSe and LE x HSe 7-KC respectively).

The interaction between vitamin E, selenium and cooking method indicated that 7-KC was the only oxysterol significantly reduced by the inclusion of both antioxidants in the diet ($P = 0.0104$). There was no significant difference in the percentage of reduction among dietary treatments. A 64% reduction in 7-KC was found in meat from the HE x LSe dietary treatment compared to the amount of 7-KC found meat from low-antioxidants dietary

treatment (5.8 *vs*. 2.1 μg/g of fat). Similarly, a 46% decrease was obtained by using the LE x HSe (5.8 *vs*. 3.2 μg/g of fat). When both antioxidants were included, a 39% decrease in 7-KC was recorded. However, total oxysterols, as defined by the sum of all the detected oxysterols, were similar among samples from all treatments (Figure 3.1).

3.3.3.2 Oxysterols in Cooked Meat

Cooking leaded to a significant increase in the oxysterols formation with the exception of α-CE (Table 3.5). Among all the cooking methods, roasting generated a greater increase in the oxysterols. For instance, 7-β-HC presented a 3.5-fold increase compared to oxysterols in frozen-raw meat, while pan-fried and roasted meat presented a 2.8- and 2.4-fold increase. The most common oxysterol found in foods, 7-KC, was significantly higher in boiled meat obtained from the LE x LSe dietary treatment (13.3 *vs*. 5.1, 5.6 and 3.7 μg/g of fat for HE x LSe, LE x HSe and HE x HSe diets respectively). This result indicates that high levels of antioxidants supplementation inhibited 7-KC formation during boiling. Interestingly, the level of 7-KC in pan-fried meat did not increase during cooking compared to frozen-raw meat from other dietary treatments. In roasted meat, 7-KC was significantly higher in samples from the LE x HSe diet (12.60 μg/g of fat). 7α-HC and 7β-HC presented a similar trend to 7-KC, having the highest amount in roasted meat from the LE x HSe diet (13.4 and 16.6 μg/g of fat for 7α-HC and 7β-HC respectively). These two oxysterols were also significantly higher in boiled samples derived from the LE x LSe dietary group compared to the other antioxidant-enriched diets. α-CE and β –CE behaved similar to the other oxysterols, indicating an enhanced oxidative stability in boiled meat due to the presence of high levels of antioxidants. Total oxysterols in boiled meat (Figure 3.1) were significantly lower (2.6-fold) when high levels of vitamin E, SeMet or both were included in the diet ($P = 0.0086$, $P = 0.0042$, $P = 0.0365$ for HE x LSe, LE x HSe and HE x HSe respectively). Roasted meat had significantly less oxysterol formation in meat from HE x LSe and HE x HSe dietary treatments. Conversely, total oxysterols in pan-fried meat were not significantly increased during cooking.

In the current study, total oxysterols in boiled and roasted meat increased when low levels of antioxidants were included in the diet (Figure 3.1). Thurner et al. (2007) reported similar increases in oxysterols in cooked meat of different species. The oxidative stability of meats was affected not only by the FA composition, but also by the cooking treatments,

including time, temperature and heat transfer conditions. The average level of total oxysterols in frozen-raw ω-3 enriched dark chicken meat was 19.05 μg/g of fat which is almost 7 times higher than total oxysterols in non-enriched breast chicken meat (2.88 μg/g of fat) (Conchillo et al., 2003).

Although it is not immediately clear why pan-frying did not increase total oxysterols compared to frozen-raw meat, several factors are worth considering. These results could indicate that some compounds, other than vitamin E and SeMet, may act as antioxidants during pan-frying. For example, released peptides from either myofibrillar or collagen proteins are possible (Palka, 2003; Sims and Bailey, 1992). Chicken dark meat contains around 4 mg of collagen /g of meat while breast meat contains 2.5 mg of collagen/g of meat (Voutila et al., 2008). Bauchart (2006) found that small peptides (92.0 and 72.9 kDA) significantly increased in beef when cooking at 75 °C. Cooking implies protein denaturation, myofibril fragmentation, gel formation and collagen peptide bond breakage (Palka, 2003). It is possible that the pan-frying cooking conditions favour the generation of these small peptides. It is known that different cooking methods result in a variation in heat flow (Hallstrom et al., 1988) which could explain our findings in pan-fried meat. In boiled and roasted meat, most of the heat is transferred to the meat by convection through water and air, respectively. In contrast, during pan-frying, heat is transferred by conduction from one side through the aluminum pan which has a much higher thermal conductivity compared to air and water (237 *vs*. 0.02 and 0.6 J/sec-m-K for air and water, respectively) (Cengel, 2002). The heat flow is calculated as follows: $Q=kA(\Delta T)$ where k is the thermal conductivity, A is the surface area of the meat and ΔT is the difference between the hot outside and cool inside. Thus, the heat flow is higher in pan-fried meat compared to the other cooking treatments and this may facilitate the formation of these thermally derived peptides with antioxidant activity.

The effect of antioxidants in cooked meat was only detected in total oxysterols in boiled and roasted meat, with the exception of roasted meat from the LE x HSe dietary treatment. This result was mainly influenced by the β-CE content in boiled meat obtained from the LE x LSe dietary treatment (11.2 μg/g of fat). The β-CE content in boiled meat obtained from the HE x LSe, LE x HSe and HE x HSe dietary treatments was not as high as in meat from the LE x LSe diet. Thus, antioxidants appear to have reduced the formation

of β-CE. Vitamin E supplementation has been shown to inhibit the formation of oxysterols in pork, chicken and veal following cooking and storage (Engeseth et al., 1993; Galvin et al., 1998). In contrast, O'Grady (2001) suggested that dietary selenium has limited potential for increasing the oxidative stability of meat. In addition, the enzyme glutathione peroxidase is part of an *in vivo* mechanism which may be denaturated (Ganther et al., 1976) at temperatures above 80 °C. This may explain the limited antioxidant effect of high level of SeMet in roasted and boiled meat, above this temperature, suggesting that vitamin E may have more protection against lipid oxidation in cooked meat products. Whereas selenium supplementation in animal diets should be used for the enrichment of meat with this element, rather than to increase its oxidative stability based on the results of the current study.

Possible explanations for the oxysterols deviation may not only be due to the small number of replicates (n=2) but also due to the differences in the meat properties such as moisture, fat and iron content (myoglobin, hemoglobin) with the latter being catalogued as pro-oxidants in meat (Guardiola et al., 1995). Hughes et al. (1994) demonstrated in low-density lipoproteins in porcine a threshold for toxicity of 1 to 2.5 and 2.5 to 5 μg/mL for 7β−HC and 7-KC respectively. However, the connection between plasma toxic levels and oxysterol levels in meat is unknown. The level of oxysterols in ω-3 enriched food is a concern due to their damaging health effects.

3.3.4 Thiobarbituric Reactive Acid Substances

The thiobarbuturic acid reactive substances (**TBARs**) test is an empirical method use for the detection of lipid oxidation in food. The test relates to the level of aldehydes that originates from hydroperoxide decomposition (Guillen-Sans and Guzman-Chozas, 1998).

3.3.4.1 TBARs Values in Frozen-Raw Meat

After 6 mo of storage at -30 ºC, oxidative stability was significantly improved in meat from birds supplemented with HE x LSe, LE x HSe and HE x HSe compared to LVELSE (0.28 *vs*. 0.18, 0.16, 0.16 nmol of MDA/mg of meat for HE x LSe, LE x HSe and HE x HSe diets; P = 0.0004). Our study showed a 35% reduction in TBARs when high levels of vitamin E were used in the diet (HE x LSe) while a reduction of 41% was obtained with high level of SeMet (Figure 3.2). When both antioxidants were in the diet, the percentage of reduction was similar to the LE x HSe-enriched diet. No differences in

TBARs were detected among the different antioxidant treatments. As expected, TBARs increased after 12 mo of storage, ranging from 0.66 to 0.76 nmol of MDA/mg of meat. However, TBARs were not significantly reduced by any of the dietary treatments (Figure 3.3). This increase in TBARs value after storage is consistent with other studies (Ajuyah et al., 1993; Cortinas et al., 2005).

This study showed that dietary antioxidants inhibit TBARs formation in frozen-raw meat after 6 mo of storage even with high levels of ω-3 PUFA in the meat (Figure 3.2). In contrast, after 12 mo of storage, these antioxidants did not inhibit TBARs formation. This could be explained by the low levels of vitamin E found in raw meat after 12 mo of storage (Figure 3.4). For instance, frozen-raw meat from the HE x LSe-enriched diet meat presented 2.95 μg of vitamin E per g of meat, being the highest compared to the amount of vitamin E found in meat obtained from the other dietary treatments (1.31 and 1.32 μg/g of meat for LE x LSe and LE x HSe-enriched diet respectively). Thus, long-term freezer storage leads to significantly reduced vitamin E levels as oxidation slowly proceeds and depletes this antioxidant (Eitemiller and Junsoo, 2004). In the present study, unidentified peaks were detected in the HPLC chromatograms which may be α-tocopherolquinones formed from vitamin E auto-oxidation (Murphy et al., 1992). Selenium was still detected after 12 mo of frozen storage at – 30 ˚C (Figure 3.5), but there was not effect on TBARs formation.

3.3.4.2 TBARs Values in Cooked Meat

The rate of oxidation was accelerated during cooking after 6 mo of storage (Figure 3.2). For example, TBARs values in boiled, pan-fried and roasted meat from LE x LSe diet were 1.5-, 1.7- and 1.5-fold respectively, higher than in frozen-raw meat. The highest TBARs value was identified in roasted meat coming from the LE x HSe-enriched diet (0.74 nmol of MDA/mg of meat). An increase of TBARs during cooking has also been reported by Cortinas et al. (2005). Pikul et al. (1984) stated that TBARs concentration in cooked meat depends on the initial TBARs concentration of the raw meat. As a consequence, changes in TBARs (ΔTBARs) were calculated as TBARs in cooked meat minus TBARs in frozen-raw meat (Figure 3.6). In boiled meat, the ΔTBARs did not significantly decrease by the addition of high levels of antioxidants into the broiler diets. Similarly, in roasted meat

none of the high antioxidant treatments significantly reduced the ΔTBARs compared to the low antioxidants level based diet.

After 12 mo of storage at -30 ˚C, a significant increase of TBARs was found in frozen-raw meat samples. However, TBARs in cooked meat significantly decreased compared to frozen-raw chicken meat. Aubourg (1993) reported that malonaldehydes (**MDA**) form dimers or trimers of MDA, which decreases the amount of MDA available to react with thiobarbituric acid (**TBA**) decreasing in this way the TBARs value. This could be the reason why TBARs did not increase in cooked meat after 12 mo of storage.

