THE PENNSYLVANIA STATE UNIVERSITY
SCHREYER HONORS COLLEGE

DEPARTMENT OF VETERINARY AND BIOMEDICAL SCIENCE

EFFECTS OF DIETARY LINOLEIC ACID ON CONVERSION OF OMEGA-3 FATTY ACIDS TO LONG CHAIN FATTY ACIDS

ELIZABETH ANN FLAHERTY
FALL 2018

A thesis
submitted in partial fulfillment
of the requirements
for a baccalaureate degree
in Veterinary and Biomedical Science
with honors in Veterinary and Biomedical Science

Reviewed and approved* by the following:

Dr. Kevin Harvatine
Associate Professor of Nutritional Physiology
Thesis Supervisor

Dr. Robert VanSaun
Professor of Veterinary Science
Honors Adviser

* Signatures are on file in the Schreyer Honors College.

ABSTRACT

With the modern diet that is high in total fats, high in omega-6 fatty acids (FA), and low in omega-3 FA, there is a high prevalence of metabolic and cardiovascular disease. Certain dietary acids are beneficial, while others may contribute to these disease processes. Eggs are an important part of the human diet as they are protein and nutrient dense and are a good source of vitamins and minerals. It is possible to manipulate the nutrients and composition of FA in the eggs by modifying the balance of the hens diets. This makes them a good target for experiments with fatty acids (FA). A diet high in omega-3 (n-3) FA has many beneficial effects including plasma lipid reduction, reduction in some types of cancer mortality, anti-inflammatory effects, antiarrhythmic effects, antithrombotic effects, antiatheromatous effects, and less severe manifestations of autoimmune diseases and inflammatory diseases. Due to the benefits of n-3 FA, it is important to understand the effects of oleic acid and linoleic acid (LA; C18:2n-6) on the deposition of n-3 FA in egg yolks. The goals of the experiment are to determine the effect of these FA on deposition of ALA and VLC n-3 FA in yolks. It is hypothesized that LA will have a negative impact on the deposition of ALA in yolks as they compete for elongase and desaturase enzymes. Additionally, it is expected that oleic acid will also have a negative effect on n-3 deposition in yolks.

Fifty hens were randomly assigned to one of five dietary treatments that included a control, two levels of high oleic safflower oil (HOSO; 2% and 4%), and two levels of high linoleic safflower oil (HLSO; 2% or 4%). All diets contained 4% flaxseed oil (FLAX) to provide α-linolenic acid (ALA; C18:3n-3) as a substrate for synthesis of the very long chain omega-3(VLC n-3) FA. Egg samples were collected on day 0 and day 28 of the experiment and were analyzed for yolk weights and FA composition. Samples of adipose and liver tissue were collected on day 28 for FA analysis.

Increasing dietary oleic acid (18:1n-9), provided by HOSO, had a positive effect on deposition of oleic acid in the yolk. Increased dietary linoleic acid (LA; C18:3n-6), provided by HLSO, had a negative effect on oleic acid deposition. HOSO and HLSO also negatively affected the deposition of ALA and

VLC n-3 FA in yolks. This supports previous experiments that showed dietary n-6 FA had a negative effect on deposition of n-3 FA in yolks as a result of competition for the elongase and desaturase enzymes. The diet that is most conducive to developing a high n-3 fatty acid egg is a diet with a high level of ALA and a low level of oleic and linoleic acid.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

List of Abbreviations

FA: Fatty Acids

LC: Long Chain (fatty acids)

VLC FA: very long chain fatty acids

PUFA: Polyunsaturated Fatty Acid

n-3 FA: Omega-3 fatty acids

n-6 FA: Omega-6 fatty acids

ALA: α-linolenic acid

EPA: eicosapentaenoic acid

DHA: docosahexaenoic acid

DPA: docosapentaenoic acid

LA: Linoleic Acid

AA: arachidonic acid

GLA: γ-linolenic acid

HOSO: high oleic safflower oil

HLSO: high linoleic safflower oil

FLAX: high n-3 flaxseed oil

ACKNOWLEDGEMENTS

I would like to thank everyone who has helped me in the completion of my thesis and through my time studying at Penn State. Thank you to Dr. VanSaun who has helped me through many semesters of scheduling, choosing courses, and staying on track for application to Vet School.

I would also like to thank Dr. Harvatine for allowing me to join his research group. I appreciate how helpful he was with providing advice for my thesis and for writing a letter of recommendation for Vet School. I would also like to thank Elaine Barnoff, Beckie Bomberger, Cesar Matamoros, Noah Shulman, Isaac Salfer, and Richie Shepardson, for helping me settle into the lab and helping familiarize me with the procedures.

I would also like to thank my parents and younger sister Katy for encouraging me to follow my dreams and apply to Vet School and for supporting me through the whole process.

