Using Alternate Omega-3 Polyunsaturated Fatty Acid Sources for the Enrichment of Broiler Meat and Table Eggs

by

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ABSTRACT

A series of experiments was conducted to explore the potential of including novel sources of omega-3 polyunsaturated fatty acids (n-3 PUFA) in broiler and laying hen diets. Camelina cake (CC), the co-product remaining after pressing of oil from camelina seed for biofuel production, was fed to broilers. Genetically modified stearidonic acid-enhanced flaxseed oil (SDAflax), was included in laying hen diets.

Increasing levels of CC (0, 8, 16 and 24%) were fed to broiler chickens to evaluate the lipid deposition response in brain, liver, breast and thigh tissue. Increasing dietary CC inclusion linearly increased the proportion of long-chain (LC) n-3 PUFA ($P<0.001$) in liver and brain tissue. In addition, the labeling claim requirement for n-3 PUFA enrichment (300 mg per 100 g of meat) was exceeded in breast and thigh by feeding the 24% camelina cake diet for 28 d or the 16% camelina cake diet for 42 d, respectively.

Stearidonic acid-flaxseed oil was fed to layers for increasing the LC n-3 PUFA in table eggs compared with conventional flaxseed oil (REGflax). The total n-3 PUFA in egg yolk from hens fed either flax oil type was not different. Egg yolk from hens fed 4% SDAflax showed a 1.5 fold increase ($P<0.001$) in LC n-3 PUFA compared with feeding REGflax. In addition, feeding SDAflax compared with REGflax resulted in greater LC n-3 PUFA deposition in thigh and breast muscle and in all other tissues (liver, heart, brain) except abdominal fat pad at d 21.

Another experiment was conducted to investigate the metabolic competition among dietary fatty acid sources (SDAflax or REGflax oil in combination with either of corn, canola or fish oil) for desaturation and elongation pathways, and their effect on the egg yolk LC n-3 PUFA content. Hens fed SDAflax oil provided 152 mg/egg of LC n-3 PUFA compared with 110 mg/egg in those fed REGflax oil ($P<0.001$). In addition, inclusion of fish oil increased ($P<0.001$)

LC n-3 PUFA, but there was no difference for yolk LC n-3 PUFA between corn oil and canola oil fed hens, suggesting a lack of lipid competition during lipid desaturation and elongation.

Camelina cake can be included in broiler diets to increase n-3 PUFA of meat; feeding SDA-enriched flax oil can increase egg LC n-3 PUFA in laying hens without negative effects on egg production and reproductive traits. The enriched poultry products (meat and table eggs) offer an alternative to increase the amount of omega-3 fatty acids in human diets.

PREFACE

The Chapter 2 of this thesis has been published in *Poultry Science* as:

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DEDICATION

This thesis is dedicated to my love of life **Himanhsu,** and my mother **Anusuiya** for their unconditional love and continuous moral support to finish my thesis.

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LIST OF ABBREVIATIONS

ALA	α-linolenic acid
AA	Arachidonic acid
β-oxidation	Beta-oxidation
CC	Camelina cake
CFIA	Canadian Food Inspection Agency
DGLA	Dihomo-γ-linolenic acid
DHA	Docosahexaenoic acid
DPA	Docosapentaenoic acid
EDA	Eicosadienoic acid
EFA	Essential fatty acids
EPA	Eicosapentaenoic acid
FDA	Food and Drug Administration US
GC-MS	Gas chromatography mass spectrometry
GLA	γ-linolenic acid
LA	Linoleic acid
LC-PUFA	Long chain n-3 polyunsaturated fatty acids
LDL	Low density lipoprotein
LNA	Linolenic acid
MUFA	Monounsaturated fatty acid
n-3 PUFA	Omega-3 polyunsaturated fatty acid
n-6 PUFA	Omega-6 polyunsaturated fatty acid
PUFA	Polyunsaturated fatty acid
SDA	Stearidonic acid
SFA	Saturated fatty acid
SEM	Standard error of mean
TFA	Total fatty acids
USDA	United States Department of Agriculture
VLDL	Very low-density lipoprotein
VLDLy	Yolk-targeted VLDL

CHAPTER 1

Literature Review

1.1 GENERAL

INTRODUCTION

Omega-3 polyunsaturated fatty acids (n-3 PUFA) include α-linolenic acid (ALA, C18:3n-3), and its long chain (LC) metabolites eicosapentaenoic acid (EPA, C20:5n-3), docosapentaenoic acid (DPA, C22:5n-3), and docosahexaenoic acid (DHA, C22:6n-3). In humans, increasing dietary LC n-3 PUFA have been linked to a reduction in the incidence of cardiovascular diseases (Tousoulis et al., 2014), atherosclerosis (Thies et al., 2003), and increased visual and neurological development in infants (Birch et al., 2010). The bioconversion of ALA to EPA and DHA through desaturation and elongation (Pereira et al., 2004) is lower in men (Burdge et al., 2002) than in women (Burdge and Wotton, 2002). The bioconversion efficiency in chickens is greater compared with mammals and fish due to increased activity of genes responsible for elongation (Gregory et al., 2013). Various agencies and health organizations, such as the Food and Agriculture Organization of the United Nations, American Dietetic Association, Dietitians of Canada, and the American Heart Association have recommended dietary intakes for total n-3 PUFA of 1.4 to 2.5 g/d, with EPA and DHA ranging from 140 to 600 mg/d. The limitation in LC n-3 PUFA bioconversion ability in humans makes it difficult to fulfill the nutritional demands of LC n-3 PUFA. Therefore, humans largely depend on consumption of dietary sources of EPA and DHA.

Total n-3 PUFA intake of vegetarians, vegans, non-fish eaters and pregnant mothers is not at adequate levels and is up to 80% lower than in fish-eaters (Welch et al., 2010). Among fish-eaters, about 25% of people consume oily fish, which contain health beneficial LC n-3

PUFA (Augood et al., 2010). The depletion of marine fish stocks has been a concern and was reflected in a United Nations resolution to restore fisheries and marine ecosystems (Domergue et al., 2005). In addition, the presence of environmental pollutants such as methyl mercury, dioxins and polychlorinated biphenyls in fish (Domingo et al., 2010) may dissuade oily fish consumption. In contrast to oily fish and other livestock meat (cattle, sheep or pork) consumption, poultry meat consumption has increased in the last few decades (Rymer and Givens, 2006). Chicken is Canada's most preferred source of animal protein with approximately a 50% increase in consumption over the past 20 years to 31.2 kg/person (Agri-food, Canada, 2013). Additionally, egg consumption has also been increasing in the last two decade in Canada reaching 21.4 dozen in 2013 (Agri-food, Canada, 2013). The enrichment of poultry products with LC n-3 PUFA may further increase consumer acceptance and therefore benefit the poultry industry. Currently, Canadian poultry and egg products are worth $4.0 billion/year (Agri-food, Canada, 2013). Given the health advantages associated with consumption of n-3 PUFA and increased consumption of poultry products (meat and table eggs), it would be wise to focus on increasing delivery of these fatty acids through poultry products to humans by feeding novel n-3 PUFA sources to egg layers and meat broilers.

Various sources of dietary n-3 PUFA such as flaxseed, fish oil, fish meal, marine algae and canola oil are fed in the diets of laying hens or broilers for enriching n-3 PUFA in eggs or meat, respectively (Cherian and Sim, 1991; Ajuyah, et al., 1991; Herber and Van Elswyk, 1996; Nitsan et al., 1999; Gonzalez-Esquerra and Leeson, 2000; Milinsk et al., 2003; Mazalli et al., 2004; Ceylan et al., 2011; Nain et al, 2012; Coorey, et al., 2015). Most of these studies have focused on the fortification of n-3 PUFA in poultry products through the diet and their effect on production parameters such as laying performance and egg characteristics for laying hens and

growth and meat yield for the broilers. It has been well established that the fatty acid composition of poultry products can be altered by dietary manipulation. However, the efficacy of deposition of n-3 PUFA into egg yolk (Antruejo et al., 2011) and chicken meat (Azcona et al., 2008) varies among various dietary n-3 fatty acid sources. More information is needed to understand the efficiency of broilers or layers to convert ALA to longer chain metabolites. The aim of this thesis was to provide insight for understanding the fatty acids type of alternate sources (camelina and stearidonic acid (SDA; C18:4n-3) enriched flaxseed) in the enrichment process of chicken meat and laying hens eggs. In addition, this thesis emphasized the bioconvertion to LC n-3 PUFA from SDA compared with ALA and what would be the effect on the ALA/SDA bioconversion when fed together with other dietary lipid sources.

1.1 Fatty Acid: Structure and Nomenclature

A fatty acid is a molecule with a hydrocarbon chain of variable length, with a carboxyl group (COOH) at one end and a methyl (CH_3) group at the other end, the latter is also known as the omega (n) end (Figure 1.1). Fatty acids can be classified based on the presence or absence of double bonds in the carbon chain. Saturated fatty acids (SFA) do not have double bonds, while monounsaturated fatty acids (MUFA) have one double bond, and polyunsaturated fatty acids (PUFA) have more than one double bond with in the carbon chain. The PUFA can be further classified on the basis of the position of the first double bond in the chemical structure relative to the methyl end, into two groups: omega-3 (n-3) fatty acids and omega-6 (n-6) fatty acids.

1.2 LIPID DIGESTION AND METABOLISM

1.2.1 Fatty Acid Digestion and Absorbtion

Lipid digestion in the newly hatched fowl is limited and is mainly facilitated by lipases secreted from the yolk sac (Carew et al., 1972; Noy et al., 1996). However, in growing birds other factors such as bile salt secretion, fatty acid binding protein synthesis and pancreatic lipase and colipase secretion play important roles in increasing lipid digestibility in birds (Krogdahl and Sell, 1985). The basic mechanism involved in lipid digestion is the breaking down of dietary large, complex molecules into smaller molecules which can be absorbed across the intestinal wall, carried in the blood, and diffused into body tissues. The majority of dietary lipids in poultry are in the form of triglycerides (Schjeide et al., 1963). Dietary fat digestion starts with the initiation of triglyceride emulsification in the gizzard (gastric lipase from proventriculus) (Krogdahl and Sell, 1985). However, most of the digestion and absorption of fats occurs at the duodenum and jejunum in chickens (Sklan et al., 1978). Pancreatic lipase with the aid of colipase, hydrolyses the triglyceride molecules into monoglycerides and free fatty acids. The monoglycerides, free fatty acids and dietary phospholipids are then emulsified by bile and form a complex known as micelles (Garrett and Young, 1975). Bile contains amphiphilic molecules with a hydrophobic surface that interacts with the monoglycerides and free fatty acids of the emulsion, and a hydrophilic surface that interacts with water to form micelles (Borgstrom, 1967). The micelles are absorbed through the brush border and enter the enterocyte in the intestine. In the enterocyte, micelles dissociate and lipids re-form into triglycerides and phospholipids. Triglycerides are packaged into portomicrons, which are lipoproteins that directly enter the portal venous system, and via the liver are sent to other tissue (Hermier et al. 1996).

1.2.2 Fatty AcidTransport

Following absorption, the dietary fatty acids undergo many metabolic transformations in the chicken. Dietary fat is transported from the avian intestine to the liver as portomicrons

(Fraser et al., 1986). The absorbed fatty acids from the diet, as well as *de novo*-synthesized fatty acids from the liver are then re-packaged and transported to other tissues in the form of very low density lipoproteins (VLDL; Leclercq et al., 1990). The fatty acids are released from VLDL after hydrolysis by lipoprotein lipase, and are taken up into the tissues and integrated into triacylglycerols or phospholipids in the tissue. In adipose tissue, the majority of fatty acids are stored in the form of triacylglycerol (Katz et al., 1966) whereas in breast muscle, there is greater proportion of phospholipids compared with triacylglycerol (Pikul and Kummerow*,* 1989*).* Fatty acids can be oxidized to yield ATP, be deposited for storage of energy (fat depot) or serve as precursors for the synthesis of the longer chain PUFA (Poureslami et al., 2010).

In laying hens, estrogen stimulates the liver to synthesize specialized yolk-targeted VLDL (VLDLy) and transport them through the blood to the ovary for incorporation into the growing oocyte (Luskey et al., 1974; Walzem et al., 1999). The VLDLy provides about 93% of the yolk lipids (Speake et al., 1998). The VLDLy particles secreted by laying hens have some very specific structural and biochemical differences from regular VLDL that allow them to be transported from the liver to the ovary rather than be used to fuel cellular metabolism or be deposited in fat depots (Bacon et al., 1978). The regular VLDL has at least six apolipoproteins (including apoA-I, apoB and apoC; Chan et al., 1976; Kudzma et al., 1979; Lin et al., 1986). In contrast VLDLy has a specific apoliprotein (VLDL-II) on its surface in addition to the standard apoliprotein B found on regular VLDL particles (Nimpf et al., 1988). The apoliprotein VLDL-II found on each VLDLy particle prevents hydrolysis by lipoprotein lipase (Aydin, 2005). In addition, VLDLy has a much smaller and more uniform size than that of regular VLDL. The small size of about 25 to 30 nm allows yolk VLDL to cross the basal lamina surrounding the

ovarian follicle (Evans et al., 1979), as well as pass through the interstitial space between individual granulosa cells (Griffin and Perry, 1985).

1.2.3 Fatty Acid Transformation

Absorption and transportation of diet-derived fatty acids in the chicken does not change the composition. Thus dietary fatty acids have major effect on fat deposition in eggs or tissues (Scaife et al., 1994). However, the effect of dietary fatty acids on overall fatty acid composition of tissues (Crespo and Esteve-Garcia., 2002) or eggs (Cruickshank, 1934) can be diluted with the de novo fatty acid synthesized by the bird. In addition, fatty acids can be either oxidized or transformed into longer chain metabolites through elongation and desaturation.

1.2.3.1 De Novo Fatty Acid Synthesis

The primary site of de novo fatty acid synthesis in chickens is the liver, with a very limited ability in adipose tissue (Leveille et al., 1968; Saadoun and Leclercq 1987). Fatty acid synthesis occurs in two distinct steps in the cytosol of liver or adipose cells. In the first step, acetyl-CoA is converted to malonyl-CoA, catalyzed by biotin-containing acetyl-CoA carboxylase (Figure 1.2). The second step is conversion of malonyl-CoA to palmitate via successive acetyl-CoA linkages (a sequential addition of two carbons to a fatty acid chain) catalysed by a multifunctional complex called fatty acid synthase (Wakil et al., 1983). The first product of de novo fatty acid synthesis in layer or broilers is C16:0. However, SFA beyond the 16-carbon length can be further elongated to C18:0 and C20:0; or desaturated to the MUFA, C16:1, C18:1, and C20:1 by desaturase and elongase.

1.2.3.2 Elongation and Desaturation

Fatty acid chain elongation beyond 16 carbon SFA and MUFA occurs primarily in the endoplasmic reticulum and mitochondria of liver, brain and other tissues (Wakil, 1989).

Chickens have a greater ability to elongate fatty acid chains than other species, linked to greater activity of Elovl 5 that has ability to elongate DPA to 24:5n–3 in addition to Elovl 2, unlike mammals in which only Elovl 2 has elongation ability (Figure 1.3; Gregory et al., 2013).

The desaturation process, which occurs in the endoplasmic reticulum, involves the removal of two hydrogens, resulting in the introduction of a double bond in the fatty acid chain Ntambi, 1999). The desaturases act at specific locations in the fatty acid chain (Nakamura and Nara, 2004). For example, Δ5 desaturase and Δ9 desaturase insert a double bond in between 5th and 6th carbon and 9th and 10th carbon from the carboxyl end of the fatty acid chain, respectively. Animals have Δ9 desaturase, Δ6 desaturase and Δ5 desaturase, but lack the ability to desaturate beyond the 9th carbon from the carboxyl end (Nakamura and Nara, 2004). Plants and insects each have Δ12 desaturase and Δ15 desaturase, which allows them to synthesize n-6 PUFA and n-3 PUFA, respectively (Griffiths et al., 1996; Sayanova et al., 2006). Linoleic acid (18:2 n-6; LA) and ALA and are precursors for long chain n-6 PUFA and n-3 PUFA, respectively (Emken et al., 1994). Chickens lack Δ12 desaturase and Δ15 desaturase. Therefore both LA and ALA are essential fatty acids for laying hens and broilers and thus must be provided through dietary supplementation (Balnave, 1971).

The Δ9 desaturase is the most expressed desaturase enzyme in the chicken and its activities are tissue-specific and gender-dependent (Dridi et al., 2007). Female chickens have greater Δ9 desaturase activity in kidney, breast muscle, proventriculus, and intestine than males (Dridi et al., 2007). In addition, the Δ9 desaturase actions are sensitive to the nutritional state and dietary fatty acid type with high PUFA inhibiting the Δ9 desaturase activity (Ntambi, 1999).

The other two desaturases in chickens, the Δ6 desaturase and the Δ5 desaturase are involved in long chain PUFA synthesis (Watkins, 1991). Birds can desaturate and elongate

dietary LA and ALA. The details of the metabolic pathways of desaturation and elongation of essential fatty acids occurring in poultry are shown in Figure 1.3. Linoleic acid is converted into γ-linolenic acid (GLA; 18:3n-6) by Δ 6-desaturase and then GLA can be elongated to dihomo-γ-linolenic acid (DGLA; 20:3n-6). The DGLA can then be desaturated further by Δ5-desaturase to yield arachidonic acid (AA; 20:4n-6; Schmitz and Ecker, 2008). Using exactly the same set of enzymes as used to metabolize LA, ALA can be converted into EPA. In addition, EPA can be further converted to DHA following two elongations, one step of desaturation by Δ6-desaturase and a peroxisomal β-oxidation step (Sprecher, 1981). The pathway shown in Figure 1.3 shows that there is step-wise competition for the same set of enzymes between the metabolites of LA and ALA for the further desaturation and elongation. The Δ6 desaturase is the rate-limiting factor for the bioconversion of ALA to SDA, and desaturation of 24:5n-3, to form DHA (Huang et al., 2011; Gregory et al., 2011). Interestingly, LA and ALA compete for Δ5 and Δ6 desaturase for the bioconversion to long chain n-6 PUFA and n-3 PUFA, respectively (Rodriguez et al., 2001). Although ALA has about 10 times greater affinity for both desaturases as compared with LA (Mohrhauer et al., 1967), the predominance of LA in western diets is causing health concerns due to an increased need of dietary long chain n-3 PUFA by humans (Simopoulos, 1991).

1.2.3.3 Beta-Oxidation of Fatty Acids

Dietary fatty acids, as well as de novo synthesized fatty acids can undergo β-oxidation. Fatty acid β-oxidation is a multi-step breakdown of a fatty acid to yield energy by various tissues. The mitochondria are the site for most β-oxidation in poultry (Bartlett and Eaton, 2004). However, β-oxidation of very long chain fatty acids occurs in the peroxisome as fatty acids greater than 22 carbons in length cannot enter the mitochondria (Wanders et al. 2001). The β-oxidation pathway is functionally similar in both the mitochondria and the peroxisome, and

consists of four subsequent steps: (1) dehydrogenation, (2) hydration, (3) dehydrogenation again and (4) thiolytic cleavage (Bartlett and Eaton, 2004). Avian mitochondria have the capability to select fatty acids for β-oxidation to release energy as a preferred substrate compared with glycolytic fuel (pyruvate) in mammalian mitochondria (Kuzmiak-Glancy and Willis, 2014). However, the rate of β-oxidation is inversely proportional to the chain length but directly proportional to the number of double bonds in the fatty acid chain in rat liver (Lopes-Cardozo and Van den Bergh, 1974). In addition, the rate of β-oxidation of PUFA is greater than SFA in broilers (Sanz et al., 2000). Furthermore, the dietary intake of n-3 PUFA up-regulates the genes for mitochondrial proteins responsible for increasing β-oxidation and decreasing fatty acid synthesis in mice (Flachs et al., 2005). Dietary n-3 PUFA compared with MUFA increase the mitochondrial as well as peroxisomal β-oxidation of SFA in the rat (Halvorsen et al., 2001). In addition, the rate of β-oxidation of ALA is negatively correlated with the efficiency of bioconversion into LC n-3 PUFA metabolites (Vermunt et al., 2000). Overall, the extent of fatty acid deposition in chicken is the result of absorption, de novo synthesis, and β-oxidation of fatty acids (Poureslami et al., 2010). However, there are some differences in laying hens as compared with broilers regarding fatty acid transport to tissues.

1.2.3 Lipid Metabolism: Broiler versus Layer Chickens

Broilers are genetically selected for rapid growth and high meat yield whereas for laying hens, the target is high egg production (Whitehead et al, 1984). Therefore there are some physiological differences in energy utilization in broilers and layers, especially with regards to the efficiency of lipid mobilization from the adipose tissue (Griffin et al., 1991). The differences in lipid metabolism start as early as the embryonic stage, with 27% greater yolk utilization in broilers chicks as compared with layer chicks (Sato et al., 2006). Broilers have greater uptake of

fatty acids into adipose tissue compared with laying hens because of a higher number of adipose cells and a greater adipose lipase activity (Hermier et al., 1989). In addition, broilers have greater hepatic Δ9 desaturase activity, which is thought to facilitate the incorporation of fatty acids into VLDL and their subsequent deposition into the extra hepatic tissues (Legrand et al. 1987). On the contrary, laying hens, probably due to the effects of estrogen, have a greater capacity for liver de novo fatty acid synthesis to meet the increased lipid demand for yolk formation (Lushkey et al., 1974; Dashti et al., 1983). In conclusion, the greater lipogenic capabilities in laying hens compared with broilers (Calabotta et al., 1985) provides greater efficiency in lipid turnover and lipid deposition.

1.2.4 Factors Affecting PUFA Metabolism in the Chicken

The transformation of PUFA into long chain metabolites from 18-carbon chain precursor in poultry is influenced by the metabolic demand of tissues, dietary fatty acid composition and hepatic activity of desaturation and elongation enzymes (Pourslami et al, 2010; Kartikasari et al., 2012). The absolute amount of substrate (ALA) in the diet is a prime factor in determining the rate of bioconversion efficiency in chickens (Goyens et al. 2006). However, there is enzymatic competition between LA and ALA for the same enzymes for the desaturation and elongation steps to synthesize AA and EPA, respectively (Figure 1.2). Therefore, a greater ratio of LA/ALA is expected to lower the conversion efficiency of ALA to LC n-3 PUFA (Goldberg et al. 2012). In addition, increases in dietary LC n-3 PUFA also decrease bioconversion efficiency in laying hens, presumably due to product down-regulation of desaturation and elongation (Cachaldora et al., 2008). In addition, broilers fed flax oil (high in n-3 PUFA) had increased rate of lipid oxidation along with increased de novo fatty acid synthesis compared with those fed with olive oil (high MUFA) or tallow (high SFA; Crespo and Estve-Garcia, 2002). Chickens have greater

elongase activity than mammals and fishes (Gregory et al., 2013; Gregory and James, 2014). In addition, the genes activities of the desaturase and elongase increases with bird age and also increases with a lower LA:ALA ratio in diets (Jing et al., 2013). Therefore, the maximum bioconversion efficiency in laying hens or broilers is expected with diets higher in ALA, lowered in both LA and in LC n-3 PUFA.

1.3 OMEGA-3 PUFA ENRICHMENT SOURCES IN POULTRY

Various sources of n-3 PUFA can be included in the diets of laying hens or broilers for n-3 PUFA enrichment of eggs or meat. The oils from land-based plant sources of n-3 PUFA are good source of medium chain n-3 PUFA, mainly ALA, (Schuman et al., 2000; Jia et al., 2008; Nain et al., 2012), whereas certain marine plants or fishes are good sources of long chain n-3 PUFA such as EPA and DHA (González-Esquerra and Leeson, 2001; Herber and Van Elswyk, 1996).

1.3.1. Marine Omega-3 PUFA Enrichmnet Source

Cold-water marine fishes, most notably herring, halibut, mackerel, sardine and salmon are predominant sources of EPA and DHA in human diets (Kris-Etherton et al., 2000). The dietary inclusion of oil from anchovy, herring, tuna or menhaden has been widely used enrichment of LC n-3 PUFA in laying hens (Hargis et al., 1991; Cachaldora et al., 2006) as well as broilers (Miller et al., 1969; Haung et al., 1990). However, oil from these fishes varies in EPA and DHA content and hence influences the level of enrichment in the poultry products (Cachaldora et al., 2006). Marine algae rich in EPA, such as *Nannochloropsis* and *Phaeodactylum* and rich in DHA, such as *Schizochytrium* and *Thraustochytrium* (Adarme-Vega et al., 2012) have been included in laying hen rations to increase LC n-3 PUFA proportions in

eggs (Bruneel et al., 2013; Lemahieu et al., 2013) and broiler breast muscle (Mooney et al., 1998). The decline in global fish stocks, issues of contamination with heavy metals and environmental pollutants such as dioxins and polychlorinated biphenyls in fish, and the off-flavors associated with feeding fish oil to laying hens cause problems for the enrichment of eggs in LC n-3 PUFA (Fraeye et al., 2012). In addition, aquaculture consumes most of the fish oil and fishmeal harvested (Naylor et al., 2000), thus increasing the cost of fish oil indicating the dire need of lower cost alternative sources of LC n-3 PUFA (Tacon and Metian, 2008). The dietary inclusion of marine algae oil provides an alternate to fish oil dependency. However, the production of oil from algae is still not cost effective (Halim et al., 2010). Therefore, novel LC n-3 PUFA enrichment sources are needed to achieve recommended intakes of these fatty acids.

1.3.2. Land-Based Omega-3 PUFA Enrichment Sources

The sources of land based n-3 PUFA are oil from flaxseed, camelina, canola, chia, and perilla which contain about 59.8%, 38.1%, 10.1%, 58.2%, and 60.9% of ALA, respectively (Ciftci et al., 2012). Flaxseed is the major source of n-3 PUFA enrichment used in the poultry industry (Schuman et al., 2000). Humans can obtains ALA in small proportions from nuts (butternuts and walnuts), fruits (strawberries and raspberries), vegetables (purslane, spirulina), legumes (beans and peas) and grain products (wheat germs; Kris-Etherton et al., 2000).

1.3.2.1. Camelina in Poultry

There is increased interest for finding non-traditional, low-cost feedstuffs to enrich n-3 PUFA in poultry products, not only for economic reasons but also for their valuable nutritional properties. *Camelina sativa*, also known as false flax, gold-of-pleasure, wild flax, linseed dodder, German sesame, or Siberian oilseed, is an ancient European crop rich in n-3 PUFA (Ghamkhar et al., 2010). Camelina is early maturing and frost resistant (Plessers et al., 1962). In addition,

camelina offers greater disease and drought tolerance, provides consistent yields and therefore has lower-cost production compared with other oilseed crops (Gugel and Falk, 2006). Camelina oil contains less than 1% of free fatty acid making it suitable for jet fuel, biodiesel and high-value industrial lubricants (Drenth et al., 2014). Camelina cake, the co-product obtained after oil extraction, has a crude protein content of 35% and a residual oil content of 10 to 20% (Nain et al., 2015). Approximately 30% of the total fatty acids in the camelina cake is ALA. The use of co-products from biofuel production, such as camelina cake in poultry diets, can be an effective strategy in reducing feed cost along with increasing n-3 PUFA enrichment.

The evaluation of anti-nutritional factors present in novel feedstuffs intended for inclusion in poultry rations is of utmost important. Camelina seed contains anti-nutritional factors such as glucosinolates and erucic acid (C22:1; Thacker and Widyaratne, 2012). Glucosinolates may affect thyroid and liver function (Tripathi and Mishra 2007). However, glucosinolates in camelina cake (22 μmol/g) are lower in quantity than in rapeseed meal (118 μmol/g; de Lange et al., 2000). In addition, the erucic acid is about the one-third level of the maximum permissible limit of 5% of total fatty acids allowed for human consumption by European authorities (Eur. Communities, 1976). Erucic acid has been associated with myocardial lipidosis of heart muscle in rats (Kramer et al., 1992) and nursing pigs (Kramer et al., 1990). Several studies have been conducted using various levels of camelina cake in broiler chickens (Acamovic et al., 1999; Aziza et al., 2010; Nain et al., 2015) and laying hens diets (Rokka et al., 2002; Cherian et al., 2009; Kakani et al., 2012), aiming to assess production performance and efficiency of enrichment of n-3 PUFA in poultry products. These studies indicate that feeding camelina cake at up to 10% of the diet did not affect growth, performance and feed consumption, nor meat and egg quality. However, the maximum inclusion level of camelina in the diets for the

n-3 PUFA requirement needs to be evaluated. Camelina cake has not been registered as a livestock feed in Canada. However, recently 12% inclusion of camelina cake has been approved for broiler ration by the Canadian Food Inspection Agency (CFIA, 2015).

1.3.2.2. Genetically Modified Flaxseed in Poultry

During the past several years, great progress has been made in characterizing and isolating the plant genes responsible for desaturation and elongation that regulate overall fatty acid composition. The genes encoding Δ15-desaturase, Δ12-desaturase Δ5-desaturase, Δ6 desaturase (Betancor et al., 2015) and elongase (Meyer et al. 2004) can now be introduced into oilseed plants to change their oil fatty acid composition (Ruiz-Lopez et al., 2014). Flax can be genetically altered to express a high level of SDA in the oil. The Δ5-desaturase gene from *Pythium irregular* and elongase gene from *Thraustochytrium* were isolated, re-amplified and inserted into a plant expression vector (Wu et al., 2005). The SDA-enhanced flax line was transformed by *Agrobacterium*-mediated plant transformation, and the plants were grown to subsequent generations in environmentally controlled growth chambers (Subedi et al., 2014).

Genetically modified SDA-enriched soybean has already received "generally recognized as safe" status in the USA (FDA: GRN 000283, 2009). There are few studies using genetically modified SDA-soybean in diets for the LC n-3 PUFA enrichment in broiler meat (Rymer et al., 2011) as well as table eggs (Elkin et al., 2015). However, feeding the SDA-rich oil to broilers had negative effects on the sensory quality (fishy odor) of reheated leg meat but not in freshly cooked leg meat (Rymer et al., 2011). Genetically modified oil seeds with greater LC n-3 PUFA contents are an alternative to the decreasing stocks of oily fish (Ruiz-Lopez et al., 2014). The production of genetically modified canola, corn, cotton and soybean has increased in last two decades (James, 2013). However, the global acceptability of genetically modified crops has been

an issue in past. A genetically modified flaxseed (tolerant to herbicide residues in soil) was approved for food and feed use by the Canadian government in 1996, but after concerns from the Flax Council of Canada over export to European countries, it was deregistered by the CFIA in 2001 (Vanella et al., 2014).

1.4 ESSENTIAL FATTY ACIDS AND HEALTH IMPACTS IN HUMANS

Many government and scientific organizations have recommended reduced SFA consumption and increased PUFA consumption in human diets for the prevention of cardiovascular disease (Gebauer et al., 2006). Both ALA and LA are essential fatty acids and are the precursors for long chain n-3 PUFA and n-6 PUFA respectively (Emken et al., 1994). The metabolites of n-3 and n-6 PUFA form plasma membrane structure, and modulate expression of genes involved in lipogenesis, glycolysis, and inflammatory mediators required for optimal growth, development and response to injury and infection (Marik et al., 2009). However, the eicosanoid metabolites resulting from oxygenation of n-3 and n-6 PUFA (prostaglandins, thromboxanes, leukotrienes and lipoxins) modulate less-inflammatory and more-inflammatory responses, respectively (Tapiero et al., 2002). The eicosanoid metabolites of n-6 PUFA causes irreversible aggregation of human platelets, and induction of smooth muscle contraction that may affect various physiological and pathological conditions in human (Funk, 2001). However, the bioactive mediators derived from LC n-3 PUFA are less potent in their inflammatory actions than those derived from n-6 PUFA (Wada et al., 2007). Therefore, decreasing dietary n-6 PUFA (Wood et al., 2014) or increasing dietary n-3 PUFA could be a helpful strategy for the prevention of cardiovascular disease, cancer, diabetes, and neurodegenerative diseases in humans (Yashodhara et al., 2009). The LC n-3 PUFA exert anti-inflammatory effects in adipose tissue

and prevent metabolic disorders by inhibiting the nuclear factor-κB, responsible for chronic inflammation (Siriwardhana et al., 2012).

The n-3 and n-6 PUFA have more double bonds than MUFA, making them more susceptible to oxidative damage (Narciso-Gaytan et al., 2011). Peroxidation of n-3 and n-6 PUFA results in formation of aldehydes, 4-hydroxy-2-hexenal and 4-hydroxy-2-nonenal respectively, which can damage proteins, DNA, and phospholipids (Guichardant et al., 2006). Consumption of oxidized LC n-3 PUFA can cause inflammation in the upper intestine (Awada et al., 2012). Inclusion of vitamin E in the diets can prevent the oxidative damage to the LC n-3 PUFA in the egg yolk (Ren et al., 2013). However, the excessive intake of n-3 PUFA can cause health problems associated with increased bleeding times (Thorngren and Gustafson, 1981) and increased risk for hemorrhagic stroke (Pedersen et al., 1999). Therefore, the FDA had recommended that daily intakes of EPA and DHA from supplements should not exceed 3 g/d (FDA, 2004).

1.4.1. Recommendations for Humans Regarding Omega-3 PUFA Intake

Most of the proven health benefits of n-3 PUFA in humans are associated with the LC n-3 PUFA, EPA and DHA. However, ALA and LA are essential fatty acids and must be provided through the diet in humans. The typical diet of modern Western society is highly skewed with a greater proportion of n-6 PUFA compared with n-3 PUFA leading to imbalance in the ratio of n-6 PUFA to n-3 PUFA (15 to 20:1; Simopoulos, 2006). A ratio of 4:1 or lower of n-6 to n-3 PUFA in the diet can contribute to a reduction of chronic diseases, including coronary heart disease, diabetes, arthritis, cancer, osteoporosis and age-related macular degeneration in humans (Benatti et al., 2004; Simopoulos, 2008). DHA is an important constituent of phospholipids of the brain grey matter (25% of the total fatty acids) and in retinal rod segments (50% of the total

fatty acids; Anderson, 1970). Therefore, the DHA demand is increased in pregnant and nursing women to fulfill the metabolic need for fetal visual and brain development (Helland et al., 2003). The dietary intake recommendation for n-3 PUFA varies with gender, age, or disease conditions. A summary of dietary recommendations issued by various health and nutrition organizations for the n-3 PUFA intakes in humans is presented in Table 1.1. The Dietitians of Canada recommended an intake of 1.6 g/day for male and 1.1 g/day for females with 500 mg/day of LC n-3 PUFA in their diets (Kris-Etherton et al., 2007).

In Canada, for a product to be labeled as a dietary source of n-3 PUFA it must have 300 mg of total n-3 PUFA per serving (CFIA, 2003). However, other countries such as Australia, New Zealand and the Europe Union require specification of the amount of LC n-3 PUFA (EPA and DHA) along with total n-3 PUFA. For example, for a product to be labeled as a dietary source of n-3 PUFA, the European Union requires that it must contain a total of 40 mg of EPA + DHA along with 300 mg of ALA per serving. A summary of regulatory conditions to be fulfilled in order to sell or claim n-3 PUFA enriched products in various countries is presented in Table 1.2.