In this study, vitamin E and SeMet reduced lipid oxidation in frozen-raw meat mainly during 6 mo of frozen storage. During cooking, antioxidants did not inhibit TBARs formation at this high level of ω-3 PUFA (total ω-3 in frozen-raw meat ranging from 1,522 to 1,265 mg FA/100 g of meat), with the exception of pan-fried meat from the HE x HSe diet. As previously discussed, the lack of antioxidant efficiency during heat processing may be related to the high level of ω-3 PUFA and to the degradation of antioxidants, *in vivo* (animals) and during heating. In fact, selenium works as an antioxidant via the gluthathione peroxidase enzyme which decreases its activity by 95% above 80 ºC (Hoac et al., 2006). Moreover, Steinhart and Rathjen (2003) reported that roasting of meat resulted in a 50 to 70% loss of tocopherols due to oxidation. Grau et al. (2001) reported loss of endogenous α-tocopherol in dark chicken meat during cooking. Vitamin E may be lost during cooking because it is a lipid soluble vitamin and our results indicated that a significant amount of lipid is lost during most cooking processes (Table 3.3). Similar to the oxysterols results, the amount of TBARs formed during cooking varied according to the cooking conditions. The main effect for cooking showed that TBARs formation was lower during pan-frying compared to other cooking methods (0.42 *vs*. 0.63 and 0.50 nmol of MDA/mg of meat for boiled and roasted meat respectively). This may indicate that other compounds, possibly small peptides, have the potential to reduce lipid oxidation. Overall, the results are in agreement with others where the high degree of unsaturation in the fat makes the meat more susceptible to oxidative damage (Higgins et al., 1999).

3.4 CONCLUSIONS

This study has demonstrated that high levels of ω-3 PUFA in frozen-raw and cooked dark chicken meat following 40 days of dietary enrichment (1,522 – 1,265 mg per 100 g of meat) induces cholesterol oxidation resulting in formation of oxysterols. After 6 mo of frozen storage, the oxysterol concentration in frozen-raw meat was unchanged, with a tendency to be lower when high vitamin E diets were used. However, a high level of antioxidant supplementation, particularly vitamin E, did reduce the level of oxysterols during cooking. Inconsistent results were found for selenomethionine supplementation in relation to cooking methods (i.e. roasting). A protective effect on TBARs formation in frozen-raw meat was found after 6 mo of storage when either antioxidant (alone or in combination) was supplemented. However, after 12 mo of storage, no protective effect on TBARs formation was observed with any dietary treatment. As a result, the shelf life of ω-3 enriched frozen meat should not exceed 6 to 12 mo. In ω-3 enriched cooked thigh chicken meat, after 6 mo of storage neither high levels of vitamin E or SeMet inhibited TBARs formation. This study was able to demonstrate that LC ω-3 PUFA are retained in the muscle membranes when meat is being cooked. However, oxysterol and TBARs levels in ω-3 enriched chicken meat should be minimize to ensure consumer safety. Further research is needed to evaluate the evolution of TBARs and oxysterols of value-added food products during storage especially after cooking.

3.5 TABLES

Table 3.1. Diet composition and nutrient content (g/ kg)[1].

	Starter	Grower	Finisher			
	(0 to 11 d)	(11 to 21 d)	(22 to 61 d)			
Enrichment			LE x LSe	HE x LSe	LE x HSe	HE x HSe
Wheat	43.67	10.32	65.50	65.50	65.50	65.50
Linpro[2]	---	---	20.00	20.00	20.00	20.00
Canola meal	---	---	4.00	4.00	4.00	4.00
Soybean meal	26.80	22.67	3.90	3.90	3.90	3.90
Canola oil	6.97	9.66	2.40	2.40	2.40	2.40
Choline chloride premix	---	---	0.50	0.50	0.50	0.50
L-Lysine	0.33	0.32	0.52	0.52	0.52	0.52
D,L-Methionine	0.29	0.37	0.16	0.16	0.16	0.16
L-Threonine	0.08	0.08	0.16	0.16	0.16	0.16
Dicalcium phosphate	1.82	1.50	1.26	1.26	1.26	1.26
Calcium carbonate	1.43	1.36	1.48	1.48	1.48	1.48
Salt, plain (NaCL)	0.47	0.49	0.28	0.28	0.28	0.28
Selplex[3] (mg)	---	---	---	---	0.3	0.3
Vit E mix (IU)	---	---	---	200	---	200
Microingrdients[4]	3.83	3.83	3.83	3.83	3.83	3.83
ME, kcal/kg	3086	3110	3200	3200	3200	3200
Crude protein, %	23	19	19.00	19.00	19.00	19.00
Crude fat, %	8.69	12	7.57	7.57	7.57	7.57
Crude fibre %	3.30	3.70	3.40	3.40	3.40	3.40
Ca %	1.00	0.90	0.90	0.90	0.90	0.90
Available P %	0.50	0.45	0.40	0.40	0.40	0.40
Met + Cys %	0.97	0.85	0.75	0.75	0.75	0.75
Met %	0.64	0.64	0.44	0.44	0.44	0.44
Lys %	1.35	1.18	1.05	1.05	1.05	1.05
Try %	0.31	0.26	---	---	---	---
Thr %	0.87	0.76	0.68	0.68	0.68	0.68

[1]LE x LSe: Dietary Treatment with low level of vitamin E and low level of selenomethionine.

HE x LSe: Dietary Treatment with high vitamin E and low level of selenomethionine.

LE x HSe: Dietary Treatment with low vitamin E and high level of selenomethionine.

HE x HSe: Dietary Treatment with high vitamin E and high level of selenomethionine.

[2]Provided by O&T Farms.

[3]Selplex 600 and Vit E were added at 50 and 400 g per 100 kg of feed

[4]The microingredient mix contained (per kg of diet): iron, 100 mg; zinc, 80 mg; manganese, 70 mg; copper, 8.5 mg; iodine, 0.5 mg; selenium, 0.1 mg; vitamin A, 10000 IU; vitamin D3, 2500 IU; vitamin E, 50 IU; vitamin K (menadione), 2 mg; niacin, 65 mg; D-pantothenic acid, 14 mg; riboflavin, 5 mg; pyridoxine, 4 mg; thiamine, 2 mg; folic acid, 0.8 mg; biotin, 0.18 mg; vitamin B12, 0.015 mg; Avizyme 1300 (Danisco Animal Nutrition, Marlborough, Wiltshire, UK), 500 mg; Bacitracin methylene Disalicylate (BMD 110 G, Alpharma, Mississauga, ON, Canada), 500 mg; Choline Cl, 400 mg; Sacox 120 (Intervet Canada), 500 mg; 0.50 g broiler premix; 0.05 g Avizyme 1302; 0.05 g Amprol; 0.05 g Zinc bacitracin.

Table 3.2. Fatty acid composition of experimental diets (mg/g of feed)[1].

Fatty Acids	LE x LSe	LE x HSe	HE x LSe	HE x HSe	SEM[2]
C16:0	6.6	6.1	5.1	4.9	0.6
C16:1 ω-7	0.1	0.1	0.1	0.1	0.01
C18:0	2.0	1.7	1.4	1.4	0.2
C18:1 ω-7	1.6	1.5	1.3	1.2	0.2
C18:1 ω-9	26.1	24.3	20.2	19.4	2.7
C18:2 ω-6	21.1	19.9	16.7	16.1	1.8
C18:3 ω-3	30.7	27.7	22.5	22.8	2.8
C18:3 ω-6	0.1	0.1	0.09	0.09	0.01
C20:0	0.2	0.2	0.2	0.2	0.03
C20:2 ω-6	0.05	0.05	0.04	0.04	0.004
C20:3 ω-6 & C22:1	0.04	0.1	0.03	0.04	0.02
C22:0	0.2	0.2	0.1	0.1	0.02
C24:1 & C22:3	0.04	0.04	0.03	0.03	0.01
Saturated	9.3	8.6	7.2	6.9	0.9
Monounsaturated	28.7	26.7	22.4	21.5	2.9
Polyunsaturated	52.1	49.9	39.4	39.1	4.6
Total ω-3	30.7	27.7	22.5	22.8	2.7
Total ω-6	21.3	20.1	16.8	16.3	1.8

[1]LSMean values obtained by using tukey's adjustment. C20:4 ω-6, C20:5 ω-3, C22:1 ω-9, C22:4 ω-6, C22:5 ω-3 and C22:6 ω-3 were not detected in the diets.

LE x LSe: Dietary Treatment with low level of vitamin E and low level of seleniomethionine.

HE x LSe: Dietary Treatment with high level of vitamin E and low level of seleniomethionine.

LE x HSe: Dietary Treatment with low level of vitamin E and high level of seleniomethionine.

HE x HSe: Dietary Treatment with high level of vitamin E and high level of seleniomethionine.

[2]SEM = standard error of the means.

Table 3.3. Lipid content in raw and cooked dark chicken meat from 61 d old broilers fed a finisher diet enriched with ω-3 PUFA and combinations of antioxidants [1].

Cooking Method	Vitamin E	SeMet	Lipid Content (%)
Frozen-Raw			8.6^{a}
Boiled			6.1^{b}
Pan-Fried			7.3^{b}
Roasted			8.7^{a}
SEM[2]			0.1
	Low		7.6^{b}
	High		8.1^{a}
		Low	7.7^{a}
		High	7.9^{a}
	SEM[2]		0.1
	Low	Low	7.6^{b}
	High	Low	7.8^{ab}
	Low	High	7.6^{b}
	High	High	8.3^{a}
	SEM[2]		0.1
Frozen-Raw	Low	Low	8.5^{abcd}
	High	Low	8.9^{ab}
	Low	High	8.1^{abcdef}
	High	High	9.1^{a}
Boiled	Low	Low	6.5^{h}
	High	Low	6.8^{gh}
	Low	High	7.1^{efgh}
	High	High	7.6^{bcdefgh}
Pan-Fried	Low	Low	7.4^{cdefgh}
	High	Low	7.2^{defgh}
	Low	High	6.8^{fg}
	High	High	8.0^{abcdefg}
Roasted	Low	Low	8.2^{abcde}
	High	Low	8.5^{abc}
	Low	High	8.3^{abcde}
	High	High	8.4^{abcde}
	SEM[2]		0.2
Source of variation	----------Probability----------		
Cooking method (C)			<.0001
SeMet (S)			0.1242
Vitamin E (E)			0.0007
C x S			0.0729
C x E			0.4653
S x E			0.0493
C x S x E			0.1367

[1]LSMean values obtained by using tukey's adjustment. Values in the same column within effect or interaction with no common superscript differ (p <0.05)
[2]SEM = standard error of the means

Table 3.4. Fatty acids (mg/100 g) in frozen-raw and cooked dark chicken meat from 61 d old broilers fed a finisher diet enriched with ω-3 PUFA and combinations of antioxidants from 61 d old broilers fed a finisher diet enriched with ω-3 PUFA and combinations of antioxidants [1].