Chapter 1

INTRODUCTION

Omega-3 fatty acids (n-3 FA) are a type of unsaturated FA that has a double bond located 3 carbons from the methyl terminal carbon. They are beneficial to human health as they reduce plasma triglyceride levels, reduce mortality rates in some types of cancers, have anti-inflammatory, antiarrhythmic, antithrombotic, and antiatheromatous effects, and can help patients with autoimmune and inflammatory diseases. These beneficial n-3 FA are obtained from the diet; ALA is commonly found in leafy green vegetables and fish, seafood, and fish oils contain high levels of eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3). Other dietary sources of n-3 FA include chicken, eggs, flaxseed, wheat grass, and canola, soybean, hemp seed, and flaxseed oils. Although fish oil is a reliable source of the VLC PUFA EPA and DHA, fish oil has major issues including that it is not sustainable and has a significant impact on aquatic ecosystems and fish populations, many people do not routinely eat fish or take fish oil supplements, and fish oil is easily oxidized. This has led to increased interest in finding alternative food sources high in n-3 FA in order to reduce environmental impact and increase consumption of VLC n-3 FA. Another source that has been thoroughly researched are eggs. Eggs are an important part of the human diet as they are protein and nutrient dense and are a good source of vitamins and minerals including vitamins A, B_{12}, E, D, and K, folate, choline, and carotenoids. They are also nutrient dense and are important especially in the diet of low-income families and the elderly because they have high quality protein and are affordable.

The goals of the experiment are to observe the effects of the added dietary oils high in C18:1 (high oleic safflower oil; HOSO) and C18:2 (high linoleic safflower oil; HLSO) on synthesis of VLC n-3 FA and FA deposition in egg yolks. Following a previous observation, it is expected that oleic acid, fed through HOSO, will reduce the deposition of ALA and derived VLC n-3 FA in egg yolks. The degree to

which LA reduces yolk deposition of ALA was also observed in order to research competition for elongase and desaturase enzymes. The eventual goal of future experiments is to increase the deposition of ALA in yolks and to enhance its conversion to VLC PUFA in order to develop a higher VLC n-3 egg. This will be beneficial to human health as it is a more sustainable source of n-3 FA and its addition to the human diet is expected to help reduce the dietary ratio of n-6:n-3 and make this beneficial dietary adjustment accessible to a wider range of consumers.

Chapter 2

LITERATURE REVIEW

Chemical Properties of Fatty Acids

Fatty acids (FA) are molecules composed of a carbon chain of between 4 and 36 carbons that have a carboxyl group (-COOH) at one end and a methyl group (-CH_3) at the other end (Oliviera et al. 2010). They are the building block for triglycerides, phospholipids, and cholesterol esters and are a precursor to several signaling molecules including the eicosanoids. Fatty acids in the form of triglycerides provide 25 to 35% of the energy for the average western diet (Kremmyda et al., 2011). These triglycerides or triacylglycerols are composed of three FA bound at the carboxyl ends through a dehydration reaction to a glycerol backbone. The individual FA can be linked to cholesterol, glycerol, and sphingosine via an esterification reaction. This leads to a variety of lipid-based biomolecules including: tri-, di- and mono-acylglycerols, cholesteryl esters, ether lipids, glycerophospholipids, ceramides, wax esters, and sphingophospholipids. Phospholipids are composed of a hydrophilic head group and one or two FA esterified to a glycerol molecule, and these molecules arrange to form bilayer cell membranes. The FA composition of phospholipids, including the degree of FA saturation and double bond conformation in the *cis*- or *trans*- orientation, affects the stability or fluidity of the membrane (Kremmyda et al., 2011). In addition, lipids circulate in the blood packaged in lipoproteins, which are groupings of lipids and proteins (Tvrzicka et al., 2011). These lipoproteins and fats also help in the transfer and storage of the fat-soluble vitamins A, D E and K (Kremmyda et al., 2011).

Fatty acids are classified by a series of biochemical characteristics including: chain length, degree of unsaturation, and location and orientation of double bonds.

Although there is no official categorization, short chain saturated FA generally consist of acetic acid (2:0), butyric acid (3:0), and propionic acid (4:0). Due to their short length, they are easily absorbed in the body. Propionic and acetic acid are transported to the liver where they are converted to glucose and FA, respectively. Medium chain saturated FA include caproic acid (6:0), caprylic acid (8:0), capric acid (10:0) and lauric acid (12:0). Long chain (LC) saturated FA include myristic acid (14:0), palmitic acid (16:0), and stearic acid (18:0). Very long chain saturated FA (VLC FA) include arachidonic acid (20:0), behenic acid (22:0), lignoceric acid (24:0), cerotic acid (26:0), montanic acid (28:0), and melissic acid (30:0). The presence of large amounts of VLC saturated FA can be linked to some metabolic diseases including Zellweger syndrome, Menke's disease, and X-linked adrenoleucodystrophy (Tvrzicka et al., 2011).

Another FA category separate from saturated FA are unsaturated FA, which have one or more double bonds. They can be divided into several groups based on their number of double bonds which separate monounsaturated FA and polyunsaturated FA (PUFA). The unsaturated FA can be further divided based on the location of the terminal double bond and these categories include omega-3 FA (n-3 FA), omega-6 FA (n-6 FA), and omega-9 FA (Tvrzicka et al., 2011).