1.4.2. Effects of Omega-3 PUFA on Broiler and Layers

Camelina and flaxseed in poultry diets can supply the essential fatty acid ALA, but also increase the LC n-3 PUFA (EPA, DPA and DHA) in laying hens (Nain et al., 2012) and broilers (Nain et al., 2015). High dietary ALA in laying hen diets increased immunoglobin (IgG) production in the hatchling (Wang et al., 2004). Additionally, increasing the maternal dietary n-3 PUFA may reduce the inflammatory-related disorders in the chicks (Hall et al., 2007). Dietary inclusion of fish oil (high in LC n-3 PUFA) in the broiler chicken increased the BW gain relative to broilers fed tallow (Korver and Klasing, 1997; Al-Khalifa et al., 2012). In addition, there was

an increase in the immune response in broiler chicks fed fish oil compared with those fed corn oil to simulated infectious challenges (Korver et al., 1998; Maroufyan et al., 2012). The supplementation of LC n-3 PUFA to broiler breeders resulted in elevated hepatic EPA and DHA concentrations of the offspring at hatch (Koppenol et al., 2015). In addition, an increased incidence of liver hemorrhage and greater oxidative status (Schumann et al., 2000) has been reported with 10 months feeding laying hens with flaxseed or oil (Bean and Leeson, 2003). However, inclusion of an anti-oxidant such as lutein (Leeson et al., 2007) or vitamin E along with n-3 PUFA source helps in increasing whole body antioxidant capability against the oxidative damages associated with high dietary levels of n-3 PUFA and liver functions in birds (Lu et al., 2014).

1.5 CHALLENGES FOR N-3 PUFA ENRICHMENT

Market survey results suggest that consumers are willing to pay a premium for the perceived health benefits from the n-3 PUFA-enriched products in Canada (Pickering, 2003). The poultry industry can capitalize on consumer preferences by formulating diets for chickens to create specialty enriched products that have the potential to contribute to human health. In addition, increasing the efficiency of existing enrichment methods could also increase the economic profitability of the functional food market that is globally estimated up to $33 billion US (Menrad, 2003).

The biggest challenge when feeding novel alternative sources of n-3 PUFA enrichment is to quantify the minimum enrichment levels of ingredients needed in the base feed to ensure enrichment of the product. The appropriate duration and inclusion level when added to poultry diets to enrich the product to a specified level of enrichment is also necessary (Lopez-Ferrer et

al., 2001; Kralik et al., 2008). The characterization of inclusion levels of dietary n-3 PUFA and feeding duration can help in reducing the cost of production in addition to achieving the required enrichment level in meat (Zuidhof et al., 2009) or eggs (Nain et al., 2012). In addition, the potential interaction of dietary n-3 PUFA source with other dietary lipid classes in order to enrich the egg or meat need to be addressed. The efficiency of deposition of n-3 PUFA in product depends on the dietary level of n-3 PUFA, as well as the dietary ratios of n-6 to n-3 PUFA, and LC n-3 PUFA to total n-3 PUFA too (García-Rebollar et al., 2008; Cachaldora et al., 2008).

It is also necessary to analyze the effect of enrichment source on the bird‘s growth, production performance, and overall health. The presence of anti-nutritional factors such as glucosinolates and erucic acid (C22:1) in camelina oil (Thacker and Widyaratne, 2012) and mucilage and linatine in flaxseed (Bhatty, 1993) might have negative effect on birds health and performance. The negative effects of flaxseed are associated with the feeding flaxseed meal rather than including flax oil in broilers (Lee et al., 1991; Olomu et al., 1991). However, the use of processing techniques such as pelleting, autoclaving, microwave roasting (Shen, et al., 2005) and extrusion (Wu et al., 2008) increases nutrient utilization and potentially reduces the negative effect of anti-nutritional factors present in flaxseed for broilers (Alzueta et al., 2003, Thacker et al., 2005).

1.6 RESEARCH APPROACH

During the 20th century, a 1,000-fold increase in the estimated per capita consumption of soybean oil (high in n-6 PUFA) has skewed the dietary ratio of n-6 PUFA to n-3 PUFA in the USA resulting in a net decrease in the levels of n-3 PUFA (EPA and DHA) in human tissues

(Blasbalg et al., 2011). Research focused on alternate sources of n-3 PUFA enrichment for the production of value-added poultry products (egg or meat) through dietary manipulation of the poultry rations is a growing area. The most common sources included for the n-3 PUFA enrichment process in laying hen and broiler industries are either flaxseed for ALA or fish oil for the LC n-3 PUFA. Previous studies have focused on flaxseed (Nain et al., 2012) or inclusion of alternate sources such as chia seed (Ayerza and Coates, 2001; Azcona et al., 2008), or marine algae (Herber and Van Elswyk. 1996; Fredriksson et al., 2006) in the poultry ration. The aim of this thesis was to develop n-3 PUFA-enriched chicken meat and eggs by incorporation of alternative sources such as camelina cake for ALA or SDA-enhanced flaxseed for increasing LC n-3 PUFA in products. This thesis provides insight for understanding the efficacious use of novel n-3 PUFA sources for n-3 PUFA enrichment of chicken meat and laying hens eggs.

1.6.1 Objectives

The overall objective of this research was to investigate inclusion of novel n-3 PUFA enrichment sources in the broiler or laying hen ration. This thesis focuses on increasing the LC n-3 PUFA proportion in the final enriched product (breast and thigh meat in broilers and table eggs in layers). In addition, the dietary effects on tissues were also evaluated. To achieve the objectives, a series of experiments including camelina cake and SDA-enhanced flaxseed in the diets were conducted.

Experiment 1: Purpose: To analyze the enrichment responses to increasing inclusions of camelina cake in typical broiler rations on the fatty acid composition of breast meat and thigh meat. The aim was to analyze if the increased ALA in the diet might have been equally available for its desaturation and chain elongation in different tissues.

Description: The study was designed to assess the novel feedstuff, camelina cake, a co-product of biodiesel production, to enrich n-3 PUFA in broiler tissues. Diets were formulated with increasing level of camelina cake (CC) in broiler diets and fed in each of 3 growth phases (starter (0 to 14d); grower (15 to 28d); and finisher (29 to 42d). The idea was to determine the combination of CC level and duration of feeding to achieve the labeling claim of 300 mg n-3 PUFA/100 g meat. The dietary treatments consisted of 4 inclusion levels of screw-pressed CC at 0, 8, 16 or 24%. On each of d 14, 28 and 42, 3 birds were randomly selected from each test pen (total of 18 birds/treatment) and euthanized to collect thigh, breast, liver and brain tissue samples. The tissue samples (n=6/treatment) were analyzed for fatty acid composition.

Experiment 2: Purpose: To examine the potential for including a novel SDA-enhanced flaxseed compared with traditional flaxseed to enrich table eggs in n-3 PUFA with special focus on the long-chain n-3 PUFA (EPA, DPA and DHA).

Description: A genetically-modified flaxseed oil enriched in SDA (25%) and GLA (16%), was compared to regular flaxseed oil for its ability to contribute to increase LC n-3 PUFA in table eggs. Twenty-four individually-caged White Leghorn laying hens (47 wk) were fed one of 3 diets for 21 d as follows: Control (basal diet + 4% corn oil), REGflax (basal diet + 4% standard flax oil) or SDAflax (basal diet + 4% SDA-enriched flax oil). At d 0, 7, 14 and 21, eggs from each hen were analyzed for fatty acid composition. After 21 d of feeding, liver, thigh, breast, heart, brain, and abdominal fat pad were analyzed for fatty acid composition to determine wheather the potential n-3 PUFA enrichment for egg yolk might have been diverted to other tissues in the body.

Experiment 3: Purpose: To analyze the lipid interaction between the n-3 PUFA in the SDA-enriched flax oil or regular flax oil with the n-3 and n-6 PUFA from canola, corn or fish oil fed.

Description: This study was designed to explore the effect of feeding SDA-enhanced flaxs oil on egg LC n-3 PUFA enrichment, when fed in dietary combination with canola corn or fish oil. A total of 120 individually-caged White Leghorn hens (47 wk old) were fed one of 15 dietary combinations. Three Control diets contained 4.5% of either canola oil (CAN), corn oil (CO), or fish oil (FO), with no flax oil. To investigate competition among fatty acids during enrichment, three sets of diets with 2% CAN, CO or FO in addition to either 2.5 or 5% of regular flax oil (Regflax) or SDA-enriched flax oil (SDAflax) were formulated. Production traits such as egg production and egg quality (albumen height and shell thickness), feed intake, total egg mass and BW change were measured. The eggs yolks and livers at 35 d were collected for fatty acid analysis. In addition, At 35 d, the ovary weight and number of large yellow follicles were recorded to assess reproductive status. The outcomes of this research will broaden knowledge of whether the inclusion of SDA enhanced flax oil can provide a viable alternative to marine oils for LC n-3 PUFA enrichment of eggs, and whether there will be an interaction of the presence of high n-6 PUFA (corn oil), or balanced n-6 to n-3 PUFA (canola oil) or high LC n-3 PUFA (fish oil) in layer diets on egg enrichment.

Table 1.1: Recommendations for the omega-3 polyunsaturated fatty acid consumption in humans.

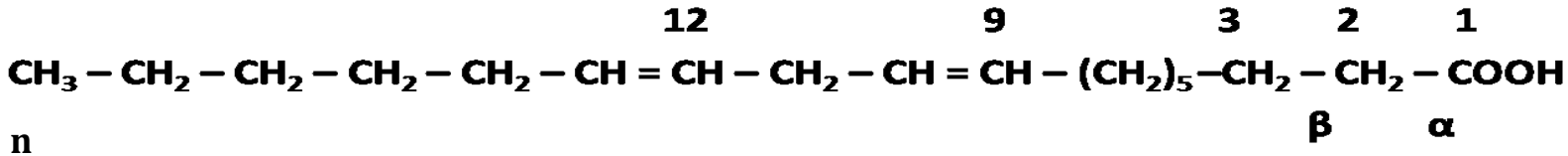
Organization/ Institute	Total n-3 PUFA[1]	Total LC n-3 PUFA[2]
American Dietetic Association and Dieticians of Canada (Kris-Etherton et al., 2003)	1.3 to 2.7 g/day	0.5 g/day
Male	1.6 g/ day	0.5 g/day
Female	1.1 g/ day	0.5 g/day
American Heart Association (AHA, 2011).		
General population		0.5 g/day
Coronary heart disease patients		1 g /day
Patients with high triacylglycerol		2-4 g/ day
National Heart Foundation of Australia (NHF, 2008)	2 g/ day	0.5 g/day
FAO/WHO Expert Consultation (WHO, 2003).		
Adult males and females	0.25- 2.0 g/ day	0.25 g/day
Pregnant and lactating females	0.25- 2.0 g/ day	0.30 g/day
National Health and Medical Research Council of Australia (NHMRCA, 2006)		
Male adults	1.3 g /day	0.19 g /day
Female adults	0. 8 g/day	0.09 g/day
Pregnant and Lactating Females	1.0 g/day	0.115 - 0.145 g/day
Scientific Advisory Committee on Nutrition (SACN, 2004)		0.45 g/day

[1]Total omega -3 polyunsaturated fatty acid
[2]Total long chain omega-3 polyunsaturated fatty acid

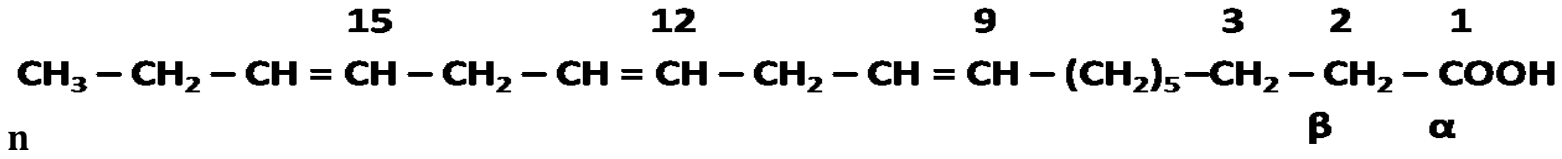
Table 1.2: The labeling requirement for the omega-3 polyunsaturated fatty acid enriched products.

Labeling regulation authority	Total n-3 PUFA[1]	Total LC n-3 PUFA
Canadian Food Inspection Agency (CFIA, 2003)		
"Good source"	≥ 300 mg / 100 g of meat	
Food and Drug Administration (FDA, 2000)		
"High source"	≥ 320 mg of ALA	≥ 32 mg / 100 g
"Good source"	≥ 160 mg of ALA	
European Commission Regulation on Nutrition and Health Claims (European Commision, 2012)		
"Source of omega-3 fatty acids"	≥ 300 mg of ALA / 100g	≥ 40 mg / 100g
"High omega-3 fatty acids"	≥ 600 mg of ALA / 100g	≥ 80 mg / 100g

[1]Total omega -3 polyunsaturated fatty acid
[2]Total long chain omega -3 polyunsaturated fatty acid



Linoleic acid C18:2 Δ9,12; n-6 polyunsaturated fatty acid



α-Linolenic acid C18:3 Δ9,12,15; n-3 polyunsaturated fatty acid

Figure 1.1: The structure of both essential polyunsaturated fatty acids, linoleic acid (n-6 PUFA) and α-linolenic acid (n-3 PUFA), depicting the omega nomenclature, where '**n**' is used to describe the position of double bonds from the methyl end of the fatty acid. The first carbon in carboxyl group is alpha **(α)** and second carbon, following the carboxyl carbon, is the beta **(β)** carbon. The last carbon in the chain, farthest from the carboxyl group, is the omega (n) carbon.

(https://www.uio.no/studier/emner/matnat/farmasi/FRM2041/v06/undervisningsmateriale/fatty_acids.pdf)

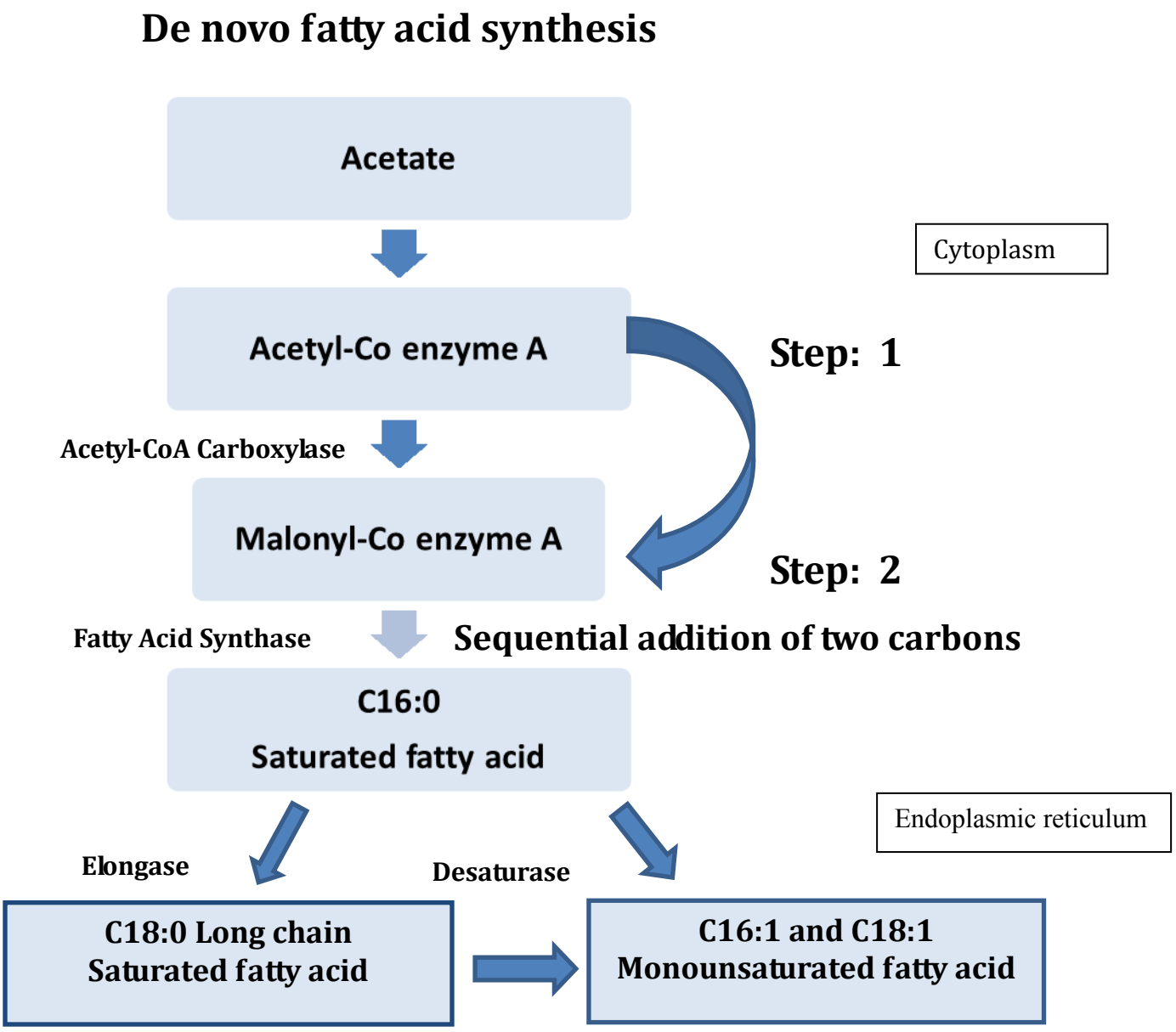


Figure 1.2: The summary of de novo synthesis of fatty acid in chickens.

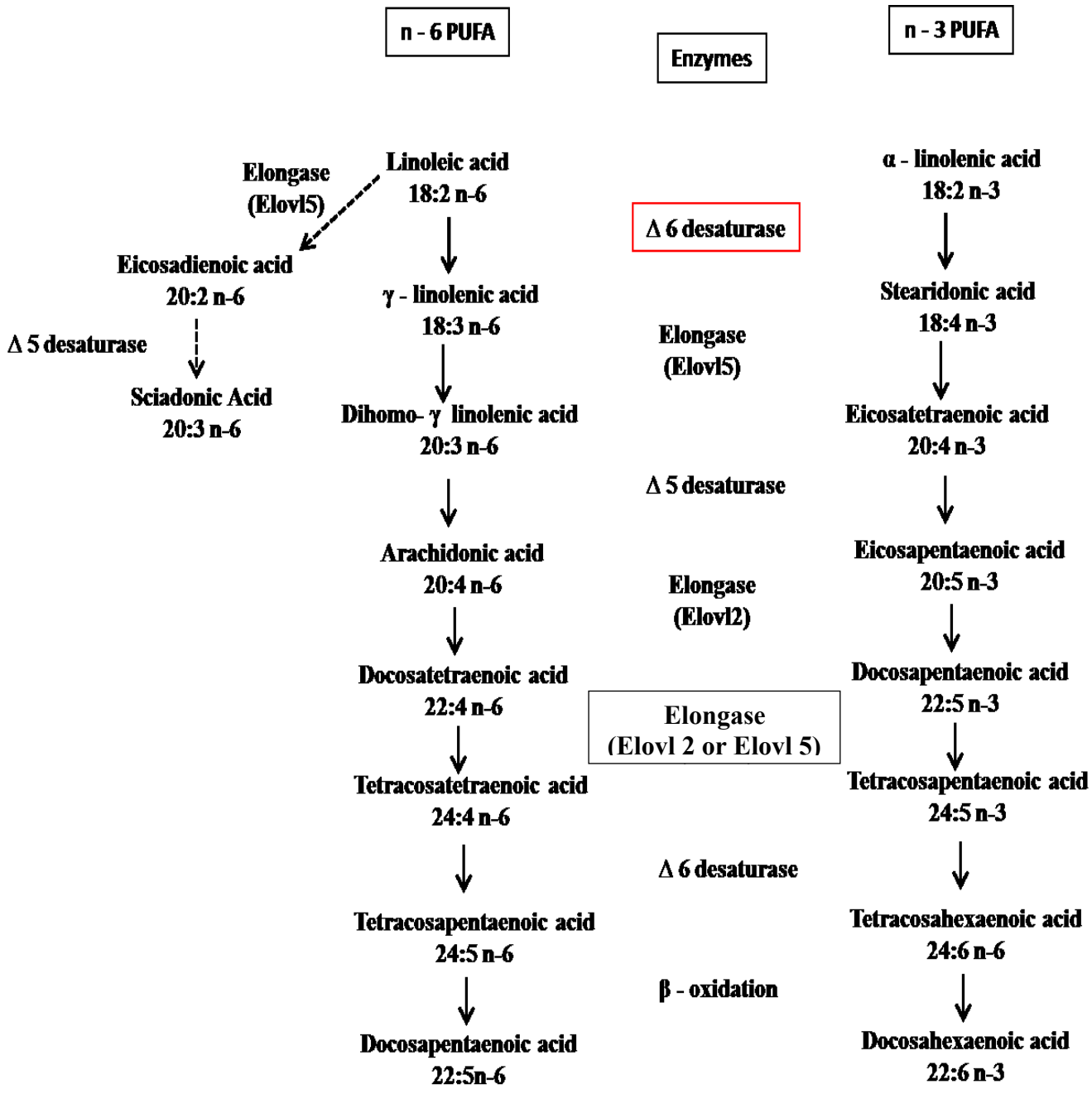


Figure 1.3: The metabolism of n−3 and n−6 polyunsaturated fatty acid involving enzymes of desaturation, elongation, and β-oxidation. The dotted line represent the alternative metablosm of linoleic acid in case of deficiency of Δ6-desaturase.
(Adapted from from Schmitz and Ecker, 2008; Huang et al., 2011; Gregory et al., 2013).

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Chapter 2

Camelina sativa cake for broilers: Effects of increasing dietary inclusion from 0 to 24% on tissue fatty acid proportions at 14, 28, and 42 days of age[#]

ABSTRACT: The benefits to human from the consumption of omega-3 polyunsaturated fatty acids (n-3 PUFA) have been recognized. *Camelina sativa* is an oilseed crop grown for biofuel production. Feeding its cake with 10-20% remaining oil (28-30% α-linolenic acid [ALA]) has potential for n-3 PUFA enrichment of poultry products. An experiment was conducted to assess fatty acid deposition in brain, liver, breast and thigh tissue by increasing inclusions of camelina cake (CC) fed to broiler chickens. Male chicks (744, Ross 308) housed in 24 cages were fed 1 of 4 dietary regimens including 0, 8, 16, 24% CC for 42 d, 6 replicates per CC level. At the end of the starter (14 d), grower (28 d) and finisher (42 d) phases, brain, liver, breast and thigh samples were collected from 3 birds per cage and diets were analyzed for fatty acid content.

Increasing CC inclusion from 0 to 8, 16, and 24%, increased dietary ALA (5.3, 11.1, 15.2, 17.8, respectively) as proportion of total fatty acid content. All diets provided a similar level of long chain (LC) n-3 PUFA (about 0.9%). Increasing dietary CC inclusion linearly increased ($P<0.001$) the proportion of ALA in breast, thigh and liver (by 76, 128, 288%, respectively), but not in brain tissue. Increasing dietary CC inclusion linearly increased ($P<0.001$) the proportion of long-chain n-3 PUFA including docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA) in liver (by 109 and 80%, respectively) and brain (24 and 6%, respectively) tissue. However, in breast and thigh tissue, increasing dietary CC inclusion only increased ($P<0.005$) DPA (by 24 and 27%, respectively). The predominant n-3 PUFA in liver and brain tissue feeding 24% CC was DHA (by 48% and 88%, respectively) unlike breast and thigh meat where it was ALA (65% and 86% respectively). The labeling claim requirement for

n-3 PUFA enrichment (300 mg per 100 g of meat) was exceeded in breast and thigh by feeding the 24% CC diet for 28 d or the 16% CC diet for 42 d, respectively.

Keywords: broiler chicken, camelina, fatty acids, omega-3 PUFA

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2.1 INTRODUCTION

Human dietary intake of the long-chain (LC), omega-3 polyunsaturated fatty acids (n-3 PUFA) eicosapentaenoic acid (**EPA**; 20:5 n-3) and docosahexaenoic acid (**DHA**; C22:6 n-3) can be effective in preventing cardiovascular disease (Din et al., 2013), rheumatoid arthritis (Berbert et al., 2005), and aid in child eye and brain development (SanGiovanni et al., 2000). In addition, evidence from epidemiological studies and clinical trials in humans indicates that dietary supplementation of omega-3 fatty acids may be beneficial in the prevention of some psychiatric conditions and attention deficit hyperactivity disorders (Richardson, 2004, Gillies et al., 2012).

Chicken meat has the highest per capita consumption of any meat in the US (USDA, 2012). The United States Department of Agriculture (USDA) projections further suggest that chicken will be in greater demand in the next decade compared with other meats because of its nutrient value, affordable price, and expected supply (USDA, 2012). In addition, there is a lower energy cost to produce poultry meat (kcal of fossil fuel/kcal of protein) and a lower environmental impact (water needed to produce 1 kg meat) compared with other livestock products (Pimentel and Pimentel et al., 2003). The growth in health conscious consumer markets provides an incentive for fatty acid enrichment of poultry meat, which can be easily enhanced through manipulation of feedstuff composition in their rations (Crespo and Esteve-Garcia, 2002).

Interest is growing in novel feedstuffs to enrich n-3 PUFA in poultry products, not only for economic reasons but also for their valuable nutritional properties. *Camelina sativa*, also known as false flax, gold-of-pleasure, wild flax, linseed dodder, German sesame, or Siberian oilseed is an ancient European crop related to oilseeds crops of the *Brassica* family like mustard, canola, and rapeseed (Ghamkhar et al., 2010). Camelina offers greater disease and drought tolerance and provides consistent yields in brown and dark brown soils. Camelina therefore

offers the potential for lower cost production compared with other oilseed crops (Gugel and Falk, 2006). Camelina seed has a high oil content (>40%) and has potential to be cultivated in the western Prairies (Gugel and Falk, 2006) or Maritime provinces of Canada (Urbaniak et al., 2008). In addition, camelina is early maturing in a short growing season and frost resistant (Plessers et al., 1962), which makes it ideal for cultivation on the marginal lands of the North American Great Plains.

Camelina cake (CC), the co-product after pressing the oil for biofuel production, can benefit the poultry industry as nutrient source for poultry feeding (Acamovic et al., 1999) because of its high crude protein (34%) and gross energy (5,730 kcal/kg; Table 1). In addition, CC contains 10–22% residual oil, of which approximately 30% is alpha-linolenic acid (**ALA**; 18:3 n-3). Camelina cake is also rich in antioxidants such as tocopherols (Budin et al. 1995) and phenolic compounds (Terpinc et al., 2012) compared with other oilseed crops. The protein in CC is a good dietary source of essential amino acids for poultry such as arginine, cystine, lysine (approximately 4.8% of crude protein), methionine and threonine (Zubr, 1997).

Camelina seed, however, contains anti-nutritional factors like glucosinolates and erucic acid (C22:1). Glucosinolates in the cake may affect thyroid and liver function (Tripathi and Mishra 2007). However, glucosinolates in CC (22 μmol/g) are different and in lower quantity than in rapeseed meal (118 μmol/g; de Lange et al., 2000). Erucic acid in camelina oil is present at one-third the maximum permissible limit of 5% in the oils allowed for human consumption by European authorities (Eur. Communities, 1976). Erucic acid has been associated with myocardial lipidosis of the heart muscle in rats (Kramer et a1., 1992). It is not known whether the low levels of glucosinolates and erucic acid in CC could have adverse effects on broilers. However, feeding broilers 25% or higher levels of of rapeseed meal with 4-fold greater glucosinolates level (79

μmol/g) and erucic acid (37 mg/g of meal) compared with broilers fed control (soybean meal) reduced liver weight and BW (Taraz et al., 2004).

There is little information on what a safe inclusion and duration of feeding of CC in broiler rations should be. The inclusion of CC has previously been tested in diets fed to broiler chickens (Ryhanen et al., 2007; Pekel et al., 2009; Aziza et al., 2010; Thacker and Widyaratne, 2012). In these trials, the inclusion of CC in broiler rations was only 10 to 15%. In the present trial, we formulated diets to include up to 24% CC along with a typical western Canadian broiler ration ingredients. The present experiment was conducted to evaluate the lipid deposition response to increasing CC inclusion in broiler diets on fatty acid proportions of brain, liver, breast, and thigh tissue. We hypothesized that increasing CC inclusion in broiler diets would result in a concomitant increase in n-3 (LC) PUFA in these tissues. We also investigated what combination of CC inclusion and length of feeding to broilers would achieve the labelling requirement claim of n-3 PUFA product enrichment (300 mg per 100 g meat; CFIA, 2003).

2.2 MATERIALS AND METHODS

The procedures of trial conduct were reviewed by the University of Alberta's Animal Care and Use Committee, and followed principles established by the Canadian Council on Animal Care (2009).

2.2.1 Experimental Management

Male chicks (n=744: Ross 308) were brought to the Poultry Research Centre at the Edmonton Research Station (Edmonton, AB) on the day of hatching. Upon arrival, chicks were randomly distributed among 24 cages in a modified pullet battery (Specht ten Elsen GmbH; Sonsbeck, Germany), 31 birds per cage. Broilers in each cage were provided *ad libitum* access to one of 4 dietary regimens for the 42 d experiment. Test cages were divided into 6 blocks based

on location within the battery. Each dietary regimen appeared once per area block for a randomized complete block design with 6 replicate cages per treatment.

Test cages measured 53.3 cm in width by 119.4 cm in length by 43.2 cm in height. Water was supplied by a nipple drinker line to which birds had continuous access. Broilers had *ad libitum* access to the assigned test diet from troughs that ran the front length of the cage. A lighting program of 23L:1D was implemented for the entire 42-d growing period.

On days 14, 28 and 42, 3 birds were randomly selected from each test cage, euthanized by cervical dislocation, weighed and then dissected to collect samples of brain, liver, breast, and thigh tissue. Specimens from individual birds were packaged separately and frozen at -20°C until fatty acid analysis were conducted. Remaining bird viscera and carcasses were disposed of by incineration.

2.2.2 Test Ingredients and Experimental Diets

Camelina seed was sourced from a commercial supplier (Mercer Seeds; Lethbridge, AB, Canada) and brought to Agri-Food Discovery Place at the Edmonton Research Station (Edmonton, AB, Canada). Seed was pressed using a Komet single-screw press (model CA59G, IBG Monforts, Mönchengladbach, Germany). The cake was ground thereafter using a Jacobson hammer mill (model P160, Series 1, Carter Day International, Minneapolis, MN) through a 3.2 mm screen.

Test diets were mixed at the Environment and Metabolism Unit at the Edmonton Research Station (Edmonton, AB, Canada) in a 300 kg horizontal paddle mixer (Model SPC-2748, Marion Mixers; Marion, IA) and were then cold-pelleted using a flat-die pellet press (Model PM1230, Buskirk Engineering, Ossian, IN). Test diets for each of the 3 growth phases (starter, d 0 to 14; grower, d 15 to 28; and finisher, d 29 to 42) consisted of 76% of a phase-

specific concentrate and increasing but reciprocal amounts of CC and cornstarch in 8 percentage point increments to make up the remaining 24% of each test diet (Table 2.1; 2.2). Our strategy in formulating the test diets was to substitute CC for a substance (cornstarch) that would have minimal impact on fatty acid profiles.

2.2.3 Fatty Acid Analysis

Test diet samples were analyzed in duplicate for dietary fatty acid content. Following grinding for 1 min, 500 mg of fine powdered feed were placed in a 50-mL Teflon-lined, screw-capped tube and kept overnight in 30 mL of Folch solution (chloroform: methanol; 2:1 v/v) at 21°C. The extraction of fat and derivatization procedure was described by Nain et al. (2012a).

For liver, brain, skinless breast and skinless thigh tissue, 1-g samples collected from 3 broilers per cage were pooled (6 samples/treatment) in 25 x 150 mm Teflon-lined, screw-capped test tubes. Total fat from tissue was extracted with a modified Folch method (Nain et al., 2012a). The tissue mixture with Folch solution was homogenized for 30 s using a homogenizer (Power Gen 1000S1, Fisher Scientific). The extracted fat was then derivatized as described by Nain et al. (2012b). Fatty acid methyl esters were analyzed by gas chromatography (GC; model 7890A, Agilent Technologies, Palo Alto, CA). The GC was equipped with a flame ionization detector (Agilent Technologies, Palo Alto, CA) and fitted with a DB-23 capillary column (50%-Cyanopropyl-methylpolysiloxane) 60-m × 0.25-mm × 0.15-µm, (Agilent Technologies, Palo Alto, CA). Each sample (1µL) was analyzed in splitless mode with helium as a carrier gas. The temperature of the front injector and back detector was 230°C. The following temperature program was applied: 60°C for 2 min, increased by 15°C min^{-1} to 180°C for 8 min and held at 180°C for 10 min, and then again increased by 2°C min^{-1} to 220°C for 20 min and held at 220°C for 10 min (total runtime = 50 min). The fatty acid peak integration and analysis was performed

using Agilent Chemstation software, rev B.04.02. (Agilent Technologies, Palo Alto, CA). The fatty acids were quantified using known amounts of heptadecanoic acid (17:0) as an internal standard (Varian Walnut Creek, California 94598-1675, USA) and were identified by comparison to standards (GLC-463, GLC-421A, NU-CHEK Prep, Inc. Elysian, MN). The fatty acid proportions were expressed relative to the sum of identified peaks.

Saturated fatty acids **(SFA**) were calculated as C12:0 + C14:0 + C16:0 + C18:0 + C20:0 + C22:0. Monounsaturated fatty acids (**MUFA**) were calculated as C16:1 n-7 + C18:1 n-7/C18:1 n-9 C20:1 n-9 + C22:1 n-9. Omega-3 fatty acids were calculated as ALA + eicosatrienoic acid (C20:3 n-3; **ETE)** + EPA + docosapentaenoic acid (22:5 n-3; **DPA)** + DHA. Omega-6 fatty acids were calculated as linoleic acid (C18:2; **LA**) + C18:3 n-6 + eicosadienoic acid (C20:2 n-6; **EDA**) + isomers of C20:3 n-6 ((Δ8,11,14-20:3; dihomo-γ-linolenic acid; **DGLA**) and Δ5,11,14-20:3; sciadonic acid (**SCA**)) + arachidonic acid (C20:4 n-6; **AA**) + C22:3 n-6 + C22:4 n-6. Polyunsaturated fatty acids (**PUFA**) were calculated as sum of n-3 PUFA + n-6 PUFA. Total fatty acid was the sum of SFA + MUFA + PUFA.

2.2.4 Statistical Analysis

Fatty acid data were analyzed using the MIXED procedure of SAS (ver. 9.2, SAS Institute; Cary, NC). Statistical models included the fixed effect of dietary CC inclusion (0, 8, 16 or 24%), duration of feeding (14, 28 or 42 d) and the 2-way interaction, with block as the random term. A contrast statement tested whether increasing dietary CC inclusion had a linear effect on fatty acids composition in tissues. If significantly linear ($P<0.05$), response increases were calculated using the slope.