	Frozen-Raw Meat				Boiled Meat				Pan-Fried Meat				Roasted Meat				SEM[2]
	LExLSe	HExLSe	LExHSe	HExHSe	LExLSe	HExLSe	LExHSe	HExHSe	LExLSe	HExLSe	LExHSe	HExHSe	LExLSe	HExLSe	LExHSe	HExHSe	
14:0	29.1^{abcd}	34.6^{ab}	32.5^{abc}	37.3^{a}	20.3^{e}	24.2^{de}	23.8^{de}	22.6^{de}	23.5^{de}	23.0^{de}	23.5^{de}	23.4^{de}	24.7^{cde}	26.3^{cde}	24.7^{cde}	27.3^{bcde}	1.45
14:1 ω-7	8.0^{bcd}	10.3^{a}	8.7^{abc}	9.7^{ab}	5.3^{f}	7.2^{cdef}	6.3^{def}	6.1^{ef}	6.7^{def}	6.4^{def}	6.6^{def}	5.8^{ef}	7.4^{cdef}	7.9^{bcde}	6.5^{def}	7.2^{cdef}	0.36
16:0	1102^{bcd}	1324^{ab}	1241^{abc}	1425^{a}	761^{e}	940^{cde}	885de	889de	952^{cde}	838de	911de	820de	1017^{bcde}	1015^{bcde}	918de	991^{cde}	55.7
16:1 ω-7	244^{abcd}	304^{a}	265^{abc}	298^{ab}	161^{f}	218^{cdef}	191^{def}	187^{def}	203^{cdef}	193^{def}	197^{def}	176^{ef}	225^{cdef}	237^{bcde}	197^{def}	216^{cdef}	11.6
18:0	285^{bc}	334^{ab}	337^{ab}	389^{a}	215^{c}	251^{bc}	251^{bc}	253^{bc}	268^{bc}	229^{c}	260^{bc}	241^{c}	277^{bc}	267^{bc}	262^{bc}	278^{bc}	15.3
18:1 ω-7	138^{bcd}	164^{ab}	158^{abc}	179^{a}	95.1^{e}	120^{cde}	115de	112de	121^{cde}	107de	116de	106de	130^{bcde}	130^{bcde}	117de	129^{bcde}	7.18
18:1 ω-9	2412^{bcd}	2838^{ab}	2793^{abc}	3170^{a}	1622^{e}	2029de	1986de	2076^{cde}	1787de	1787de	2006de	1803de	2258^{bcde}	2201^{bcde}	2030de	2237^{bcde}	127
18:2 ω-6	1001^{abcd}	1087^{abc}	1105^{ab}	1245^{a}	692^{e}	821^{cde}	820^{cde}	809de	870^{bcde}	728de	843^{bcde}	772de	942^{bcde}	888^{bcde}	847^{bcde}	935^{bcde}	48.7
18:3 ω-3	1189^{abc}	1185^{abc}	1236^{ab}	1429^{a}	805^{c}	918^{bc}	930^{bc}	951^{bc}	1006^{bc}	799^{c}	991^{bc}	868^{bc}	1122^{abc}	780^{c}	997^{bc}	1073^{abc}	73.4
18:3 ω-6	4.8^{abc}	5.0^{ab}	4.8^{ab}	6.1^{a}	3.2^{d}	3.8^{bcd}	3.6^{bcd}	3.8^{bcd}	3.9^{bcd}	3.4^{cd}	3.9^{bcd}	3.5^{cd}	4.3^{bcd}	4.3^{bcd}	4.0^{bcd}	4.3^{bcd}	0.24
20:3 ω-3	6.7^{bc}	7.0^{bc}	7.1^{bc}	8.5^{ab}	5.8^{c}	6.6^{c}	6.2^{c}	9.8^{a}	7.1^{bc}	6.4^{c}	6.9^{bc}	6.7^{bc}	7.0^{bc}	6.9^{bc}	7.0^{bc}	6.9^{bc}	0.33
20:3 ω-6	8.2^{abcd}	9.3^{ab}	8.6^{abc}	10.2^{a}	6.0^{d}	6.9^{bcd}	6.7^{cd}	6.3^{cd}	7.4^{bcd}	6.3^{cd}	7.1^{bcd}	6.9^{bcd}	7.9^{abcd}	7.5^{bcd}	7.4^{bcd}	7.9^{abcd}	0.45
20:4 ω-6	9.6^{bc}	10.0^{bc}	10.8^{b}	11.8^{b}	7.4^{c}	9.4^{bc}	8.6^{bc}	20.0^{a}	9.2^{bc}	8.9^{bc}	10.2^{bc}	8.76^{bc}	9.6^{bc}	10.7^{bc}	10.3^{bc}	9.6^{bc}	0.60
20:5 ω-3	18.1^{b}	19.5^{b}	18.3^{b}	21.6^{ab}	19.2^{b}	21.2^{ab}	19.2^{b}	25.0^{a}	21.8^{ab}	21.2^{ab}	22.1^{ab}	22.9^{ab}	20.5^{ab}	21.4^{ab}	22.5^{ab}	21.1^{ab}	0.97
22:5 ω-3	25.6^{ab}	25.9^{ab}	25.2^{ab}	30.1^{a}	22.4^{b}	24.9^{ab}	22.1^{ab}	28.4^{ab}	26.8^{ab}	23.5^{ab}	26.7^{ab}	25.4^{ab}	27.1^{ab}	26.5^{ab}	27.1^{ab}	26.2^{ab}	1.35
22:6 ω-3	27.4^{a}	28.0^{a}	28.3^{a}	32.7^{a}	26.6^{a}	29.2^{a}	27.4^{a}	11.1^{b}	31.2^{a}	28.3^{a}	30.9^{a}	32.7^{a}	29.9^{a}	30.1^{a}	31.4^{a}	31.0^{a}	1.49
SFA[3]	1418^{bcd}	1693^{ab}	1612^{abc}	1852^{a}	997^{e}	1215^{cde}	1160de	1165de	1246^{cde}	1091de	1194de	1085de	1322^{bcde}	1309^{bcde}	1205^{cde}	1297^{bcde}	72.3
MUFA[4]	2802^{bcd}	3318^{ab}	3225^{abc}	3658^{a}	1884^{e}	2374de	2298de	2270de	2408^{cde}	2093de	2326de	2091de	2621^{bcde}	2576^{bcde}	2351de	2590^{bcde}	146
PUFA[5]	2290^{abc}	2377^{abc}	2444^{ab}	2796^{a}	1587^{d}	1841^{bcd}	1845^{bcd}	1865^{bcd}	1983^{bcd}	1625^{d}	1942^{bcd}	1746^{cd}	2170^{abcd}	1776^{cd}	1954^{bcd}	2115^{bcd}	117
Total ω-3	1267^{abc}	1265^{abc}	1315^{ab}	1522^{a}	878^{c}	999^{bc}	1006^{bc}	1026^{bc}	1092^{abc}	878^{c}	1078^{bc}	954^{bc}	1206^{abc}	865^{c}	1085^{bc}	1158^{abc}	76.3
Total ω-6	1023^{abcd}	1112^{abc}	1129^{ab}	1274^{a}	708^{e}	841^{cde}	839^{cde}	840^{cde}	890^{bcde}	747de	864^{bcde}	791de	964^{bcde}	910^{bcde}	869^{bcde}	957^{bcde}	49.9
ω-6:ω-3	0.8^{a}	0.9^{a}	0.9^{a}	0.8^{a}	0.8^{a}	0.8^{a}	0.8^{a}	0.8^{a}	0.8^{a}	0.8^{a}	0.8^{a}	0.8^{a}	0.8^{a}	1.1^{a}	0.8^{a}	0.8^{a}	0.07

[1]LSMean values obtained by using tukey's adjustment. Values in the same row with no common superscript differ ($P < 0.05$). LE x LSe: Dietary Treatment with low level of vitamin E and low level of selenomethionine. HE x LSe: Dietary Treatment with high vitamin E and low level of selenomethionine. LE x HSe: Dietary Treatment with low vitamin E and high level of selenomethionine. HE x HSe: Dietary Treatment with high vitamin E and high level of selenomethionine.
[2]SEM: Standard Error.
[3]SFA: Saturated Fatty Acids
[4]MUFA: Monounsaturated Fatty Acids
[5]PUFA: Polyunsaturated Fatty Acids

Table 3.5. Levels of lipid oxidation (μg/g of fat) frozen-raw and cooked ω-3 enriched chicken meat after 4 mo of storage from 61 d old broilers fed a finisher diet enriched with ω-3 PUFA and combinations of antioxidants[1].