Unsaturated FA can be separated based on the orientation of the groups around the double bond into *cis*- and *trans*- conformations. The orientation of the double bond in unsaturated FA is categorized into two conformations: *cis*-and *trans*-. *Cis*- refers to the conformation where the two groups of the same type are on the same side of the molecule, in this case, the hydrogen atoms. The common *cis*-monounsaturated FAs include oleic acid (18:1 n-9*c*), *cis*-vaccenic acid (18:1 n-7*c*), and palmitoleic acid (16:1 n-7*c*). Oleic acid has antithrombotic and antiatherogenic properties and can be found in olive oil, canola oil, rapeseed oil, peanut oil, almond oil and avocado oil. Another conformation found in FA is *trans*- where the same groups, or hydrogen atoms are on the opposite sides of the double bond. *Trans*- FA include elaidic acid (18:1 n-9*t*) and *trans*-vaccenic acid (18:1 n-7*t*). The industrially produced *trans*- FA tend to increase low density lipoprotein (LDL) cholesterol and decrease high density lipoprotein (HDL)

cholesterol in humans. They previously were commonly used in creating shortening and in the processing of fast food[3], although this trend has recently fallen out of favor as more research was done on the negative health effects of high dietary levels of synthesized *trans*- FA (Tvrzicka et al., 2011).

Polyunsaturated fatty acids have more than one double bond and are commonly categorized based on their omega designation. Omega-3 FA have a double bond located 3 carbons from the methyl terminal carbon and include α-linolenic acid (ALA; 18:3 n-3) and its elongation and desaturation products. It is mostly converted to eicosapentaenoic acid (EPA; 20:5n-3), docosapentaenoic acid (DPA; 22:5n-3), and docosahexaenoic acid (DHA; 22:6n-3). This conversion is not very efficient in humans and production animals. These FA are important in the effective function and regulation of the cardiovascular system, nervous system, and in optic tissues (Oliviera et al., 2010). Conversely, n-6 FA have a carbon-carbon double bond in the sixth position from the terminal end and are mainly derived from linoleic acid (LA; 18:3 n-6). The main products are γ-linolenic acid (GLA; 18:3n-6), dihomo-γ-linolenic acid (20:3n-6), arachidonic acid (AA; 20:4n-6), and docosapentaenoic acid (DPA; 22:5n-6). The n-3 and n-6 FA affect the production of eicosanoids, as n-6 derived eicosanoids can lead to activation of inflammation (Tvrzicka et al., 2011). Eicosanoids include prostaglandins, leukotrienes, thromboxanes, prostacyclins, and hydroxyacids and play a part in the control and function of the paracrine system (Oliviera et al., 2010).

The LC PUFA are either incorporated into the phospholipid membrane or processed in the body through β-oxidation to provide a source of energy for cells. After phospholipase A_2 breaks down the phospholipids, two enzyme complexes, lipooxygenase and cyclooxygenase, alter the free FA. This leads to production of components that have either inflammatory properties if modified from an n-6 FA or anti-inflammatory properties if derived from n-3 FA. Both FA classes use the same enzymes, so there is enzymatic competition for these enzymes as well as Δ-desaturase enzymes (Lenihan-Geels et al., 2013; Yannakoupoulos, 2007).

Based on biochemical pathways in humans, there are certain FA that are essential, or must be provided in the diet. There are FA that can be synthesized in humans using desaturase enzymes up to the

Δ9 position, but not in the n-3 or n-6 position. Humans do not have the enzymes in the categories Δ12 and Δ15 desaturases, so they must obtain these from the diet, including LA (18:2n-6) and ALA (18:3n-3). In addition, humans are not efficient at using elongation and desaturation to form VLC PUFAs including EPA (20:5n-3), DHA (22:6n-3), and AA (20:4n-6) from ALA found in the diet. As a result, enrichment of EPA and DHA is difficult from dietary ALA (Tvrzicka et al., 2011). Some consider EPA and DHA as conditionally essential FA as although they can be synthesized from ALA, but optimal quantities for healthful may not be naturally produced, and thus must be provided in the diet.

Modern Diet Trends

As a species, diet composition of humans has changed drastically in regards to the amount and ratio of n-6 to n-3 FA. The diet has changed from being lower in total fats, lower in saturated fats, and about equal in n-3 FA and n-6 FA to a diet that is higher in total fat, higher in saturated fat, and higher in n-6 FA and lower in n-3 FA. This comes as a result of changing food sources in the human diet, as well as changing the feedstuffs for livestock that transfer to the diet of humans via the food chain (Simopoulos, 2002). In the modern diet, sources for essential FA include: LA in most seeds except coconut, cacao, and palm, ALA in leafy green vegetables, and EPA and DHA in fish, seafood, and fish and algae oil supplements (Simopoulos, 1991; Lewis et al., 2000). Other dietary sources of n-3 FA include chicken, eggs, canola oil, soybean oil, wheat grass, hemp seed oil, flaxseed, flaxseed oils (Lewis et al., 2000).