2.3 RESULTS AND DISCUSSION

2.3.1 Feed Fatty Acids

A fatty acid analysis of CC indicated that ALA (C18:3 n-3) comprised 29% of total FA and was the predominant FA (Table 1). Linoleic acid comprised 21%, whereas oleic acid (C18:ln-9) and gonodic acid (C20:1) made up 17% and 15% each, respectively. Researchers studying the fatty acid profile of CC have previously reported 31% ALA, 23% LA, and 17% oleic acid (Putnam et al. 1993). Budin et al. (1995) reported 31% ALA, 21% LA, and 16% oleic acid in camelina oil. Erucic acid (22:1), which could be a concerning anti-nutritional factor because of its negative effects on rat heart muscle (Kramer et al., 1992), made up 1.7% of fat in CC (Table 1) and less than 1.4% of fat in feed (Table 3). This level was well below the 5% permitted for human food consumption (Eur. Communities, 1976). Variation in cultivar, soil type, growing season (Abramovic and Abram, 2005), and oil extraction technology are factors contributing to reported differences in the fatty acid profiles of CC.

As dietary CC inclusion increased from 0% to 8%, 16% and 24% (Table 2), the average concentration of dietary ALA increased from 5.3% to 11.1%, 15.2%, and 17.8 %, respectively (Table 2.3). However, LC n-3 PUFA in diets averaged 1.1%, 0.9%, 0.8%, and 0.7% for the 0%, 8%, 16% and 24% CC diets, respectively. Although these results (Table 2.3) show variation likely due to limited sampling, the supply of fatty acid was far more consistent as broilers consumed multiple meals per day over the 14 d duration of each phase vs. a 1 g diet sample collected once and analyzed duplicates averaged.

With increasing proportion of n-3 PUFA, the proportion of n-6 PUFA decreased in the diets. Therefore the ratio of n-6 to n-3 PUFA averaged 5.0, 2.5, 1.7 and 1.4 for the 0%, 8%, 16% and 24% CC diets, respectively. A lower ratio of n-6 to n-3 PUFA in poultry diets decreases the competition of ALA with LA for enzymes involved in bioconversion to LC n-3 PUFA resulting in increased tissue content (Nain et al., 2012a; Riediger et al., 2009). The reduction

in the ratio of n-6 PUFA to n-3 PUFA in enriched foods has an important role in the prevention of chronic inflammatory diseases in humans (Pischon et al., 2003). In recent years, there has been an increase in human clinical studies that have analyzed the positive health effects of dietary ALA (Djoussé et al., 2005; Campos et al., 2008; Dai et al., 2010). However, the established claims for heart health still focus on the importance of long chain n-3 PUFA such as EPA and DHA.

2.3.2 Liver Fatty Acids

There was no interaction of dietary CC inclusion level and duration of feeding on the proportions of SFA, PUFA, n-6 PUFA and ratio of n-6 to n-3 PUFA in liver (Table 2.4). However, increasing dietary CC inclusion as well as duration of feeding affected ($P<0.05$) most fatty acids in the liver. Increasing dietary CC inclusion, linearly increased ($P<0.001$) the proportion of liver SFA, but this proportion decreased ($P<0.001$) with length of feeding from 14 to 42 d. The predominant SFA, palmitic acid (C16:0), linearly decreased ($P<0.001$) with increasing dietary CC inclusion level and followed the trend that was observed for the experimental diets (Table 2.3). However, the linear increase in liver C18:0 and C22:0 ($P<0.01$) with increasing dietary CC inclusion level differs from that observed for these fatty acids in diets. Feeding a diet enriched with n-3 PUFA increases the proportion of SFA to maintain membrane fluidity by keeping a constant ratio of saturated to unsaturated fatty acids (Huang et al., 2011). Epidemiologic studies have shown strong positive correlations between intake of SFA leading to an increase in serum low-density-lipoprotein cholesterol and an increase in the incidence of cardiovascular disease (Posner et al., 1991). Increases in dietary SFA, like C12:0, C14:0, C16:0, have proven to raise total cholesterol in humans

whereas longer chain FA, like C18:0, have a neutral effect on total serum cholesterol level (Karupaiah et al., 2011).

There was an interaction ($P<0.001$) between increasing dietary CC inclusion level and length of feeding on liver MUFA proportion as with increasing CC level in diet increased MUFA from d 14 to 28 and decreased by d 42. Among the MUFA, C18:1 and C16:1 were the two predominant fatty acids, which proportions linearly decreased ($P<0.001$) in liver as dietary CC inclusion level increased. The decrease in C18:1 and C16:1 when broilers were fed high PUFA diets was possibly due to an inhibition effect of increased dietary n-3 PUFA on the Δ9-desaturase enzyme, resulting in lower *de novo* synthesis of MUFA (Villaverde et al., 2006). Pachikian et al. (2008) reported an increase in liver C18:1 in mice fed an n-3 depleted diet due to greater hepatic β-oxidation capacity. The proportion of C20:1, a prominent MUFA in CC, linearly increased ($P<0.001$) in liver as dietary CC inclusion level increased. The proportion of erucic acid was not different among broilers fed 0, 8 and 16% CC, but was greater for those fed 24% CC.

Linear increases ($P<0.001$) were observed in both n-3 PUFA and n-6 PUFA in liver when feeding increasing dietary CC inclusions (Table 2.4). Increasing dietary CC inclusion level and length of feeding interacted ($P= 0.015$) on the proportion of LC n-3 PUFA. Length of CC feeding did not change the proportions of LC n-3 PUFA in 0% CC fed broilers. The proportion of LC n-3 PUFA increased in broilers fed 8, 16 and 24% CC compared with controls at 14 d. There was, however, no change in LC n-3 PUFA proportions at 28 and 42 from 14 d. At 24% dietary CC inclusion, liver LC n-3 PUFA proportions increased at 28 d, but they decreased at 42 d. Because diets had similar proportions of LC n-3 PUFA (averaged 0.9%), the increase in liver LC n-3 PUFA may have been due to greater

bioconversion of ALA into LC n-3 PUFA. In addition, there were concomitant linear increases ($P<0.001$) of all the metabolic products of the n-3 PUFA pathway with the exception of EPA as dietary CC inclusion level increased. These results confirm the ability of broilers to elongate ALA to LC n-3 PUFA. Gregory et al. (2013) reported that broilers have greater capability for bioconversion of ALA to LC n-3 PUFA as compared with the human and rat liver because of greater expression of *Elovl2* and *Elovl5* genes, which results in greater DHA synthesis.

There was a linear increase ($P<0.001$) in the proportion of n-6 PUFA in liver with increasing dietary CC inclusion level (Table 2.4). Interestingly, there were linear increases ($P<0.001$) in liver of ARA and C22:3 n-6 also with increasing dietary CC inclusion, which suggests possible LA bioconversion to its n-6 PUFA long chain derivatives. It is known that there is a competitive mechanism between LA and ALA, which utilize the same desaturase and elongase enzymes for bioconversion into their respective longer chain PUFA (Watkin 1991; Holman, 1998). Furthermore, ALA is generally efficient in suppressing LA bioconversion (Holman, 1998) and usually results into decreased concentration of ARA even when supplied in an equal proportion as LA in the diets. However, if ALA is present at lower concentrations relative to LA, as was the case in our diets (Table 2.3), it might not be effective in suppressing the increases in ARA bioconversion. Wolff et al. (1999) has reported a possible alternative LA bioconversion mechanism (when ALA is slightly greater than LA) leading to increased proportion of EDA and SCA (elongation products of LA) contrary to the normal LA bioconversion to ARA. In the present study, we noticed linear increases ($P<0.001$) of EDA and SCA levels in liver. Eicosadienoic acid has been shown to act as a weaker pro-inflammatory agent compared with ARA whereas SCA is considered to

be a potent anti-inflammatory agent (Huang et al., 2011). Therefore, increased dietary supplementation of ALA through increasing dietary CC inclusion may have not only increased the amount of LC n-3 PUFA (EPA and DHA), but also stimulated the production of n-6 PUFA, such as EDA and SCA.

2.3.3 Brain Fatty Acids

There were no interactions between increasing dietary CC inclusion level and length of feeding on brain fatty acid composition (Table 2.5). Brain medium chain fatty acids proportions were generally not affected by dietary CC inclusion level. Linear responses were similar to those observed in liver fatty acid: SFA, n-3 PUFA, and LC n-3 PUFA proportions linearly increased ($P<0.05$) whereas MUFA, n-6 PUFA and n-6 to n-3 PUFA ratio linearly decreased ($P<0.05$) with increasing dietary CC inclusion. However, the effect of feeding increasing dietary CC inclusions was mostly evident on long chain fatty acids, especially those with ≥20 carbons. The proportion of brain ALA was not different ($P>0.05$) in broilers fed increasing dietary CC inclusions. The effect of length of feeding CC was particularly evident at 28 and 42 d. At d 14, the proportions were lowest for the total MUFA, n-3 PUFA, LC n-3 PUFA and were highest for SFA. Consistent with our observations for d 28 and 42, Poureslami et al., (2010) also reported a decrease in brain DPA and DHA from levels at 21 vs. 42 d in broilers fed diets including linseed or fish oil.

The brain from broilers fed the control diet had lower ($P<0.001$) n-3 PUFA, total LC n-3 PUFA and greater ($P<0.001$) total n-6 PUFA compared with brains from broilers fed increasing dietary CC inclusions (Table 2.5). Among the LC n-3 PUFA, feeding increasing dietary CC inclusions linearly decreased ($P<0.001$) EPA, whereas DPA and DHA linearly increased ($P<0.001$). Among the brain n-6 PUFA, increasing dietary CC inclusions in

broiler diets linearly increased (P<0.001) LA. However, the predominant n-6 fatty acid in brain, AA, was unaffected by increasing dietary CC inclusion. Increases in AA can aggravate neuroinflammation, which is a crucial element in the pathogenesis of Alzheimer's disease in humans (Esposito et al., 2008). The brain has a very low ratio of n-6 PUFA to n-3 PUFA (less than 1) and feeding increasing dietary CC inclusion from 0 to 24% linearly lowered (P<0.001) the n-6:n-3 ratio. Simopolous (2011) reviewed evidence that humans had a 1:1 ratio of n-6 to n-3 PUFA in brain tissue during most of their evolution. Low ratios of n-6 to n-3 PUFA are relevant for a sustained cognitive function and, thus, for the prevention of dementia (Loef and Walach, 2013).

2.3.4 Breast and Thigh Fatty Acids

Increasing CC inclusion in broiler diets resulted in changes in the fatty acid composition of breast and thigh tissue at 14, 28 and 42 d (Table 2.6 and 2.7). No interactions between increasing dietary CC inclusion and length of feeding were observed for the proportions of SFA, MUFA, PUFA, n-3 PUFA and n-6 PUFA in breast tissue (Table 2.6). Increasing dietary CC inclusion linearly decreased (P<0.001) SFA in breast and there was also a decrease with length of feeding (P<0.001). Palmitic acid (C16:0) was the predominant SFA and followed the same pattern as for total SFA and as for length of feeding. The proportion of breast MUFA linearly decreased (P<0.001) with increasing dietary CC inclusion. As the proportions of oleic acid (C18:1) in feed decreased with increasing CC inclusion (Table 2.1), there was also a linear decrease (P<0.001) in C18:1 in breast tissue. The other dominant MUFA present in CC diets, gadoleic acid (C20:1), showed a linear increase (P<0.001) and more than doubled vs. control in breast tissue with increasing dietary CC inclusion. The proportion of MUFA in breast tissue was lower at 14

d, but it was similar at 28 and 42 d of feeding (P<0.001). Increasing dietary CC inclusion linearly increased (P<0.001) n-3 PUFA, LC n-3 PUFA, as well as n-6 PUFA in breast tissue.

Increasing dietary CC inclusion and length of feeding did not interact to affect proportions of SFA, PUFA, n-3 PUFA and n-6 PUFA in thigh tissue (Table 2.7). Unlike breast tissue, there was no effect of increasing dietary CC inclusion on the proportion of SFA in thigh tissue (Table 2.7). For MUFA, however, increasing dietary CC inclusion interacted with length of feeding (P=0.039) such that the proportion of MUFA in thigh from birds fed 0 and 8 % CC was lower at 42 d compared with 14 d levels, but in birds fed 16 or 24 % CC, there was an increase in MUFA in thigh tissue at 42 d compared with d 14. The increase in MUFA in thigh tissue was unexpected. As the increase in the proportion of dietary PUFA generally decreases MUFA in tissues due to their inhibitory role on the desaturase enzyme needed for *de novo* synthesis of MUFA (Lefevre et al., 2001).

Linear increases (P≤0.02) in the proportions of thigh n-3 PUFA and n-6 PUFA were similar to that observed for breast tissue. Increasing CC inclusion in broiler diets linearly increased (P<0.001) the proportion of ALA in both breast and thigh tissue. Increased ALA in both breast and thigh muscle in turn linearly increased (P<0.001) ETE and DPA only, whereas, DHA and EPA were not affected. The observed linear increase in ETE, the first metabolite of ALA bioconversion, in breast and thigh tissue might be a result of increased bioconversion of dietary ALA. All diets contained similar proportions of fish meal and consequently resulted in similar proportions of DHA in the breast and thigh tissue. Breast DPA linearly increased (P<0.001) in broilers fed increasing dietary CC inclusion. Increasing dietary CC inclusion interacted (P<0.05) with length of feeding for DPA in thighs. The increasing CC inclusion in diet increased the DPA proportion in broiler fed for 14 and 28

but had no difference among 42 d. Docosapentaenoic is usually the major LC n-3 PUFA in broilers meat fed plant-based sources with high ALA content (Betti et al., 2009). Aziza et al. (2010) reported similar findings for EPA, DPA and DHA in breast and thigh meat feeding 10% CC in broiler rations. The chicken liver has greater genetic ability to convert ALA to DHA compared to mammels (Gregory et al., 2013) even though muscle deposition appears to be quite low (Lopez-Ferrer, et al., 2001). We observed an increase in brain DHA with increasing dietary provision of ALA, whereas increasing dietary CC inclusion resulted in muscle tissue with DHA proportion not different from 0% CC. Molecular-level studies in rat brain have shown that DHA in the brain is mainly derived from liver while most of the ALA, after uptake from the circulation across the blood-brain barrier, is usually oxidized (DeMar et al., 2005; Igarashi et al., 2007). The differential depositions of fatty acids from liver to other tissues in broilers had been reported by Poureslami et al., (2010) and Aziza et al., (2010). Other factors such as strain difference (muscle DHA in Cobb 500 > Ross 308; Rymer et al. 2009) and fatty acid classes (triglycerides or phospholipids; Betti et al., 2009) may also contribute to the final fatty acid composition of muscle or tissue.

In the present study we observed linear increases of AA in liver tissue only feeding increasing dietary levels of CC, with no effect on AA in brain, breast and thigh tissue. Adding the same proportion of fishmeal to the diets, a source of LC n-3 PUFA, might have increased deposition of LC n-3 PUFA in tissue somewhat, while simultaneously acting as a negative feedback mechanism for ALA metabolism and therby allowing LA to utilize enzymes for their bioconversion, but this does not explain response differences among CC inclusion levels. Previous researchers have also reported a reduction in bioconversion with

dietary fishmeal (Cachaldora et al., 2006) or decreased elongase and desaturase (enzymes needed for bioconversion) activity with dietary fish oil inclusion (Turchini et al., 2012).

The pattern of deposition of different n-3 PUFA types was variable in liver and brain tissues or breast and thigh tissue. Docosahexaenoic acid was the predominant n-3 PUFA in liver and brain tissue (48% and 88% of n-3 PUFA, respectively) whereas ALA was the major n-3 PUFA in breast and thigh tissue (65% and 86% of n-3 PUFA, respectively). The accumulation efficiency of essential fatty acids (LA and ALA) in breast and thigh tissue along with the deposition of n-3 PUFA showed a variable affinity of each fatty acid for the different tissue types. Thigh tissue showed a greater accumulation capability of both essential fatty acids, LA and ALA as compared with breast (Table 2.6 and Table 2.7). The greater concentration of LC n-3 PUFA in breast meat is mostly associated with a greater phospholipid fraction compared with thigh meat (Betti et al. 2009). The differences in the tissue specific distribution of n-3 PUFA in broilers (Poureslami et al., 2010) or in rabbits (Ander et al., 2010) have been reported previously. The brain has a lower n-6 to n-3 ratio as compared with liver or skeletal tissue. The magnitude of change in n-6 to n-3 PUFA ratio, however, was lower in brain as compared with liver or muscle suggesting that the brain might have preference in biological development and that the brain maintains the balance between n-6 to n-3 PUFA close to 1:1 (Simopolous, 2011).

The Canadian Food Inspection Agency (CFIA) allows labeling of a food product as a "source of n-3" if the total n-3 PUFA is ≥300 mg per reference amount (100 g meat; CFIA, 2003). In the present study, broilers exceeded the required labeling level for n-3 PUFA in breast tissue when the 24% CC diet was fed for 28 d or 42 d (Table 2.8). The greatest n-3 PUFA deposition achieved was 349 mg/100g of breast tissue when the 24% CC diet was fed

for 42 d equivalent to 119 mg per 100g of LC n-3 PUFA. In comparison, for thigh tissue the inclusion of even 16% CC in the diet for 42 d enriched the tissue above the CFIA labeling requirement. The highest level for thigh n-3 PUFA deposition achieved through feeding the 24% CC diet was 674 mg per 100 g, but with only 76 mg per 100 g of LC n-3 PUFA. These results indicate that enrichment of thigh tissue for total n-3 PUFA is easier than skinless breast, but that breast tissue provides greater opportunities for LC n-3 PUFA enrichment compared with thigh tissue. Feeding CC with lower oil content (10 to 12%) resulting from more effective seed pressing, (e.g., expeller-pressing) may lower the rate of breast and thigh n-3 PUFA enrichment perhaps requiring longer feeding.

The outcomes of the present study indicate that there is potential for the inclusion of CC in broiler rations. The currently preferred plant-based n-3 PUFA source, flaxseed, has been shown to increase enrichment in breast as well as thigh meat (Betti et al., 2009). Expanding availability of camelina cake as a co-product of biofuel production and the lack of competition for human food uses offers potential to develop camelina cake as a livestock feed ingredient for poultry meat n-3 PUFA enrichment. Moreover, feeding diets with greater ALA concentration increased the overall n-3 PUFA status in broilers. Greater concentration of tocopherols and phenolic compounds (anti-oxidants) in camelina oil compared with flax oil might offer advantages regarding decreased oxidative potential and greater storage stability of the camelina cake. Effects of n-3 PUFA from increasing camelina cake inclusions on immune status were not evaluated in the present experiment, and thus constitute a research opportunity for future studies.

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Table 2.1. Nutrient (% as-is, unless otherwise noted) and fatty acid composition (% of total fatty acids) of screw-pressed camelina cake.

Item	Camelina cake
Moisture	8.05
Gross energy, kcal/kg	5,730
Crude protein	34.25
Crude fiber	9.37
Acid detergent fibre	15.04
Neutral detergent fibre	26.32
Ash	5.38
Calcium	0.23
Phosphorus	1.01
Ether extract	21.0
Fatty acid, % of total fatty acids	
Saturated fatty acids	11.9
Myristic acid (C14:0)	0.1
Palmitic acid (C16:0)	7.4
Stearic acid (C18:0)	2.7
Arachidic acid (C20:0)	1.4
Behenoic acid (C22:0)	0.3
Monusaturated fatty acids	34.2
Oleic acid (C18:1)	17.2
Gonodic acid (C20:1)	15.3
Erucic acid (C22:1)	1.7
n-3 Polyunsaturated fatty acids	30.1
α-Linolenic acid (C18:3 n-3)	28.8
Eicosatrienoic acid (C20:3 n-3)	1.2
Eicosapentaenoic acid (C20:5 n-3)	0.1
n-6 Polyunsaturated fatty acids	23.8
Linoleic acid (C18:2 n-6)	21.1
γ-Linolenic acid (C18:3 n-6)	0.2
Arachidonic acid (C20:4 n-6)	2.5
n-6:n-3 Polyunsaturated fatty acids	0.79

Table 2.2 Ingredient composition of the phase test diets (% as fed).

	Screw-pressed camelina cake inclusion, %											
	Starter phase (d 0 – 14)				Grower phase (d 14 – 28)				Finisher phase (d 28 – 42)			
Ingredient	0	8	16	24	0	8	16	24	0	8	16	24
Cornstarch	24.00	16.00	8.00	0.00	24.00	16.00	8.00	0.00	24.00	16.00	8.00	0.00
Camelina cake	0.00	8.00	16.00	24.00	0.00	8.00	16.00	24.00	0.00	8.00	16.00	24.00
Wheat, ground	32.05	32.05	32.05	32.05	39.92	39.92	39.92	39.92	43.51	43.51	43.51	43.51
Soybean meal	20.10	20.10	20.10	20.10	12.17	12.17	12.17	12.17	11.33	11.33	11.33	11.33
Corn, ground	13.47	13.47	13.47	13.47	13.51	13.51	13.51	13.51	11.26	11.26	11.26	11.26
Canola oil	2.82	2.82	2.82	2.82	2.52	2.52	2.52	2.52	3.10	3.10	3.10	3.10
Fish meal	2.24	2.24	2.24	2.24	3.75	3.75	3.75	3.75	2.63	2.63	2.63	2.63
Mono-di-calcium phosphate	1.55	1.55	1.55	1.55	1.01	1.01	1.01	1.01	1.08	1.08	1.08	1.08
Limestone	1.50	1.50	1.50	1.50	1.06	1.06	1.06	1.06	1.07	1.07	1.07	1.07
Vitamin/mineral premix[1]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Choline premix[2]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Salt	0.43	0.43	0.43	0.43	0.34	0.34	0.34	0.34	0.36	0.36	0.36	0.36
Vitamin E premix[3]	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
D,L – methionine	0.17	0.17	0.17	0.17	0.08	0.08	0.08	0.08	0.07	0.07	0.07	0.07
L – lysine HCl	0.17	0.17	0.17	0.17	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11
L-threonine	0.04	0.04	0.04	0.04	0.08	0.08	0.08	0.08	0.02	0.02	0.02	0.02
Enzyme[4]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Coccidiostat[5]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Antibiotic[6]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05

[1]Provided the following per kg of feed: 10,000 IU vitamin A, 4,000 IU vitamin D_3, 50 IU vitamin E, 4 mg menadione, 4 mg thiamine, 10 mg riboflavin, 15 mg pantothenic acid, 2 mg folic acid, 65 mg niacin, 5 mg pyridoxine, 0.2 mg biotin, 0.02 mg vitamin B_{12}, 120 mg manganese, 80 mg iron, 100 mg zinc, 20 mg copper, 1.65 mg iodine and 0.3 mg selenium.

[2]Provided 100 mg of choline per kg of mixed feed

[3]Provided 15 IU of tocopherol per kg of mixed feed

[4]Provided the following enzyme activities per kg of mixed feed: 150 units xylanase, 125 units glucanase, 4,000 units amylase, 1,750 units protease and 5,000 units invertase

[5]Coban, Elanco (Guelph, ON, Canada), Canadian Food Inspection Agency, Medicating Ingredient Brochure #57, Claim 1.

[6]BMD 110, Alpharma (Leduc, AB, Canada). Canadian Food Inspection Agency, Medicating Ingredient Brochure #48, Claim 2.

Table 2.3. Fatty acid composition (% of total fatty acids) of the phase test diets[1].

	Screw-pressed camelina cake inclusion, %											
	Starter (d 0 – 14)				Grower (d 14 – 28)				Finisher (d 28 – 42)			
Item	0	8	16	24	0	8	16	24	0	8	16	24
Saturated fatty acids	21.60	16.77	14.29	13.58	14.18	13.00	13.18	14.17	14.2	14.5	12.55	12.98
Lauric acid (C12:0)	0.50	0.31	0.26	0.23	0.30	0.26	0.29	0.29	0.34	0.33	0.24	0.19
Myristic acid (C14:0)	0.11	0.07	0.06	0.05	0.17	0.06	0.07	0.05	0.06	0.06	0.05	0.04
Palmitic acid (C16:0)	16.54	12.41	10.21	9.34	11.40	9.15	9.33	9.46	11.22	10.99	8.89	8.77
Stearic acid (C18:0)	2.91	2.61	2.42	2.42	1.67	1.76	2.04	2.32	1.72	2.02	2.00	2.11
Arachidic acid (C20:0)	0.87	0.77	0.81	0.99	0.20	1.35	1.01	1.60	0.47	0.66	0.95	1.42
Behenic acid (C22:0)	0.34	0.31	0.31	0.33	0.23	0.23	0.27	0.28	0.16	0.26	0.25	0.28
Lignoceric acid (C24:0)	0.33	0.29	0.22	0.22	0.21	0.19	0.17	0.17	0.23	0.18	0.17	0.17
Monounsaturated fatty acids	47.85	47.49	43.32	42.38	44.34	42.78	41.90	40.23	43.24	41.82	43.53	42.42
Palmitoleic acid (C16:1)	0.43	0.36	0.30	0.27	0.49	0.47	0.39	0.34	0.47	0.41	0.35	0.27
Oleic acid (C18:1)	45.26	40.51	33.53	31.33	41.91	36.44	33.35	29.15	41.17	34.77	34.87	32.45
Gadoleic acid (C20:1)	1.87	5.43	7.84	8.85	1.53	4.55	6.47	8.81	1.32	5.37	6.74	7.91
Erucic acid (C22:1)	nd[2]	0.79	1.14	1.34	0.10	0.83	1.15	1.38	0.04	0.81	1.07	1.23
Nervonic acid (C24:1 n-9)	0.15	0.31	0.39	0.47	0.24	0.37	0.43	0.44	0.17	0.35	0.39	0.45
n-3 Polyunsaturated fatty acids	4.93	9.42	15.46	18.12	6.69	12.6	16.29	19.20	7.81	13.86	16.35	18.39
α-Linolenic acid (C18:3 n-3)	4.13	8.63	14.58	17.40	5.19	11.57	15.41	18.29	6.69	13.05	15.74	17.82
Long-chain n-3 PUFA[2]	0.80	0.79	0.88	0.72	1.50	1.03	0.88	0.91	1.12	0.81	0.51	0.57
Eicosatrienoic acid (C20:3 n-3)	0.04	0.08	0.02	0.02	nd	nd	nd	nd	nd	nd	nd	nd
Eicosapentaenoic acid (C20:5 n-3)	0.21	0.24	0.24	0.22	0.44	0.32	0.26	0.21	0.39	0.26	0.20	0.13
Docosapentaenoic acid (C22:5 n-3)	nd	nd	0.02	0.02	0.09	0.05	0.06	0.04	nd	0.04	0.04	0.01
Docosahexaenoic acid (C22:6 n-3)	0.55	0.47	0.60	0.46	0.97	0.66	0.56	0.66	0.73	0.51	0.37	0.43
n-6 Polyunsaturated fatty acids	25.62	26.32	26.93	25.92	34.79	31.62	28.63	26.40	34.75	29.82	27.57	26.41
Linoleic acid (C18:2 n-6)	25.27	25.95	26.63	25.67	33.86	30.98	28.13	25.97	34.09	29.31	27.24	26.19
Arachidonic acid (C20:4 n-6)	0.35	0.37	0.30	0.25	0.93	0.64	0.50	0.43	0.66	0.51	0.33	0.22
n-6:n-3 Polyunsaturated fatty acids	5.20	2.79	1.74	1.43	5.20	2.51	1.76	1.38	4.45	2.15	1.69	1.44

[1]Based on duplicate analysis of a 1g sample per diet taken once per growth phase.
[2]PUFA = Polyunsaturated fatty acids
nd = Non-detectable

Table 2.4. Effect of increasing dietary inclusion level of screw-pressed camelina cake and length of feeding on fatty acid composition (% of total fatty acids) of liver tissue from broilers[1].

	Camelina cake inclusion, %					Length of feeding, d				P - value			
Item	0	8	16	24	SEM	14	28	42	SEM	Lev	Day	Level x day	Linear (level)
Saturated fatty acids	47.71^{b}	50.80^{a}	52.10^{a}	51.98^{a}	0.85	57.30^{a}	44.37^{c}	50.28^{b}	0.73	0.002	<0.001	0.200	<0.001
Lauric acid (C12:0)	1.33^{b}	1.74^{a}	1.96^{a}	2.04^{a}	0.11	1.74	1.94	1.62	0.09	<0.001	0.063	0.166	<0.001
Myristic acid (C14:0)	0.45^{a}	0.46^{a}	0.39^{b}	0.31^{c}	0.02	0.42^{a}	0.36^{b}	0.43^{a}	0.02	<0.001	0.009	0.011	<0.001
Palmitic acid (C16:0)	29.83^{a}	29.76^{a}	27.73^{b}	23.78^{c}	0.69	30.82^{a}	23.85^{c}	28.66^{b}	0.61	<0.001	<0.001	0.340	<0.001
Stearic acid (C18:0)	15.10^{d}	17.18^{c}	19.74^{b}	22.65^{a}	0.45	22.01^{a}	16.01^{c}	17.98^{b}	0.40	<0.001	<0.001	0.020	<0.001
Arachidic acid (C20:0)	0.14^{b}	0.23^{ab}	0.22^{ab}	0.27^{a}	0.03	0.33^{a}	0.15^{b}	0.17^{b}	0.03	0.036	<0.001	0.439	0.011
Behenic acid (C22:0)	0.86^{d}	1.43^{c}	2.07^{b}	2.93^{a}	0.13	1.98^{a}	2.07^{a}	1.42^{b}	0.12	<0.001	<0.001	0.023	<0.001
Monounsaturated fatty acids	39.92^{a}	33.18^{b}	25.83^{c}	17.15^{d}	1.14	22.36^{c}	34.47^{a}	30.22^{b}	1.03	<0.001	<0.001	0.016	<0.001
Palmitoleic acid (C16:1)	5.11^{a}	4.14^{b}	2.75^{c}	1.10^{d}	0.21	2.41^{b}	3.73^{a}	3.67^{a}	0.19	<0.001	<0.001	0.059	<0.001
Oleic acid (C18:1)	33.93^{a}	27.86^{b}	21.61^{c}	14.01^{d}	1.00	18.24^{c}	29.61^{a}	25.21^{b}	0.90	<0.001	<0.001	0.019	<0.001
Gadoleic acid (C20:1)	0.47^{d}	0.66^{c}	0.84^{b}	1.35^{a}	0.05	0.84	0.86	0.79	0.04	<0.001	0.447	0.019	<0.001
Erucic acid (C22:1)	0.10^{b}	0.14^{ab}	0.14^{ab}	0.19^{a}	0.03	0.18	0.12	0.14	0.03	0.027	0.099	0.487	0.008
n-3 Polyunsaturated fatty acids	2.12^{d}	3.40^{c}	5.28^{b}	8.18^{a}	0.32	4.49^{b}	5.36^{a}	4.39^{b}	0.30	<0.001	0.002	0.055	<0.001
α-Linolenic acid (C18:3 n-3)	0.36^{d}	0.83^{c}	1.49^{b}	2.46^{a}	0.09	1.08^{b}	1.38^{a}	1.40^{a}	0.08	<0.001	0.001	0.551	<0.001
Long-chain n-3 PUFA[2]	1.75^{d}	2.57^{c}	3.79^{b}	5.72^{a}	0.26	3.41^{b}	3.98^{a}	2.98^{b}	0.24	<0.001	<0.001	0.015	<0.001
Eicosatrienoic acid (C20:3 n-3)	0.05^{d}	0.13^{c}	0.24^{b}	0.42^{a}	0.02	0.26^{a}	0.17^{b}	0.20^{b}	0.02	<0.001	0.002	0.410	<0.001
Eicosapentaenoic acid (C20:5 n-3)	0.08	0.10	0.11	0.11	0.01	0.16^{a}	0.06^{b}	0.08^{b}	0.01	0.358	<0.001	0.144	0.117
Docosapentaenoic acid (C22:5 n-3)	0.35^{d}	0.56^{c}	0.94^{b}	1.32^{a}	0.06	0.73^{b}	0.90^{a}	0.75^{b}	0.05	<0.001	0.013	0.006	<0.001
Docosahexaenoic acid (C22:6 n-3)	1.28^{d}	1.78^{c}	2.52^{b}	3.91^{a}	0.20	2.25^{b}	2.88^{a}	1.98^{b}	0.19	<0.001	<0.001	0.007	<0.001
n-6 Polyunsaturated fatty acids	10.18^{d}	12.54^{c}	16.69^{b}	22.61^{a}	0.73	15.73	15.75	15.04	0.67	<0.001	0.551	0.203	<0.001
Linoleic acid (C18:2 n-6)	6.51^{d}	8.65^{c}	11.25^{b}	15.53^{a}	0.48	10.55	10.61	10.29	0.43	<0.001	0.821	0.681	<0.001
γ-Linolenic acid (C18:3 n-6)	0.13^{c}	0.17b^{c}	0.22^{b}	0.29^{a}	0.02	0.25^{a}	0.20^{b}	0.16^{b}	0.02	<0.001	0.007	0.219	<0.001
Eicosadienoic acid (C20:2 n-6)	0.62^{c}	0.56^{c}	0.76^{b}	0.92^{a}	0.05	0.71	0.75	0.68	0.04	<0.001	0.314	0.003	<0.001
DGLA/SCA[3] (C20:3 n-6)	0.63^{b}	0.72^{b}	0.91^{a}	0.94^{a}	0.05	0.82^{a}	0.87^{a}	0.71^{b}	0.05	<0.001	0.002	<0.001	<0.001
Arachidonic acid (C20:4 n-6)	2.19^{c}	2.29^{c}	3.38^{b}	4.70^{a}	0.20	3.21	3.18	3.03	0.18	<0.001	0.641	0.075	<0.001
Docosotrienoic acid (C22:3 n-6)	0.11^{c}	0.15^{bc}	0.18^{ab}	0.21^{a}	0.01	0.18	0.15	0.16	0.01	<0.001	0.103	0.226	<0.001
Adrenic acid (C22:4 n-6)	0.07	0.09	0.11	0.11	0.01	0.13^{a}	0.05^{c}	0.10^{b}	0.01	0.099	<0.001	0.006	0.032
n-6:n-3 Polyunsaturated fatty acids	5.03^{a}	3.80^{b}	3.34^{c}	2.83^{d}	0.17	3.97^{a}	3.28^{b}	3.99^{a}	0.15	<0.001	<0.001	0.534	<0.001

[1] Based on 1 g samples collected from 3 broilers per cage that were pooled (6 samples per treatment).
[2]PUFA = Polyunsaturated fatty acids.
[3]dihomo-γ-linolenic acid (DGLA), and sciadonic acid (Δ5,11,14–20:3; SCA) were calculated as sum for the both isomers of C 20:3 n-6.
[a-c]Means within row with different superscript differ (P<0.05).

Table 2.5. Effect of increasing dietary inclusion level of screw-pressed camelina cake and length of feeding on fatty acid composition (% of total fatty acids) of brain tissue from broilers[1].