Cooking Method	Vitamin E	SeMet	7 - KC	7-α-HC	7-β-HC	α-CE	β-CE
Frozen-Raw			3.7^{c}	4.9^{b}	3.1^{c}	3.0^{a}	4.5^{c}
Boiled			6.9^{ab}	7.8^{a}	8.6^{b}	2.0^{b}	5.8^{b}
Pan-Fried			5.6^{b}	7.3^{a}	7.6^{b}	2.2^{b}	5.4^{bc}
Roasted			8.3^{a}	9.2^{a}	10.8^{a}	2.5^{ab}	6.9^{a}
SEM[2]			0.4	0.7	0.6	0.2	0.3
	Low		7.4^{a}	8.7^{a}	9.0^{a}	3.0^{a}	6.9^{a}
	High		4.8^{b}	5.9^{b}	6.1^{b}	1.9^{b}	4.4^{b}
		Low	6.3^{a}	7.9^{a}	7.7^{a}	2.5^{a}	5.8^{a}
		High	6.0^{a}	6.7^{a}	7.4^{a}	2.4^{a}	5.5^{a}
	SEM[2]		0.3	0.4	0.4	0.2	0.2
	Low	Low	8.1^{a}	9.8^{a}	9.4^{a}	3.2^{a}	7.4^{a}
	High	Low	4.5^{b}	6.0^{b}	5.9^{b}	1.7^{b}	4.3^{c}
	Low	High	6.8^{a}	7.6^{b}	8.6^{a}	2.8^{a}	6.4^{b}
	High	High	5.1^{b}	5.8^{b}	6.2^{b}	2.0^{b}	4.5^{c}
	SEM[2]		0.5	0.6	0.7	0.2	0.3
Frozen-Raw	Low	Low	5.8^{bc}	7.1^{cdef}	3.7^{ef}	2.9^{abcde}	4.8^{cde}
	High	Low	2.1^{g}	3.8^{ef}	2.6^{f}	2.5^{bcdef}	3.9^{de}
	Low	High	3.2^{fg}	5.2^{ef}	3.3^{f}	3.4^{ab}	5.1^{cd}
	High	High	3.6^{efg}	3.4^{f}	2.8^{f}	3.3^{abc}	4.4^{cde}
Boiled	Low	Low	13.3^{a}	14.8^{a}	16.9^{a}	4.0^{a}	11.2^{a}
	High	Low	5.0^{cdef}	6.6^{cdef}	5.6^{def}	1.3^{fg}	4.2^{cde}
	Low	High	5.6^{bcdef}	5.7^{def}	6.9^{cde}	1.8^{defg}	4.6^{cde}
	High	High	3.7^{defg}	4.3^{ef}	5.0^{def}	0.9^{g}	3.1^{e}
Pan-Fried	Low	Low	4.8^{cdefg}	7.5^{cde}	5.5^{def}	3.2^{bcd}	6.2^{bc}
	High	Low	5.3^{cdef}	6.9^{cdef}	7.6^{cde}	1.6^{efg}	4.7^{cde}
	Low	High	5.7^{cde}	6.2^{def}	7.4^{cd}	2.2^{cdef}	5.2^{cd}
	High	High	6.8^{bc}	8.8^{cd}	9.9^{bc}	2.0^{cdefg}	5.7^{bcd}
Roasted	Low	Low	8.4^{b}	9.7^{bc}	11.6^{b}	2.8^{abcde}	7.4^{b}
	High	Low	5.7^{bcde}	6.7^{cdef}	7.9^{bcd}	1.5^{efg}	4.5^{cde}
	Low	High	12.6^{a}	13.4^{ab}	16.6^{a}	3.8^{ab}	10.7^{a}
	High	High	6.5^{bcd}	6.8^{cdef}	7.2^{cde}	1.8^{defg}	5.0^{cde}
SEM[2]			0.1	1.4	1.3	0.5	0.7
Source of Variation			----------Probability----------				
Cooking method (C)			<.0001	0.0015	<.0001	0.0337	0.0008
SeMet (S)			0.4765	0.0941	0.6865	0.7655	0.2789
Vitamin E (E)			<.0001	0.0004	0.0002	<.0001	<.0001
C x S			0.0003	0.0052	0.0020	0.0261	<.0001
C x E			0.0010	0.0166	0.0001	0.1053	0.0004
S x E			0.0535	0.1555	0.3748	0.1579	0.0871
C x S x E			0.0104	0.0926	0.0070	0.2707	0.0036

[1]LSMean values obtained by using tukey's adjustment. Values in the same column within effect or interaction with no common superscript differ ($P < 0.05$).

7-KC = 7-keto cholesterol (μg/g fat)

7-α-HC = 7-α-hydroxycholesterol (μg/g fat)

7-β-HC = 7-β-hydroxycholesterol (μg/g fat)

β-CE = β-cholestanetriol, (μg/g fat)

α-CE = α-cholestanetriol, (μg/g fat)

[2]SEM = standard error of the means.

3.6 FIGURES

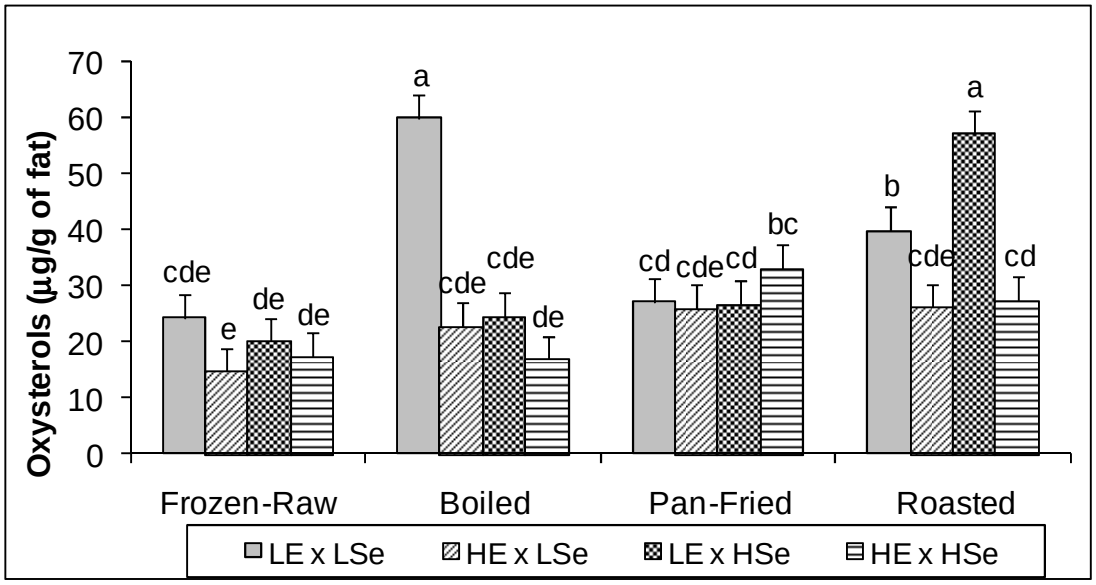

Figure 3.1. Total oxysterols detected in omega-3 in frozen-raw and cooked enriched dark chicken meat after 4 mo of frozen storage. Total oxysterols were calculated as the sum of 7-KC, 7-α-HC, 7-β-HC, α-CE and β-CE. [a-e] LSMean values obtained by using tukey's adjustment. Values with no common superscript differ ($P < 0.05$). LE x LSe: Dietary treatment with low level of vitamin E and low level of selenomethionine; HE x LSe: Dietary treatment with high vitamin E and low level of selenomethionine; LE x HSe: Dietary treatment with low vitamin E and high level of selenomethionine; HE x HSe: Dietary treatment with high vitamin E and high level of selenomethionine.

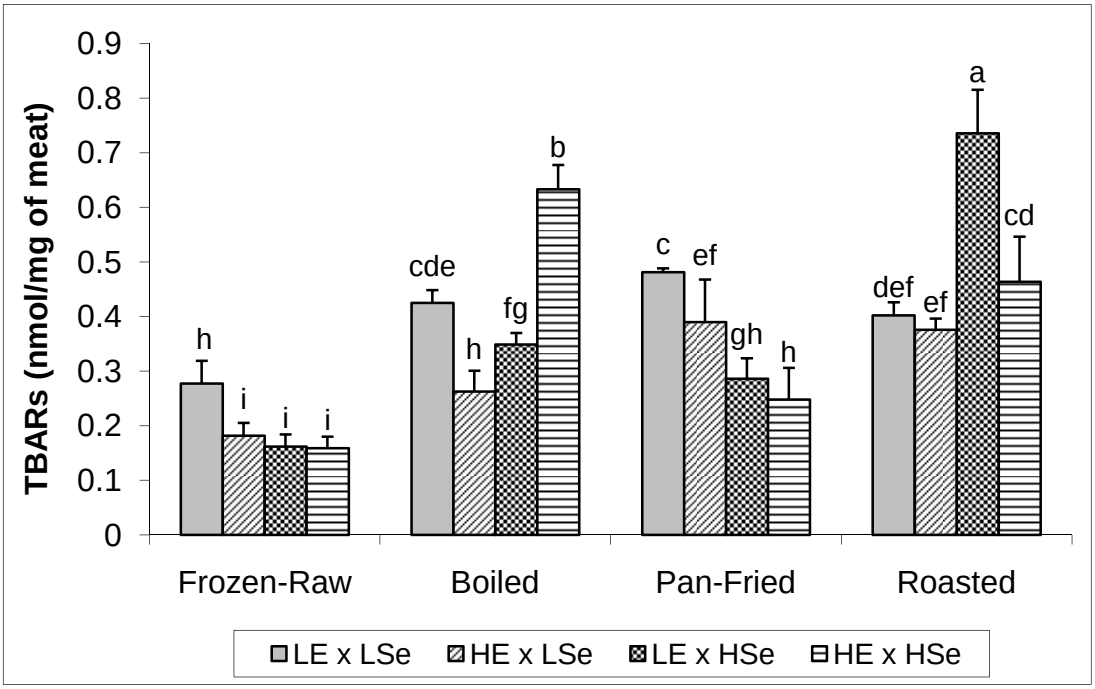

Figure 3.2. Thiobarbituric acid reactive substances (TBARs) in raw and cooked dark chicken meat after 6 mo of frozen storage. [a-h]LSMean values obtained by using tukey's adjustment. Values with no common superscript differ ($P < 0.05$). LE x LSe: Dietary treatment with low level of vitamin E and low level of selenomethionine; HE x LSe: Dietary treatment with high vitamin E and low level of selenomethionine; LE x HSe: Dietary treatment with low vitamin E and high level of selenomethionine; HE x HSe: Dietary treatment with high vitamin E and high level of selenomethionine.