Humans originally lived as hunter-gatherers and the majority of their dietary fats (2.3g/d) were LC n-3 and n-6 FA. They also had a lower n-6:n-3 ratio, which was close to 2.4. Originally, the Paleolithic diet included green leafy vegetables, nuts, berries, fish, lean meat, and fruits. Cereal grains were introduced during the Agricultural Revolution when humans changed from hunter-gatherers to an agriculturally based society. The diet changed to include corn, wheat, and rice, which are higher in n-6 FA and carbohydrates and low in n-3 FA. This trend was continued in the modern diet in the USA, where

a lower portion of dietary fats (0.2 g/d) were LC n-3 FA. The ratio of n-6:n-3 is currently close to 12, which is over 6 times the ratio in the hunter-gatherer lifestyle. The modern diet includes cereal grains introduced during the Agricultural Revolution, and is more calorically dense. Soybeans and soybean oil products over the past 75 years. The modern diet is higher in saturated fat, n-6 FA, and *trans*- FA and lower in n-3 FA. Until 15 years ago, it was common to partially hydrogenate plant oils to reduce oxidation during storage, distribution, and frying (Simopoulos, 2002). Partial hydrogenation results in formation of *trans*-FA, which can cause issues because *trans*- FA interact with the reactions of n-6 and n-3 FA which reduces the amount of available AA (20:4n-6), EPA (20:5n-3), and DHA (22:6n-3) (Simopoulos, 2002). In addition, in the modern era, vegetable oils have been used more frequently and the amount of hydrogenation has decreased. These hydrogenated oils also increased the consumption of *trans*- FA for many years (Simopoulos, 1991). In recent years, however, consumption of *trans*- FA has decreased as the oils are fully hydrogenated.

The feed sources for livestock also have an impact on the FA in the human diet. Modern livestock are fed a high grain diet that is high in n-6 FA and low in n-3 FA, and this leads to lower n-3 deposition in fats. This change in n-3 reduction is also consistent in farm-raised fish. Eggs from free-range chickens in one study were shown to have an n-6: n-3 ratio of 1.3 and eggs from a USDA survey had a ratio closer to 19.9 (Simopoulos, 2002).

The LC PUFA AA, EPA, and DHA which are essential in the human diet, are precursors to molecules, eicosanoids, which consist of leukotrienes, prostaglandins, and thromboxanes. DHA and EPA are precursors to 5 series leukotrienes and 3 series prostanoids, while AA is a precursor to prostaglandins, thromboxanes, and 4 series leukotrienes. EPA and AA compete for cyclooxygenase and lipoxygenase, the enzymes that synthesize leukotrienes and prostaglandin. This means a diet high in the n-3 FA inhibits use of these pathways by AA. This leads to less thromboxane A_2, which is a strong vasoconstrictor and leads to platelet aggregation, less production of prostaglandin E_2, less production of leukotriene B_2, which induces chemotaxis of leukocytes, and increased production of thromboxane A_3, which is a weaker

vasoconstrictor and weaker platelet aggregator (Simopoulos, 1991). This dietary change leads to beneficial effects including lipid reduction, reduction in some types of cancer mortality, and anti-inflammatory, antiarrhythmic, antithrombotic, and antiatheromatous effects (Simopoulos, 1991; Lewis et al., 2000). A diet higher in n-3 FA has also helped patients with autoimmune diseases and inflammatory diseases including: arthritis, rheumatoid arthritis, psoriasis, lupus erythematosus, asthma, and ulcerative colitis (Simopoulos, 1991).

Importance of Eggs

Eggs are an important part of the human diet as they are protein and nutrient dense and are a good source of vitamins and minerals. Egg yolks contain about 6g of fats including mainly phospholipids, triglycerides, and cholesterol, and to a lesser extent free FA and cholesterol esters (Yannakoupoulos, 2007). They are a good source (10 to 19% daily value) of vitamin B_{12}, vitamin D, vitamin A, folate, and phosphorus and they are an excellent source (20% daily value) of riboflavin, protein, vitamin K, and selenium (Applegate, 2000). They are also considered one of the best dietary sources of vitamin D and K (Applegate, 2000). In adults who consume eggs, it was found that eggs contribute 10 to 20% daily folate and 20 to 30% vitamins A, B_{12}, and E (Applegate, 2000). Adults who did not eat eggs were more likely to have lower levels of these vitamins (Applegate, 2000). Eggs also contribute large amounts of carotenoids, including lutein and zeaxanthin, which can help in the prevention of disease. They are also a good source of choline, a nutrient that is important to memory development and brain development in utero and in young children (Applegate, 2000; McNamara, 2015). A deficiency in choline during pregnancy can lead to an increased risk of spina bifida and neural tube development disorders. In addition, most adults are deficient in choline. Increasing choline intake by one egg per day can decrease plasma levels of inflammatory mediators and reduces breast cancer incidence (McNamara, 2015).