	Camelina cake inclusion, %					Length of feeding, d				P - value			
Item	0	8	16	24	SEM	14	28	42	SEM	Level	Day	Level x day	Linear (level)
Saturated fatty acids	52.68	53.66	54.05	55.11	0.75	58.14^{a}	48.29^{c}	55.19^{b}	0.65	0.156	<0.001	0.957	0.030
Lauric acid (C12:0)	2.04	2.10	2.06	2.25	0.13	1.42^{b}	3.31^{a}	1.61^{b}	0.11	0.646	<0.001	0.837	0.349
Myristic acid (C14:0)	0.46	0.43	0.45	0.43	0.03	0.38^{b}	0.59^{a}	0.37^{b}	0.02	0.824	<0.001	0.763	0.666
Palmitic acid (C16:0)	29.60	30.40	30.55	31.22	0.59	34.19^{a}	25.69^{c}	31.45^{b}	0.51	0.286	<0.001	0.983	0.072
Stearic acid (C18:0)	19.80	19.87	20.08	20.25	0.29	21.32^{a}	17.82^{b}	20.87^{a}	0.25	0.675	<0.001	0.877	0.225
Arachidic acid (C20:0)	0.47	0.43	0.44	0.42	0.05	0.40	0.45	0.47	0.04	0.910	0.455	0.993	0.596
Behenic acid (C22:0)	0.31^{c}	0.43^{b}	0.46^{ab}	0.53^{a}	0.02	0.44	0.43	0.42	0.02	<0.001	0.851	0.275	<0.001
Monounsaturated fatty acids	24.58^{a}	23.62^{a}	22.66^{ab}	21.51^{b}	0.69	21.33^{b}	24.53^{a}	23.41^{a}	0.60	0.018	0.001	0.905	0.002
Palmitoleic acid (C16:1)	1.50	1.44	1.42	1.54	0.07	1.45^{b}	1.67^{a}	1.31^{b}	0.06	0.574	<0.001	0.591	0.755
Oleic acid (C18:1)	22.02^{a}	21.22^{a}	20.46^{ab}	19.18^{b}	0.61	19.08^{b}	21.82^{a}	21.25^{a}	0.53	0.020	0.003	0.900	0.002
Gadoleic acid (C20:1)	0.90^{a}	0.80^{ab}	0.64^{b}	0.60^{b}	0.09	0.66	0.86	0.69	0.07	0.055	0.117	0.274	0.007
Erucic acid (C22:1)	0.17	0.17	0.14	0.19	0.02	0.15	0.18	0.17	0.02	0.445	0.619	0.651	0.962
n-3 polyunsaturated fatty acids	10.85^{c}	11.94^{b}	12.77^{ab}	12.84^{a}	0.31	10.32^{c}	14.38^{a}	11.60^{b}	0.27	<0.001	<0.001	0.741	<0.001
α-Linolenic acid (C18:3 n-3)	0.31	0.35	0.32	0.40	0.05	0.34	0.36	0.33	0.04	0.502	0.899	0.911	0.312
Long-chain n-3 PUFA[2]	10.55^{b}	11.59^{a}	12.45^{a}	12.44^{a}	0.32	9.98^{c}	14.02^{a}	11.27^{b}	0.28	<0.001	<0.001	0.752	<0.001
Eicosapentaenoic acid (C20:5 n-3)	0.47^{a}	0.28^{b}	0.21^{c}	0.21^{c}	0.02	0.29	0.32	0.26	0.02	<0.001	0.142	0.069	<0.001
Docosapentaenoic acid (C22:5n-3)	0.51^{c}	0.66^{b}	0.76^{b}	0.90^{a}	0.03	0.65^{b}	0.81^{a}	0.66^{b}	0.03	<0.001	<0.001	0.673	<0.001
Docosahexaenoic acid (C22:6 n-3)	9.56^{b}	10.65^{a}	11.49^{a}	11.34^{a}	0.31	9.03^{c}	12.89^{a}	10.35^{b}	0.27	<0.001	<0.001	0.724	<0.001
n-6 Polyunsaturated fatty acids	11.89^{a}	10.78^{b}	10.53^{b}	10.54^{b}	0.19	10.21^{b}	12.80^{a}	9.79^{b}	0.16	0.004	<0.001	0.207	0.003
Linoleic acid (C18:2 n-6)	0.64^{c}	0.81^{b}	0.82^{b}	1.01^{a}	0.04	0.90^{a}	0.81^{ab}	0.74^{b}	0.04	<0.001	0.014	0.646	<0.001
γ-Linolenic acid (C18:3 n-6)	0.36	0.40	0.31	0.37	0.05	0.28^{b}	0.55^{a}	0.25^{b}	0.04	0.556	<0.001	0.702	0.636
Eicosadienoic acid (C20:2 n-6)	2.20^{a}	1.11^{b}	0.84^{c}	0.68^{c}	0.09	1.23^{b}	1.50^{a}	0.90^{c}	0.08	<0.001	<0.001	0.130	<0.001
DGLA/SCA[3] (C20:3 n-6)	0.64	0.63	0.62	0.66	0.03	0.62^{b}	0.72^{a}	0.59^{b}	0.02	0.728	<0.001	0.807	0.672
Arachidonic acid (C20:4 n-6)	5.98	6.07	6.25	6.26	0.14	5.41^{c}	7.25^{a}	5.76^{b}	0.12	0.406	<0.001	0.871	0.098
Docosotrienoic acid (C22:3 n-6)	1.23	1.26	1.26	1.22	0.04	1.22^{b}	1.33^{a}	1.18^{b}	0.03	0.799	0.005	0.534	0.932
Adrenic acid (C22:4 n-6)	0.88^{a}	0.51^{b}	0.43^{bc}	0.35^{c}	0.04	0.60^{a}	0.65^{a}	0.38^{b}	0.03	<0.001	<0.001	0.368	<0.001
n-6:n-3 Polyunsaturated fatty acids	1.02^{a}	0.87^{b}	0.80^{c}	0.80^{c}	0.02	0.95^{a}	0.86^{b}	0.82^{b}	0.02	<0.001	<0.001	0.149	<0.001

[1] Based on 1 g samples collected from 3 broilers per cage that were pooled (6 samples per treatment).
[2]PUFA = Polyunsaturated fatty acids.
[3]dihomo-γ-linolenic acid (DGLA), and sciadonic acid (Δ5,11,14–20:3; SCA) were calculated as sum for the both isomers of C 20:3 n-6.
[a-c]Means within row with different superscript differ (P<0.05).

Table 2.6. Effect of increasing dietary inclusion level of screw-pressed camelina cake and length of feeding on fatty acid composition (% of total fatty acids) of breast tissue of broilers[1].

	Camelina cake inclusion, %					Length of feeding, d				P - value			
Item	0	8	16	24	SEM	14	28	42	SEM	Level	Day	Level x day	Linear (level)
Saturated fatty acids	34.75^{a}	33.72^{ab}	32.64^{bc}	32.02^{c}	0.59	35.00^{a}	33.65^{a}	31.19^{b}	0.52	0.010	< 0.001	0.314	0.001
Lauric acid (C12:0)	4.37	4.11	3.97	4.31	0.22	3.03^{c}	5.12^{a}	4.42^{b}	0.19	0.557	< 0.001	0.303	0.712
Myristic acid (C14:0)	0.51	0.51	0.53	0.49	0.05	0.66^{a}	0.43^{b}	0.44^{b}	0.04	0.921	< 0.001	0.645	0.850
Palmitic acid (C16:0)	21.21^{a}	20.65^{ab}	19.42^{bc}	18.19^{c}	0.46	20.26^{a}	20.31^{a}	19.04^{b}	0.40	<0.001	0.046	0.617	<0.001
Stearic acid (C18:0)	7.43	6.99	7.08	7.40	0.24	8.99^{a}	6.47^{b}	6.22^{b}	0.21	0.334	< 0.001	0.314	0.968
Arachidic acid (C20:0)	0.15	0.17	0.18	0.20	0.02	0.15	0.19	0.19	0.02	0.238	0.073	0.879	0.054
^{B}ehenic acid (C22:0)	1.07^{b}	1.29^{ab}	1.47^{a}	1.42^{a}	0.08	1.91^{a}	1.14^{b}	0.88^{c}	0.07	0.007	< 0.001	0.048	0.002
Monounsaturated fatty acids	44.82^{a}	41.99^{b}	39.09^{c}	36.27^{d}	1.00	37.70^{b}	42.34^{a}	41.59^{a}	0.86	<0.001	0.001	0.691	<0.001
Palmitoleic acid (C16:1)	5.37^{a}	4.92^{ab}	4.22^{b}	3.23^{c}	0.29	3.71^{b}	5.17^{a}	4.43^{b}	0.25	<0.001	< 0.001	0.579	<0.001
Oleic acid (C18:1)	36.67^{a}	34.01^{b}	31.56^{c}	29.38^{c}	0.86	30.34^{b}	34.29^{a}	34.09^{a}	0.74	<0.001	< 0.001	0.764	<0.001
Gadoleic acid (C20:1)	0.85^{c}	1.24^{b}	1.43^{b}	1.89^{a}	0.12	1.36^{a}	1.30^{a}	1.40^{a}	0.10	<0.001	0.797	0.400	<0.001
Erucic acid (C22:1)	0.12	0.15	0.18	0.19	0.03	0.15	0.16	0.16	0.02	0.170	0.936	0.873	0.034
n-3 Polyunsaturated fatty acids	4.46^{c}	6.83^{b}	8.80^{a}	10.24^{a}	0.54	7.15	7.20	8.39	0.47	<0.001	0.112	0.373	<0.001
α-Linolenic acid (C18:3 n-3)	1.93^{c}	3.99^{b}	5.31^{ab}	6.65^{a}	0.49	3.22^{b}	4.52^{a}	5.67^{a}	0.42	<0.001	0.001	0.392	<0.001
Long-chain n-3 PUFA[2]	2.53^{b}	2.84^{b}	3.48^{a}	3.59^{a}	0.22	3.94^{a}	2.68^{b}	2.72^{b}	0.19	0.002	< 0.001	0.526	<0.001
Eicosatrienoic acid (C20:3 n-3)	0.20^{c}	0.32^{b}	0.53^{a}	0.57^{a}	0.04	0.47	0.37	0.38	0.03	<0.001	0.119	0.588	<0.001
Eicosapentaenoic acid (C20:5 n-3)	0.11	0.12	0.08	0.11	0.02	0.13^{a}	0.13^{a}	0.06^{b}	0.02	0.475	0.005	0.228	0.639
Docosapentaenoic acid (C22:5 n-3)	0.89^{b}	1.15^{b}	1.47^{a}	1.53^{a}	0.10	1.54^{a}	1.08^{b}	1.16^{b}	0.09	<0.001	< 0.001	0.336	<0.001
Docosahexaenoic acid (C22:6 n-3)	1.33	1.26	1.41	1.41	0.09	1.80^{a}	1.14^{b}	1.12^{b}	0.08	0.547	< 0.001	0.769	0.256
n-6 Polyunsaturated fatty acids	14.92^{c}	16.28^{c}	18.33^{b}	20.25^{a}	0.62	19.62^{a}	15.20^{c}	17.52^{b}	0.54	<0.001	< 0.001	0.861	<0.001
Linoleic acid (C18:2 n-6)	11.26^{d}	12.86^{c}	14.67^{b}	16.39^{a}	0.57	14.72^{a}	12.26^{b}	14.41^{a}	0.49	<0.001	< 0.001	0.882	<0.001
γ-Linolenic acid (C18:3 n-6)	0.24	0.26	0.27	0.29	0.02	0.37^{a}	0.22^{b}	0.20^{b}	0.02	0.436	< 0.001	0.329	0.108
Eicosadienoic acid (C20:2 n-6)	0.66^{b}	0.62^{b}	0.67^{b}	0.78^{a}	0.03	0.82^{a}	0.61^{b}	0.62^{b}	0.03	0.011	< 0.001	0.586	0.011
DGLA/SCA[3] (C20:3 n-6)	0.62	0.57	0.65	0.56	0.05	0.74^{a}	0.53^{b}	0.53^{b}	0.04	0.483	< 0.001	0.973	0.848
Arachidonic acid (C20:4 n-6)	1.89	1.69	1.79	1.96	0.12	2.62^{a}	1.36^{b}	1.52^{b}	0.10	0.401	< 0.001	0.570	0.518
Docosotrienoic acid (C22:3 n-6)	0.25	0.28	0.28	0.27	0.02	0.35^{a}	0.22^{b}	0.24^{b}	0.02	0.849	< 0.001	0.547	0.680
n-6:n-3 Polyunsaturated fatty acids	3.66^{a}	2.47^{b}	2.18^{b}	2.13^{b}	0.12	2.98^{a}	2.41^{b}	2.44^{b}	0.11	<0.001	< 0.001	0.148	<0.001

[1] Based on 1 g samples collected from 3 broilers per cage that were pooled (6 samples per treatment).
[2] PUFA = Polyunsaturated fatty acids.
[3] dihomo-γ-linolenic acid (DGLA), and sciadonic acid (Δ5,11,14–20:3; SCA) were calculated as sum for the both isomers of C 20:3 n-6.
[a-c]Means within row with different superscript differ (P<0.05).

Table 2.7. Effect of increasing dietary inclusion level of screw-pressed camelina expeller and length of feeding on fatty acid composition (% of total fatty acids) of thigh tissue of broilers[1].

Item	Camelina cake inclusion, %					Length of feeding, d				P - value			
	0	8	16	24	SEM	14	28	42	SEM	Level	Day	Level x day	Linear (level)
Saturated fatty acids	38.09	39.07	34.92	34.23	1.14	34.13	40.63	35.52	0.99	0.249	0.146	0.869	0.085
Lauric acid (C12:0)	1.37	1.51	1.55	1.65	0.17	1.71	1.55	1.30	0.15	0.615	0.086	0.127	0.205
Myristic acid (C14:0)	3.49	3.45	3.45	3.40	0.03	0.48	0.41	0.45	0.03	0.290	0.192	0.420	0.093
Palmitic acid (C16:0)	27.66	28.59	23.71	23.29	1.23	23.01	28.92	25.75	1.08	0.173	0.604	0.944	0.036
Stearic acid (C18:0)	5.12	4.89	5.38	5.16	0.43	5.40^{a}	5.87^{a}	4.45^{b}	0.39	0.768	0.014	0.261	0.573
Arachidic acid (C20:0)	0.09	0.12	0.38	0.17	0.11	0.11	0.31	0.15	0.10	0.258	0.328	0.379	0.237
Behenic acid (C22:0)	0.36^{b}	0.47^{ab}	0.49^{ab}	0.56^{a}	0.05	0.42^{b}	0.57^{a}	0.42^{b}	0.05	0.034	0.012	0.047	0.006
Monounsaturated fatty acids	46.11^{a}	44.44^{a}	41.95^{ab}	39.21^{b}	1.91	44.84	41.46	37.89	2.72	0.024	0.219	0.039	0.003
Palmitoleic acid (C16:1)	9.75^{a}	9.06^{a}	7.69^{ab}	5.89^{b}	0.96	7.56	7.74	6.99	0.85	0.022	0.070	0.068	0.003
Oleic acid (C18:1)	34.31	32.94	31.38	30.07	1.58	30.60	30.98	32.95	1.38	0.231	0.535	0.025	0.041
Gadoleic acid (C20:1)	1.50^{d}	1.86^{c}	2.25^{b}	2.63^{a}	0.10	2.09^{a}	1.83^{b}	2.26^{a}	0.09	<0.001	0.001	0.057	<0.001
Erucic acid (C22:1)	0.55^{b}	0.58^{ab}	0.63^{a}	0.62^{a}	0.02	0.59	0.61	0.59	0.02	0.021	0.599	0.631	0.003
n-3 Polyunsaturated fatty acids	2.63^{d}	4.70^{c}	7.31^{b}	9.72^{a}	0.44	5.75^{b}	5.00^{b}	7.53^{a}	0.39	<0.001	0.001	0.117	<0.001
α-Linolenic acid (C18:3 n-3)	1.88^{d}	3.62^{c}	6.09^{b}	8.34^{a}	0.44	4.83^{b}	3.72^{c}	6.40^{a}	0.39	<0.001	0.001	0.020	<0.001
Long-chain n-3 PUFA[2]	0.75^{b}	1.08^{ab}	1.22^{a}	1.38^{a}	0.17	0.91	1.28	1.13	0.16	0.010	0.089	0.091	<0.001
Eicosatrienoic acid (C20:3 n-3)	0.04^{c}	0.12^{b}	0.24^{a}	0.27^{a}	0.03	0.15	0.19	0.17	0.03	<0.001	0.511	0.830	<0.001
Eicosapentaenoic acid (C20:5 n-3)	0.03	0.02	0.03	0.03	0.00	0.02^{b}	0.03^{a}	0.02^{b}	0.01	0.789	0.001	0.945	0.902
Docosapentaenoic acid (C22:5 n-3)	0.27^{b}	0.45^{a}	0.48^{a}	0.54^{a}	0.07	0.34	0.50	0.47	0.07	0.012	0.083	0.042	0.003
Docosahexaenoic acid (C22:6 n-3)	0.41	0.48	0.47	0.55	0.08	0.40	0.56	0.47	0.07	0.494	0.126	0.059	0.180
n-6 Polyunsaturated fatty acids	13.17	11.78	15.82	16.84	1.83	15.30	12.94	14.97	1.67	0.077	0.390	0.827	0.020
Linoleic acid (C18:2 n-6)	11.23^{bc}	10.31^{c}	13.66^{ab}	15.06^{a}	1.42	13.32	10.90	13.47	1.30	0.019	0.132	0.848	0.004
γ-Linolenic acid (C18:3 n-6)	0.66	0.08	0.67	0.11	0.40	0.53	0.54	0.08	0.35	0.571	0.567	0.335	0.682
Eicosadienoic acid (C20:2 n-6)	0.23^{b}	0.28^{b}	0.38^{a}	0.41^{a}	0.03	0.29	0.34	0.34	0.03	<0.001	0.211	0.473	<0.001
DGLA/SCA[3] (C20:3 n-6)	0.19	0.22	0.23	0.24	0.03	0.19^{b}	0.26^{a}	0.20^{b}	0.03	0.506	0.021	0.139	0.156
Arachidonic acid (C20:4 n-6)	0.77	0.79	0.80	0.92	0.12	0.87	0.81	0.78	0.11	0.747	0.761	0.060	0.366
Docosotrienoic acid (C22:3 n-6)	0.08	0.10	0.09	0.11	0.01	0.10	0.09	0.09	0.01	0.393	0.596	0.163	0.221
n-6:n-3 Polyunsaturated fatty acids	5.53^{a}	2.54^{b}	2.59^{b}	1.90^{b}	0.63	3.58	3.23	2.61	0.55	<0.001	0.421	0.331	<0.001

[1] Based on 1 g samples collected from 3 broilers per cage that were pooled (6 samples per treatment).
[2] PUFA = Polyunsaturated fatty acids.
[3] dihomo-γ-linolenic acid (DGLA), and sciadonic acid (Δ5,11,14–20:3; SCA) were calculated as sum for the both isomers of C 20:3 n-6.
[a-c]Means within row with different superscript differ (P<0.05).

Table 2.8. Effect of increasing dietary inclusion level of screw-pressed camelina cake on fatty acid composition (mg/g of wet tissue) of thigh and breast meat of 14, 28, and 42 d-old broilers[1].

		Camelina cake inclusion, %					P-value	
Item	Day	0	8	16	24	SEM	Level	Linear
Breast								
Total fatty acid	14	11.70	10.99	11.37	10.78	0.59	0.153	0.062
	28	17.32	17.70	21.12	27.87	6.97	0.608	0.228
	42	14.35	9.30	14.91	30.72	7.45	0.240	0.123
Saturated fatty acids	14	4.08	3.96	3.93	3.70	0.20	0.611	0.228
	28	6.08	6.08	7.08	8.91	2.32	0.731	0.314
	42	4.99	2.83	4.44	9.26	2.34	0.279	0.185
Monounsaturated fatty acids	14	5.04	4.35	3.94	3.69	0.36	0.079	0.014
	28	8.22	7.82	8.51	10.50	2.74	0.865	0.503
	42	6.51	4.05	6.80	11.11	2.83	0.386	0.212
n-6 Polyunsaturated fatty acids	14	1.99	1.92	2.42	2.42	0.16	0.066	0.015
	28	2.16	2.50	3.40	4.86	1.10	0.250	0.060
	42	2.06	1.56	2.42	6.39	1.53	0.136	0.065
n-3 Polyunsaturated fatty acids	14	0.55^{b}	0.70^{ab}	1.04^{a}	0.92^{a}	0.12	0.041	0.010
	28	0.59	1.02	1.74	3.06	0.67	0.068	0.013
	42	0.63^{b}	0.77^{b}	1.12^{b}	3.49^{a}	0.72	0.043	0.029
Long-chain n-3 polyunsaturated fatty acids	14	0.36^{b}	0.40^{b}	0.53^{a}	0.45^{ab}	0.04	0.016	0.011
	28	0.35	0.42	0.63	0.93	0.23	0.234	0.053
	42	0.31^{c}	0.61^{bc}	0.82^{b}	1.19^{a}	0.21	0.024	0.042
Thigh								
Total fatty acid	14	44.37	34.43	29.99	19.91	10.74	0.311	0.077
	28	32.84	31.56	31.07	32.91	2.20	0.848	0.953
	42	32.02	32.51	55.97	55.01	13.19	0.180	0.041
Saturated fatty acids	14	9.85	7.67	8.24	4.91	2.60	0.300	0.119
	28	9.11	8.13	7.77	7.72	0.76	0.252	0.078
	42	8.52	8.21	13.04	11.61	3.23	0.472	0.187
Monounsaturated fatty acids	14	17.60	17.92	12.12	6.99	5.10	0.302	0.077
	28	14.16^{a}	12.31^{ab}	9.76^{b}	11.03^{b}	1.39	0.038	0.013
	42	13.54	13.30	24.06	20.56	5.94	0.354	0.132
n-6 Polyunsaturated fatty acids	14	11.21	3.88	4.64	3.51	4.39	0.563	0.301
	28	3.30	3.49	5.04	4.61	0.75	0.237	0.072
	42	3.80^{b}	4.19^{b}	8.08^{ab}	9.92^{a}	1.81	0.022	<0.001
n-3 Polyunsaturated fatty acids	14	0.90	1.63	2.33	2.01	0.71	0.408	0.150
	28	0.77^{c}	1.39^{b}	1.69^{b}	2.43^{a}	0.24	<0.001	<0.001
	42	1.10^{b}	1.87^{b}	4.98^{a}	6.74^{a}	1.16	<0.001	<0.001
Long-chain n-3 polyunsaturated fatty acids	14	0.13	0.19	0.24	0.20	0.04	0.121	0.065
	28	0.31^{b}	0.31^{b}	0.42^{ab}	0.55^{a}	0.07	0.014	0.002
	42	0.27^{c}	0.31^{c}	0.53^{b}	0.76^{a}	0.07	0.031	0.022

[1] Based on 1 g samples collected from 3 broilers per cage that were pooled (6 samples per treatment).
[a-c]Means within row with different superscript differ (P<0.05).

Chapter 3

Feeding stearidonic acid-enhanced flaxseed oil to egg layers increases long-chain omega-3 PUFA in the table eggs

Abstract: Feeding flaxseed oil enriched in stearidonic acid (SDA; 25% of total fatty acids) and g-linolenic acid (GLA; 16% of total fatty acids) content was compared to traditional flaxseed oil and corn oil in laying hens for its ability to increase long chain (LC) n-3 polyunsaturated fatty acids (PUFA) in table eggs. White Leghorn laying hens (47 wk old) were fed a basal diet including 4% corn oil (Control), 4% regular flax oil (REGflax) or 4% SDA-enriched flax oil (SDAflax). Egg yolks collected on day 0, 7, 14 and 21 were analyzed for fatty acid composition. Fatty acid composition of liver, thigh, breast, heart, brain, and abdominal fat pad were analyzed after 21 d of feeding.

Eggs from hens fed SDAflax for 21 d were enriched with SDA to 0.30% of total yolk fatty acids. The SDAflax eggs also had an 8-fold increase in yolk GLA by 7 d of feeding. Feeding both REGflax and SDAflax increased yolk total n-3 PUFA by 6-fold compared with Control eggs at 7 d ($P<0.001$). Although there was no difference in total n-3 PUFA content between SDAflax and REGflax yolks at 21d, the increase in LC n-3 PUFA in egg yolks from hens fed SDAflax oil was double that of those fed REGflax. There was a 6-fold increase in eicosatetraenoic acid (ETA; 0.11% *vs*. 0.66%), a 1.5-fold increase in EPA (0.17% *vs.* 0.25%), and a 2.2-fold increase in DPA (0.18% *vs*. 0.39%), in SDAflax compared with REGflax yolk lipids after 21 d. Feeding SDAflax oil compared with REGflax oil resulted in more LC n-3 PUFA deposition in thigh and breast muscle and in all other tissues tested except abdominal fat pad at d 21. In the abdominal fatpad, LC n-3 PUFA were minimal indicating that enrichment was likely in the phospholipid rather than the triglyceride fraction. These results suggest that SDA-

enriched flaxseed can be included in egg layer diets to increase LC n-3 PUFA in table eggs to enhance human health benefits.

Keywords: Egg, gamma-linolenic acid, modified flax, long chain n-3 polyunsaturated fatty acids, stearidonic acid.

3.1 INTRODUCTION:

Health organizations are advising consumers to increase consumption of omega-3 polyunsaturated fatty acids (**n-3 PUFA**), specifically long chain (**LC**) n-3 PUFA: eicosapentaenoic (20:5 n-3; **EPA**) and docosahexaenoic acids (22:6 n-3; **DHA**). Increased intake of LC n-3 PUFA by humans has been positively linked to a reduction in the incidence of cardiovascular diseases (Tousoulis et al., 2014), atherosclerosis (Thies et al., 2003), and increased visual and neurological development in infants (Birch et al., 2010).

Enrichment of poultry products with LC n -3 PUFA is mainly achieved by feeding fish oil or marine algae oil (Rymer and Givens, 2005; Lemahieu, et al., 2015). However, by 2010 aquaculture was consuming 73% of global production of fishmeal and 71% of fish oil from only 10% of fishmeal and 16% fish oil in 1990 (FAO, 2012). Alternative sources of LC n-3 PUFA are needed to ensure sustainability of poultry product enrichment. Flaxseed, with about 58% of total fatty acids as α-linolenic acid (18:3n-3; **ALA),** is the major plant source included in poultry diets for egg enrichment of n-3 PUFA (Fraeye et al., 2012).

The β-oxidation pathway is the predominant fate of dietary medium chain PUFA such as ALA in rodents and humans. Further, the β-oxidation of ALA is negatively correlated with its bioconversion into LC n-3 PUFA metabolites (Vermunt et al., 2000). However, laying hens do not preferentially oxidize ALA and hence are able to bioconvert a greater proprotion into LC n-3 PUFA (Reiser et al., 1951). The bioconversion of ALA to LC n-3 PUFA, although greater in poultry than in mammals, is still only moderate in avian species due to the dependency on the rate limiting Δ6-desaturase (Poureslami et al., 2010). Flaxseed has been genetically altered to express a high content of stearidonic acid (18:4 n-3; **SDA;** Abbadi et al., 2004; Subedi et al.,

2015), which is a metabolic intermediate on the biosynthetic pathway of ALA to EPA (Ruiz-Lopez et al., 2012). Bypassing the rate-limiting initial Δ 6-desaturation of ALA to SDA, SDA can readily be converted to EPA and to a limited extend to DHA, more efficiently than ALA in humans (James et al., 2003) or in chickens (Kitessa and Young, 2009). Dietary SDA increased omega-3 index (EPA + DHA, measured as % of total blood fatty acid) by 19.5% in the SDA group *vs.* 25.4% in the EPA group compared with a control group in humans (Harris et al., 2008). In addition, SDA has an inhibitory effect on mice fibroblast cell growth (Cantrill et al., 1993). Furthermore, SDA-enriched flax oil has anti-tumor activity against breast cancer cells (Subedi et al., 2015).

A diet with 4% SDAflax oil also contains 13.3% gamma linolenic acid (18:3 n-6; **GLA**). Several clinical studies, mostly with borage seed oil (24% GLA) or evening primrose oil (8 to 10% GLA), have shown beneficial effects in atopic dermatitis (Stewart et al., 1991; Simon et al., 2014) and rheumatoid arthritis in humans (Leventhal et al., 1993). The aim of the present study was to examine the potential of feeding layers a novel SDA/GLA-enhanced flaxseed compared to a regular flaxseed to enrich table egg n-3 PUFA proportions and specifically to enrich LC n-3 PUFA (DHA, DPA and EPA). We also examined the sites of enrichment lipid deposition within the hen and whether potential enrichment for the egg was lost to other tissues or organs of the body.Therefore, the fatty acid composition of economically important breast and thigh muscles along with the non-meat compartments such as liver, heart, brain and abdominal fatpad were analyzed.

3.2 MATERIALS AND METHODS

3.2.1 Birds and Management

Animal use and experimental procedures were reviewed by the University of Alberta Animal Care and Use Committee livestock and were in accordance with the Canadian Council on Animal Care (2009). The novel test ingredient, SDA-enhanced flaxseed, was grown at the University of Alberta's Faculty of Agricultural, Life, and Environmental Sciences land in St. Albert, AB, Canada as described by Subedi et al. (2015). The oil from SDA-enhanced flaxseed was extracted using a Komet single-screw press (Model CA 59 G; IBG Monforts, Mönchengladbach, Germany). Processing conditions were: screw speed = 45 rpm, barrel temperature = 60°C, and processing rate ranged from 4 to 6 kg/h.

A group of 45-wk-old Lohmann White Leghorn laying hens was selected from the Poultry Research Centre flock based on a high level of egg production and uniformity of body weight. Selected hens (n=24) were moved to individual laying cages within the same barn, two weeks before the start of the trial to allow the birds to become acclimatized to the cage environment. Eight hens were then randomly allocated to each of the 3 experimental treatments. The birds had ad libitum access to feed and water throughout the 21 d trial. A lighting program of 16 h of light and 8 h of dark was used for the entire experiment.

A basal diet was formulated to meet or exceed the nutrient requirements (Layer Management Guide, Lohmann LSL-Classic, 2004; NRC, 1994) for laying hens (47 weeks) and 4% of one of the three test oil sources was added (Control = corn oil; REGflax = regular flax oil and SDAflax = SDA-enriched flax oil; Table 3.1). Representative diet samples were collected to measure the dietary fatty acid profile. At days 0, 7, 14 and 21, eggs were collected from each hen and separated yolk samples were frozen (-20°C) until fatty acid analysis. At the end of the trial, all layers were euthanized by cervical dislocation, and tissues (liver, skinless thigh, skinless

breast, heart, brain, abdominal fatpad) were collected and stored at -20°C until fatty acid analysis.

3.2.2 Fatty Acid Analysis.

Fatty acid profiles of yolk were analyzed using GC-MS. The fatty acids were extracted and derivitized as outlined by Nain et al., (2012). For each yolk, 1-g sample (8 samples/treatment) was homogenized with Folch solution for 30 s using a homogenizer (Power Gen 1000S1, Fisher Q1 Scientific) in 25 × 150-mm Teflon lined screw-capped test tubes. The extraction of fat and derivatization from tissue samples was performed as described by Nain et al., (2015). The resultant fatty acid methyl esters were then analyzed by gas chromatography (GC; model 7890A, Agilent Technologies, Palo Alto, CA) fitted with a 5975 inert XL Mass Selective Detector. The fatty acid methyl esters were separated using a DB-23 capillary column (30 m × 0.25 mm × 0.25 μm) with a constant helium flow of 1.2 mL/min. The following temperature program was applied: 90°C, hold for 4 min, increase by 10°C/min to 180°C, then hold for 2 min, and increase by 5°C/min to 220°C, finally hold for 2 min (total runtime = 25 min). The fatty acid peak integration and analysis was performed using Agilent Chemstation software, rev B.04.02. (Agilent Technologies, Palo Alto, CA). The fatty acids were identified by comparison to standards (GLC-463, and GLC-421A, NU-CHEK Prep, Inc. Elysian, MN).

Individual fatty acid content was expressed as percentage of total fatty acids (TFA). Saturated fatty acids (SFA) were the sum of 14:0 + 16:0 + 18:0 + 20:0. Monounsaturated fatty acids (MUFA) were the sum of 16:1n-7 + 18:1n-7 + 18:1n-9 + 20:1n-9. Total n-3 fatty acids were the sum of ALA + SDA + 20:4n-3 (ETA) + EPA + DPA + DHA. Total n-6 fatty acids were the sum of 18:2n-6 (LA) + GLA + 20:2n-6 + 20:3n-6 (DGLA) + 20:4n-6 (AA) + 22:4n-6.

3.2.3 Statistical Analysis

The experimental unit was considered to be the cage, and treatments were assigned to cage units in a completely randomized fashion. Fatty acid proportions of egg yolk were analyzed as a two way ANOVA including oil type (Control, REGflax and SDAflax) and sampling days (0d, 7d, 14d and 21d) as fixed effects. Least squares means were compared using Tukey's test, and reported as significant if $P < 0.05$. Fatty acid proportions of liver, skinless thigh, skinless breast, heart, brain, and abdominal fatpad were analyzed as a one-way ANOVA using the MIXED procedure of SAS with dietary oil (Control, REGflax and SDAflax) as fixed effect.

3.3 RESULTS AND DISCUSSION

Control, REGflax oil and SDAflax oil diets contained 19.3%, 21.8% and 21.9% SFA and 26.8%, 21.7% and 20.5% MUFA, respectively (Table 3.2). Diets containing either flax oil had nearly one third of fatty acids as n-3 PUFA wheras Control diet contained one half of total fatty acid as n-6 PUFA. The two flax oil diets had nearly a 1:1 n-6 to n-3 PUFA ratio, drastically different from corn oil control (22:1). However, they differed in the type of n-6 PUFA and n-3 PUFA proportions. The SDAflax oil diet had 13.8% SDA and 16.9% ALA compared to 31.7% of ALA in the REGflax oil diet. In addition, the diet with SDAflax oil had 13.3% GLA and 9.7% LA whereas in the REGflax oil diet, LA was the predominant n-6 PUFA at 24.4% of total fatty acids. Fatty acid composition of diets can modify the fatty acid composition of egg yolk (Oliveira et al., 2010). However, yolk fatty acid composition is also affected by the rates of de novo fatty acid synthesis, beta-oxidation, and elongation and desaturation (Pourslami et al., 2010; Kartikasari et al, 2012). A greater proportion of ALA (Nain et al., 2012) or lower ratio of n-6 to n-3 PUFA increases the bioconversion process (Goldberg et al., 2012) and thus play an important role in the fatty acid composition of yolk and other tissues.

3.3.1 Egg Yolk Fatty Acid Profile

3.3.1.1 Saturated and monounsaturated fatty acids

The total fatty acid in egg yolk was not affected by oil type, duration of feeding, nor were there any interactions (Table 3.3). There was oil by day interactions ($P < 0.05$) for egg yolk total SFA and MUFA. Total SFA proportion in egg yolks from hens fed REGflax oil decreased more with duration of feeding than layers fed corn oil, which were intermediate. In contrast, SFA proportion increased by d 7 and remained elevated in yolks of layers fed SDAflax oil (Table 3.3). Yolk C16:0 proportions remained relatively constant over time for controls, but it progressively decreased for layers fed REGflax oil, whereas it increased by d 7 and remained greater until d 21 for layers fed SDAflax oil. Yolk C18:0 proportions remained relatively constant over feeding day for both Control and REGflax oil layers, whereas it increased by d 7 and were greater up to d 21 for layers fed SDAflax oil. An inverse trend to that observed for yolk SFA was evident for MUFA. Yolk total MUFA, C18:1 and C16:1 remained relatively constant for Control hens; proportions progressively increased with duration of feeding for layers fed REGflax oil, but decreased by d 7 and remained lower for layers fed SDAflax oil.