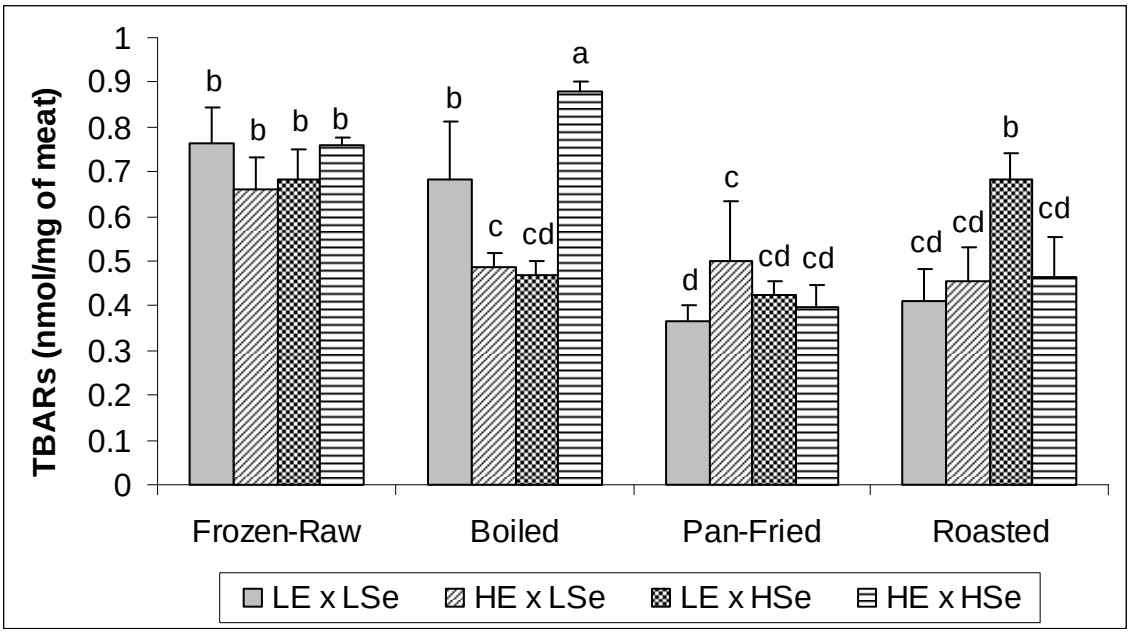

Figure 3.3. Thiobarbituric acid reactive substances (TBARs) in raw and cooked dark chicken meat after 12 mo of frozen storage. [a-d]LSMean values obtained by using tukey's adjustment. Values with no common superscript differ ($P < 0.05$). LE x LSe: Dietary treatment with low level of vitamin E and low level of selenomethionine; HE x LSe: Dietary treatment with high vitamin E and low level of selenomethionine; LE x HSe: Dietary treatment with low vitamin E and high level of selenomethionine; HE x HSe: Dietary treatment with high vitamin E and high level of selenomethionine.

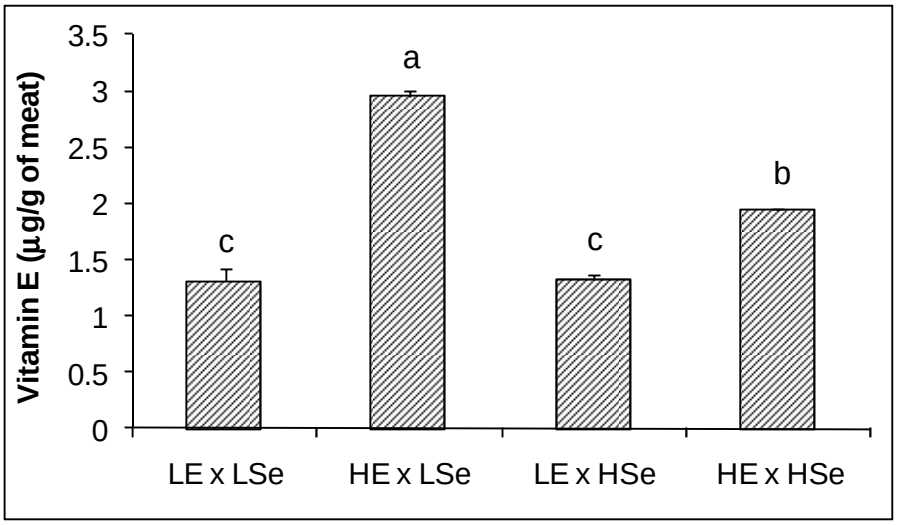

Figure 3.4. Vitamin E levels in frozen-raw dark chicken meat after 12 mo of frozen storage. [a-c]LSMean values obtained by using tukey's adjustment. Values with no common superscript differ ($P < 0.05$). LE x LSe: Dietary treatment with low level of vitamin E and low level of selenomethionine; HE x LSe: Dietary treatment with high vitamin E and low level of selenomethionine; LE x HSe: Dietary treatment with low vitamin E and high level of selenomethionine; HE x HSe: Dietary treatment with high vitamin E and high level of selenomethionine.

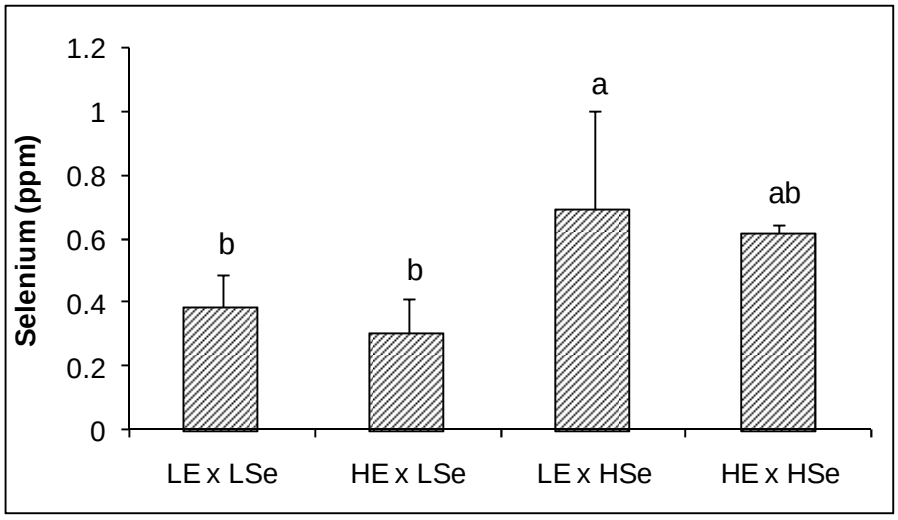

Figure 3.5. Selenium levels in frozen-raw dark chicken meat after 12 mo of storage. [a-b]LSMean values obtained by using tukey's adjustment. Values with no common superscript differ ($P < 0.05$). LE x LSe: Dietary treatment with low level of vitamin E and low level of selenomethionine; HE x LSe: Dietary treatment with high vitamin E and low level of selenomethionine; LE x HSe: Dietary treatment with low vitamin E and high level of selenomethionine; HE x HSe: Dietary treatment with high vitamin E and high level of selenomethionine.

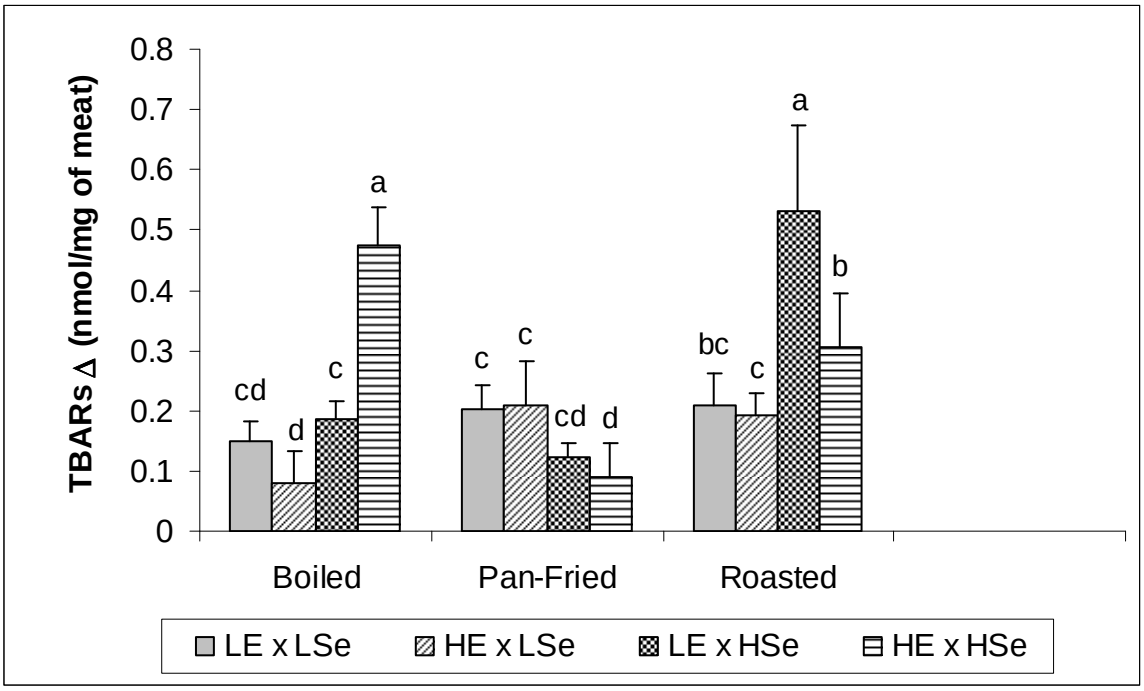

Figure 3.6. Change in thiobarbituric acid reactive substances (ΔTBARs) formation during cooking after 6 mo of storage. Change was calculated as TBARs in cooked meat minus TBARs in frozen-raw meat. [a-d]LSMean values obtained by using tukey's adjustment. Values with no common superscript differ ($P < 0.05$). LE x LSe: Dietary treatment with low level of vitamin E and low level of selenomethionine; HE x LSe: Dietary treatment with high vitamin E and low level of selenomethionine; LE x HSe: Dietary treatment with low vitamin E and high level of selenomethionine; HE x HSe: Dietary treatment with high vitamin E and high level of selenomethionine.

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4 EFFECTS OF ANTIOXIDANTS ON CARCASS YIELD AND MUSCLE LIPIDS OF OMEGA-3 ENRICHED BREAST BROILER MEAT

4.1 INTRODUCTION

A strategy to increase ω-3 polyunsaturated fatty acids (**ω-3 PUFA**) intake in the human diet includes consumption of enriched products. Omega-3 PUFA are linked with positive health benefits. Eicosapentanoic (20:5 ω-3; **EPA**) and docosahexaenoic ω-3 fatty acids (22:6 ω-3; **DHA**) are related to the prevention of various cancers and cardiovascular heart diseases (**CHD**; Yashodhara et al., 2009), whereas consumption of saturated fatty acids (**SFA**) has been correlated with blood cholesterol concentration and CHD (Mensink et al., 2003).

The primary ω-3 PUFA source used in enrichment of chicken meat is α–linolenic acid (18:3 ω–3; **LNA**; Villaverde et al., 2006). The ω-3 fatty acid LNA is converted to longer chain ω–3 PUFA, EPA, DHA and 22:5 (**DPA)** by various elongase and desaturase enzymes (Schmitz and Ecker, 2008). Age and sex may influence the fatty acid composition of meat; however, few studies have focused on the effect of sex on fatty acid composition and deposition of older broilers. In pigs, the concentration of LNA and PUFA in back-fat increases in the order: males<females<barrows, while the concentration of SFA increases (Nurnberg and Ender, 1989). In broiler chickens, females contain a greater amount of abdominal or carcass fat than males (Pym and Solvyns, 1979). Differences in the fatty acid deposition due to sex are also seen in lambs (Nurnberg et al., 1996), pigs (Nurnberg and Ender, 1992) and cattle (Malau-Aduli et al., 1998).