Due to their nutritional value, eggs have many health and social benefits. Because of their nutrient density and low cost, they are important especially in the diet of low-income families (Applegate, 2000). Eggs, and their proteins, help increase satiety and can lead to less snacking and better weight management and loss. They are also a helpful source of food to the elderly because they have high quality protein, are affordable, and are easier to prepare and chew. By using eggs as an affordable source of protein, this can also help the elderly maintain lean muscle and reduce progression of sarcopenia (McNamara, 2015). A diet high in carotenoids from eggs (lutein and zeaxanthin) helps reduce the risk of developing macular degeneration, cataracts, some cancers, and atherosclerosis. In addition, lutein can also help increase the optical density of macular pigment (McNamara, 2015).

The public perception of eggs as a "healthy" food or "unhealthy" food has changed over the years. In 1968, the American Heart Association (AHA) suggested that people eat three or less eggs per day to limit their cholesterol intake (McNamara, 2015). The main basis for placing cholesterol limits (300 mg) were animal studies, epidemiological studies, and clinical studies that found connections between high dietary cholesterol, high serum cholesterol and high dietary cholesterol (McNamara, 2015). There was not a clear scientific reasoning as to why the limit for cholesterol of 300 mg/day was chosen in 1968, but it was supposedly an estimate of half of the average daily cholesterol intake (McNamara, 2015). Although eggs were condemned as an "unhealthy" food as they are a source of dietary cholesterol, it was recently found that eggs have little to no effect on blood cholesterol levels (Applegate, 2000). The diets that showed a correlation between dietary cholesterol and heart disease were also high in fat, saturated fat, and low in whole grains, a composition which can also affect blood cholesterol levels. This suggests that the correlation was confounded by other factors. Kritchevsky and Kritchevsky also suggested that there was no direct relationship between dietary cholesterol and total serum cholesterol or development of cardiovascular disease (Kritchevsky and Kritchevsky, 2000; Applegate, 2000). Past experiments that originally found a supposed link failed to take into consideration multiple confounding factors such as age, smoking, blood pressure, dietary intake of fiber and fat, and high levels of daily variation of

cholesterol in one person (Kritchevsky and Kritchevsky, 2000; McNamara, 2000). Other experiments had weaknesses in their arguments that eggs had a significant effect on developing cardiovascular disease. One issue is many species have differences in their deposition of cholesterol and may have high variation in daily levels; for example, humans have mostly LDL cholesterol but most animal models have HDL (McNamara, 2000). This means the results in one species on lipid deposition and conversion may not be as accurate with respect to the same response in humans.

Synthesis of Egg Yolks

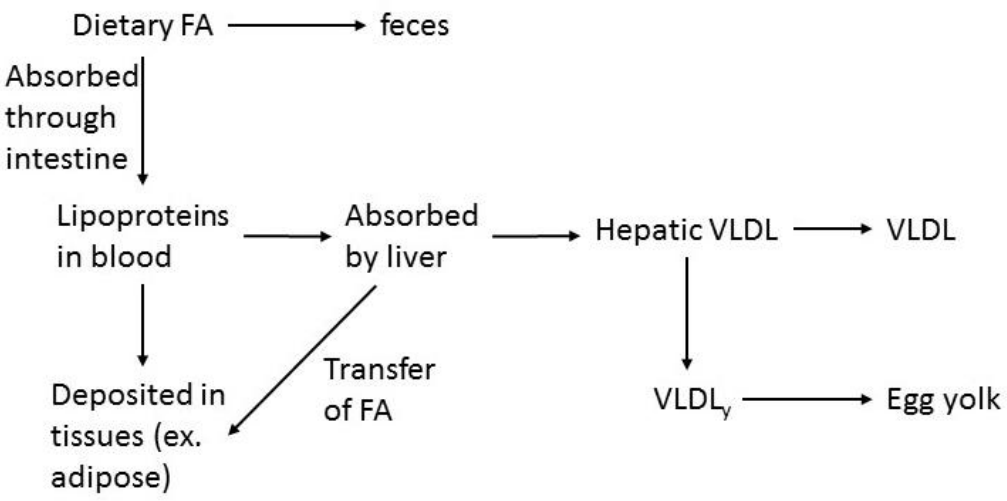
Egg development occurs in three stages. In the first stage the oocytes increase from 60 μm to 2 mm in diameter. During the second stage the oocytes undergo slow growth. In the third step, which occurs when the yolk is 6 to 7 mm, some yolks enter a hierarchical rapid growth phase where the yolk increases in size to about 35 mm and are destined for ovulation and the others enter atresia. The oocytes destined for ovulation are preovulatory follicles and are arranged in a hierarchy designated F1 to F5, F1 being closest to ovulation. F1 then undergoes ovulation and the albumen, shell membrane and shell are deposited over the next 25 hours. The ovulation of F1 triggers the rapid growth phase of the next oocyte (Bujo et al., 1997).