High dietary PUFA has been associated with the inhibition of Δ-9 desaturase (**SCD**; Stearoyl-CoA desaturase), the enzyme responsible for the conversion of C16:0 and C18:0 to their corresponding MUFA (Garg et al., 1988; Lefevre et al., 2001) in laying hens (Kim et al., 2007) and broilers (Royan et al., 2011). In the present study, the Control, REGflax oil and SDAflax oil diets contained 54, 57 and 58% total PUFA (Table 3.2), so an expected result would have been a reduction in yolk MUFA. However, we observed that feeding SDAflax oil reduced yolk MUFA compared with Controls after only 7 d of feeding, but REGflax oil in contrast resulted in increased yolk MUFA after 14 d of feeding. It seems that SDAflax oil may have reduced endogenous lipid synthesis in laying hens whereas REGflax oil increased it compared with

Control. There was no difference in yolk MUFA when high PUFA diets including sunflower oil, soybean or flaxseed oil were fed to laying hens (Mazalli et al., 2004). Similarly, Elkin et al., (2015) also indicated an increased C16:0 and C18:0 and reduced C16:1 and C18:1 in egg yolks from hens fed SDA-soybean oil compared with regular flax oil despite equal dietary inclusion (5%). However, the SDAflax oil used in the present study had greater n-3 PUFA compared with the SDA-soybean (53 vs. 37.4% of total fatty acids) used by Elkin et al., (2015). Therefore, the reason for increased SFA, C16:0 and C18:0 and decreased C16:1 and C18:1 in laying hens fed SDAflax oil compared with REGflax oil needs to be further elucidated through analyzing the effect of SDAflax oil inclusion on expression of other enzymes, such as SCD (Shang et al., 2005), expression of peroxisome proliferators-activated receptor α or carnitine palmitoyl transferase and acyl-coenzyme A oxidase in *de novo* synthesis of fatty acid (Tang et al., 2007).

3.3.1.2 Polyunsaturated fatty acids

There were oil by day interactions ($P < 0.05$) for total n-6 PUFA in egg yolk (Table 3.4). Feeding either type of flax oil decreased total n-6 PUFA and LA by 7 percentage points by d 7. Yolk C20:4n-6 (AA) remained constant over time for Controls, but decreased in eggs from layers fed REDflax oil by d 7 comapred with 0 d and remained constant thereafter. Yolk AA was decreased in the eggs from hens fed SDAflax oil by 21 d comapred with 0 d. Yolk GLA increased 8-fold by d 7 and remained elevated in hens fed SDAflax oil compared with the Control and REGflax oil treatments. The reduction in yolk AA over time with dietary REGflax oil in laying hens is in agreement with prior research (Cherian and Sim, 1993; Nain et al., 2012). Inclusion of SDAflax oil in laying hen rations resulted in an 8-fold increase in egg yolk GLA after only 7 d of feeding compared with Control and REGflax oil. The reduction in yolk AA in hens fed with increased dietary n-3 PUFA is associated with reduced Δ6-desaturase availability

for n-6 PUFA substrates (Cherian and Sim, 2001). In addition, the increased proportion of GLA and DGLA in SDAflax oil compared with REGflax did not contribute to increasing the AA proportion in yolk. The reduction in AA despite higher dietary GLA and DGLA might be due to competition for desaturation and and elongation for bioconversion of GLA to AA. The desaturase and elongase might have preferred n-3 PUFA substrate over n-6 PUFA substrate as observed for the use of Δ6-desaturase, where ALA has greater affinity compared with LA (Brenner, 1971).

There was an oil by day interaction for yolk total n-3 PUFA (Table 3.5). Inclusion of either flax type oil in diet increased total n-3 PUFA in egg yolk by 6-fold by d 7 of feeding compared with corn oil, but there were no differences between SDAflax and REGflax oil groups at any of the sampling days. The same pattern was observed for yolk LC n-3 PUFA, but the increase for layers fed SDAflax oil was double that of those fed REGflax oil by d 7 and remained greater to d 21. Yolk ALA increased 7.5-fold for layers fed either flax oil vs. corn oil by d 7, and increased 2 percentage points more by d 14 for layers fed REGflax vs. SDA flax. Yolk DHA proportion doubled by d 7 feeding either flax oil whereas it remained low in layers fed corn oil at all sampling days. Yolk ETA increased 5-fold by d 7, for layers fed SDAflax oil, but not in the other treatments groups. There was an oil treatment effect ($P<0.05$) on SDA and EPA. Feeding 4% SDAflax oil increased SDA from non-detectable levels to 0.30% of the total yolk fatty acids whereas it was absent in yolk of layers fed REGflax oil or corn oil. Yolk EPA increased in layers fed either flax oil type, but the increase was 0.1 percentage point greater for those fed SDAflax oil.

The increased egg LC n-3 PUFA in both flax oil groups compared with Control demonstrates that laying hens are capable of increasing the long chain fatty acid metabolites in

the egg from medium chain n-3 PUFA in the diets. In addition, the additional increase (57% greater) in yolk LC n-3 PUFA in hens fed SDAflax oil compared with REGflax oil indicates increased bioconversion with the presence of SDA in SDAflax oil compared with ALA in REGflax oil. However, in the current study, the greatest increase among long chain LC n-3 PUFA was for ETA (211.7% increase) in SDAflax oil eggs after only 7 d, suggesting the need for identification of a new rate-limiting steps in the bioconversion chain from SDA to DHA. Birds have a greater capability of elongation (Gregory et al., 2013). However, the conversion of ETA to EPA is Δ5-desaturase-dependent, and therefore the increased proportion of ETA relative to EPA and DPA in yolks from hens fed SDAflax oil at 21 d compared to other treatments might be an indication of a potential rate limiting step in LC n-3 PUFA bioconversion.

The omega-3 index (percentage of EPA+DHA in red cell lipids) was increased in humans fed SDA (Harris et al., 2003; Lemke et al., 2010). Further, James et al., (2003) suggested that intake of 1 g SDA is as effective as dietary intake of 300 mg of EPA for the efficacy of increasing tissue EPA in humans. The n-6 PUFA, GLA has anti-inflammatory effects similar to n-3 PUFA, probably by increasing DGLA and suppressing the biosynthesis of AA, which is associated with the generation of inflammatory metabolites (Johnson et al. 1997). Humans can consume up to 4,200 mg SDA/day for 12 weeks, and up to 1,700 mg GLA/day for 28 days and 9,100 mg ALA/day for four weeks without any adverse effect (EFSA, 2010). The eggs enriched with inclusion of SDAflax oil in layers diet provide a dual advantage for the egg enrichment strategies. The SDAflax oil increased the LC n-3 PUFA in egg yolk and it also provided increased SDA proprotions in the egg yolk. Therefore, the egg enriched with SDAflax oil may be a novel food source in the supplementation strategy for increasing LC n-3 PUFA levels in the general population.

The ratio of n-6 to n-3 PUFA in egg yolks decreased from 19.6 to 4.3 and from 17.2 to 1.9 by d 7 and decreased further to 1.61 and 1.55 by d 21 of feeding REGflax oil and SDAflax oil, respectively, whereas it increased from 17.7 to 24.0 by d 14 of feeding corn oil (Table 3.5). In humans, diets with a lower n-6 to n-3 PUFA ratio down-regulate the expression of genes for pro-inflammatory cytokine synthesis (Weaver et al., 2009). A low n-6 PUFA to n-3 PUFA ratio led to increased mental performance in children 3 to 10 years of age (Sheppard and Cheatham, 2013). The eggs from layer fed either REGflax oil or SDAflax oil had ratios closer to the suggested historical ratio of 1:1 in humans (Simopoulos, 2011).

3.3.2 Fatty Acid Composition in Tissues

The fatty acid composition of different anatomical compartments in chickens responds differently to dietary fatty acid changes for the preference of fatty acid accumulation and overall fatty acid composition (Pourslami et al., 2011). In the present study, we analyzed the fatty acid composition of liver due to its prominant role in n-3 PUFA metabolism (Cherian and Sim, 2001); abdominal fatpad contains most of the stored fatty acids (Wood et al., 2008). Fatty acid composition of economically important skeletal muscle (i. e. breast and thigh meat), whereas the heart, composed of smooth muscle, was also analyzed (Bonen et al., 2009). Brain has preferential deposition of LC n-3 PUFA especially DHA in birds as well as mammals (Cherian and Sim, 1991). The detailed fatty acid profiles of tissues are included as appendix in the thesis. There was no dietary oil effect on total SFA and MUFA in breast, thigh (Table 3.6), brain (Table 3.7) or heart tissue (Table 3.8). In liver (Table 3.7) and abdominal tissue (Table 3.8), there was no difference for total SFA between layers fed corn oil and REGflax oil. However, there was increased SFA in these tissues for hens fed SDAflax oil compared with REGflax oil. In addition, there was decreased n-6 PUFA in all tissues tested except heart in hens fed REGflax oil and

SDAflax oil compared with corn oil. Except in the brain, there were increases in total n-3 PUFA in breast and thigh muscle, liver and heart tissues and abdominal fatpad from hens fed SDAflax oil and REGflax oil as compared with hens fed corn oil Control (Tables 3.6, 3.7 and 3.8). The increase in total n-3 PUFA in most of the tissues is in accordance with previous studies in that the fatty acid proportions of different tissues reflect dietary fatty acid composition (Celebi et al., 2011).

However, there was a greater proportion of LC n-3 PUFA in liver (54% greater) and heart tissue (5% greater) ($P<0.05$) for hens fed SDAflax oil as compared to tissues from hens fed REGflax oil, respectively. There was no effect of dietary oil treatment on LC n-3 PUFA proportion in abdominal fat pad. The LC n-3 PUFA deposit preferably in the phospholipid rather than in the triglyceride fraction of the tissue (Betti et al., 2009) and the abdominal fatpad contains more than 90% of fatty acids as triglycerides (Wood et al., 2008). Therefore, there was no dietary oil effect on the LC n-3 PUFA proportions in abdominal fatpad.

Stearidonic acid was present in the muscle, liver, heart, fatpad and brain tissue of hens fed SDAflax oil (Figure 3.1), and was not detected in tissues from birds fed corn oil or REGflax oil. Previously, SDA-enriched diets containing echium oil (Kitessa and Young, 2003) or SDA-enriched soybean oil (Rymer et al., 2011) have enriched broiler tissues with SDA. In the present study, however, we observed that the abdominal fat had the greatest proportion of SDA among all the tissues studied, suggesting that a greater proportion of SDA might have escaped metabolism and was stored in abdominal fatpad. It is important to note that the medium chain n-3 PUFA (ALA and SDA) have greater affinity for the beta–oxidation pathway compared with the LC n-3 PUFA (Brenner and Norum 1982) and therefore increased LC n-3 PUFA resulted from n-3 PUFA bioconversion was observed throughout body compartments in laying hen.

Laying hens efficiently transfer fatty acid from diets through highly specialized mechanism of packaging into "yolk-targeted" very low density lipoprotein (VLDLy) particles in the liver (Walzem et al., 1999). These VLDLy are deposited in the growing ovarian follicle without changing fatty acid composition via receptor-mediated endocytosis (Holdsworth, et al., 1974). In the current study, the difference for LC n-3 PUFA among hens fed SDAflax oil compared with REGflax oil was most evident in liver and egg yolks. Higher estrogen in women relative to men increases bioconversion of ALA to DHA via stimulation of the Δ5 and Δ6 desaturases (Giltay et al., 2004). Estrogen in laying hens stimulates the biosynthesis of VLDLy (Walzem et al., 1999). In addition, laying hens are highly efficient in incorporation of LC n-3 PUFA into the developing ovarian follicle (Speake et al., 1998). We speculate that the known link between estrogen and ALA bioconversion to long chain n-3 PUFA (Burdge, 2002) might be enhanced in laying hens, resulting in an even greater bioconversion of SDA to long chain n-3 PUFA by the hen than what may be possible in humans consuming SDA. In addition, broiler chickens have a greater capability to elongate medium chain to long chain fatty acids compared with most mammals, and even some fishes (Gregory et al., 2013). However, the effect of high n-6 PUFA or high LC n-3 PUFA on enzymatic expression for n-3 PUFA bioconversion in the laying hens needs to be addressed. Numerous factors apart from the conversion to longer chain metabolites, such as mitochondrial beta-oxidation and fatty acid excretion (Turchini et al., 2007) might play an important role for the final concentration of fatty acids in the eggs.

In conclusion, feeding layers 4% REGflax oil increased egg yolk LCn-3 PUFA after only 7 d compared with Controls fed corn oil. Furthermore, feeding 4% SDAflax oil to laying hens increased each of the LC n-3 PUFA (ETA, EPA, DPA and DHA) in egg yolk at 7, 14 and 21 d compared with feeding REGflax oil to hens. Therefore, it can be concluded that including 4%

SDAflax oil in layer diets, by bypassing the rate-limiting Δ6-desaturase-dependent metabolic step, provided greater LC n-3 PUFA in eggs compared with feeding regular flax oil. The efficacy of inclusion of SDAflax and its effect on egg quality and reproductive status needs to be elucidated in future studies. The inclusion of SDAflax in laying hen diets has the potential to become a viable alternative to current but depleting marine sources of LC n-3 PUFA (fish oil). In addition, further investigation is needed to understand the effect of SDAflax oil on the enzymes of lipogenesis and beta-oxidation of fatty acid in the laying hens.

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Table 3.1 Ingredient composition of the basal diet

Ingredients	Basal diet
Corn grain	47.9
Soybean meal	21.6
Wheat bran	14.8
Calcium carbonate	8.5
Dicalcium phosphate	1.1
Layer vitamin/mineral premix[1]	0.5
choline chloride[2]	0.5
Vitamin E[3] premix	0.5
Sodium chloride	0.3
D,L – methionine	0.2
Treatment oil[4]	4.0
Calculated Nutrient Composition:	
ME, kcal/kg	2,720
Crude Protein, %	17.0
Crude Fat, %	6.5
Ca, %	3.6
P, available %	0.4
Met + cys, %	0.7
Lysine, %	0.9

[1]Provided the following per kilogram of diet: vitamin A (retinyl acetate), 12,000 IU; cholecalciferol, 3,000 IU; vitamin E (DL-α-tocopheryl acetate), 40 IU; vitamin K, 2.0 mg; pantothenic acid, 14 mg; riboflavin, 6.5 mg; folacin, 1.0 mg; niacin, 40 mg; thiamine, 3.3 mg; pyridoxine, 6.0 mg; vitamin B12, 0.02 mg; biotin, 0.2 mg; iodine, 0.5 mg; Mn, 75 mg; Cu, 15 mg; Zn, 80 mg, Se, 0.1 mg; and Fe, 100 mg;

[2]Provided 1000 mg of choline chloride.. per kg of diet

[3]Provided vitamin E, 50 IU per kg.of diet. 125 mg of ethoxyquin.

[4]The experimental diets were: Control–basal diet + 4% corn oil; REGflax oil –basal diet + 4% regular flax oil; Stearidonic acid-enriched flax (SDAflax) –basal diet + 4% SDA-enriched flax oil.

Table 3.2: Analyzed fatty acid composition of the experimental diets[1].

Fatty acid (% of total fatty acids)	Control	REGflax	SDAflax
Saturated fatty acids (SFA)	19.3	21.8	21.9
C14:0	0.4	0.5	0.5
C16:0	16.5	15.6	14.1
C18:0	1.7	5.3	6.9
C20:0	0.4	0.3	0.3
C22:0	0.3	0.1	0.1
Monounsaturated fatty acids (MUFA)	26.8	21.7	20.5
C16:1	1.1	0.6	0.5
C18:1	25.3	20.9	19.8
C20:1	0.4	0.2	0.2
n-6 Polyunsaturated fatty acids	51.6	24.6	26
C18:2n-6 (LA)	49.8	24.4	9.7
C18:3n-6 (GLA)	1.1	ND	13.3
C20:3n-6	0.7	0.2	1.8
C20:4n-6	ND	ND	1.2
n-3 Polyunsaturated fatty acids	2.3	31.9	31.6
C18:3n-3 (ALA)	2.2	31.7	16.9
C18:4n-3 (SDA)	ND	ND	13.8
C20:4n-3 (ETA)	ND	0.1	0.4
C22:6n-3(DHA)	0.1	0.1	0.5
Long Chain n-3 Polyunsaturated fatty acids	0.1	0.2	0.9
Ratio of n-6 / n-3 Polyunsaturated fatty acids	22.4	0.8	0.8

[1]Control–basal diet + 4% corn oil, REGflax oil –basal diet + 4% regular flax oil, Stearidonic acid-enriched flax (SDAflax) –basal diet + 4% SDA-enriched flax oil.

Table 3.3. Saturated and monounsaturated fatty acids (% of total fatty acids) in egg yolk from laying hens fed corn oil, regular flax oil or SDAflax oil diets[1] for 21 d.

Treatment	Control				REGflax				SDAflax					P-value		
Duration	0 d	7 d	14 d	21 d	0 d	7 d	14 d	21 d	0 d	7 d	14 d	21 d	SEM	Oil	Day	Oil*Day
TFA[2]	406.38	414.01	400.80	407.56	408.43	382.20	408.43	400.07	404.30	392.20	407.25	382.30	15.14	0.536	0.309	0.253
Total SFA[3]	37.33^{b}	37.11^{b}	37.31^{b}	36.75^{bc}	36.61^{bc}	35.98^{bcd}	35.19^{cd}	34.73^{d}	37.17^{b}	42.27^{a}	43.19^{a}	42.41^{a}	0.376	<0.001	<0.001	<0.001
C14:0	0.26	0.24	0.27	0.28	0.23	0.23	0.24	0.24	0.26	0.29	0.32	0.30	0.012	<0.001	0.003	0.138
C16:0	26.77^{bcd}	26.48^{cd}	27.49^{bcd}	26.15^{de}	25.90^{def}	25.83^{def}	25.12^{ef}	24.10^{f}	26.58^{cde}	28.67^{ab}	29.53^{a}	28.09^{ab}	0.417	<0.001	0.001	0.001
C18:0	10.18^{b}	10.28^{b}	9.46^{b}	10.19^{b}	10.36^{b}	9.83^{b}	9.76^{b}	10.26^{b}	10.21^{b}	13.20^{a}	13.26^{a}	13.91^{a}	0.293	<0.001	<0.001	<0.001
C20:0	0.05	0.05	0.04	0.11	0.05	0.04	0.05	0.06	0.06	0.07	0.05	0.07	0.014	0.272	0.005	0.385
Total MUFA[4]	38.70^{bc}	38.93^{bc}	39.31^{b}	39.34^{b}	39.07^{bc}	40.60^{ab}	41.82^{a}	42.37^{a}	39.36^{b}	35.83^{d}	35.61^{d}	36.70^{cd}	0.597	0.001	0.127	<0.001
C14:1	0.07	0.06	0.05	0.07	0.07	0.07	0.05	0.09	0.07	0.06	0.05	0.05	0.005	0.074	0.058	0.001
C16:1	2.39^{bc}	2.24^{c}	2.52^{bc}	2.49^{bc}	2.13^{c}	2.91^{ab}	3.03^{a}	3.07^{a}	2.45^{bc}	2.08^{c}	2.19^{c}	2.26^{c}	0.114	<0.001	<0.001	0.005
C18:1	36.14^{bcd}	36.52^{bc}	36.63^{abc}	36.67^{abc}	36.78^{abc}	37.56^{ab}	38.70^{ab}	39.18^{a}	36.76^{abc}	33.67^{d}	33.36^{d}	34.38^{cd}	1.238	<0.001	0.287	0.001
C20:1	0.17^{a}	0.17^{a}	0.16^{ab}	0.18^{a}	0.17^{a}	0.13^{bc}	0.09^{de}	0.11^{cd}	0.16^{ab}	0.08^{e}	0.06^{e}	0.07^{e}	0.007	<0.001	<0.001	<0.001

[a-f]Means within row with different superscript differ (P<0.05). LSmeans based on 8 egg layers per dietary treatment.
[1]Control–basal diet + 4% corn oil, REGflax oil –basal diet + 4% regular flax oil, Stearidonic acid-enriched flax (SDAflax) –basal diet + 4% SDA-enriched flax oil.
[2]Total fatty acids (TFA) = Sum of all the fatty acids in the egg yolk.[3]Saturated fatty acids (SFA) = C14:0 + C16:0 + C18:0 + C20:0.
[4]Monounsaturated fatty acids (MUFA) = C16:1 n-7 + C18:1 n-7 + C18:1 n-9 + C20:1 n-9.

Table 3.4. Omega-6 polyunsaturated fatty acids (% of total fatty acids) in egg yolk from laying hens fed corn oil, regular flax oil or SDAflax oil diets[1] for 21 d.

Treatment	Control			REGflax				SDAflax					P-value		
Duration	0 d	7 d	14 d	21 d	0 d	7 d	14 d	21 d	0 d	7 d	14 d	21 d	SEM	Oil	Day	Oil*Day
18:2n-6 (LA)	19.67^{a}	19.63^{a}	20.02^{a}	20.14^{a}	19.96^{a}	14.61^{b}	13.06^{bc}	12.88^{bc}	18.58^{a}	11.59^{c}	10.70^{c}	10.57^{c}	0.545	<0.001	<0.001	<0.001
18:3n-6 (GLA)	0.08^{b}	0.09^{b}	0.08^{b}	0.05^{b}	0.09^{b}	0.06^{b}	0.05^{b}	0.09^{b}	0.08^{b}	0.67^{a}	0.70^{a}	0.64^{a}	0.011	<0.001	<0.001	<0.001
C20:2	0.05^{ab}	0.05^{b}	0.04^{b}	0.11^{a}	0.05^{ab}	0.04^{b}	0.04^{ab}	0.06^{ab}	0.06^{ab}	0.07^{ab}	0.04^{ab}	0.04^{ab}	0.013	<0.001	<0.001	<0.001
C20:3n6	0.07^{bc}	0.07^{bc}	0.05^{c}	0.06^{bc}	0.06^{c}	0.10^{b}	0.07^{bc}	ND	0.07^{bc}	0.53^{a}	ND	ND	0.019	<0.001	<0.001	<0.001
20:4n-6 (AA)	2.30^{ab}	2.28^{ab}	2.06^{ab}	1.83^{ab}	2.45^{a}	1.07^{cd}	1.02^{d}	0.90^{d}	2.25^{ab}	1.69^{bc}	1.70^{bc}	1.12^{cd}	0.139	<0.001	<0.001	<0.001
C22:2n-6	0.06	0.06	0.04	0.06	0.06	0.04	0.05	0.06	0.06	0.05	0.04	0.06	0.004	0.4235	0.008	0.586
C22:4n-6	0.29^{ab}	0.33^{a}	ND	0.34^{a}	0.33^{a}	0.15^{ab}	ND	ND	0.33^{a}	0.12^{b}	ND	ND	0.048	0.0242	0.008	0.016
Total n-6 PUFA[2]	22.48^{a}	22.46^{a}	22.22^{a}	22.38^{a}	22.94^{a}	15.89^{b}	14.16^{bc}	13.83^{bc}	21.36^{a}	13.91^{bc}	13.13^{bc}	12.43^{c}	0.648	<0.001	<0.001	<0.001

$^{a-d}$Means within row with different superscript differ (P<0.05). LSmeans based on 8 egg layers per dietary treatment. [1]Control–basal diet + 4% corn oil, REGflax oil –basal diet + 4% regular flax oil, Stearidonic acid-enriched flax (SDAflax) –basal diet + 4% SDA-enriched flax oil.
[2]Total n-6 polyunsaturated fatty acids (PUFA) = 18:2n-6 (LA) + 18:3n-6 (GLA) + 20:2n-6 + 20:3n-6 + 20:4n-6 (AA) + 22:2n-6 + 22:4n-6.

Table 3.5. Omega-3 polyunsaturated fatty acids (% of total fatty acids) in egg yolk from laying hens fed corn oil, regular flax oil or SDAflax oil diets[1] for 21 d.

Treatment	Control				REGflax				SDAflax					P-value		
Duration	0 d	7 d	14 d	21 d	0 d	7 d	14 d	21 d	0 d	7 d	14 d	21 d	SEM	Oil	Day	Oil*Day
18:3n-3 (ALA)	0.32^{c}	0.33^{c}	0.33^{c}	0.34^{c}	0.34^{c}	5.51^{ab}	7.07^{a}	6.94^{a}	0.99^{c}	4.67^{b}	5.02^{b}	5.08^{b}	0.341	<0.001	<0.001	<0.001
18:4n-3 (SDA)	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.29	0.30	0.30	0.017	<0.001	0.840	0.734
20:4n-3 (ETA)	0.14^{b}	0.14^{b}	0.11^{b}	0.12^{b}	0.15^{b}	0.13^{b}	0.10^{b}	0.11^{b}	0.15^{b}	0.72^{a}	0.63^{a}	0.66^{a}	0.019	<0.001	<0.001	<0.001
20:5n-3 (EPA)	ND	ND	ND	ND	ND	0.16^{c}	0.16^{c}	0.17^{bc}	ND	0.26^{a}	0.25^{a}	0.25^{ab}	0.014	<0.001	0.651	0.943
22:5n-3 (DPA)	0.10^{cd}	0.04^{d}	0.05^{d}	0.03^{d}	0.05^{d}	0.16^{bcd}	0.15^{cd}	0.18^{bcd}	0.07^{d}	0.53^{a}	0.35^{abc}	0.39^{ab}	0.053	<0.001	0.001	0.002
22:6n-3 (DHA)	0.72^{bc}	0.66^{c}	0.45^{c}	0.54^{c}	0.65^{c}	1.26^{a}	1.20^{ab}	1.45^{a}	0.76^{bc}	1.44^{a}	1.41^{a}	1.62^{a}	0.094	<0.001	<0.001	<0.001
Total n-3 PUFA[2]	1.28^{b}	1.17^{b}	0.93^{b}	1.00^{b}	1.19^{b}	7.02^{a}	8.51^{a}	8.58^{a}	1.91^{b}	7.56^{a}	7.71^{a}	8.05^{a}	0.427	<0.001	<0.001	<0.001
Total LC n-3 PUFA[3]	0.97^{c}	0.85^{c}	0.60^{c}	0.66^{c}	0.85^{c}	1.64^{b}	1.60^{b}	1.89^{b}	0.97^{c}	2.87^{a}	2.65^{a}	2.91^{a}	0.108	<0.001	<0.001	<0.001
n-6:n-3 Ratio	17.65^{bc}	19.46^{abc}	24.22^{a}	22.48^{ab}	19.59^{abc}	4.28^{d}	1.67^{d}	1.61^{d}	17.22^{c}	1.90^{d}	1.71^{d}	1.55^{d}	1.122	<0.001	<0.001	<0.001

$^{a-d}$Means within row with different superscript differ (P<0.05). LSmeans based on 8 egg layers per dietary treatment.

[1]Control–basal diet + 4% corn oil, REGflax oil –basal diet + 4% regular flax oil, Stearidonic acid-enriched flax (SDAflax) –basal diet + 4% SDA-enriched flax oil.

[2]Total n-3 polyunsaturated fatty acids (PUFA) = 18:3n-3 (ALA) + 18:4n-3 (SDA) + 20:4n-3 (ETA) + 20:5n-3 (EPA) + 22:5n-3 (DPA) + 22:6n-3 (DHA).

[3]Long chain n-3 polyunsaturated fatty acids (PUFA) = 20:4n-3 (ETA) + 20:5n-3 (EPA) + 22:5n-3 (DPA) + 22:6n-3 (DHA).

Table 3.6: Fatty acid composition (% of total fatty acids) of breast and thigh muscles from laying hens fed corn oil, regular flax oil or SDAflax oil diets[1] for 21 d.

Tissue	Breast					Thigh				
Treatment	Control	REGflax	SDAflax	SEM	P-value	Control	REGflax	SDAflax	SEM	P- value
SFA[2]	27.95	29.84	30.60	2.122	0.665	27.96	25.69	26.53	1.481	0.589
MUFA[3]	39.17	38.71	38.86	2.176	0.989	38.05	44.00	43.88	1.688	0.099
n-6 PUFA[4]	30.27^{a}	26.74^{b}	26.00^{b}	0.715	0.001	32.68^{a}	25.00^{b}	25.38^{b}	0.684	0.002
n-3 PUFA[5]	2.07^{b}	4.92^{a}	4.61^{a}	0.559	<0.001	1.30^{b}	5.31^{a}	4.21^{a}	0.371	0.002
LC n-3 PUFA[6]	1.04^{b}	1.36^{ab}	1.78^{a}	0.149	0.019	0.31^{b}	0.42^{ab}	0.56^{a}	0.058	0.034
n-6:n-3 Ratio	12.97^{a}	5.53^{b}	6.08^{b}	0.551	<0.001	25.78^{a}	4.73^{b}	6.10^{b}	2.238	0.004

$^{a\text{-}b}$Means within row and tissue with different superscript differ (P<0.05). LSmeans based on 8 egg layers per dietary treatment.

[1]Control–basal diet + 4% corn oil, REGflax oil –basal diet + 4% regular flax oil, Stearidonic acid-enriched flax (SDAflax) –basal diet + 4% SDA-enriched flax oil.

[2]Saturated fatty acids (SFA) = C14:0 + C16:0 + C18:0 + C20:0.

[3]Monounsaturated fatty acids (MUFA) = C16:1n-7 + C18:1n-7 + C18:1n-9 + C20:1n-9.

[4]Total n-6 polyunsaturated fatty acids (PUFA) = 18:2 n-6 (LA) + 18:3n-6 (GLA) + 20:2n-6 + 20:3n-6 + 20:4n-6 (AA) + 22:2n-6 + 22:4 n-6.

[5]Total n-3 polyunsaturated fatty acids (PUFA) = 18:3n-3 + 18:4n-3 + 20:4n-3 + 20:5n-3 + 22: n-3 + 22:6n-3.

[6]Long chain n-3 polyunsaturated fatty acids (PUFA) = 20:4n-3 + 20:5n-3 + 22:5n-3 + 22:6n-3.

Table 3.7: Fatty acid composition (% of total fatty acids) of liver and brain from laying hens fed corn oil, regular flax oil or SDAflax oil diets[1] for 21 d.

Tissue	Liver					Brain				
Treatment	Control	REGflax	SDAflax	SEM	P- value	Control	REGflax	SDAflax	SEM	P- value
SFA[2]	35.93[b]	34.57[b]	40.00[a]	0.982	0.027	46.24	46.03	48.13	1.615	0.617
MUFA[3]	41.61[ab]	46.70[a]	39.41[b]	2.297	0.008	30.35	30.86	27.75	2.512	0.657
n-6 PUFA[4]	21.30[a]	12.45[b]	14.33[b]	1.854	0.002	12.26[a]	10.61[b]	10.66[b]	0.331	0.010
n-3 PUFA[5]	1.16[b]	6.28[a]	6.26[a]	1.019	<0.001	11.92	13.09	14.04	0.610	0.097
LC n-3PUFA[6]	0.66[c]	2.29[b]	3.54[a]	0.268	<0.001	11.81[b]	12.62[ab]	13.67[a]	0.421	0.032
n-6:n-3 Ratio	18.36[a]	1.98[b]	2.29[b]	1.343	<0.001	1.04[a]	0.81[b]	0.76[b]	0.035	0.008

[a-b]Means within row and tissue with different superscript differ (P<0.05). LSmeans based on 8 egg layers per dietary treatment.

[1]Control–basal diet + 4% corn oil, REGflax oil –basal diet + 4% regular flax oil, Stearidonic acid-enriched flax (SDAflax) –basal diet + 4% SDA-enriched flax oil.

[2]Saturated fatty acids (SFA) = C14:0 + C16:0 + C18:0 + C20:0.

[3]Monounsaturated fatty acids (MUFA) = C16:1n-7 + C18:1n-7 + C18:1n-9 + C20:1n-9.

[4]Total n-6 polyunsaturated fatty acids (PUFA) = 18:2 n-6 (LA) + 18:3n-6 (GLA) + 20:2n-6 + 20:3n-6 + 20:4n-6 (AA) + 22:2n-6 + 22:4 n-6.

[5]Total n-3 polyunsaturated fatty acids (PUFA) =18:3n-3 + 18:4n-3 + 20:4n-3 + 20:5n-3 + 22: n-3 + 22:6n-3.

[6]Long chain n-3 polyunsaturated fatty acids (PUFA) = 20:4n-3 + 20:5n-3 + 22:5n-3 + 22:6n-3.

Table 3.8: Fatty acid composition (% of total fatty acids) of heart and abdominal fatpad from laying hens fed corn oil, regular flax oil or SDAflax oil diets[1] for 21 d.

Tissue	Heart					Abdominal fatpad				
Treatment	Control	REGflax	SDAflax	SEM	P-value	Control	REGflax	SDAflax	SEM	P- value
SFA[2]	30.15	30.45	30.69	1.402	0.958	18.84^{ab}	16.85^{b}	20.34^{a}	0.701	0.007
MUFA[3]	33.07	31.20	33.40	2.009	0.708	48.94	50.09	47.99	0.798	0.211
n-6 PUFA[4]	35.17^{a}	32.10^{ab}	30.48^{b}	1.029	0.013	28.89^{a}	25.79^{b}	24.56^{b}	0.810	<0.001
n-3 PUFA[5]	1.61^{b}	6.25^{a}	5.42^{a}	0.324	<0.001	3.128^{b}	7.13^{a}	6.97^{a}	0.753	0.001
LC n-3 PUFA[6]	0.42^{b}	1.29^{a}	1.33^{a}	0.142	<0.001	0.29	0.27	0.25	0.062	0.828
n-6:n-3 Ratio	22.21^{a}	5.45^{b}	5.77^{b}	0.843	<0.001	12.26^{a}	4.84^{b}	3.59^{b}	1.311	<0.001

$^{a.b}$Means within row and tissue with different superscript differ (P<0.05). LSmeans based on 8 egg layers per dietary treatment.

[1]Control–basal diet + 4% corn oil, REGflax oil –basal diet + 4% regular flax oil, Stearidonic acid-enriched flax (SDAflax) – basal diet + 4% SDA-enriched flax oil.

[2]Saturated fatty acids (SFA) = C14:0 + C16:0 + C18:0 + C20:0.