Sex can also affect meat technological properties such as water holding capacity. Differences in meat quality due to sex are related to differences in circulating levels of sex hormones (Aberle et al., 2001). In broilers, sex can affect the final pH of breast meat, thus color and water holding capacity, since there is a negative correlation between these parameters (Barbut, 1996; Le Bihan-Duval et al., 1998).

Typically, dietary treatments which are used to produce poultry ω-3 enriched meat are also fortified with antioxidants such as selenium, which is part of the glutathione peroxidase complex, and vitamin E. Due to the presence of longer fatty acids with a higher

number of double bonds (unsaturation), the oxidative stability of ω-3 enriched meat is lower compared to non-enriched meat (Barroeta, 2007). Lipid oxidation compromises meat quality and nutritional value due to the formation of potentially toxic compounds such as oxysterols (Cortinas et al., 2005). In order to reduce lipid oxidation in omega-3 enriched chicken meat, antioxidant supplementation is used in poultry diets.

The purpose of this study was to evaluate the effect of sex on carcass yield, fatty acid composition and drip loss of breast meat enriched with omega-3 fatty acids and a combination of antioxidants. Relationships among these parameters were assessed on an individual basis.

4.2 MATERIALS AND METHODS

4.2.1 Study Design

Animals used in this project were managed according to the Guide of Use of Experimental Animals (Canadian Council on Animal Care, 1984). The finisher diet (ME=3,200 kcal/kg; CP=19%) fed from 21 d of age was enriched with 20% linPRO (50% flaxseed, O&T Farms, Regina, Saskatchewan, Canada) as the source of α-linolenic acid (**LNA**). This ration was fortified with two levels of each of the antioxidants vitamin E and selenomethionine in a 2 x 2 factorial arrangement, with dietary vitamin E provided at 50 or 250 IU/kg, and selenomethionine at 0 or 0.3 mg/kg. All diets contained 0.1 g/kg sodium selenite. Birds were randomly distributed into 8 pens (32 birds per pen). Carcass yield, drip loss and fatty acid analysis of breast meat were done on male and female broilers, whose sex was verified at processing.

4.2.2 Stock, Management, and Sampling

A total of two hundred fifty-six mixed-sex Ross x Ross 308 broilers were reared on standard starter (0 to 11 d; ME=3,086 kcal/kg; CP=23%) and grower diets (to 21d; ME=3,110 kcal/kg; CP=19%). Diet composition and fatty acid profile is presented elsewhere (Chapter 3). Birds were provided *ad libitum* access to feed and water. Following an initial 24 h light period, the lighting program was 23L:1D for the duration of the experiment. On day 61, 32 birds per dietary treatment (16 males and 16 females) were slaughtered after a 12-h feed withdrawal period (n=128). Carcasses were cut up after reaching an internal temperature of 4 ºC. Eviscerated carcass weight (no head, neck and

feet) and weight of the *Pectoralis major*, *Pectoralis minor*, thighs, drums and wings were recorded. Yields were determined as a percentage of eviscerated carcass weight. Carcass yield was calculated as a percentage of live body weight after feed withdrawal.

Drip loss was determined on all birds using core samples (25x50 mm) that were cut in the same direction as the muscle fibers at the thickest part from the left side of the breast. A rectangular cutting tool was used to ensure uniform sample size. Breast muscle samples were suspended on cheese cloth in sealed plastic containers, and stored at 4 ºC for 72 h. Percentage drip loss was calculated as ((initial weight of the core – final weight of the core) / initial weight of the core) x 100.

4.2.3 Fat Extraction and Fatty Acid Profile of Breast Meat

Breast muscle fat extraction was done following the procedure described by Betti et al. (2009a). Briefly, total lipids were extracted using chloroform for 24 hours on 1 g of freeze-dried meat. The divertisation reaction was done using methanolic HCl (hydrochloric acid in methanol) in a water bath at 55 °C for 1 h. Fatty acid methyl ester composition was determined with a Varian 3400 gas chromatograph (Varian Walnut Creek, California, USA) equipped with a SGE BP20 capillary column (30m x 0.25 mm ID x 0.25 µm film thickness; Scientific Instrument Services In., Ringoes, NJ, USA) with a 30.20 min running time. Gas chromatograph operating conditions were as follows: an initial temperature of 50 °C then increased to 120 °C at a rate of 20 °C/min and a final temperature of 230 °C which increased at a rate of 10 °C/min. A Cool-on-Column injection method was used, with an initial and final injector temperature (CO_2) of 60 °C and 230 °C respectively, increasing at a rate of 150 °C/min. The temperature of the detector was 240 °C and the column head pressure of the carrier gas (helium) was 25 psi. Heptadecanoic acid (17:0) was used as an internal standard (Nu-chek prep Inc. Elysian, Mn, USA). Twelve samples were analyzed per each dietary treatment (6 per sex).

Saturated fatty acid (**SFA**) levels were calculated as 14:0 + 16:0 + 18:0 + 22:0. Monounsaturated fatty acid (**MUFA**) levels were calculated as 14:1 ω-7 + 16:1 ω-7 + 17:1 + 18:1 ω-7 + 18:1 ω-9 + 19:1 + 20:1 ω-15. Polyunsaturated fatty acid (**PUFA**) levels were calculated as linoleic acid (18:2 ω-6; **LA**) + LNA + 20:2 ω-6 + 20:3 ω-3 + 20:3 ω-6 + arachidonic acid (20:4 ω-6; **AA**) + EPA + 22:4 + DPA + DHA. Long chain ω-3 PUFA were calculated as EPA + DPA + DHA. Total ω-3 fatty acid levels were calculated as LNA + 20:3

ω-3 + EPA + DPA + DHA. Total ω-6 fatty acid levels were calculated as LA + 20:2 ω-6 + + 20:3 ω-6 + AA.

4.2.4 Statistical Analysis

All data were subjected to a nested (split-plot) ANOVA using the MIXED procedure of SAS (SAS System, 2002). Four dietary treatments were analyzed and sex was nested within pen (Sex (Vitamin E x Selenium)). Least squares means separation was performed using Tukey's test. Pearson correlation coefficients among yield, body weight and individual fatty acid parameters were calculated using the CORR procedure of SAS (SAS System, 2002). Significance was assessed at the $P \leq 0.05$ level.

4.3 RESULTS AND DISCUSSION

4.3.1 Body Weight and Yield

Body weight at 61 d was significantly lower in females than in males (3,788 *vs*. 4,395 g, respectively; Table 4.1). Similarly, carcass yield was lower in female than in male birds (68.65% *vs*. 69.51%, respectively). Breast muscle yield was significantly higher in female than in male birds (30.08% *vs*. 29.20%). Breast muscle yield in males ranged from 26.04% to 32.45% and from 23.81% to 34.78% in females. These sex-related yield differences are typical in broilers. These findings concur with Ricard and Touraille (1988) and Zuidhof (2005), who reported that males had a slightly lower breast yield than females. Thigh and wing yields were not affected by sex. Drums yield was higher in male birds (14.22%) compared to female birds (13.55%).

Inclusion of dietary selenium affected thigh yield (17.19% *vs*. 16.76%), which increased from 16.76% to 17.19% when broilers were fed the higher level of selenium (Table 4.1). The lack of effect of antioxidants on body weight is consistent with Choct et al. (2004), in their comparison of selenium-enriched treatments (either inorganic or organic form) in 38 d old broilers. Other studies have found increased body weights and feed efficiency when selenium yeast (Sel-Plex) and vitamin E were incorporated in the diets (Mahmoud and Edens, 2005; Bobade et al., 2009). Daun and Akesson (2004) compared the selenium content of two muscle types of five different species: chicken, duck, turkey, ostrich and lamb, and concluded that the selenium content was significantly higher in

oxidative muscles than in glycolytic muscle. This may have contributed to the increased thigh yield reported in the current experiment.

4.3.2 Drip Loss in Breast Meat

Breast meat drip loss was significantly lower in male birds (2.67%) than in female birds (3.02%) (Table 4.2). Drip loss percentage ranged from 2.50 to 3.12, which concurs with the percentages reported by Betti et al. (2009b). Higher female breast meat drip losses have also been reported by Fanatico et al. (2005). The female breast meat is lighter in color than that of the male (Schneider, 2009), which is typically linked to lower post-morten pH which directly affects drip losses. Froning et al. (1968) also reported a lighter color in female turkeys than males.

Drip loss was not influenced by dietary antioxidant supplementation. Contradictory results about the effect of vitamin E on drip loss have been reported. Dirinck et al. (1996) and Cannon et al. (1996) did not find any differences between drip losses of vitamin E supplemented and control porcine muscles. However, in frozen pork and in lamb chops higher levels of α-tocopheryl acetate reduced drip losses (Asghar et al., 1991; Buckley et al., 1995). These results could be linked to the positive effect of vitamin E on the integrity of muscle cell membranes. Vitamin E prevents the oxidation of membranal phospholipids during storage, and this could inhibit the passage of sarcoplasmic fluid through the membranes (Monahan et al., 1994).

Choct et al. (2004) reported that supplementation with organic selenium reduced drip loss compared to supplementation with sodium selenite or organic selenium (1.12% *vs*. 0.87%). Water holding capacity normally varies among genetic strains but it is not related to bird growth performance (Filus et al., 1995). Within the current study, drip loss was not correlated with body weight ($r = -0.24$; $P = 0.1177$). In general, drip loss during storage and display could be affected by age, sex, breed, diet and pre-slaughter stress (Fennema, 1990).

4.3.3 Breast Muscle Lipid Content and Fatty Acids Composition

The lipid content of breast meat ranged from 1.96 to 2.21% (Table 4.2). Sex did not affect the percentage of lipid in breast meat. Schneider (2009), who measured lipid content

in 52 d old broilers, also found no sex effect on breast lipid content. Similar to our findings, Grey et al. (1983) found no clear increase in fat content of meat (breast and thigh) between 3 and 11 wks of age. The sex effect was not significant if only meat was considered, but if fat content of skin was also considered females had a greater proportion of fat than males. Overall, females tend to have a higher body percentage of fat than males and older birds are fatter than younger birds (Leenstra, 1986). However, Lonergan et al. (2003) found a greater lipid content of breast in females than in males at 8 wks of age. Strain or diet composition effects may have contributed to differences in our findings compared to Lonergan et al. (2003), who used unique chicken populations. Individual antioxidant supplementation did not affect breast muscle lipid content (Table 4.2). However, the interaction of vitamin E and selenium had a significant effect on lipid content. In fact, lipid content of breast meat from high vitamin E and low selenium supplementation was higher compared to lipid content of breast meat obtained when both antioxidants were supplemented (2.14 *vs* 1.81 %).