This continuous and sequential egg production affects the conversion and transport of FA in chickens. Thirty percent of the yolk is lipid based as $VLDL_y$, and this high level of production means that the chicken must be able to transport large amounts of FA in the form of lipoproteins and also maintain homeostasis[13]. Due to their partially hydrophobic internal structure, and hydrophilic external structure, lipoproteins are important in the transport of water-insoluble nutrients, cholesterol, and nonessential FA (Elkin, 1997).

Ingested lipids are partially hydrolyzed and absorbed by intestinal mucosal cells. They are re-esterified in the ER and reassembled into particles with cholesterol, phospholipids, and proteins or

lipoproteins (Yannakoupoulos, 2007). These particles enter the portal system as portomicrons (Yannakoupoulos, 2007; Hermier, 1997). The liver then synthesizes the lipoproteins HDL and VLDL, which are secreted. VLDL are synthesized in the lumen of the smooth ER of the liver and secreted as lipoproteins (See Figure 1) (Oliviera et al., 2010; Tvrzicka et al., 2011). Estrogen is one of the main regulators that stimulates VLDL production mechanisms to switch to produce $VLDL_y$ (Walzem et al., 1999). $VLDL_y$ is a small particle that contains high amounts of TAGs and it is optimally between 25 and 50 nm in diameter (Walzem et al., 1999). $VLDL_y$ enters the oocytes by receptor-mediated endocytosis (Bujo et al., 1997). The receptor responsible for this transfer is oocyte vitellogenesis receptor, a 95-kDa protein, which binds both $VLDL_y$ to provide energy for the embryo if the egg is fertilized. This receptor also helps components other than lipoproteins enter the yolk, including riboflavin, and a protein α_2-M. In addition, it was found that the kidney also secretes VLDL, possibly to make up for the high level of $VLDL_y$ in the liver (Walzem et al., 1999). In addition to deposition of $VLDL_y$ in yolks, there is also lipid deposition of VLDL in adipose and liver of laying hens (Elkin, 1997; Hermier, 1997). There is only a limited amount of de novo lipid synthesis that occurs outside the liver, so most of the accumulated fat is from the diet or synthesized in the liver and transported to the tissues. With the use of lipoprotein lipase, the triglycerides in the VLDL are hydrolyzed and are either stored in the adipose as triglycerides or oxidized (Hermier, 1997; Elkin, 1997).

Figure 1. Fatty Acid Transfer and Conversion in Laying Hens



Selection of Oils

As a result of modern feeding methods, eggs currently have a higher amount of n-6 FA, but this can be adapted to a more desirable level by changing the balance of oils in the diet through supplementation. The most widely used methods are to feed fish meal or fish oil, which increases the DHA and EPA in the diet, or to use plant oils like flaxseed oil, which increases the ALA in the diet (Yannakoupoulos, 2007). In the current study, flaxseed oil was selected as a terrestrial source of n-3 FA (C 18:3 n-3). In addition, to test its interaction with n-6 FA, two types of safflower oil were used: high linoleic and high oleic. The safflower oil traditionally has high levels of n-6 FA, but varieties with high oleic acid were developed through selective breeding programs.

In the past, fish oils were used in order to increase the n-3 FA in feeds or as a dietary supplement as humans. Fish oils are a good source of DHA and EPA, which are VLC PUFAs. As mentioned previously, these FA are beneficial to human health and reduce inflammatory mediators. This leads to a reduced effect of inflammatory diseases such as inflammatory bowel disease, cardiovascular disease, arthritis, and certain types of cancer (Lenihan-Geels et al., 2013). The main fish sources for these oils include salmon, menhaden, herring, and mackerel because they have a higher level of oil (Lenihan-Geels et al., 2013; Yannakoupoulos, 2007). Menhaden and herring oils especially have a high amount of EPA and DHA (12% to 14%). Although fish oil is a reliable source of the VLC PUFA, a major issue with using fish oil as a source of n-3 FA is that the use of the fish oil has a significant impact on aquatic ecosystems and fish populations. This has led to increased interest in finding alternative sources and reducing environmental impact by researching plants high in n-3 FA. Another issue with the use of fish-based oils is that their use is limited because the food processed with these oils sometimes has a fishy taste (Yannakoupoulos, 2007). This is possibly from the susceptibility of these oils to oxidation. Oxidation leads to formation of peroxides, taste changes, changes in texture, off-flavors, and loss of nutrients of the eggs (Kassis et al., 2012).