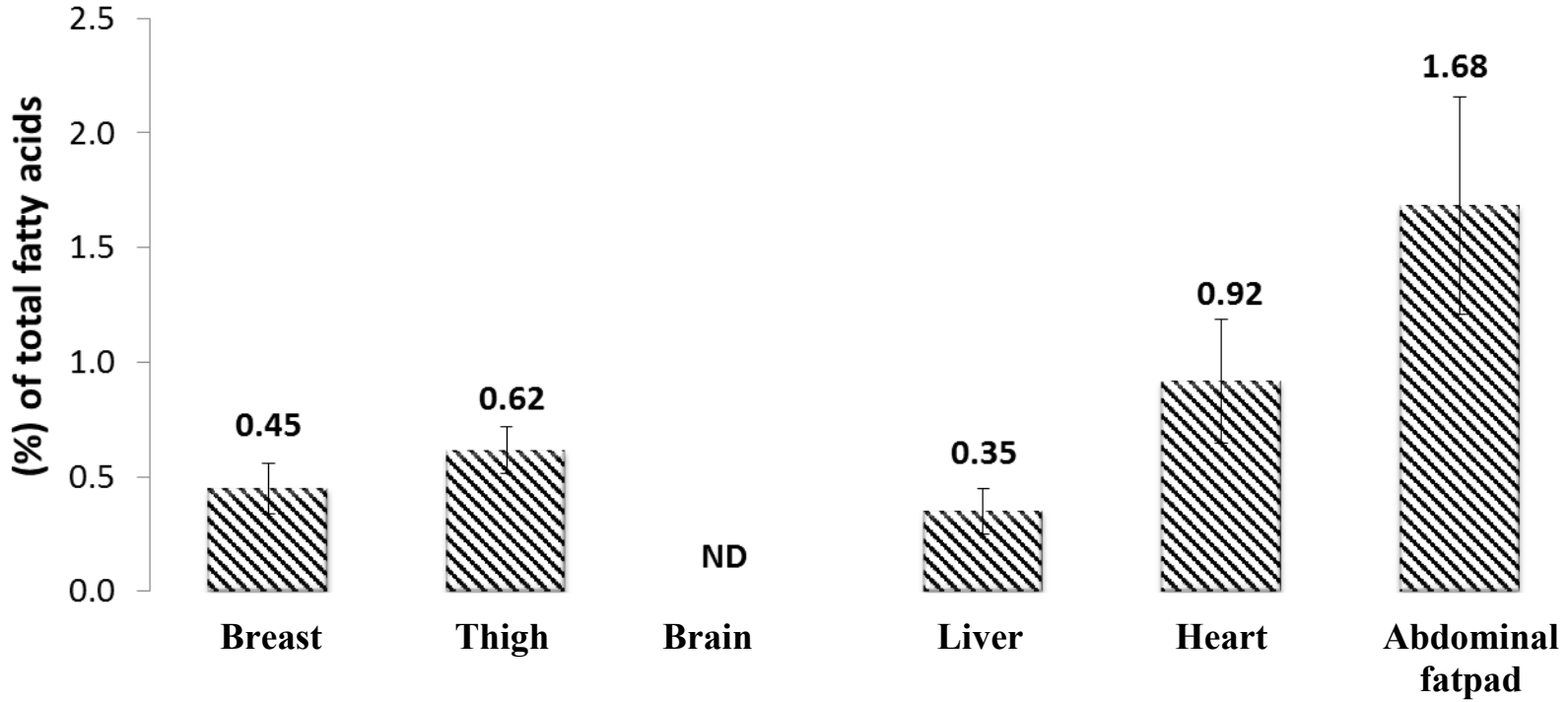
[3]Monounsaturated fatty acids (MUFA) = C16:1n-7 + C18:1n-7 + C18:1n-9 + C20:1n-9.

[4]Total n-6 polyunsaturated fatty acids (PUFA) = 18:2 n-6 (LA) + 18:3n-6 (GLA) + 20:2n-6 + 20:3n-6 + 20:4n-6 (AA) + 22:2n-6 + 22:4 n-6.

[5]Total n-3 polyunsaturated fatty acids (PUFA) = 18:3n-3 + 18:4n-3 + 20:4n-3 + 20:5n-3 + 22: n-3 + 22:6n-3.

[6]Long chain n-3 polyunsaturated fatty acids (PUFA) = 20:4n-3 + 20:5n-3 + 22:5n-3 + 22:6n-3.

Figure 3.1. Proportion of stearidonic acid (SDA) in tissues from egg layers fed SDAflax oil diet.



Stearidonic acid-enriched flax (SDAflax) –basal diet + 4% SDA-enriched flax oil.

Chapter 4

Feeding stearidonic acid-enriched flax oil or regular flax oil in combination with canola, corn or fish oil to laying hens for egg bioactive lipid enrichment

Abstract: Intake of long chain n-3 polyunsaturated fatty acids (LC n-3 PUFA) by humans is encouraged. The effect of a genetically-modified flax oil, enriched in stearidonic acid (C18:4n-3; SDA), on egg LC n-3 PUFA enrichment was studied in White Leghorn hens (47 wk old, n=120). To investigate the metabolic competition among dietary fatty acid sources for desaturation and elongation pathways, three sets of diets with 2% corn (CO), canola (CAN), or fish (FO) plus either 2.5 or 5% of either regular flax oil (REGflax) or SDA-enriched flax oil (SDAflax) were formulated. Production and egg traits were measured at 35 d; eggs and livers were collected at 35 d for fatty acid analysis. Ovary weight and the number of large yellow follicles were also measured at 35 d.

Neither reproductive traits, total egg mass, egg albumen height nor egg shell thickness were affected by diet. However, layers fed 5% oil diets had reduced feed intake and greater BW gain ($P < 0.05$) compared with those fed 2.5% oil. Increasing the inclusion of flax oil from 2.5% to 5% in hen diets resulted in a 1.6-fold increase ($P < 0.05$) in the total n-3 PUFA in liver. However, total n-3 PUFA in liver was not different between flax oil types or among the base oils, indicating that the type of base oil (CO or CAN or FO) used in addition to either type of flax oil did not affect liver total n-3 PUFA enrichment. For yolk enrichment, feeding SDAflax oil resulted in a 33.5% increase in LC n-3 PUFA compared with REGflax oil diets. Inclusion of FO increased LC n-3 PUFA; there was no difference for yolk LC n-3 PUFA between CO and CAN treatment hens, suggesting a lack of competition for enzymes during lipid desaturation and

elongation. SDA-enriched flaxseed oil increased egg LC n-3 PUFA without negative effects on egg production and reproductive traits.

Keywords: Egg, desaturation, elongation, fatty acid metbolism, stearidonic acid, flax.

4.1 INTRODUCTION

Government agencies and health organizations recommend human dietary intake of total omega-3 polyunsaturated fatty acid (n-3 PUFA) ranging from 1.4 to 2.5 g/d, with eicosapentaenoic acid (EPA; C20:5n-3) and docosahexaenoic acid (DHA; C22:6n-3) ranging from 140 to 600 mg/d (FAO; Kris-Etherton et al., 2002; Kris-Etherton and Innis, 2007). Meeting the recommendation for the high level of LC n-3 PUFA (EPA + DHA) intake for humans is a challenge. Fish is the main human source of LC n-3 PUFA. However, marine fish stocks are constantly declining (Domergue, et al., 2005). Therefore, relying on marine sources for LC n-3 PUFA alone is not a sustainable and globally applicable solution (Pauly et al., 2005).

Most oilseed plants have the Δ-12 or Δ-15 desaturase emzymes required for the biosynthetic pathway leading to accumulation of n-6 PUFA or n-3 PUFA respectively (Griffith et al., 1996). Sources of n-3 PUFA, such as flaxseed oil, camelina oil, chia oil, or perilla oil contain mainly α-linolenic acid (ALA; C18:3n-3; Ciftci et al., 2012), whereas corn oil, soybean oil, and sunflower oil contain high levels of linoleic acid (LA; C18:2n-6; Zambiazi, et al., 2007). Unlike plants, mammals and birds lack the ability to desaturate 18-carbon PUFA between the methyl end (omega) and 9th carbon of the molecule, therefore, both ALA and LA are essential fatty acids (Holman, 1986). However, dietary ALA and LA can be bioconverted to the longer chain metabolites EPA and arachidonic acid (AA; 20:4n-6), respectively, through the same set of desaturases (Δ-6 desaturase and Δ-5 desaturase) and elongases (Elovl5 and Elovl2; Wang et al., 2006).

Recent genetic advances in metabolic transformations of crops provide opportunities to modify the fatty acid composition of oil seed plants (Abbadi et al., 2004; Ruiz-Lopez et al., 2014). Genetically modified SDA-enriched soybean, (26% SDA) has been granted safety

clearance by the Food and Drug Administration (USA) with maximum intake up to 1.9 g/person/day (Hammond et al., 2008). Stearidonic acid is a metabolic intermediate of the biosynthetic pathway of ALA to DHA (Kitessa and Young, 2009). SDA can be readily bioconverted to EPA and DHA in rodents (Yamazaki et al., 1992), humans (James et al., 2002; Harris et al., 2008), and chickens (Kitessa and Young, 2009). In one such attempt, flaxseed was genetically modified to enhance stearidonic acid (28% SDA of total fatty acid; C18:4n-3; Subedi et al., 2015). In addition, the SDA enhanced flax oil also has 16% of total fatty acids as γ-linolenic acid (GLA; C18:3n-6). The γ-linolenic acid has been effective in prevention of atopic dermatitis in humans (Senapati et al., 2008).

Chickens have greater elongase activity than other animals, enabling increased LC n-3 PUFA synthesis capacity (Gregory et al., 2013). The conversion of ALA to LC n-3 PUFA is limited by substrate competition for desaturases and elongases (Tu et al., 2010). The presence of high LA in the diet limits the synthesis of n-3 LC PUFA by competitive inhibition (Lands et al. 1992). In addition, a reduction in bioconversion of n-3 PUFA to the longer-chain metabolites with increasing level of fish oil (FO), a direct dietary source of LC n-3 PUFA, in the laying hen diet has been reported (Cachaldora et al., 2006).

Results from a previous study (Chapter 3) showed that feeding 4% SDA flax oil to layers increased yolk LC n-3 PUFA compared with feeding 4% regular flax oil. The present experiment was designed to investigate the metabolic competition among dietary fatty acids for desaturation and elongation pathways, and their effect on liver and egg yolk LC n-3 PUFA proportions. We hypothesized that decreasing the dietary n-6 to n-3 PUFA ratio (corn oil *vs.* canola oil) in addition to regular flax oil (REGflax) or SDA-enriched flax oil (SDAflax) would decrease LC n-

3 PUFA enrichment in the egg, whereas the inclusion of LC n-3 PUFA (fish oil) with REGflax oil or SDAflax oil in the diet would increase LC n-3 PUFA enrichment in the egg.

4.2 MATERIALS AND METHODS

4.2.1 Birds and Management

The experiment was conducted in accordance with Canadian Council on Animal Care (2009) guidelines and was approved by the University of Alberta Animal Care and Use Committee: Livestock. The novel test ingredient in the experiment, SDA-enhanced flaxseed was grown at the University of Alberta's Faculty of Agricultural, Life, and Environmental Sciences land in St. Albert, AB, Canada and developed as described by Subedi et al., (2015). The SDA flaxseed was pressed with a Komet single-screw press (Model CA 59 G; IBG Monforts, Mönchengladbach, Germany). Processing conditions were: screw speed = 45 rpm, barrel temperature = 60°C, and processing rate ranged from 4 to 6 kg/h. The crude oil was then stored in dark bottles at -20°C to minimize oxidative damage prior to mixing with the layer diets.

Individually-caged Lohmann White Leghorn laying hens (45 wks of age) from the Poultry Research Centre flock were monitored for two weeks for egg production. A group of 120 hens were then selected and randomly allocated to one of the 12 experimental dietary treatments (n=10 per treatment). The layers had ad libitum access to feed and water throughout the 35 d experiment. A lighting program of 16 h of light and 8 h of dark was implemented.

Twelve diets were fed for 35 d as a 3*2*2 factorial arrangement of treatments. Three types of basal oils (2% of either corn (CO), canola (CAN), or fish (FO)) were added in combination with two flax oils (regular flax oil, REGflax; or SDA-enriched flax oil, SDAflax) at two levels (Low, 2.5 or High, 5%; Table 4.1). Egg production and egg weight were measured daily. Body weight and feed consumption were measured at 35 d. Egg traits including yolk

weight, albumen height and shell thickness were also measured after 35 d of feeding. At 35 d, yolk samples from individual layers were separated and frozen at -20°C for subsequent fatty acid analysis. Layers were euthanized and livers were also collected at 35 d and frozen at -20°C pending fatty acid analysis. Laying hens were assessed for reproductive tract morphology, examined to count number of large yellow follicles (>10 mm diameter) and ovary weight. Representative feed samples were collected for determination of dietary fatty acid composition.

4.2.2 Fatty Acid Analysis

Extraction of fat from yolk and feed and derivatization procedure was similar as described by Nain et al., (2012). For liver, 1-g samples (4 samples/treatment) were homogenized with Folch solution for 30s using a homogenizer (Power Gen 1000S1, Fisher Q1 Scientific) in 25 × 150-mm Teflon-lined, screw-capped test tubes. The extraction of fat from liver and derivatization were performed as described in Chapter 3. The resultant fatty acid methyl esters were analyzed by gas chromatography (GC; model 7890A, Agilent Technologies, Palo Alto, CA) fitted with a 5975 inert XL Mass Selective Detector (Agilent Technologies, Palo Alto, CA). The fatty acid methyl esters were separated using a DB-23 capillary column (30 m × 0.25 mm × 0.25 μm) with a constant helium flow of 1.2 mL·/min. The following temperature program was applied: 90°C, hold for 4 min, 10°C/min to 180°C, hold for 2 min, and 5°C/min to 220°C, hold for 2 min. (total runtime = 25 min). The fatty acid peak integration and analysis was performed using MSD ChemStation data analysis software (version F.01.00.1903, Agilent Technologies, Palo Alto, CA). The efficiency of methylation of fatty acids were measured using heptadecanoic acid (1 mg/ml; 17:0) and total fatty acids (mg/g sample) were quantified using methyl heneicosanoate (1 mg/ml; C21:0 methyl ester) as an internal standard (Varian Walnut Creek,

CA). Peaks were identified by comparison of retention time with standards (GLC-463, and GLC-421A, NU-CHEK Prep, Inc. Elysian, MN).

Fatty acids were expressed as percentage of total fatty acids. Saturated fatty acids (SFA) were the sum of 14:0 + 16:0 + 18:0 + 20:0. Monounsaturated fatty acids (MUFA) were the sum of 16:1n-7 + 18:1n-7 + 18:1n-9 + 20:1n-9. Total n-3 fatty acids were the sum of ALA + SDA + 20:4n-3 (ETA) + EPA + 22:5n-3 (DPA) + DHA. Total n-6 fatty acids were the sum of LA + GLA + 20:2n-6 + 20:3n-6 + AA + 22:4n-6. Total PUFA was the sum of n-3 and n-6 PUFA.

4.2.3 Statistical Analysis

Production traits (feed intake, BW change, total egg production and total egg mass for entire 35 d), reproductive traits (ovary weight and number of large yellow follicle), egg quality traits (albumen height, yolk weight and shell thickness) and fatty acid composition of liver and egg yolks at 35 d were analyzed as completely randomized 2*2*3 factorial arrangement of treatments. The fixed effects were 2 flax types (Regular flax oil or SDA flaxseed oil) at 2 inclusion levels (Low 2.5% and High 5%) in combination with 2% of each of 3 base oil types (CO, CAN, and FO). Separation of means among base oil types was conducted using the Pdiff option of the MIXED procedure of SAS Version 9.3 for Windows (SAS Institute Inc., Cary, NC). Data were considered significant if $P < 0.05$.

4.3 RESULTS AND DISCUSSION

4.3.1 Dietary Fatty Acid Profile

The SFA proportions in dietary treatments ranged between 11.8 and 15.3% of total fatty acids (Table 4.2). Diets with greater MUFA contained lower PUFA. The inclusion of either type of flax oil increased the total n-3 PUFA proportions in diet. The n-6 to n-3 PUFA ratio in the diets ranged between 0.6 and 1.8. Inclusion of either flax type, at either inclusion level with CAN

or CO resulted in very low LC n-3 PUFA proportions (<1%) compared with the FO combination diets (9.2% to 11.7%). In addition, the FO diets contained greater EPA (6.6 to 8.3%) and DHA (0.9 to 1.2%) proportions than the CAN and CO diets (EPA and DHA <0.09%). Inclusion of 2.5% or 5% SDA-flax oil increased dietary SDA proportions to 9.1% and 14.3% and increased the GLA proportions to 5.2% and 7.8%, respectively, of total fatty acids. The fatty acid composition of the oils dietary has a direct effect on the lipid profile of the egg yolk of laying hens (Oliveira et al., 2010). However inclusion level, feeding duration, stability of oils in diet, and interaction with other feed ingredients can affect the laying hen's ability to transfer dietary fatty acids to the egg yolk (Jia et al., 2008; Nain et al., 2012b).

4.3.2 Layer Performance and Production Traits

Laying hens fed High flax oil diets had lower (P<0.05) daily feed intake but greater BW gain compared with those fed the Low diets (Table 4.3). However, there was no difference for feed intake and change in body weight between flax types and among the type of base oil fed to the laying hens. Egg production and total egg mass were not affected by dietary oil treatment. Similarly, albumen height, yolk weight and shell thickness were also not affected by dietary oil treatment. Egg mass and yolk weight in laying hens are related to hepatic very low density lipoprotein synthesis (Salvante et al., 2007), which is highly correlated to dietary energy level and protein content (Gunawardana et al., 2008). Diets that provide lower energy result in increased feed intake and egg weight (Keshavarz and Nakajima, 1995). In the present study the diets with Low flax levels were 188 kcal lower than the High diets (Table 4.1). Laying hens fed high oil diets had 3.3% lower feed intake but 35.3% greater BW gain (116 *vs.* 157 g), suggesting that the additional energy provided by the High oil diets was used for increasing BW rather than increasing egg production or egg mass. An increase of dietary ME from 2,642 to 2,815 kcal/kg

resulted in a 4.0% decrease in feed intake (3,922 *vs.* 3,795 g) and a 55.7% increase in BW gain (140 *vs.* 218 g) without affecting the egg production or egg mass (Grobas et al., 1999). The absence of an effect of dietary fat source, either of plant origin (canola, corn or flax) or animal origin (fish oil) on egg quality traits has been previously demonstrated in studies by Jiang et al., (1992), Baucells et al., (2000), and Mazalli et al., (2004). However, Sell et al. (1987) noted that increasing animal-vegetable fat inclusion from 3 to 6% in hen diets increased egg yolk weight in layers aged 24 to 34 wks, but there was no effect of fat type on yolk weight after 36 weeks age. In the present study layers, started at 47 wks age and there was no dietary difference in neither egg nor yolk weight.

Reproductive traits (ovary weight and large yellow follicle number) were also not different among fixed effects (Table 4.3). Ovary weight and number of large yellow follicle were not different in laying hens fed up to 10% of flaxseed (Arshami et al., 2010), or 5% FO (Edeid et al., 2008). The lack of dietary effect on egg and reproductive parameters in the current study indicates that the two inclusion levels of oils from regular flax or SDA flax oil in combination with CO, CAN, or FO had no negative effect on reproductive performance of the laying hens.

4.3.3 Hepatic Fatty Acid Profile

Dietary flax oil type, flax oil level, or base oil had no effect on the total fatty acid content (mg/g) in liver (Table 4.4). There were no interactions of flax oil type, flax oil level and base oil on the liver SFA proportions. In addition, there was no difference for liver total SFA among basal oil types (CO, CAN or FO) and between flax inclusion levels (Low or High). However, liver total SFA from hens fed REGflax was 14.3% lower ($P<0.05$) than those fed SDAflax. Both, C16:0 and C18:0 were lower ($P<0.05$) in liver from hens fed REGflax compared with SDAflax. Total MUFA and C18:1n-9 were 13.3% greater ($P<0.05$) in hens fed REGflax oil compared with

hens fed SDAflax oil. In addition, hepatic MUFA proportions decreased (P<0.05) by 8.1%- in hens fed High flax oil compared with Low flax oil level.

The liver is the site of 90 to 95% of de novo fatty acid synthesis in layers (O'hea and Leveilie, 1969). De novo synthesis can contribute to the SFA and MUFA proportion of tissues or yolk (Naber and Biggert, 1989). Previous studies have suggested that dietary flax oil and fish oil can each reduce hepatic lipid synthesis, mainly by increasing the activity of the perioxysomal receptor and binding proteins associated with SFA, eventually reducing their proportions (Konig et al., 2008; Cherian and Hayat, 2009). In the current study, hepatic SFA was lower in layers fed REGflax oil diets compared to SDAflax oil diets. Therefore, SDAflax might not have suppressed SFA lipogenesis to the same extent as REGflax oil.

Dietary flax oil type or flax oil level had no effect on the total n-6 PUFA in liver (Table 4.4). However, the dietary effect of base oil was evident in hepatic total n-6 PUFA. Total n-6 PUFA and LA were greater in hens fed the diets containing CO compared with CAN or FO (P > 0.05). In addition, hepatic LA was greater (P<0.05) and AA was lower (P<0.05) in hens fed REGflax oil diets as compared with SDAflax ones, reflecting the dietary fatty acid effect on the hepatic fatty acid profile. The increase of LA and total n-6 PUFA in liver from hens fed CO might be related to a greater proportion of LA and n-6 PUFA in CO diets. Fatty acid levels in laying hen diets had a prominent effect on hepatic PUFA proportions (Cherian and Sim, 2001; Oliveira et al., 2010).

The increase from Low to High dietary flax oil inclusion in hen diets resulted in a 1.6-fold increase (P<0.05) in total n-3 PUFA in the liver (Table 4.4). However, total n-3 PUFA in liver was not different between flax oil types or due to base oil, suggesting that the type of base oil added to either type of flax oil did not affect final n-3 PUFA proportions in the liver.

Furthermore, there was no dietary flax oil, level or base oil effects on the hepatic total LC n-3 PUFA, indicating a lack of dietary effect on the lipid bioconversion ability of the liver. However, the increased ($P<0.05$) hepatic DHA in hens fed SDAflax compared with REGflax oil diet was linked to a likely increase in fatty acid bioconversion in SDAflax compared with REGflax. Among LC n-3 PUFA, liver EPA was greater ($P<0.05$) in FO fed hens compared with CO fed hens, because the FO diets contained greater EPA. Overall, total n-6 PUFA and n-3 PUFA in the liver were largely affected by the dietary fatty acid composition rather than de novo hepatic synthesis or elongation and desaturation of fatty acid in the laying hens.

4.3.4 Egg Yolk Fatty Acid Profile

4.3.4.1. Saturated and Monounsaturated Fatty Acid

Dietary flax oil type, level, and base oil type had no effect on the total fatty acid content in the egg yolk (Table 4.5). There were no interactions of flax oil type, flax oil level and base oil on total SFA proportions in egg yolk. However, there was a 17.0% increase ($P<0.05$) in yolk total SFA for hens fed SDAflax oil diet compared with REGflax oil. Yolk SFA was also greater ($P<0.05$) in hens fed diets containing FO compared with CO. The yolk C16:0 and C18:0 incresed ($P<0.05$) in hens fed SDAflax oil at High inclusion compared with High level of REGflax oil but had no diferrence at Low inclusion (Figure 4.1). In addition, SDAflax oil in layer diets with CAN or FO resulted in a greater ($P<0.05$) yolk C18:0 proportion compared with REGflax in combination of CAN or FO.

There was an interaction of flax oil type by flax oil level ($P < 0.05$) and an interaction of flax level by base oil ($P < 0.05$) for total MUFA (Figure 4.2) and C18:1 (Figure 4.3) in egg yolk. MUFA and C18:1 proportions were not different in hens fed REGflax at either level but it was reduced ($P<0.05$) in hens fed High SDAflax oil compared with Low SDAflax oil. Increasing the

flax oil level from Low to High reduced (P<0.05) yolk MUFA in hens fed CAN and CO but had no effect feeding FO. In addition, layers fed SDAflax oil with CAN or CO had a lower (P<0.05) yolk C18:1 comapred with REGflax oil with CAN or CO (Figure 4.3).

Egg yolk fatty acid profile can be altered by modifing the dietary oil source (Oliveira eta l., 2010). However, the de novo synthesis of fatty acids maintains fairly constant SFA proportions in egg yolk, but yolk MUFA proportion is reduced with an increase in dietary PUFA (Raes et al., 2002). Laying hens fed high PUFA (both n-6 and n-3 PUFA) diets have reduced yolk MUFA, likely due to a reduction in the de novo MUFA synthesis through the inhibition of Δ9-desaturase activity (Cherian and Sim 1991; Mazalli et al., 2004). Each of the oil sources included in the current experiment were high in PUFA content therefore the expected dietary treatments effect on the Δ9-desaturase activity might have been similar among hens. However, the greater yolk SFA for SDAflax-fed hens compared with REGflax and lower MUFA in High SDAflax-fed hens compared to Low SDAflax-fedd hens indicates that SDAflax oil was more effective than REGflax oil in suppressing the de novo synthesis.

4.3.4.2. Omega-6 Polyunsaturated Fatty Acid

There were no interactions of flax type, flax level and base oil on total yolk n-6 PUFA (Table 4.5). In addition, yolk n-6 PUFA proportions were not different between either flax oil type or flax oil levels. Laying hens fed CO diets had increased (P<0.05) yolk n-6 PUFA and LA compared with those fed CAN or FO diets. Yolk LA was also greater (P<0.05) in hens fed REGflax compared with SDAflax oil diets. Hens fed High SDAflax oil had increased (P<0.05) GLA compared with either level of REGflax oil. The AA proportion was greater (P<0.05) in hens fed SDAflax oil compared with REGflax with increasing level of oil inclusion (Figure 4.4). In addition, laying hens fed SDAflax oil with any of the base oils, and hens fed REGflax in

combination with CO had greater yolk AA compared with hens fed REGflax with either CAN or FO (Figure 4.4).

The increase in total n-6 PUFA and LA proportions in the layers fed CO diets might be due to the higher LA content in CO as compared with other oils fed in the study. However, the yolk AA proportion is directly proportional to the amount of its precursor LA present in hen diets (Baucells et al., 2000). Greater dietary LA and GLA content can contribute to increase yolk AA proportions (Goldberg et al., 2013). However, the lower affinity of LA compared with ALA for Δ6-desaturase poses potential competitive inhibition for LA bioconversion to AA (Cherian and Sim, 2001), and with an increase in dietary ALA, there is a reduction in yolk AA (Nain et al., 2012b). The SDAflax oil diets contained reduced ALA as compared with the REGflax oil (Table 4.2) making it less effective in inhibiting AA synthesis. Increase of AA in the human diet has been linked with generation of prostaglandin E_2 (Eilati et al., 2013). Prostaglandin E_2 exhibits a range of pro-inflammatory effects responsible for cardiovascular disease, and progression of various systemic diseases such as rheumatoid arthritis and osteoporosis (Legler et al., 2010). Contrarily, GLA exert an anti-inflammatory mechanism, not different to the metabolites of n-3 PUFA in humans (Senapati et al., 2008; Johnson et al., 1997), and GLA enriched egg yolk can be effective in prevention of atopic dermatitis in human (Park et al., 2014). Therefore, the increased level of GLA in egg yolk may account for the increased yolk AA in the hens fed High SDAflax oil.

4.3.4.3. Omega-3 Polyunsaturated Fatty Acid

There were no interactions of flax oil type, flax oil level and base oil nor effects of flax oil type or base oil on total yolk n-3 PUFA (Table 4.5). Hens fed High flax oil had a 1.4-fold increased yolk n-3 PUFA compared with Low flax oil. Yolk ALA was greater ($P<0.05$) in hens

fed REGflax oil compared with SDAflax oil. In addition, dietary SDAflax oil resulted in enrichment of SDA in egg yolk, which was not detectable in yolks from hens fed REGflax oil. There were no interactions of flax oil type, flax oil level and base oil on yolk LC n-3 PUFA (Table 4.5). Inclusion of SDAflax oil in diets resulted in 33.5% increase ($P<0.05$) in the yolk LC n-3 PUFA compared with REGflax oil diets. Inclusion of FO in laying hen diet increased the LC n-3 PUFA in egg yolk compared with those fed CO and CAN. However, there was no difference in yolk LC n-3 PUFA in hens fed CO or CAN. There was a greater ($P<0.05$) proportion of DHA in egg yolk from hens fed SDAflax oil compared with REGflax oil. Dietary flax oil type, flax oil level and base oil did not interacted, nor was there an effect of flax oil type on the n-6 to n-3 PUFA ratio in egg yolk. The layers fed CO had a greater ($P<0.05$) ratio of n-6 to n-3 PUFA in egg yolk compared to those fed CAN or FO.

Yolk ALA constituted more than half of the total n-3 PUFA proportion irrespective of the dietary oil treatments. A dietary ratio of n-6 to n-3 PUFA of 1:1 results in the greatest bioconversion from ALA to DHA in human liver cells in vitro (Harnack et al., 2009). The presence of LA in CO exerts a suppressive effect to the LC n-3 PUFA biosynthesis from ALA (Watkins, 1995; Shimizu et al., 2001). However, when fed in equal amounts, ALA is metabolized preferentially over LA in humans by desaturases and is about 10 times stronger at suppressing LA metabolism compared with the effect of LA on ALA metabolism (Holman, 1998). In our study, inclusion of 2% of CO or CAN oil in combination with 2.5% or 5% of either flax oil was not effective in inhibiting the LC n-3 PUFA metabolism in laying hens. The lack of competitive inhibition of LA for ALA bioconversion might be linked to a low ratio of n-6 to n-3 PUFA in our diets (close to 1:1). However, inclusion of FO in the diets increased yolk LC n-3 PUFA compared to CO and CAN. These results are in accordance with Poureslami et al. (2012),

that the dietary proportions of n-3 PUFAs were the major determinant of n-3 PUFA egg yolk fatty acid composition, and that n-3 PUFA (from flax or fish oil) are increased due to increased inclusion level (LOW to HIGH) and are not affected by the presence of fat type such as high in SFA (coconut oil), MUFA (sunflower oil) or n-6 PUFA (soyabean oil) in hen diet.

Laying hens are capable of transferring dietary fatty acids into the egg yolks (Hargis and Van Elswyk, 1993; Oliveira et al., 2010). Therefore, it was expected that the predominant fatty acids in the diets (n-3 PUFA or n-6 PUFA) would be reflected in the egg yolk fatty acid profile. However, the increased liver DHA and increased total LC n-3 PUFA (EPA and DHA) in yolk from hens fed SDAflax compared with REGflax indicate greater LC n-3 PUFA bioconversion with inclusion of SDAflax in laying hen. There was no difference in the total hepatic LC n-3 PUFA in the hens fed REGflax or SDAflax oil diets or among basal oils diets. However, in egg yolk, there was an increased total LC n-3 PUFA in hens fed SDAflax oil as compared to those REGflax. The difference between liver and egg yolk LC n-3 PUFA profiles might be because dietary lipid is transported via portal circulation to the liver, from the liver, is transported to the developing follicle for deposition as yolk lipid (Walzem et al., 1996). Recently, Elkin et al., (2015) also reported similar findings that inclusion of 5% SDA-soybean oil (SDA = 24% of total fatty acids) caused no difference in liver LC n-3 PUFA, but increased LC n-3 PUFA in egg yolk as compared with 5% regular flax oil. However, they also reported that the majority of dietary SDA was deposited in adipose tissue rather than being bioconverted into LC n-3 PUFA. In the present study, the SDA-flax oil (SDA = 28% of total fatty acids) was fed to White Leghorn hens (48 weeks old) compared with Hy-Line W-36 hens (38 weeks old) in their study. However, the desaturation and elongation ability in laying hen increases from 36 to 58 weeks (Scheideler et al., 1998).Therefore, we observed a 33.5% increased LC n-3 PUFA in hens fed SDAflax oil diet

compared with the REGflax oil diets. However, despite the SDAflax oil provided about 9 to 10% and 14% of dietary SDA in LOW and HIGH diets respectively, the increase in bioconvserion to DHA was only about 34.5% (1.74% of fatty acids in REGflax *vs.*2.34% in SDAflax).

In conclusion, there was no effect of dietary oil treatment on egg production, egg quality and reproductive status of laying hens after 35 d of feeding, suggesting dietary SDAflax oil had no adverse effect on laying hen production. The lack of metabolic competition among dietary fatty acid sources for desaturation and elongation pathways between CO and CAN might be linked to a close dietary ratio of n-6 to n-3 PUFA. However, to understand the effect of n-6 PUFA on the n-3 PUFA desaturation and elongation mechanism future studies needs to consider with a wider range on n-6 to n-3 PUFA ratio in the layers diet.

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Table 4.1: Ingredient composition (as-is,) of the experimental diets[1].

Ingredients, %	Corn oil				Canola oil				Fish oil			
	REGflax		SDAflax		REGflax		SDAflax		REGflax		SDAflax	
	2.5%	5.0%	2.5%	5.0%	2.5%	5.0%	2.5%	5.0%	2.5%	5.0%	2.5%	5.0%
Canola meal	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0
Soybean meal	10.1	12.6	10.1	12.6	10.1	12.6	10.1	12.6	10.1	12.6	10.1	12.6
Wheat grain	52.3	50.2	52.3	50.2	52.3	50.2	52.3	50.2	52.3	50.2	52.3	50.2
Wheat bran	11.7	8.8	11.7	8.8	11.7	8.8	11.7	8.8	11.7	8.8	11.7	8.8
Calcium carbonate	8.9	8.9	8.9	8.9	8.9	8.9	8.9	8.9	8.9	8.9	8.9	8.9
Dicalcium phosphate	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6
Sodium chloride	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
D,L – methionine	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Layer premix[2]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin E premix[3]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Choline premix[4]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Corn oil	2.0	2.0	2.0	2.0	-	-	-	-	-	-	-	-
Canola oil	-	-	-	-	2.0	2.0	2.0	2.0	-	-	-	-
Fish oil	-	-	-	-	-	-	-	-	2.0	2.0	2.0	2.0
Flax oil (REG)	2.5	5.0	-	-	2.5	5.0	-	-	2.5	5.0	-	-
Flax oil (SDA)	-	-	2.5	5.0	-	-	2.5	5.0	-	-	2.5	5.0
Calculated Nutrient												
ME, kcal/kg	2,655	2,815	2,655	2,815	2,642	2,801	2,642	2,801	2,627	2,786	2,627	2,786
Crude Protein, %	17.4	18.0	17.4	18.0	17.4	18.0	17.4	18.0	17.4	18.0	17.4	18.0
Crude Fat, %	6.4	8.8	6.4	8.8	6.4	8.8	6.4	8.8	6.4	8.8	6.4	8.8
Ca, %	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9
P, available %	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Met + cys, %	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7
Lysine, %	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8

[1]White Leghorn hens (47 wk old, n=120) were fed one of 12 diets arranged as a 2*2*3 factorial: 2 flax oil type (regular flax (REGflax), or SDA-enriched flax (SDAflax) at 2 inclusion levels, (2.5% and 5%), in combination with 2% of 3 oil types (corn, canola and fish oil) for 35 d.

[2]Provided per kilogram of diet: vitamin A (retinyl acetate), 12,000 IU; cholecalciferol, 3,000 IU; vitamin E (DL-α-tocopheryl acetate), 40 IU; vitamin K, 2.0 mg; pantothenic acid, 14 mg; riboflavin, 6.5 mg; folacin, 1.0 mg; niacin, 40 mg; thiamine, 3.3 mg; pyridoxine, 6.0 mg; vitamin B12, 0.02 mg; biotin, 0.2 mg; iodine, 0.5 mg; Mn, 75 mg; Cu, 15 mg; Zn, 80 mg, Se, 0.1 mg; and Fe, 100 mg;

[3]Provided vitamin E, 50 IU per kg of diet; 125 mg of ethoxyquin.

[4]Provided 1000 mg per kg of choline chloride.