Fatty acid analysis of the breast muscle samples indicated that the most predominant SFA, MUFA and PUFA were palmitic (16:0), oleic (18:1 ω-9) and LA or LNA respectively. Both SFA and MUFA did not differ due to sex (Table 4.3). However, the content of PUFA was significantly greater in males than females (480 *vs*. 436 mg/100g of meat, respectively). In this study, this increase was related to greater deposition of LA and LNA in males than in females. LNA was mainly deposited in the triacylglycerol fraction (Betti et al., 2009a) which is more influenced by sex than phospholipids (Warrants et al., 1999). Since long chain ω-3 PUFA are located in the phospholipid membranes (Betti et al., 2009a), they are less likely to be affected by sex. However, EPA content was 1.13-fold higher in female than in male breast meat. In pigs, Zhang et al. (2009) found that SFA was not affected by sex whereas some long chain PUFA (20:4, 22:5 and 22:6) were higher in gilts than in barrows. Also, Rymer and Givens (2005) concluded that mature birds would be expected to show a sex difference in their response to dietary LNA. In Rymer and Given's study, the regression slope of the response in dark meat LNA concentration to increasing dietary LNA concentration appeared slightly steeper in males than that of the females (0.13 *vs*. 0.11).

The size of the bird could be correlated to ω-3 PUFA deposition in tissues. In this study, there was a positive correlation of total ω-3 with carcass size (r = 0.43; $P = 0.0355$)

and yield (r = 0.44; *P* = 0.0292) in males. Perhaps bigger birds could have eaten more and deposited directly the most ω-3 PUFA in the breast muscle. In females, the proportion of ω-3 PUFA deposited did not increase with carcass weight.

Dietary supplementation with vitamin E may have reduced fatty acid oxidation. For instance, SFA (20.39%), MUFA (16.85%) and PUFA (15.32%) were higher when the higher levels of vitamin E was supplemented to the broiler diets (Table 4.3). Similarly, increases in LNA (14%) and LA (16%) were found in meat from birds fed higher vitamin E. Also, long chain ω-3 PUFA (EPA + DPA +DHA) were 15.82% higher in birds fed high levels of vitamin E compared to birds fed lower levels. Similarly, total ω-3 and total ω-6 deposition in breast muscle was higher in birds fed the high vitamin E. In sheep, low muscle vitamin E concentrations were associated with reduced ω-6 and ω-3 PUFA muscle content (Kasapidou et al., 2001). Similar findings have been reported in different type of meats such as chicken (Bou et al., 2006) and rabbit (Tres et al., 2008) when α-tocopherol was supplemented. Vitamin E may prevent oxidative loss of ω-3 PUFA in breast meat.

Dietary supplementation with organic selenium also increased levels of most fatty acids, although not as many fatty acids as vitamin E did. For instance, MUFA did not significantly increase in meat from birds fed high compared to low levels of dietary selenium (460 *vs*. 506 mg/100g of meat, respectively). Conversely, the concentration of SFA and PUFA had an increase of 12% and 10.93% respectively in birds fed the high selenium. In contrast to vitamin E, high dietary selenium did not increase LNA in breast meat. Only LA was significantly higher in birds fed high selenium compared to birds fed low levels (197 *vs*. 177 mg/100 g of meat respectively). As a consequence of DHA increase, an increase of 18% in the long chain ω-3 PUFA in breast muscle was also observed when birds were fed with selenium-enriched diet. Increases in fatty acids because of selenium supplementation have been reported in other studies (Haug et al., 2007) and this could be essential for the ω-3 meat enrichment.

4.4 CONCLUSIONS

The poultry industry could benefit economically by an appropriate selection of both broiler sex and dietary supplementation when producing ω-3 enriched chicken meat. In fact, this study demonstrated that bigger males deposited a greater proportion of ω-3 PUFA

in the breast muscle compared to smaller birds. Nevertheless, the inclusion of dietary vitamin E and selenium is also necessary when producing ω-3 enriched meat since it represents practical implications by increasing the level of valuable fatty acids such as DHA.

4.5 TABLES

Table 4.1. Body weight and breast, drums, thighs, wings and carcass yield as a percentage of carcass weight from 61 d old broilers fed a finisher diet enriched with ω-3 PUFA and combinations of antioxidants[1].

Vitamin E	Selenium	Sex	Body Weight (g)	Carcass[2] (%)	Breast[3](%)	Drums[3] (%)	Thighs[3] (%)	Wings[3] (%)
		Female	3788[b]	68.65[b]	30.08[a]	13.55[b]	16.85	10.87
		Male	4395[a]	69.51[a]	29.20[b]	14.22[a]	17.10	11.00
Low			4121	69.04	29.52	14.01	17.16	10.90
High			4063	69.12	29.75	13.77	16.78	10.96
	Low		4113	68.91	29.73	13.88	16.76[b]	10.96
	High		4070	69.25	29.54	13.89	17.19[a]	10.91
Pooled SEM			52	0.24	0.23	0.11	0.14	0.08
	Low	Female	3772[b]	68.34	30.32	13.49[b]	16.75	10.76
		Male	4454[a]	69.48	29.14	14.27[a]	16.78	11.15
	High	Female	3804[b]	68.95	29.83	13.61[b]	16.94	10.97
		Male	4336[a]	69.55	29.25	14.17[a]	17.43	10.84
SEM			74	0.34	0.32	0.15	0.20	0.12
Sources of Variation			----------Probability----------					
Sex			<.0001	0.0127	0.0080	<.0001	0.2106	0.3095
Vitamin E			0.4359	0.8252	0.4788	0.1146	0.0627	0.6728
Selenium			0.5618	0.3258	0.5656	0.9680	0.0366	0.7005
Vitamin E x Sex			0.5585	0.9132	0.3668	0.1392	0.1126	0.6535
Selenium x Sex			0.3133	0.4293	0.3552	0.4593	0.2460	0.0378
Vitamin E x Selenium			0.4943	0.3789	0.1203	0.4043	0.8944	0.5652
Vitamin E x Selenium x Sex			0.5808	0.8858	0.6509	0.1409	0.6802	0.9938

[1]Least squares means within the column with no common superscript are significantly different ($P < 0.05$).

Vitamin E at 50 IU (Low) and 250 IU (High) per kg of feed. Selenium at 0.1 mg (Low; sodium selenite) and 0.3 mg (High; selenomethionine). SEM=Standard error of the mean.

[2]Eviscerated carcass yield as a percentage of live body weight, without head, neck or feet.

[3]Percentage of eviscerated carcass weight.

Table 4.2. Percentages of drip loss and lipid content in chicken breast meat from 61 d old broilers fed a finisher diet enriched with ω-3 PUFA and combinations of antioxidants[1].

Vitamin E	Selenium	Sex	Drip Loss (%)[2]	Lipid Content (%)[3]
		Female	3.02[a]	1.96
		Male	2.67[b]	2.06
Low			2.88	2.00
High			2.82	2.02
	Low		2.83	2.06
	High		2.87	1.96
Pooled SEM			0.11	0.04
Low	Low		2.84	1.98[ab]
	High		2.92	2.06[ab]
High	Low		2.89	2.14[a]
	High		2.74	1.85[b]
SEM			0.16	0.05
Sources of Variation		----------Probability----------		
Sex			0.0272	0.0883
Vitamin E			0.6868	0.6782
Selenium			0.8070	0.0725
Vitamin E x Sex			0.7773	0.7359
Selenium x Sex			0.5525	0.2971
Vitamin E x Selenium			0.4855	0.0031
Vitamin E x Selenium x Sex			0.6342	0.0684

[1] Least squares means within the column with no common superscript were significantly different ($P < 0.05$). SEM=Standard error of the mean.

Vitamin E at 50 IU (Low) and 250 IU (High) per kg of feed. Selenium at 0.1 mg (Low; sodium selenite) and 0.3 mg (High; selenomethionine).

[2] n=128.

[3] n=48.

Table 4.3. Fatty acids composition (mg/100 g) in breast chicken meat from 61 d old broilers fed a finisher diet enriched with ω-3 PUFA and combinations of antioxidants [1].

Main Effects							
			Vitamin E		Selenium		
Fatty Acids	Female	Male	Low	High	Low	High	Pooled SEM
14:0	6.6	6.2	5.7[b]	7.0[a]	5.8[b]	6.9[a]	0.35
14:1 ω-7	0.1	0.1	0.2	0.1	0.1	0.1	0.10
16:0	247	228	209[b]	266[a]	224[b]	251[a]	9.18
16:1 ω-7	35.1	31.8	29.0[b]	38.0[a]	34.2	32.8	2.35
17:1	ND	0.1	ND	0.1	0.1	ND	0.05
18:0	79.4	76.2	70.3[b]	85.3[a]	70.7[b]	84.8[a]	2.14
18:1 ω-7	37.3	36.5	32.2[b]	41.6[a]	35.1	38.7	1.42
18:1 ω-9	416	400	373[b]	444[a]	387	430	20.0
18:2 ω-6	177[b]	197[a]	171[b]	203[a]	177[b]	197[a]	5.79
18:3 ω-3	176[b]	208[a]	178[b]	206[a]	184	200	7.75
19:1	0.3	0.3	0.4	0.2	0.5	0.1	0.17
20:1 ω-15	4.0	4.1	3.8	4.3	3.9	4.2	0.18
20:2 ω-6	1.6	1.6	0.1[b]	2.2[a]	1.1[b]	2.1[a]	0.23
20:3 ω-3	3.0	3.4	2.7[b]	3.7[a]	2.7[b]	3.7[a]	0.20
20:3 ω-6	4.2	3.8	3.6[b]	4.4[a]	3.5[b]	4.6[a]	0.23
20:4 ω-6	15.4	14.9	13.3[b]	17.0[a]	13.3[b]	16.9[a]	0.58
20:5 ω-3	17.1[a]	15.1[b]	14.9[b]	17.4[a]	14.5[b]	17.7[a]	0.70
22:0	0.1	ND	ND	0.1	0.1	ND	0.09
22:4	0.8	0.3	0.4	0.7	0.4	0.7	0.21
22:5 ω-3	28.8	25.2	25.2	28.7	25.0	29.0	2.40
22:6 ω-3	11.1	11.1	9.9[b]	13.2[a]	9.7[b]	13.4[a]	0.70
Long Chain ω-3	57.9	51.5	50.0[b]	59.4[a]	49.2[b]	60.1[a]	3.22
Saturated	333	311	285[b]	358[a]	301[b]	342[a]	11.3
Monounsaturated	494	473	439[b]	528[a]	460	506	23.2
Polyunsaturated	436[b]	480[a]	420[b]	496[a]	432[b]	485[a]	14.7
Total ω-3	237[b]	263[a]	231[b]	269[a]	236[b]	264[a]	8.71
Total ω-6	198[b]	218[a]	189[b]	227[a]	195[b]	220[a]	6.17
ω -6:ω-3	0.8	0.8	0.8	0.8	0.8	0.8	0.01

[1]Least squares means within the row with no common superscript are significantly different ($P < 0.05$). SEM=Standard error of the mean.