There has been recently been a move to using plant-based n-3 sources in feeds, which would help sustainability and reduce overfishing, but there are still some issues concerning the balance of FA in these sources. Some plants, including flax, hemp, walnut, canola, soybean, and primrose, are high in n-3 FA, but still lack EPA and DHA (Lenihan-Geels et al., 2013; Kassis et al., 2012). Baucellis et al. looked at the option of replacing fish oils with different levels of four oils: flaxseed, rapeseed, sunflower and tallow (Baucellis et al., 2000). The eggs from the chickens fed with plant based oils or tallow all had decreases in the DHA and DPA in the egg when the percent fish oil in the diets decreased. However, the eggs from hens fed flaxseed oil as a supplement had a smaller decrease in DHA and DPA as the amount of dietary fish oil decreased. Eggs from the chickens fed with rapeseed oil, sunflower oil, or tallow also all had increased AA and increased n-6:n-3 ratios as the percent fish oil in the diets decreased. The eggs from hens fed flaxseed as a supplement had a lower increase in AA as the amount of dietary fish oil decreased and there was no change in the n-6:n-3 ratio (Baucellis et al., 2000). This suggests flaxseed oil (or linseed oil in the UK) would be a good candidate for the source of n-3 FA in the experiment or as a replacement for fish oil as it is only at much higher concentrations that flaxseed oil has unpleasant off flavors. When fish oil is added, it is recommended to feed 1.5% or less fish oil to prevent an off flavor, but flaxseed oil can be fed to just under 15% before a fishy flavor can be detected (Yannakoupoulos, 2007). The supplementation of feeds with extruded flaxseed meal have the effect of increasing the amount of LA, ALA, and DHA in the diet of hens as well as decreasing the amount of AA and the overall n-6:n-3 ratio (Imran et al., 2015). The drawbacks of using some sources of flax, however, are that full flaxseed contains tannins and cyanogenic glycosides, components that can lead to decreased productivity, growth depression, nervousness, reduced feed efficiency, and decreased egg quality in hens. However, extrusion can remove many of these undesired components. This is a method of processing the flaxseed by heating and shearing by squeezing it through a die. Flaxseed can also be processed by boiling, roasting in a microwave, acid treatment, or wet autoclave (Imran et al., 2015).

Other potential sources not examined in this study may include using algal oils high in EPA or DHA and plant oils high in stearidonic acid (C18:4n-3), which is the second step in the VLC n-3 FA synthesis pathway (See Figure 2). Stearidonic acid has been shown to affect inflammation, reduce triglycerides in the blood, reduce Cox-2, which is a protein found in some tumors, and reduce risk factors for atherosclerosis (Lenihan-Geels et al., 2013). However, conversion to EPA and DHA is limited, including in the hen (Elkin et al., 2015). High EPA and DHA oils have similar oxidation issues to fish oil.

Another oil utilized in this experiment is high oleic safflower oil (HOSO). Oleic acid (18:1 n-9*c*) is a monounsaturated FA that is beneficial to the food industry as it helps extend the shelf life of products and leads to lower production costs because there is less need for hydrogenation. This lack of hydrogenation also reduces levels of *trans*- fats in the product leading to a healthier product (Pham et al., 2010). Oleic acid can be converted to LA using Δ12-desaturase-2, an enzyme encoded by FAD2 genes. Certain crops including soybeans, safflower, sunflower, peanut, and canola are genetically engineered to produce gene combinations that yield a product with increased levels of oleic acid (Pham et al., 2010). If the plant contains two mutated FAD2 genes, or is homozygous for FAD2-1A and FAD2-1B mutant alleles, there is a higher level of oleic acid, comprising of 80% of the total oil (Pham et al., 2010; Stoddard et al., 2014). If there is a mutation in one FAD2 gene or one mutant and one wild type allele, the wild type allele will prevent the high oleic acid levels (Pham et al., 2010). This selection of specific alleles can be completed by sequence specific nuclease activity (Stoddard et al., 2014).

The other oil used in the experiment was high linoleic safflower oil (HLSO). Linoleic acid (C18:2 n6), as mentioned before, is a precursor to VLC FA including AA (See Figure 3). This process uses the same Δ5 and Δ6 desaturases as the n-3 elongation pathway so addition of this FA will act as a competitor and will determine to what extent n-6 FA elongation will reduce the synthesis of VLC FA. One study (Goldberg et al., 2013) proposed that a reduced LA: ALA ratio would lead to higher level of VLC FA in the yolk, especially DHA (Goldberg et al., 2013). In the experiment, corn oil, canola,

flaxseed, and high oleic sunflower oils were used to change the levels of LA in the diet. It found that increasing levels of dietary LA led to lower oleic acid and EPA in the yolks. Increasing dietary LA also led to an increase of yolk LA, AA, DPA, GLA, and total n-6 PUFA (Goldberg et al., 2013). The use of LA in the experiment will act as a competitor for the elongation pathways and its inclusion will allow for research on a desirable balance of n-6 and n-3 FA that yield in the highest n-3 deposition in yolks.