Table 4.2. Fatty acid composition (% of total fatty acids) of experimental diets[1]

Fatty acid	Corn oil				Canola oil				Fish oil			
	REGflax		SDAflax		REGflax		SDAflax		REGflax		SDAflax	
	2.5%	5.0%	2.5%	5.0%	2.5%	5.0%	2.5%	5.0%	2.5%	5.0%	2.5%	5.0%
SFA[2]	13.95	13.08	14.19	13.53	11.76	12.30	12.71	11.75	15.11	13.88	15.26	14.43
C14:0	0.05	0.05	0.06	0.06	0.07	0.09	0.09	0.06	1.93	1.54	1.91	1.52
C16:0	11.42	10.01	11.45	10.22	9.19	8.69	9.54	8.40	10.92	9.65	10.91	9.89
C16:1n-7	0.21	0.17	0.20	0.16	0.26	0.31	0.34	0.25	2.40	1.90	2.38	1.86
C18:0	2.47	3.02	2.69	3.25	2.50	3.52	3.18	3.28	2.26	2.69	2.43	3.02
MUFA[3]	26.26	24.73	22.13	19.03	35.82	32.67	31.60	27.09	27.65	26.18	23.62	20.54
C18:1n-7	23.70	22.77	19.81	17.26	32.12	29.62	27.92	23.88	17.03	17.68	13.16	12.18
C18:1n-9	1.90	1.46	1.78	1.32	2.69	1.88	2.29	2.21	2.06	1.69	1.92	1.67
C20:1n-7	0.37	0.29	0.30	0.25	0.65	0.76	0.82	0.68	5.94	4.72	5.93	4.66
C24:1n-7	0.08	0.05	0.04	0.04	0.10	0.09	0.12	0.07	0.22	0.18	0.23	0.17
PUFA[4]	59.80	62.18	63.68	67.44	52.41	55.03	55.69	61.16	57.24	59.93	61.13	65.03
Total n-6 PUFA[5]	38.72	32.63	39.57	34.89	28.65	24.62	29.61	26.40	24.30	21.81	25.24	23.47
C18:2n-6 (LA)	38.60	32.54	34.41	27.04	28.56	24.50	24.23	18.49	24.04	21.61	19.79	15.69
C18:3n-6 (GLA)	0.00	0.00	5.04	7.71	0.00	0.00	5.26	7.80	0.06	0.04	5.25	7.68
C20:2n-6	0.06	0.03	0.06	0.06	0.05	0.07	0.02	0.00	0.09	0.08	0.10	0.00
C20:4n-6 (AA)	0.00	0.00	0.00	0.03	0.00	0.00	0.04	0.05	0.04	0.03	0.04	0.05
C22:2n-6	0.06	0.06	0.06	0.05	0.05	0.05	0.06	0.06	0.06	0.05	0.05	0.05
Total n-3 PUFA[6]	21.07	29.56	24.11	32.55	23.77	30.41	26.08	34.76	32.94	38.12	35.89	41.55
C18:3n-3(ALA)	20.55	28.92	13.80	18.02	23.20	29.81	15.81	19.84	21.18	28.46	14.06	18.08
C18:4n-3(SDA)	0.07	0.05	9.36	14.01	0.04	0.06	9.42	14.13	0.64	0.49	10.16	14.30
LC n-3 PUFA[7]	0.45	0.59	0.94	0.53	0.53	0.54	0.85	0.79	11.11	9.17	11.66	9.17
C20:3n-3	0.00	0.16	0.46	0.07	0.00	0.00	0.26	0.35	0.10	0.26	0.57	0.43
C20:4n-3	0.19	0.19	0.19	0.18	0.25	0.31	0.33	0.25	1.49	1.25	1.60	1.24
C20:5n-3 (EPA)	0.05	0.04	0.09	0.09	0.07	0.03	0.05	0.03	8.31	6.57	8.18	6.46
C22:5n-3(DPA)	0.17	0.15	0.16	0.16	0.14	0.19	0.20	0.15	0.11	0.11	0.11	0.12
C22:6n-3 (DHA)	0.04	0.04	0.04	0.03	0.07	0.01	0.01	0.01	1.11	0.98	1.20	0.93

n-6:n-3 PUFA	1.8	1.1	1.6	1.1	1.2	0.8	1.1	0.8	0.7	0.6	0.7	0.6

[1]White Leghorn hens (47 wk old, n=120) were fed one of 12 diets arranged as a 2*2*3 factorial: 2 flax oil type (regular flax (REGflax), or SDA-enriched flax (SDAflax) at 2 inclusion levels, (2.5% and 5%), in combination with 2% of 3 oil types (corn, canola and fish oil) for 35 d.
[2]SFA = saturated fatty acids; SFA = C14:0 + C16:0 + C18:0 + C20:0 + C22:0.
[3]MUFA = monounsaturated fatty acids; MUFA = C16:1 n-7 + C18:1 n-7 + C18:1 n-9 + C22:1 n-9 + C24:1 n-7.
[4]PUFA = polyunsaturated fatty acids; PUFA = C18:2 n-6 + C18:3 n-3 +C18:3 n-6 + C20:2n-6 + C20:3n-3 + C20:3n-6 + C20:4 n-6 + C20:2 n-6 + C20:5 n-3 + C22:2 n-6 + C22:6 n-3.
[6]Total n-6 PUFA = C18:2 n-6 + C18:3 n-6 + C20:2n-6 + C20:3n-6 + C20:4 n-6 + C22:4 n-6.
[5]Total n-3 PUFA = C18:3 n-3 + C20:3 n-3 + C20:5 n-3 + C22:6 n-3.
[7]LC n-3 PUFA = C20:3 n-3 + C20:5 n-3 + C22:6 n-3.

Table 4.3. Layer performance, egg quality and reproductive traits of the White Leghorn hens fed the experimental diets[1,2].

	Flax type			Flax level			Base oil				P-value		
Performance traits	REGflax	SDAflax	SEM	Low	High	SEM	Corn	Canola	Fish	SEM	Flax oil type	Flax oil Level	Base oil
Feed intake (g)	3,843	3,874	32.3	3,922	3,795	32.2	3,840	3,913	3,822	39.5	0.491	0.007	0.236
BW change (g)	132.7	140.6	12.4	116.2	157.1	12.4	139.9	0.151.0	119.0	15.2	0.654	0.022	0.324
Egg production (%)	94.0	96.0	0.76	95.5	94.8	0.76	95.5	95.1	94.7	0.93	0.195	0.513	0.816
Egg mass (g)	1,993	2,043	20.2	2,032	2,005	20.2	2,026	2,009	2,020	24.7	0.082	0.359	0.889
Egg quality traits													
Albumen height (mm)	8.44	8.35	0.14	8.48	8.31	0.14	8.49	8.35	8.35	0.17	0.684	0.418	0.798
Yolk weight (g)	16.20	16.13	0.19	16.04	16.30	0.19	16.09	16.25	16.16	0.23	0.799	0.347	0.886
Shell thickness (µm)	341.1	337.4	0.29	335.7	342.8	0.29	338.6	338.2	340.9	0.36	0.378	0.087	0.852
Reproduction traits													
Large yellow follicles[3] (n)	6.47	6.69	0.13	6.56	6.61	0.12	6.5	6.63	6.63	0.18	0.226	0.761	0.811
Ovary weight (g)	60.99	59.23	1.92	60.47	59.76	1.93	58	61.47	60.87	2.3	0.521	0.794	0.544

[a-b]Means within a row without a common superscript differ (P<0.05). LSmeans based on 10 individually housed layers per dietary treatments.
[1]White Leghorn hens (47 wk old, n=120) were fed one of 12 diets arranged as a 2*2*3 factorial: 2 flax oil type (regular flax (REGflax), or SDA-enriched flax (SDAflax) at 2 inclusion levels, (2.5% and 5%), in combination with 2% of 3 oil types (corn, canola and fish oil) for 35 d.

[2] Flax oil type *flax oil level, flax oil type * base oil, and flax oil type * flax oil level* base oil interactions were not significant (P>0.05).
[3]>10 mm diameter.

Table 4.4. Fatty acid composition (% of total fatty acids) of liver tissue collected at day 35 from White Leghorn hens fed the experimental diets[1,2].

Fatty acid	Flax oil type			Flax oil level			Base oil				P-value		
	REGflax	SDAflax	SEM	Low	High	SEM	Corn	Canola	Fish	SEM	Flax oil type	Flax oil level	Base oil
TFA[3] (mg/g)	81.25	86.31	2.89	78.75	86.31	3.75	81.27	84.48	78.6	3.42	0.240	0.367	0.113
SFA[4]	35.09	40.05	0.32	37.51	37.68	0.73	37.37	37.17	38.17	0.39	<0.001	0.796	0.181
C14:0	0.31	0.31	0.03	0.33	0.29	0.04	0.24[b]	0.23[b]	0.46[a]	0.03	0.865	0.381	0.001
C16:0	21.80	23.73	0.35	22.73	22.83	0.43	22.40	22.36	23.55	0.43	0.001	0.882	0.108
C18:0	12.98	16.01	0.35	14.45	14.56	0.54	14.73	14.58	14.17	0.43	<.0001	0.875	0.643
MUFA[5]	44.46	39.24	0.98	43.68	40.14	1.23	39.95	42.84	42.75	1.20	0.001	0.015	0.180
C16:1n-7	3.29	2.51	0.13	2.90	2.80	0.19	2.63	2.59	3.33	0.16	0.001	0.716	0.003
C18:1n-7	1.70	1.39	0.03	1.66	1.45	0.07	1.33[c]	1.58[b]	1.71[a]	0.03	<0.001	0.001	<0.001
C18:1n-9	38.76	34.68	0.94	38.42	35.16	1.08	35.43	38.00	36.73	1.15	0.006	0.017	0.298
C20:1n-7	0.319	0.31	0.03	0.32	0.31	0.04	0.17[b]	0.20[b]	0.58[a]	0.05	0.662	0.604	0.047
C24:1n-7	0.42	0.52	0.06	0.47	0.47	0.06	0.42	0.52	0.47	0.07	0.254	0.963	0.641
Total n-6 PUFA[6]	14.03	14.60	0.69	13.95	14.54	0.85	17.12[a]	13.72[b]	12.10[b]	0.85	0.562	0.464	0.002
C18:2n-6 (LA)	12.21	10.91	0.44	11.46	11.57	0.64	14.23[a]	10.64[b]	9.82[b]	0.54	0.048	0.740	<0.001
C18:3n-6 (GLA)	ND	0.51	0.04	0.24	0.65	0.08	0.27	ND	ND	0.07	0.006	0.017	0.466
C20:4n-6 (AA)	1.79	3.13	0.29	2.32	2.57	0.35	2.59	2.82	1.96	0.35	0.003	0.493	0.228
C22:2n-6	ND	0.07	0.01	0.07	0.06	0.01	0.06	ND	ND	0.01	0.871	0.144	0.120
Total n-3 PUFA[7]	6.39	6.06	0.39	4.83	7.59	0.39	5.56	6.27	6.85	0.48	0.560	<0.001	0.200
C18:3n-3 (ALA)	4.06	2.70	0.20	2.30	4.47	0.26	3.21	3.38	3.55	0.25	<0.001	<0.001	0.637
C18:4n-3 (SDA)	ND	0.39	0.05	0.16	0.58	0.07	ND	ND	ND	ND	0.546	0.001	0.534
LC n-3 PUFA[8]	2.33	2.97	0.32	2.43	2.82	0.33	2.15	2.72	3.08	0.39	0.170	0.348	0.274
C20:4n-3	0.06	ND	0.01	0.05	0.06	0.01	ND	0.06	ND	0.01	0.591	0.567	0.889
C20:5n-3 (EPA)	0.35	0.28	0.04	0.26	0.37	0.05	0.21[b]	0.30[ab]	0.45[a]	0.05	0.240	0.053	0.009
C22:5n-3(DPA)	0.18	0.28	0.03	0.20	0.26	0.03	0.20	0.24	0.25	0.03	0.021	0.118	0.467
C22:6n-3(DHA)	1.74	2.34	0.15	1.93	2.11	0.26	1.68	2.10	2.35	0.30	0.010	0.524	0.321
n-6:n-3 PUFA	2.39	2.66	0.08	3.06	1.98	0.20	3.41[a]	2.27[b]	1.90[c]	0.10	0.026	<0.001	<0.001

[a-c]Means within a row without a common superscript differ (P<0.05). LS means based on 10 individually-housed layers per dietary treatment

[1]White Leghorn hens (47 wk old, n=120) were fed one of 12 diets arranged as a 2*2*3 factorial: 2 flax oil type (regular flax (REGflax), or SDA-enriched flax (SDAflax) at 2 inclusion levels, (2.5% and 5%), in combination with 2% of 3 oil types (corn, canola and fish oil) for 35 d.
[2] Flax oil type *flax oil level, flax oil type * base oil, and flax oil type * flax oil level* base oil interactions were not significant (P>0.05).
[4]Saturated fatty acids (SFA) = C14:0 + C16:0 + C18:0 + C20:0 + C22:0.
[5]Monounsaturated fatty acids (MUFA) = C16:1n-7 + C18:1n-7 + C18:1n-9 + C22:1n-9 + C24:1n-7.
[6]Total n-6 Polyunsaturated fatty acids (PUFA) = C18:2n-6 + C18:3n-6 + C20:2n-6 + C20:3n-6 + C20:4n-6 + C22:4n-6.
[7]Total n-3 PUFA = C18:3n-3 + C20:3n-3 + C20:5n-3 + C22:6n-3.
[8]LC n-3 PUFA = C20:3n-3 + C20:5n-3 + C22:6n-3.

Table 4.5. Fatty acid composition (% of total fatty acids) of egg yolks collected at day 35 from White Leghorn hens fed the experimental diets[1].

Fatty acid	Flax type			Level			Base oil				P-value					
	REGflax	SDAflax	SEM	Low	High	SEM	Corn	Canola	Fish	SEM	Flax oil type	Flax oil level	Base oil	Flax type* Flax level	Flax type* Base oil	Flax level * Base oil
TFA[2] (mg/ g yolk)	367.92	380.28	6.57	371.65	376.55	6.75	385.27	367.53	369.49	8.37	0.198	0.606	0.249	0.122	0.564	0.576
SFA[3]	32.49	38.02	0.56	34.68	35.67	0.81	34.14^{b}	34.81^{ab}	36.81^{a}	0.69	<0.001	0.163	0.028	0.342	0.873	0.416
C14:0	0.23	0.28	0.02	0.26	0.25	0.02	0.23^{b}	0.18^{b}	0.37^{a}	0.02	0.032	0.393	<0.001	0.583	0.503	0.370
C16:0	22.99	25.58	0.20	24.35	24.2	0.38	23.78^{b}	23.78^{b}	25.31^{a}	0.24	<0.001	0.958	<0.001	0.017	0.567	0.280
C18:0	10.22	12.17	0.27	11.10	11.22	0.39	11.57	10.85	11.17	0.33	<0.001	0.610	0.309	0.011	0.006	0.059
MUFA[4]	45.08	40.95	0.39	44.87	41.22	0.72	41.27^{b}	45.13^{a}	42.65^{b}	0.48	<0.001	<0.001	<0.001	0.003	0.577	0.010
C16:1n7	2.93	2.41	0.04	2.78	2.58	0.07	2.48^{b}	2.46^{b}	3.07^{a}	0.09	<0.001	0.001	0.015	0.144	0.717	0.249
C18:1n7	1.73	1.45	0.03	1.66	1.52	0.06	1.41^{b}	1.68^{a}	1.67^{a}	0.04	<0.001	0.005	<0.001	0.002	0.022	0.144
C18:1n9	39.56	36.17	0.39	39.51	36.27	0.68	36.56^{b}	40.27^{a}	36.76^{b}	0.48	<0.001	<0.001	<0.001	0.031	0.161	0.006
C20:1n7	0.23	0.27	0.04	0.26	0.24	0.05	0.17^{b}	0.09^{b}	0.49^{a}	0.05	0.426	0.827	<0.001	0.356	0.349	0.345
C24:1n7	0.64	0.66	0.02	0.67	0.63	0.02	0.656	0.628	0.665	0.02	0.650	0.222	0.540	0.393	0.536	0.123
Total n-6 PUFA[5]	14.79	13.87	0.45	14.29	14.44	0.63	17.37^{a}	12.92^{b}	12.7^{b}	0.55	0.161	0.898	<0.001	0.098	0.793	0.732
C18:2n6 (LA)	13.77	11.83	0.43	12.83	12.86	0.62	15.68^{a}	11.43^{b}	11.29^{b}	0.53	0.003	0.916	<0.001	0.327	0.911	0.651
C18:3n6 (GLA)	ND	0.46	0.04	0.28	0.48	0.07	0.33	ND	0.27	0.06	0.001	0.188	0.591	0.007	0.250	0.885
C20:4n6 (AA)	0.92	1.52	0.06	1.22	1.20	0.10	1.35^{a}	1.22^{ab}	1.09^{b}	0.07	<0.001	0.990	0.043	0.001	0.001	0.563
C222n6	ND	ND	ND	0.06	0.06	0.01	ND	ND	0.05^{a}	0.01	0.007	0.352	0.012	0.279	0.001	0.873
C225n6	ND	0.06	0.01	0.07	0.06	0.01	0.07	ND	0.05	0.01	0.082	0.262	0.065	0.460	0.023	0.371
Total n-3 PUFA[6]	7.64	7.27	0.34	6.15	8.76	0.32	7.22	7.14	8.00	0.42	0.453	<0.001	0.299	0.894	0.714	0.776
C18:3n3 (ALA)	5.77	4.61	0.29	4.04	6.25	0.29	5.22	5.15	5.20	0.35	0.008	<0.001	0.988	0.747	0.357	0.661
C18:4n3 (SDA)	ND	0.19	0.02	0.11	0.19	0.03	0.14	ND	0.10	0.03	0.001	0.105	0.634	0.009	0.302	0.774

LC n-3 PUFA[7]	1.85	2.47	0.09	2.04	2.38	0.13	1.89[b]	1.90[b]	2.70[a]	0.12	<0.001	0.035	<0.001	0.457	0.368	0.547
C20:4n3 (ETA)	ND	0.07	0.01	0.06	0.11	0.01	0.06	0.08	ND	0.01	0.891	0.584	0.637	0.029	0.660	0.585
C20:5n3 (EPA)	0.17	0.26	0.02	0.19	0.24	0.02	0.17[b]	0.17[b]	0.31[a]	0.02	0.001	0.031	<0.001	0.502	0.868	0.792
C22:5n3 (DPA)	0.16	0.31	0.02	0.22	0.26	0.03	0.22	0.24	0.26	0.02	<0.001	0.367	0.435	0.031	0.311	0.369
C22:6n3 (DHA)	1.46	1.89	0.07	1.63	1.78	0.10	1.47[b]	1.45[b]	2.10[a]	0.09	0.001	0.084	<0.001	0.694	0.318	0.269
n-6:n-3 PUFA	2.09	2.13	0.13	2.50	1.73	0.16	2.78[a]	1.88[b]	1.69[b]	0.16	0.815	0.001	<0.001	0.366	0.941	0.159

[a-c]Means within a row without a common superscript differ (P<0.05). LSmeans based on 10 individually-housed layers per dietary treatment.

[1]White Leghorn hens (47 wk old, n=120) were fed one of 12 diets arranged as a 2*2*3 factorial: 2 flax oil type (regular flax (REGflax), or SDA-enriched flax (SDAflax) at 2 inclusion levels, (2.5% and 5%), in combination with 2% of 3 oil types (corn, canola and fish oil) for 35 d.

[2]Flax oil type * flax oil level* base oil interaction was not significant (P>0.05).

[2]Total fatty acid (TFA) (mg/ g yolk) = Amount of total fatty acid in 1 gm of egg yolk sample.

[3]Saturated fatty acids (SFA) = C14:0 + C16:0 + C18:0 + C20:0 + C22:0.

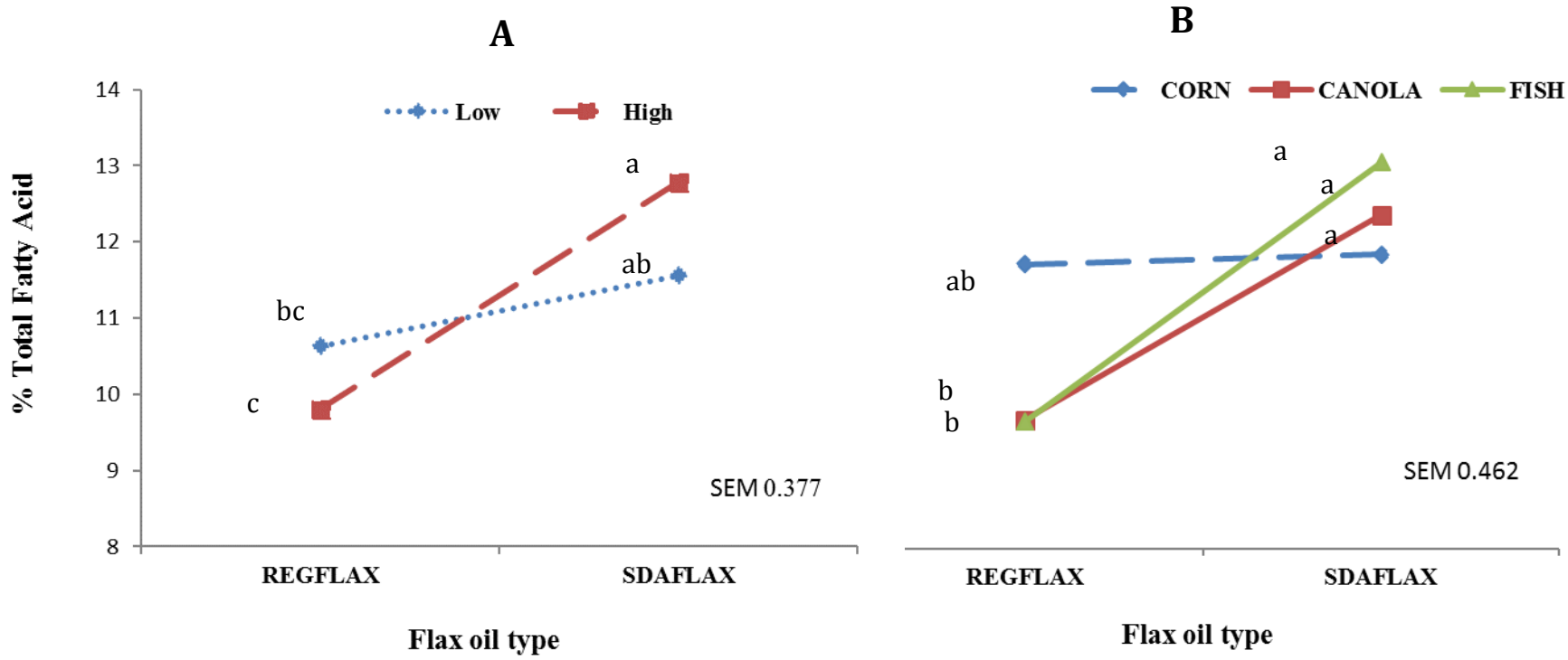
[4]Monounsaturated fatty acids (MUFA) = C16:1 n-7 + C18:1 n-7 + C18:1 n-9 + C22:1 n-9 + C24:1 n-7.

[5]Total n-6 PUFA = C18:2 n-6 + C18:3 n-6 + C20:2n-6 + C20:3n-6 + C20:4 n-6 + C22:4 n-6.

[6]Total n-3 PUFA = C18:3 n-3 + C20:3 n-3 + C20:5 n-3 + C22:6 n-3.

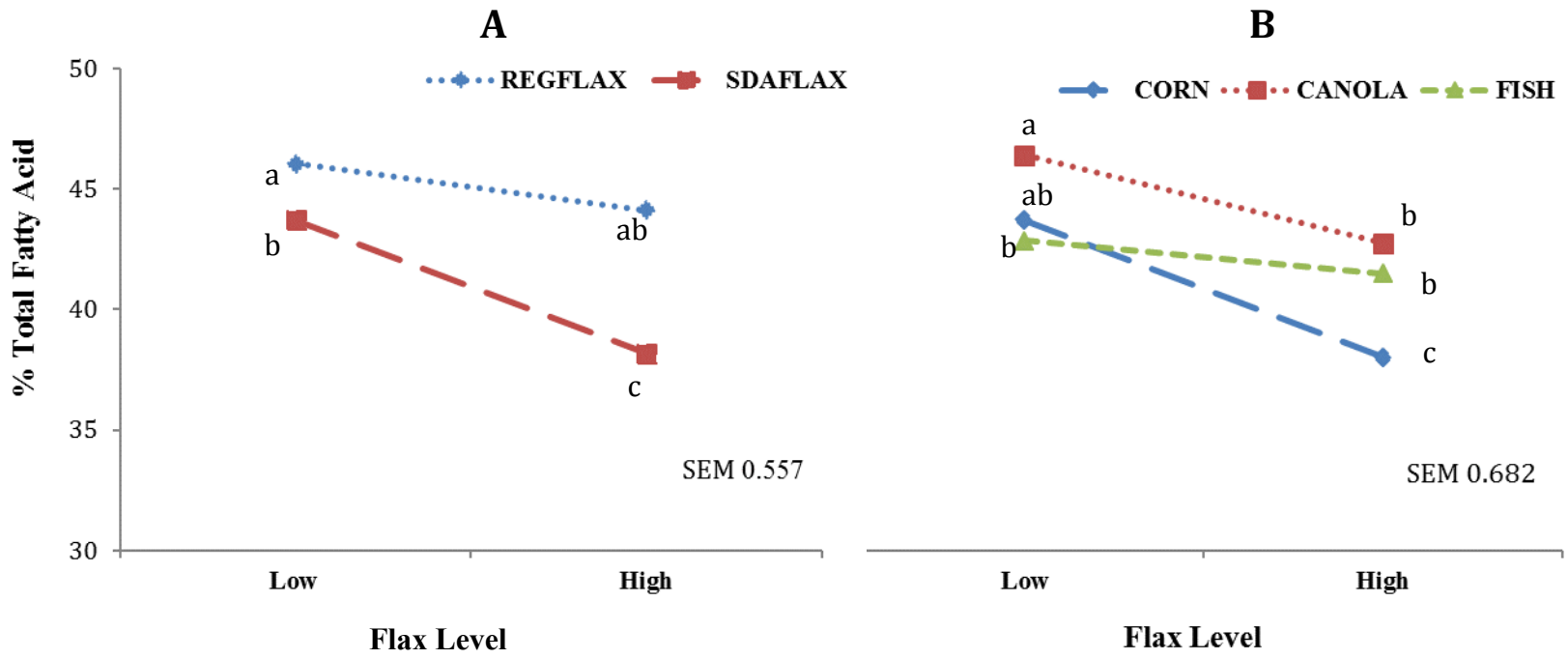
[7]LC n-3 PUFA = C20:3 n-3 + C20:5 n-3 + C22:6 n-3.

Figure 4.1: C18:0 (% of total fatty acids) in egg yolks collected after 35 d of feeding from White Leghorn hens fed experimental diets varying in oil source and inclusion level[1].



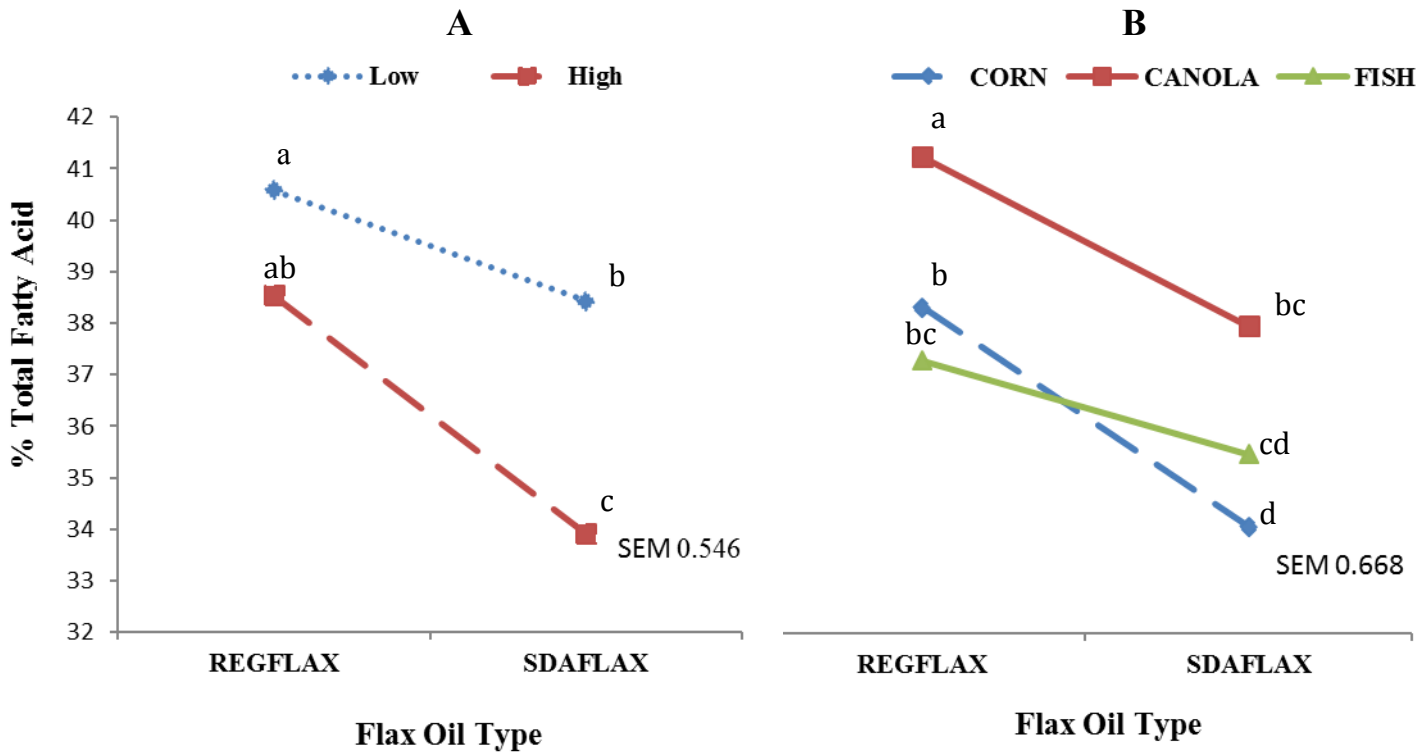
[a-c]Means without a common superscript differ (P<0.05). LSmeans based on 10 individually-housed layers per dietary treatment.
[1]White Leghorn hens (47 wk old, n=120) were fed one of 12 diets arranged as a 2*2*3 factorial: 2 flax oil type (regular flax (REGflax), or SDA-enriched flax (SDAflax) at 2 inclusion levels, (2.5% and 5%), in combination with 2% of 3 oil types (corn, canola and fish oil) for 35 d.
Panel A = Effect of interaction of flax oil type and flax oil level on yolk C18:0 (% of total fatty acids).
Panel B = Effect of interaction of flax oil type and base oil type on yolk C18:0 (% of total fatty acids).

Figure 4.2: Total monounsaturated fatty acid composition (MUFA; % of total fatty acids) of egg yolks collected at day 35 from White Leghorn hens fed the experimental diets[1].



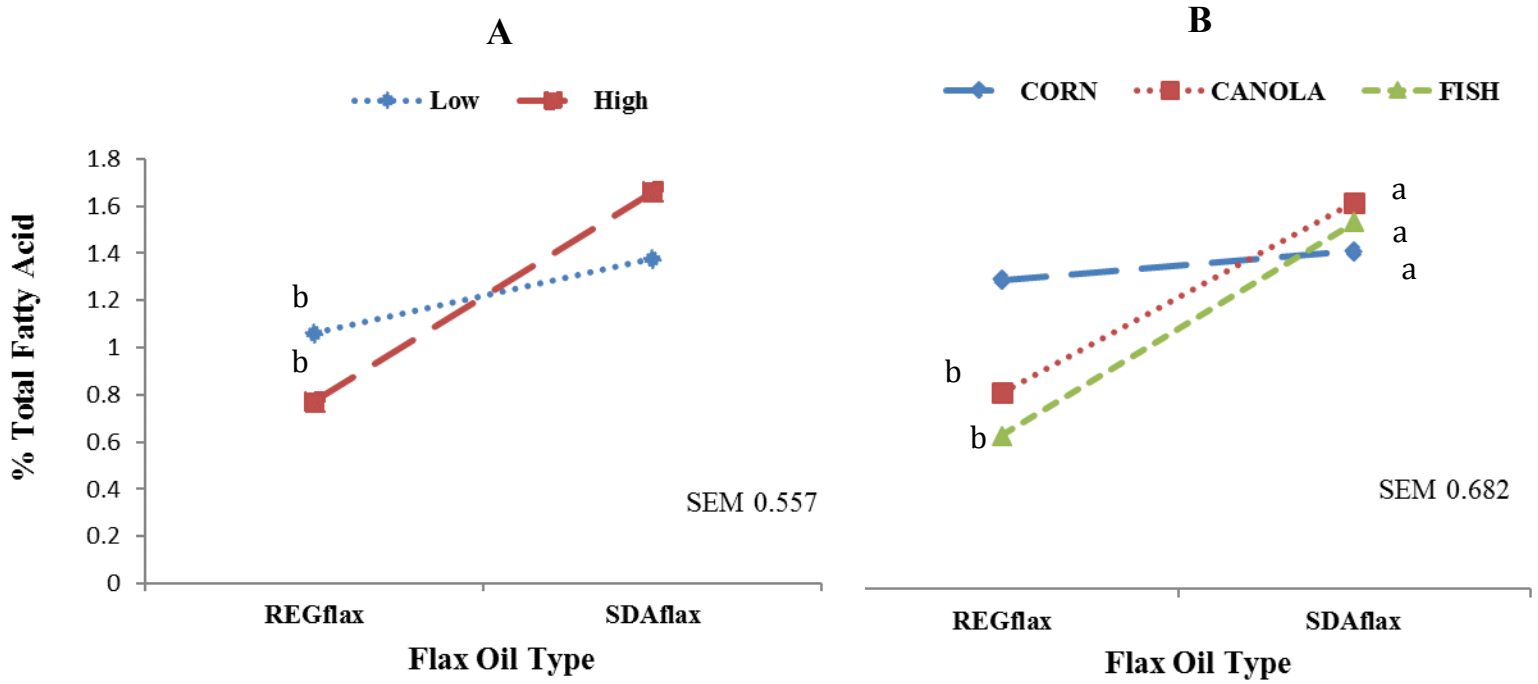
[a-c]Means without a common superscript differ (P<0.05). LSmeans based on 10 individually-housed layers per dietary treatment.
[1]MUFA = monounsaturated fatty acids; MUFA = C16:1 n-7 + C18:1 n-7 + C18:1 n-9 + C22:1 n-9 + C24:1 n-7.
[2]White Leghorn hens (47 wk old, n=120) were fed one of 12 diets arranged as a 2*2*3 factorial: 2 flax oil type (regular flax (REGflax), or SDA-enriched flax (SDAflax) at 2 inclusion levels, (2.5% and 5%), in combination with 2% of 3 oil types (corn, canola and fish oil) for 35 d.
Panel A = Effect of interaction of flax oil type and flax oil level on yolk MUFA (% of total fatty acids).
Panel B = Effect of interaction of flax oil level and base oil type on yolk MUFA (% of total fatty acids).

Figure 4.3: The C18:1 composition (% of total fatty acids) of egg yolks collected at day 35 from White Leghorn hens fed the experimental diets[1].



[a-c]Means without a common superscript differ (P<0.05). LSmeans based on 10 individually-housed layers per dietary treatment.
[1]White Leghorn hens (47 wk old, n=120) were fed one of 12 diets arranged as a 2*2*3 factorial: 2 flax oil type (regular flax (REGflax), or SDA-enriched flax (SDAflax) at 2 inclusion levels, (2.5% and 5%), in combination with 2% of 3 oil types (corn, canola and fish oil) for 35 d.
Panel A = Effect of interaction of flax oil type and flax oil level on yolk C18:1 (% of total fatty acids).
Panel B = Effect of interaction of flax oil type and base oil type on yolk C18:1 (% of total fatty acids).