Vitamin E at 50 IU (Low) and 250 IU (High) per kg of feed. Selenium at 0.1 mg (Low; sodium selenite) and 0.3 mg (High; selenomethionine).

Table 4.4. Analysis of variance table and probabilities for antioxidants and broiler sex for the amount of fatty acids in breast muscle.

Probabilities	Main Effects			Interaction Effects			
Fatty Acids	Sex (S)	Vitamin E (VE)	Selenium (Sel)	S x Sel	S x VE	VE x Sel	S x VE x Sel
14:0	0.3931	0.0086	0.0252	0.7192	0.2314	0.8112	0.2759
14:1	0.6330	0.5161	0.6330	0.0903	0.5554	0.5554	0.5161
16:0	0.1608	<.0001	0.0486	0.3720	0.7959	0.4080	0.7087
16:1 ω-7	0.3266	0.0089	0.6862	0.6476	0.6842	0.0702	0.3246
17:1	0.3223	0.3223	0.3223	0.3223	0.3223	0.3223	0.3223
18:0	0.2923	<.0001	<.0001	0.2729	0.4986	0.8438	0.5820
18:1 ω-7	0.6705	<.0001	0.0756	0.3314	0.4922	0.2488	0.9994
18:1 ω-9	0.5731	0.0161	0.1284	0.5383	0.2197	0.7171	0.8584
18:2 ω-6	0.0155	0.0003	0.0232	0.0161	0.1506	0.8739	0.9131
18:3 ω-3	0.0055	0.0146	0.1546	0.0147	0.1953	0.7060	0.5892
19:1	0.9782	0.3222	0.0732	0.5067	0.1264	0.7627	0.3946
20:1 ω-15	0.7467	0.0810	0.1659	0.1849	0.0525	0.9776	0.8421
20:2 ω-6	0.8835	0.0004	0.0056	0.2891	0.0563	0.5874	0.4440
20:3 ω-3	0.2057	0.0011	0.0006	0.1572	0.0176	0.7621	0.0592
20:3 ω-6	0.1685	0.0144	0.0017	0.7467	0.1298	0.4036	0.1645
20:4 ω-6	0.5176	<.0001	<.0001	0.9393	0.3521	0.2471	0.1811
20:5 ω-3	0.0494	0.0157	0.0024	0.5165	0.5163	0.2102	0.8457
22:0	0.3223	0.3223	0.3223	0.3223	0.3223	0.3223	0.3223
22:4	0.0710	0.2566	0.2904	0.0395	0.6740	0.7248	0.5401
22:5 ω-3	0.2901	0.3059	0.2528	0.5369	0.4355	0.1898	0.7694
22:6 ω-3	0.4090	0.0012	0.0004	0.9060	0.3021	0.0383	0.3779
Long Chain ω-3	0.1616	0.0441	0.0203	0.7685	0.8280	0.0893	0.9878
Saturated	0.1683	<.0001	0.0128	0.3491	0.7076	0.5153	0.8671
Monounsaturated	0.5421	0.0092	0.1684	0.5055	0.2526	0.5733	0.7914
Polyunsaturated	0.0359	0.0006	0.0139	0.0239	0.1817	0.4857	0.8397
Total ω-3	0.0414	0.0032	0.0283	0.0343	0.2572	0.3364	0.6671
Total ω-6	0.0302	<.0001	0.0066	0.0205	0.1188	0.7568	0.9158
ω -6:ω-3	0.4066	0.0844	0.7745	0.6525	0.4290	0.0277	0.1471

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5 PROJECT SUMMARY AND IMPLICATIONS

Enrichment of poultry meat with ω-3 polyunsaturated fatty acids (**PUFA**) is an emerging method to increase the consumption of these health promising fatty acids. Benefits of long chain ω-3 fatty acids (EPA and DHA) include decreased risk of cardiovascular heart disease, brain development and prevention of cancers. The study of ω-3 enrichment has been clearly established in raw meat. However, little attention has been paid to the stability of these enriched products during storage and cooking. I believe it is important to assess both how much omega-3 remains in the final cooked product and the quantity of breakdown products that can appear during cooking processes. The use of antioxidants in broiler diets is an alternative way to mitigate the incidence of genotoxic compounds.

In this project, I used an omega-3 PUFA enriched diet fortified with antioxidants. The implications of these diets on broiler growth and yield traits were tested. Thigh and breast muscle samples were taken to further analysis of lipid content, composition and oxidative status. The implications of key findings are discussed below.

Variables related to the dietary treatments of the broilers, such as feeding period and ω-3 dietary source, should be considered. Between two broiler trials, for example, maximum levels of ω-3 in thigh meat were 1,000 mg/100 g of meat (17% flaxseed) and 1,267 mg/100g of meat (20% linPRO) by 35d and 61d respectively. It is recommended that poultry meat producers establish in advance target levels of enrichment in the different tissues since these levels determine the feeding period of the broilers. For instance, to achieve 300 mg of ω-3 per 100 g of breast meat 11.3 d are required when using 17% flaxseed. Overall, factors that are important include feed enrichment level, time on enriched diet and source of enrichment.

Sex of the broilers also affected the ω-3 enrichment of broilers' tissues. This study demonstrated that males deposited more ω-3 than females did. As a result, the poultry industry should grow males in order to optimize meat enrichment. Furthermore, one of the parameters to establish the quality of the meat is based on the drip loss which is linked to water holding capacity. Breast meat from females had a higher drip loss than breast meat from males at 61 d of age. The birds' sex was driving both enrichment and meat quality. This could be particularly important in omega-3 enriched chicken meat, since some PUFA

can increase the membranes' disruption caused by lipid oxidation, and contribute to exudative loss from meat.

In addition to these factors, the distribution of omega-3 PUFA differs among lipid classes. This study demonstrated that a-linolenic acid is mainly found in the triacylglyerols fraction whereas, long chain ω-3 PUFA are deposited in the phospholipid membranes. In fact, EPA, DHA and DPA were synthesized within the birds since they were not detected in the fatty acid composition of the diets. However, the precursor of the long chain ω-3 PUFA, α-linolenic acid, came mostly from the dietary source. This project was designed to explain ω-3 PUFA meat stability by using a single ω-3 dietary source. Probably a higher and better enrichment may be achieved by including long chain ω-3 PUFA in the poultry diet.

A second theme in my research was the stabilization of omega-3 enriched chicken meat. Besides an omega-3 source, broiler diets should also be fortified with antioxidants in order to protect the fatty acids against oxidation. Polyunsaturated fatty acids are more susceptible to oxidation because of the presence of the more reactive doubles bonds in their structure. The inclusion of ingredients like vitamin E and selenium in the diets improved the antioxidant status of the meat and increased the level of PUFA in raw breast and thigh meat in broilers of 61 d of age.

The effects of lipid oxidation products on human health are known to be adverse. However, further characterization of these effects is needed. The presence of ω-3 PUFA was correlated with a higher oxidation rate. My findings confirmed that theory. The level of oxysterols in thigh meat was higher than published values for non-enriched meat. Antioxidants have been successfully used to reduce the formation of lipid oxidation products. In this study, both vitamin E and selenium dietary supplementation, alone or in combination, reduced the thiobarbituric reactive substances in raw thigh meat. Based on my results, TBARs formation in frozen-raw thigh meat stored for 4 mo can be reduced by 35% when vitamin E is incorporated in the broiler diet. However, oxysterols formation in frozen-raw thigh meat was not inhibited by any of the antioxidant combinations.

It is possible that the oxysterols are continuing to degrade. For example, in frozen-raw 12 mo stored samples the level of oxysterols did not change compared to 4 mo stored

samples (data not shown). However, after 12 mo new or unkown peaks appeared in the chromatograms which may be other unidentified oxysterols. In this study, oxysterols were choosen based on previous works done on raw meat products. In future studies, consideration may be needed for the identification of further breakdown products (i.e. oxysterols) during storage and cooking.

Food products are exposed to high temperatures during cooking which contributes to increased lipid oxidation. Cooking methods can also have a large impact on the rate of ω-3 breakdown and the production of genotoxic compounds such as oxysterols due to time, temperature and heat exposure effects. My results demonstrated that frying resulted in the lowest amount of oxidation products compared to roasting or boiling. The generation of lipid oxidation products appears to be influenced by the heat flow rate and temperature. In frying, the level of oxysterols was similar to the levels found in raw meat. In constrast, the level of oxysterols in boiled and roasted meat increased after cooking, especially in meat fortified with low levels of antioxidants.

Further research should focus on finding appropriate ω–3 poultry dietary sources. The enrichement could possibly be optimized by mixing α-linolenic and long chain ω-3 fatty acid sources in the broiler diets. Also, the lipid oxidative stability of ω-3 enriched chicken meat could be evaluated by using the right amount and combination of antioxidants in the broiler diets. The ω-3 PUFA enrichment in the second experiment (Chapter 3) was much higher than that of the first experiment (Chapter 2) or in published examples. It may be that the best alternative to avoid side effects (i.e. sensory quality) is a combination of birds' dietary treatments and post-slaughter processing methods. Methods could include injection, marination or modified atmosphere packaging.

Overall, increased attention to broiler nutrition can improve the quality and nutritional value of ω-3 enriched meat. Companies who are willing to launch ω-3 enriched chicken meat should address most of the variables that were studied in this project. For the consumers it is nutritionally desirable to increase the ω-3 intake while reducing the exposure to dietary mutagenic factors such as those derived from cholesterol oxidation. This thesis characterized the degree of breakdown of ω-3 enriched products and contributes to the development of a strategy to reduce the generation of harmful compounds. When marketing ω-3 enriched chicken, it is important to recognize that the lipid fraction is

unevenly distributed in the different tissues of poultry. This may be an interesting opportunity that could allow the development of this market segment to proceed at the further processed or specialized cut level rather than the less profitable whole bird level.