Figure 4.4: Arachidonic acid composition (AA; % of total fatty acids) of egg yolks collected at day 35 from White Leghorn hens fed the experimental diets[1].



[a-b]Means without a common superscript differ (P<0.05). LSmeans based on 10 individually-housed layers per dietary treatment.
[1]White Leghorn hens (47 wk old, n=120) were fed one of 12 diets. The diets were fed as a 2*2*3 factorial arrangement with 2 flax oil type (regular flax (REGflax), or SDA-enriched flax (SDAflax) at 2 inclusion levels, (Low=2.5% and High= 5%), in combination with 2% of 3 oil types (corn, canola and fish oil) for 35 d.
Panel A = Effect of interaction of flax oil type and flax oil level on yolk arachidonic acid composition (AA; % of total fatty acids).
Panel B = Effect of interaction of flax oil type and base oil type on yolk arachidonic acid composition (AA; % of total fatty acids).

Chapter 5

Summary and Implications

5.1 SUMMARY AND IMPLICATIONS

The health benefits of long chain (LC) omega-3 polyunsaturated fatty acids (n-3 PUFA; eicosapentaenoic acid (EPA, C20:5n-3), and docosahexaenoic acid (DHA, C22:6n-3)) have been linked with decreased incidence of cardiovascular disease, increase in brain and neural development in infants and prevention of cancer in humans (Calder, 2014). Due to increased consumer awareness of the health effects of n-3 PUFA, there has been rise in the n-3 PUFA products market and it is expected to be $4.3 billion by 2019 (Global Trend and Forecast report, 2014). Chicken meat constitutes the one-third of animal protein in the world (FAO, 2012), suggesting a strong market potential for n- 3 PUFA enriched chicken meat.

Only about one-fourth of pregnant women in Alberta consume more than 200 mg/day of LC n-3 PUFA (Jia et al., 2015), as recommended by the European Commission with the International Society for the Study of Fatty Acids and Lipids for pregnant and lactating (Koletzko et al. 2008). Salmon and other seafood, fish, and seaweed products (79%) followed by poultry meat and eggs (13%) are major source of LC n-3 PUFA consumed by humans (Jia et al., 2015). However, with depleting availability of marine sources (fish oil) the demand for alternate sources of LC n-3 PUFA is rising (Betencor et al., 2015). Microalgae, depending on their origin species, are good source of EPA and DHA, and have potential to be used to enrich table eggs (Lemahieu et al., 2015) or broiler meat (Rymer et al., 2010) for LC n-3 PUFA. However, due to low supply and high production cost, economically feasible large-scale production of microalgae for LC-PUFA is a big hurdle (Haslam et al., 2012). In addition, carotenoids in the microalgae can increase redness of the yolk, which may be a concern for consumers in some markets (Lemahieu

et al., 2013). Alternatively, oils extracted from genetically modified crops high in EPA and DHA can become a viable alternative to fish oils (Betancore et al., 2015). The enrichment of poultry products using novel n-3 PUFA sources can be a successful strategy to fulfill human nutritional needs.

Feed constitutes more than two-third of the cost for poultry production (Willems et al., 2013). Camelina cake, being a co-product from biofuel production, offers great potential as a cost-effective n-3 PUFA alternative for broiler chicken or laying hen feeding (Aziza et al., 2010; Kakani et al., 2012). The first study of this thesis established the potential for the inclusion of camelina cake in broiler rations for n-3 PUFA enrichment of chicken meat. The Canadian labeling claim requirement for n-3 PUFA-enriched meat products (300 mg/100g meat) was exceeded in breast and thigh by feeding a 24% camelina cake diet for 28 d or 16% camelina cake diet for 42 d, respectively. The enrichment of thigh muscle with total n-3 PUFA was greater than for skinless breast, but breast muscle had a greater proportion of LC n-3 PUFA enrichment compared with thigh muscle. In addition, there was a linear increase in the proportion of LC n-3 PUFA in liver (DPA increased by 109% and DHA by 80%) and brain (DPA increased by 24% and DHA increased by 6%) for each 8% increase in dietary camelina cake inclusion. In 2009, the US Food and Drug Administration approved the inclusion of 10% camelina cake in laying hen or broiler feed in the USA (FDA, 2009). Recently, the Canadian Food Inspection Agency has approved 12% inclusion of camelina cake for the broilers in Canada (Roberts, 2015).

Inclusion of 10 to 12% camelina cake might be not sufficient to meet the labeling requirement for broiler meat n-3 PUFA enrichment. However, camelina cake has lower cost of production, (Acamovic et al., 1999) and greater vitamin E content compared with other oil seeds (Quezada and Cherian et al., 2012). Therefore, the inclusion of camelina cake along with dietary

LC n-3 PUFA sources might be a practical approach for the production of n-3 PUFA enriched broiler meat. Alternatively, 10% inclusion of camelina cake in laying hens increased total yolk n-3 PUFA (160 mg/egg; Kakani et al., 2012), and daily consumption of two of these enriched eggs would exceed the 300 mg of n-3 PUFA recommendation for humans (Cherian et al., 2009). Further, the egg is an ideal delivery system for enrichment fatty acids because of its more stable environment than those from many meat sources (Ren et al., 2013). Nutrients such as lutein, folic and choline contained in the egg are much more bioavailable than those from plant sources (Chung et al., 2004). In addition, the fatty acids are concentered in egg yolk, unlike marketable meat portions in which they are unevenly distributed (Gonzalez-Esquerra and Lesson, 2001) and consumers also trim off fat from meat.

The bioconversion of α-linolenic acid (ALA, C18:3n-3) to LC n-3 PUFA, although greater in poultry species than in mammals (Gregory et al., 2013) is still only moderately effective in poultry due to dependency on the rate limiting factor Δ6-desaturase (Poureslami et al., 2010). A genetically modified flaxseed plant (SDAflax) enriched in stearidonic acid (SDA; 18:4 n-3), and gamma linolenic acid (18:3 n-6; GLA) was included in laying hen diets. We hypothesized that feeding SDAflax oil to laying hens, thereby bypassing the rate-limiting initial Δ 6-desaturation of ALA to SDA, could increase LC n-3 PUFA proportions in egg yolk. Strategically, this was the first ever experiment to establish the inclusion of high SDA/GLA flaxseed in any livestock-feeding regime. The results from Chapter 3 showed that SDAflax oil and regular flax oil had no difference in potential to enrich eggs in total n-3 PUFA, but hens fed SDAflax oil had greater LC n-3 PUFA enrichment in eggs. Inclusion of 4% flax oil in laying hen diets increased each of the long chain n-3 PUFA (ETA, EPA, DPA and DHA) in egg yolk compared with regular flax oil. SDA-enriched flax oil has anti-tumor activity against breast

cancer cells (Subedi et al., 2015). In addition, GLA has shown potential to decrease conditions such as atopic dermatitis and rheumatoid arthritis in humans (Leventhal et al., 1993; Simon et al., 2014). Therefore, consumption of the eggs enriched with inclusion of SDAflax oil in layers diet not only increased LC n-3 PUFA but also provided an increased SDA and GLA in the eggs and hence these eggs may be well positioned to increase the long-term well-being of humans.

Omega-3 enriched eggs have been available in the market and are sold at a premium price. Omega-3 eggs currently in the market are mostly from hens fed flaxseed or flaxseed oil and contain the majority of n-3 PUFA as ALA (Fraeye et al., 2012). The eggs from hens fed 4% conventional flax oil as in Chapter 3 were greater in their n-3 PUFA proportions to those omega-3 enriched eggs available in market (Yannakopoulos, 2005). Results from Chapter 3 showed that regular flax oil had a greater total n-3 PUFA as well as LC n-3 PUFA in yolks compared with control eggs. In addition, LC n-3 PUFA in egg yolk from hens fed SDAflax oil was double that of those fed REGflax, suggesting that consumption of two of SDAflax oil enriched eggs will be equivalent to three of regular flax oil enriched eggs for LC n-3 PUFA proportions.

A second experiment with SDAflax oil was conducted to investigate the metabolic competition among dietary fatty acid sources for desaturation and elongation pathways, and their effect on egg yolk LC n-3 PUFA. Three sets of diets with 2% corn (CO), canola (CAN), or fish (FO) added with either 2.5 or 5% of regular flax oil (REGflax) or SDA-enriched flax oil (SDAflax) were fed to laying hens for 35 d. The increase from 2.5% to 5% inclusion level of flax in hen diets had increase total n-3 PUFA in liver by 1.6-fold. However, total n-3 PUFA in liver was not different among the base oils or between flax types, suggesting that presence of base oils (CO or CAN or FO) in addition to either type of flax oil did not affect final n-3 PUFA enrichment in the liver. For yolk enrichment, inclusion of SDAflax oil in diets resulted in an

additional 33.5% (2.47% *vs.* 1.85%) increase in the LC n-3 PUFA compared with REGflax oil diets. In addition, inclusion of FO in diets with flax oil has increased LC n-3 PUFA in egg yolk. However, contrary to our hypothesis that the presence of high dietary n-6 PUFA from corn oil in diet would decrease the LC n-3 PUFA enrichment of egg yolk, we did not observe any difference between canola and corn oil for LC n-3 PUFA enrichment. A possible reason for the lack of difference in yolk LC n-3 PUFA from hens fed SDAflax along corn oil compared with canola oil might be the ratio of n-6 to n-3 PUFA in the diets, which was 0.8 to 1.2:1 in all diets. A 1:1 ratio of n-6 to n-3 PUFA in the culture medium resulted in the greatest bioconversion from ALA to DHA in human liver cells in vitro (Harnack et al., 2009). ALA is about 10 times stronger at suppressing LA metabolism compared with the effect of LA on ALA metabolism (Holman, 1998). Therefore in our study, inclusion of CO or CAN oil in combination with either flax oil was not effective in inhibiting ALA metabolism in laying hens.

Consumption of one egg from hens fed SDAflax oil would provide 151.5 mg of LC n-3 PUFA (2.47% * 16.13 g of yolk * 380.28 mg/g of yolk) compared with the eggs from hens fed REGflax oil with 110.3 mg of LC n-3 PUFA (1.85% * 16.20 g of yolk * 367.9 mg/g of yolk) (Table 5.1). Consumption of eggs enriched with n-3 PUFA derived from SDAflax oil would be helpful to increase the LC n-3 PUFA status in the human body. The eggs from hens fed SDAflax oil and REGflax provided about 445.9 and 455.6 mg/egg of total n-3 PUFA respectively, that constitute about one-quarter of recommended daily intake for n-3 PUFA in males (1.6 g/day) and one-third in females (female 1.1 g/day; Kris-Etherton et al., 2007). The LC n-3 PUFA enriched eggs can be helpful for the pregnant and lactating women, where increased consumption of fish should be limited due to concerns of mercury contamination (Oken et al., 2003). The eggs from hens fed SDAflax oil contained 282.8 of ALA, 11.7 mg/egg of SDA, 15.9 mg/egg of EPA and

115.9 mg/egg of DHA.The global acceptance for genetically modified plants and the enriched product is still a big concern and may limit the adoption of such approach in commercial production. Health Canada has issued “no objection” for the inclusion of SDA-enriched soybean in human food (Health Canada, 2015) or animal feed (CFIA, 2012). The guidelines for the use of SDAflax oil in human or animal feed needs to be further elucidated through comprehensive assessments.

In summary, camelina cake can be included in broiler diets to increase n-3 PUFA of meat; feeding SDA-enriched flax oil can increase egg LC n-3 PUFA in laying hens without negative effects on egg production and reproductive traits. Inclusion of FO increased LC n-3 PUFA; there was no difference for yolk LC n-3 PUFA between CO and CAN treatment hens, suggesting a lack of competition for enzymes during lipid desaturation and elongation. Therfore, the enriched poultry products (meat and table eggs) offer an alternative to increase the amount of total n-3 PUFA in human diets. The outcomes from the researches in this thesis provide the opportunity for the poultry producers targeting for the enrichment of their products in n-3 PUFA. However, future studies with the collaboration of plant biotechnologists and animal nutritionist are needed to accelerate development and commercialization of these value-added plants with modified oil composition.

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Table 5.1: The amount of omega-3 polyunsaturated fatty acid enrichment (mg /egg) in yolks collected at day 35 from White Leghorn hens fed the experimental diets[1]

	Flax oil type		Flax oil Level		Base oil type		
Fatty acid (mg/egg)	REGflax	SDAflax	Low	High	Corn	Canola	Fish
Total n-3 PUFA[2]	455.4	445.9	366.6	537.7	447.6	426.4	477.7
C18:3n3 (ALA)	343.9	282.8	240.8	383.6	323.6	307.6	310.5
C18:4n3 (SDA)	ND	11.7	6.6	11.7	8.7	ND	6.0
LC n-3 PUFA[3]	113.5	151.5	121.6	146.1	117.2	113.5	161.2
C20:4n3 (ETA)	ND	4.3	3.6	6.8	3.7	4.8	ND
C20:5n3 (EPA)	10.4	15.9	11.3	14.7	10.5	10.2	18.5
C22:5n3 (DPA)	9.8	19.0	13.1	16.0	13.6	14.3	15.5
C22:6n3 (DHA)	89.6	115.9	97.2	109.3	91.1	86.6	125.4

[1]White Leghorn hens (47 wk old, n=120) were fed one of 12 diets arranged as a 2*2*3 factorial: 2 flax oil type (regular flax (REGflax), or SDA-enriched flax (SDAflax) at 2 inclusion levels, (2.5% and 5%), in combination with 2% of 3 oil types (corn, canola and fish oil) for 35 d.
[2]Total n-3 polyunsaturated fatty acids (PUFA) = 18:3n-3 (ALA) + 18:4n-3 (SDA) + 20:4n-3 (ETA) + 20:5n-3 (EPA) + 22:5n-3 (DPA) + 22:6n-3 (DHA).
[3]Long chain n-3 polyunsaturated fatty acids (PUFA) = 20:4n-3 (ETA) + 20:5n-3 (EPA) + 22:5n-3 (DPA) + 22:6n-3 (DHA).

APPENDIX

Table A-1: Fatty acid composition of breast tissue (% of total fatty acids) collected from hens fed experimental diets[1] for 21 d (Chapter 3).

	Breast				
Fatty acid	Control	REGflax	SDAflax	SEM	P-value
SFA[2]	27.95	29.84	30.69	2.12	0.665
C14:0	0.40	0.37	0.34	0.02	0.173
C16:0	22.16	20.70	21.02	0.70	0.308
C18:0	9.30	8.79	9.23	0.76	0.863
C20:0	0.06	0.09	0.07	0.03	0.761
C22:0	0.05	ND	0.02	0.01	0.212
MUFA[3]	39.17	38.71	38.86	2.18	0.989
C14:1	2.51	2.64	2.86	0.64	0.931
C16:1n7	0.56	0.59	0.53	0.05	0.631
C16:1n9	1.37	1.45	1.59	0.20	0.754
C18:1n7	32.64	32.31	31.79	2.19	0.962
C18:1n9	2.73	2.91	2.57	0.16	0.277
C20:1	0.28	0.37	0.26	0.02	0.493
n-6 PUFA[4]	30.27^{a}	26.74^{b}	26.00^{b}	0.70	0.001
C18:2n-6 (LA)	23.19^{a}	20.96^{ab}	18.84^{b}	0.73	0.002
C18:3n-6 (GLA)	ND	ND	0.201^{a}	0.07	0.393
C20:2n-6	0.28^{a}	0.143^{b}	0.14^{b}	0.02	0.001
C20:4n-6	5.47^{a}	4.567^{a}	5.349^{a}	0.85	0.674
C20:3n-6	0.40^{b}	0.259^{b}	0.65^{a}	0.07	0.002
C22:3n-6	0.69^{a}	0.35^{b}	0.37^{b}	0.09	0.022
C22:4n-6	0.19	0.14	0.22	0.07	0.664
n-3 PUFA[5]	2.07^{b}	4.92^{a}	4.61^{a}	0.559	<0.001
LC n-3 PUFA[6]	1.04^{b}	1.36^{ab}	1.78^{a}	0.149	0.019
C18:3n-3 (ALA)	1.33^{b}	3.67^{a}	2.26^{ab}	0.41	0.002
C18:4n-3 (SDA)	0.05^{ab}	0.05^{b}	0.46^{a}	0.18	0.009
C20:3n-3	0.12	0.117	0.19	0.07	0.445
C20:4n-3 (ETA))	0.06	0.10	0.19	0.06	0.205
C22:5n-3(DPA)	0.23	0.45	0.69	0.13	0.066
C22:6n-3(DHA)	0.72	0.97	1.07	0.13	0.149
n-6:n-3 Ratio	12.97^{a}	5.53^{b}	6.08^{b}	0.551	<0.001

[1]Experimental diets: Control–basal diet + 4% corn oil; REGflax oil –basal diet + 4% standard flax oil; SDAflax oil –basal diet + 4% SDA-enriched flax oil.
[2]Saturated fatty acids (SFA) = C14:0 + C16:0 + C18:0 + C20:0 + C22:0.
[3]Monounsaturated fatty acids (MUFA) = C16:1n-7 + C16:1t + C18:1n-9 + C18:1n-9 + C20:1n-9.
[4]Total n-6 polyunsaturated fatty acids (PUFA) = 18:2n-6 (LA) + 18:3n-6 (GLA) + 20:2n-6 + 20:4n-6 (AA) + 22:2n-6 + 22:4n-6.
[5]Total n-3 polyunsaturated fatty acids (PUFA) = 18:3n-3 (ALA) + 18:4n-3 (SDA) + 20:4n-3 (ETA) + 20:5n-3 (EPA) + 22:5n-3 (DPA) + 22:6n-3 (DHA).

[6]Long chain n-3 polyunsaturated fatty acids (PUFA) = ETA + EPA + DPA + DHA.
[a-b]Means within a row without a common superscript differ (P<0.05). LSmeans based on 8 individually-housed layers per dietary treatment.

Table A-2: Fatty acid composition of thigh tissue (% of total fatty acids) collected from hens fed experimental diets[1] for 21 d (Chapter 3).

	Thigh				
Fatty acid	Control	REGflax	SDAflax	SEM	P-value
SFA[2]	27.96	25.69	26.53	1.48	0.589
C14:0	0.26	0.32	0.33	0.03	0.202
C16:0	19.06	18.31	18.94	0.59	0.642
C18:0	8.64	7.07	7.26	0.92	0.473
MUFA[3]	38.05	44.00	43.88	1.69	0.100
C16:1n7	1.54	2.57	2.27	0.34	0.203
C16:1n9	0.58	0.61	0.67	0.06	0.498
C18:1n7	2.19	2.28	2.43	0.07	0.108
C18:1n9	33.34	38.16	37.97	1.40	0.110
C20:1	0.31[b]	0.31[b]	0.48[a]	0.03	0.014
C22:1n7	0.09	0.07	0.06	0.01	0.153
PUFA	33.99[a]	30.30[b]	29.59[b]	0.51	0.006
n-6 PUFA[4]	32.68[a]	25.00[b]	25.38[b]	0.68	0.002
C18:2n-6 (LA)	29.50[a]	23.38[b]	22.82[b]	0.55	0.002
C18:3n-6 (GLA)	0.08[b]	0.08[b]	0.58[a]	0.06	0.003
C20:4n-6 (AA)	2.87[a]	1.45[a]	1.83[a]	0.49	0.220
C20:3 n-6	0.23[a]	0.08[a]	0.15[a]	0.03	0.074
n-3 PUFA[5]	1.30[b]	5.31[a]	4.21[a]	0.37	0.002
LC n-3 PUFA[6]	0.31[b]	0.42[ab]	0.56[a]	0.06	0.034
C18:3n-3 (ALA)	1.06[c]	4.89[a]	3.03[b]	0.28	0.002
C18:4n-3 (SDA)	ND	0.04[b]	0.62[a]	0.17	0.106
C20:3n-3	ND	0.05[a]	0.05[a]	0.01	0.986
C20:4n-3 (ETA))	0.04	0.04	0.07	0.01	0.045
C22:5n-3(DPA)	0.09[b]	0.11[b]	0.16[a]	0.01	0.018
C22:6n-3(DHA)	0.17	0.22	0.27	0.05	0.365
n-6:n-3 Ratio	25.78[a]	4.73[b]	6.10[b]	2.24	0.004

[a-c]Means within a row without a common superscript differ (P<0.05). LSmeans based on 8 individually-housed layers per dietary treatment.

[1]Experimental diets: Control–basal diet + 4% corn oil; REGflax oil –basal diet + 4% standard flax oil; SDAflax oil –basal diet + 4% SDA-enriched flax oil.

[2]Saturated fatty acid (SFA) = C14:0 + C16:0 + C18:0.

[3]Monounsaturated fatty acid (MUFA) = C16:1n-7 + C16:1t + C18:1n-9 + C20:1n-9 + C18:1n-9 + C22:1n-9 + C24:1n-9.

[4]Total n-6 polyunsaturated fatty acid (PUFA) = 18:2n-6 (LA) + 18:3n-6 (GLA) C22:1n-9.+ 20:4n-6 (AA) + 22:2n-6.

[5]Total n-3 polyunsaturated fatty acids (PUFA) = 18:3n-3 (ALA) + 18:4n-3 (SDA) + 20:4n-3 (ETA) + 20:5n-3 (EPA) + 22:5n-3 (DPA) + 22:6n-3 (DHA).

[6]Long chain n-3 polyunsaturated fatty acids (PUFA) = ETA + EPA + DPA + DHA.

Table A-3: Fatty acid composition of liver tissue (% of total fatty acids) collected from hens fed experimental diets[1] for 21 d (Chapter 3).

	Liver				
Fatty acid	Control	REGflax	SDAflax	SEM	P-value
SFA[2]	35.93[b]	34.57[b]	40.00[a]	0.98	0.008
C14:0	0.29	0.32	0.27	0.04	0.249
C16:0	23.39[ab]	21.59[b]	24.71[a]	0.59	0.017
C18:0	12.27[b]	12.66[b]	16.02[a]	0.92	0.006
MUFA[3]	41.61[ab]	46.70[a]	39.41[b]	2.29	0.015
C16:1n7	1.75	2.53	1.34	0.34	0.334
C16:1n9	0.83	0.88	0.82	0.04	0.682
C18:1n7	1.46	1.83	1.44	0.07	0.308
C18:1n9	36.93	40.72	33.99	2.24	0.197
C20:1	0.23	0.34	0.34	0.04	0.334
C22:1n7	0.42	0.44	0.48	0.02	0.457
PUFA	22.46	18.73	20.59	2.01	0.645
n6 PUFA[4]	21.32[a]	12.45[b]	14.33[b]	1.65	0.001
C18:2n-6 (LA)	17.34[a]	11.10[b]	10.32[b]	0.95	0.001
C18:3n-6 (GLA)	0.08	ND	0.69	0.06	0.433
C20:4n-6 (AA)	3.83[a]	1.31[b]	2.87[ab]	0.59	0.008
C22:4n-6	0.07	0.06	0.45	0.13	0.738
n-3 PUFA[5]	1.15[b]	6.68[a]	6.39[a]	0.84	0.007
LC n-3 PUFA[6]	0.67[c]	2.29[b]	3.54[a]	0.23	0.014
C18:3n-3 (ALA)	0.39[c]	4.46[a]	2.50[b]	0.58	0.002
C18:4n-3 (SDA)	ND	ND	0.35	0.17	
C20:3n-3 (ETA)	ND	0.05	0.32	0.02	0.667
C20:5n-3 (EPA))	0.06[b]	0.31[a]	0.25[a]	0.09	0.044
C22:5n-3(DPA)	0.19	0.54	0.62	0.18	0.076
C22:6n-3(DHA)	0.42[b]	1.39[ab]	2.35[a]	0.55	<0.001
n-6:n-3 Ratio	18.36[a]	1.86[b]	2.24[b]	1.04	0.001

[a-b]Means within a row without a common superscript differ (P<0.05). LSmeans based on 8 individually-housed layers per dietary treatment

[1]Experimental diets: Control–basal diet + 4% corn oil; REGflax oil –basal diet + 4% standard flax oil; SDAflax oil –basal diet + 4% SDA-enriched flax oil.

[2]Saturated fatty acids (SFA) = C14:0 + C16:0 + C18:0.

[3]Monounsaturated fatty acids (MUFA) = C16:1n-7 + C16:1n-9 + C18:1n-9 + C18:1n-9 + C20:1n-9 + C24:1n-9.

[4]Total n-6 polyunsaturated fatty acids (PUFA) = 18:2n-6 (LA) + 18:3n-6 (GLA) + 20:2n-6 + 20:4n-6 (AA) + 22:4n-6.

[5]Total n-3 polyunsaturated fatty acids (PUFA) = 18:3n-3 (ALA) + 18:4n-3 (SDA) + 20:4n-3 (ETA) + 20:5n-3 (EPA) + 22:5n-3 (DPA) + 22:6n-3 (DHA).

[6]Long chain n-3 polyunsaturated fatty acids (PUFA) = ETA + EPA + DPA + DHA.

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Table A-4: Fatty acid composition of brain tissue (% of total fatty acids) collected from hens fed experimental diets[1] for 21 d (Chapter 3).

	Brain				
Fatty acid	Control	REGflax	SDAflax	SEM	P-value
SFA[2]	46.24	46.03	48.13	1.62	0.617
C14:0	0.79	0.86	0.77	0.03	0.200
C16:0	24.62	25.52	25.94	0.66	0.398
C18:0	20.83	19.64	21.42	0.99	0.469
MUFA[3]	30.35	30.86	27.75	2.51	0.657
C16:1n7	0.55	0.61	0.55	0.03	0.338
C16:1n9	3.46	3.57	3.39	0.30	0.908
C18:1n7	6.31	6.25	5.92	0.40	0.762
C18:1n9	18.32	18.81	16.57	1.39	0.543
C20:1	0.47	0.29	0.27	0.12	0.431
C24:1n7	1.24	1.33	1.05	0.24	0.703
PUFA	23.88	23.40	24.39	0.88	0.739
n-6 PUFA[4]	12.26[a]	10.61[b]	10.66[b]	0.12	0.010
C18:2n-6 (LA)	0.57	0.40	0.34	0.06	0.085
C20:3n-6	0.29	0.30	0.32	0.05	0.098
C20:4n-6 (AA)	8.92	7.73	7.84	0.39	0.527
C22:4n-6	2.48	2.17	2.16	0.16	0.306
C22:5n-6	0.96[a]	0.39[b]	0.37[b]	0.12	0.009
n-3 PUFA[5]	11.92	13.09	14.04	0.61	0.098
C18:3n-3 (ALA)	0.19[b]	0.25[a]	0.24[ab]	0.01	0.032
LC n-3 PUFA[6]	11.81[b]	12.62[ab]	13.67[a]	0.05	0.012
C20:3n-3 (ETA)	0.13	0.16	0.14	0.02	0.541
C20:5n-3 (EPA)	0.19	0.21	0.17	0.04	0.717
C22:5n-3 (DPA)	0.35	0.29	0.39	0.06	0.385
C22:6n-3 (DHA))	11.13	11.96	12.97	0.77	0.284
n-6:n-3 Ratio	1.04[a]	0.81[b]	0.76[b]	0.04	0.001

[a-b]Means within a row without a common superscript differ (P<0.05). LSmeans based on 8 individually-housed layers per dietary treatment.

[1]Experimental diets: Control–basal diet + 4% corn oil; REGflax oil –basal diet + 4% standard flax oil; SDAflax oil –basal diet + 4% SDA-enriched flax oil.

[2]Saturated fatty acids (SFA) = C14:0 + C16:0 + C18:0.

[3]Monounsaturated fatty acids (MUFA) = C16:1n-7 + C16:1n-9 + C18:1n-7 + C18:1n-9 + C20:1n-9 + C24:1n-9.

[4]Total n-6 polyunsaturated fatty acids (PUFA) = 18:2n-6 (LA) + 18:3n-6 (GLA) + 20:2n-6 + 20:4n-6 (AA) + 22:4n-6 +22:5n-6.

[5]Total n-3 polyunsaturated fatty acids (PUFA) = 18:3n-3 (ALA) + 18:4n-3 (SDA) + 20:4n-3 (ETA) + 20:5n-3 (EPA) + 22:5n-3 (DPA) + 22:6n-3 (DHA).

[6]Long chain n-3 polyunsaturated fatty acids (PUFA) = ETA + EPA + DPA + DHA.

Table A-5: Fatty acid composition of heart (% of total fatty acids) collected from hens fed experimental diets[1] for 21 d (Chapter 3).

	Heart				
Fatty acid	Control	REGflax	SDAflax	SEM	P-value
SFA[2]	30.15	30.45	30.69	1.40	0.962
C14:0	0.27	0.25	0.29	0.02	0.216
C16:0	18.52[a]	16.71[b]	18.99[a]	0.37	0.006
C18:0	11.48	13.73	13.27	0.86	0.180
MUFA[3]	33.07	31.20	33.4	2.00	0.705
C16:1n7	1.74	1.39	1.72	0.14	0.187
C16:1n9	0.23	0.35	0.25	0.08	0.514
C18:1n9	30.61	29.00	31.06	1.85	0.714
C20:1n9	0.42	0.38	0.35	0.03	0.189
PUFA	36.78	38.35	35.90	0.81	0.118
n-6 PUFA[4]	35.17[a]	32.10[ab]	30.48[b]	1.03	0.013
C18:2n-6 (LA)	28.96	23.32	23.34	1.92	0.078
C18:3n-6 (GLA)	0.15[b]	0.14[b]	0.81[b]	0.07	<0.001
C20:4n-6 (AA)	5.44	5.54	6.79	0.62	0.275
C20:2 n-6	0.33[a]	0.21[b]	0.17[b]	0.02	<0.001
C22:4n-6	0.34[a]	0.17[b]	0.20[b]	0.03	0.001
C22:5n-6	0.17[b]	0.24[b]	0.99[a]	0.08	<.0001
n-3 PUFA[5]	1.61[b]	6.25[a]	5.42[a]	0.32	<.0001
C18:3n-3 (ALA)	1.12[c]	4.78[a]	3.17[b]	0.26	<.0001
C18:4n-3 (SDA)	ND	ND	0.92	0.12	
LC n-3 PUFA[6]	0.42[b]	1.29[a]	1.33[a]	0.20	<0.001
C20:3n-3 (ETA)	0.24	0.44	0.28	0.10	0.342
C20:5n-3 (EPA)	ND	0.39	0.39	0.05	0.964
C22:5n-3 (DPA)	0.17[b]	0.16[b]	0.30[a]	0.04	0.040
C22:6n-3 (DHA))	0.17[b]	0.35[a]	0.40[a]	0.04	0.001
n-6:n-3 Ratio	22.21[a]	5.45[b]	5.77[b]	0.84	<0.001

[a-b]Means within a row without a common superscript differ (P<0.05). LSmeans based on 8 individually-housed layers per dietary treatment.

[1]Experimental diets: Control–basal diet + 4% corn oil; REGflax oil –basal diet + 4% standard flax oil; SDAflax oil –basal diet + 4% SDA-enriched flax oil.

[2]Saturated fatty acids (SFA) = C14:0 + C16:0 + C18:0.

[3]Monounsaturated fatty acids (MUFA) = C16:1n-7 + C16:1n-9 + C18:1n-9 + C20:1n-9.

[4]Total n-6 polyunsaturated fatty acids (PUFA) = 18:2n-6 (LA) + 18:3n-6 (GLA) + 20:2n-6 + 20:4n-6 (AA) + 22:2n-6+ 22:4n-6.

[5]Total n-3 polyunsaturated fatty acids (PUFA) = 18:3n-3 (ALA) + 18:4n-3 (SDA) + 20:4n-3 (ETA) + 20:5n-3 (EPA) + 22:5n-3 (DPA) + 22:6n-3 (DHA).

[6]Long chain n-3 polyunsaturated fatty acids (PUFA) = ETA + EPA + DPA + DHA.

Table A-6: Fatty acid composition of abdominal fatpad (% of total fatty acids) collected from hens fed experimental diets[1] for 21 d (Chapter 3).

	Abdominal fatpad				
Fatty acid	Control	REGflax	SDAflax	SEM	P-value
SFA[2]	18.84[ab]	16.85[b]	20.34[a]	0.70	0.007
C14:0	0.30[ab]	0.28[b]	0.32[a]	0.01	0.041
C16:0	16.32[a]	14.90[b]	16.55[a]	0.31	0.002
C18:0	3.41[ab]	3.18[b]	3.87[a]	0.17	0.029
C20:0	0.09	0.08	0.08	0.01	0.810
MUFA[3]	48.94	50.09	47.99	0.81	0.211
C16:1n7	3.25	3.23	2.92	0.31	0.701
C16:1n9	0.34	0.27	0.26	0.05	0.448
C18:1n9	45.04	46.24	44.49	0.78	0.288
C20:1n9	0.32	0.35	0.32	0.02	0.514
PUFA	32.22	33.06	31.67	0.69	0.380
n-6 PUFA[4]	28.89[a]	25.79[b]	24.56[b]	0.81	<0.001
C18:2n-6 (LA)	28.48[a]	25.36[b]	23.17[c]	0.58	<0.001
C18:3n-6 (GLA)	0.20[b]	0.09[b]	1.14[a]	0.09	0.029
C20:4n-6 (AA)	0.19	0.22	0.18	0.05	<0.001
C20:2n-6	0.20	0.16	0.23	0.05	0.856
C22:4n-6	0.10	0.10	0.08	0.03	0.854
C22:5n-6	0.07	0.08	0.07	0.01	0.456
n-3 PUFA[5]	3.13[b]	7.13[a]	6.97[a]	0.75	0.001
C18:3n-3 (ALA)	2.83[b]	6.89[a]	5.04[ab]	0.72	0.003
C18:4n-3 (SDA)	ND	ND	1.68	0.13	
LC n-3 PUFA[6]	0.29	0.27	0.24	0.06	0.828
C20:5n-3 (EPA)	0.17	0.16	0.14	0.03	0.736
C22:5n-3 (DPA)	0.14	0.07	0.13	0.03	0.266
C22:6n-3 (DHA)	0.12	0.06	0.10	0.02	0.456
n-6:n-3 Ratio	12.26[a]	4.845[b]	3.59[b]	1.31	<0.001

[a-b]Means within a row without a common superscript differ (P<0.05). LSmeans based on 8 individually-housed layers per dietary treatment.

[1]Experimental diets: Control–basal diet + 4% corn oil; REGflax oil –basal diet + 4% standard flax oil; SDAflax oil –basal diet + 4% SDA-enriched flax oil.

[2]Saturated fatty acids (SFA) = C14:0 + C16:0 + C18:0.

[3]Monounsaturated fatty acids (MUFA) = C16:1n-7 + C16:1n-9 + C18:1n-9 + C18:1n-9 + C20:1n-9 + C24:1n-9.

[4]Total n-6 polyunsaturated fatty acids (PUFA) = 18:2n-6 (LA) + 18:3n-6 (GLA) + 20:2n-6 + 20:4n-6 (AA) + 22:4n-6.

[5]Total n-3 polyunsaturated fatty acids (PUFA) = 18:3n-3 (ALA) + 18:4n-3 (SDA) + 20:4n-3 (ETA) + 20:5n-3 (EPA) + 22:5n-3 (DPA) + 22:6n-3 (DHA).

[6]Long chain n-3 polyunsaturated fatty acids (PUFA) = ETA + EPA + DPA + DHA.

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