Figure 2. β-oxidation, chain elongation, and desaturation of α-Linolenic acid

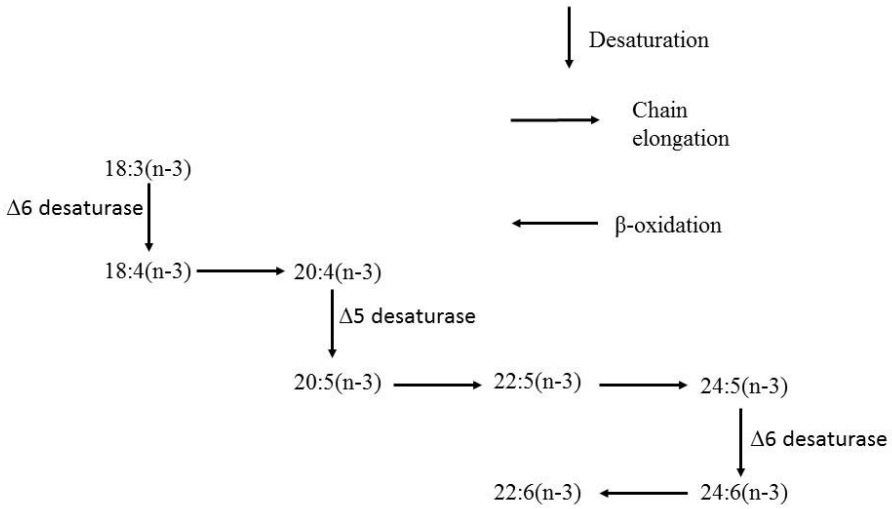
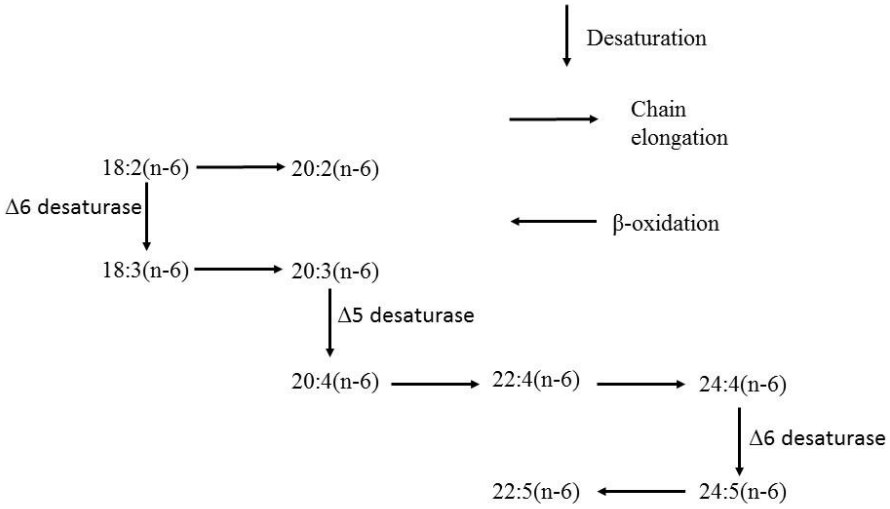


Figure 3. β-oxidation, chain elongation, and desaturation of Linoleic acid



Chapter 3

MATERIALS AND METHODS

Birds

All animal protocols were approved by the Institutional Animal Care and Use Committee of The Pennsylvania State University. Fifty Hy-Line W-36 hens (~1 year of age) were used in this study and were selected from a group of 100 hens based on egg production over five days prior to the experiment. Before the start of the experiment, the hens were fed a standard layer mash (Wengers Feeds, Elizabethtown, PA) with soybean meal, coarse chopped corn, and degermed cornmeal (Agricor Inc, Marion, IN). Hens were blocked by egg production and ten hens were randomly assigned to one of four dietary treatments and ten hens were assigned to a control diet. The study consisted of a 28-day test period. The experiment design (Table 1) consisted of a diet plan with 4% FLAX alone as a control and two amounts of high oleic safflower oil HOSO (2% and 4%), or two amounts of high linoleic safflower oil HLSO (2% or 4%) as fed. Starch and cellulose were included in the diets to balance energy density as fat concentration increased.

Egg weights were recorded daily for each hen and all eggs were collected on day 0 and day 28 of the experiment for determination of yolk weights and FA composition. Yolks were separated from the albumin, lyophilized, and ground with a mortar and pestle. Feed intake for each hen was observed over the entire duration of the study. At the end of the study, the hens were euthanized following IUCUC protocol and samples of adipose and liver were collected for fatty acid composition analysis. The entire liver was removed, weighed and subsamples collected from more than three locations. The entire abdominal fat pad was removed, weighed, and subsamples collected from more than three locations. Subsamples were snap frozen in liquid nitrogen and stored at -80°C. Tissue was pulverized by tapping with hammer after removal from liquid nitrogen.

Table 1. Diet set makeup testing different dietary levels of high oleic safflower oil (HOSO) or high linoleic safflower oil (HLSO) as fed.

Dietary sets	FLAX	HOSO	HLSO
1	4%	0%	0%
2	4%	2%	0%
3	4%	4%	0%
4	4%	0%	2%
5	4%	0%	4%

Analysis of Fatty Acid Profile by Gas Chromatography

Fatty acid samples were methylated and quantified by gas chromatography. A two-step methylation procedure was conducted on the yolks, liver, and adipose samples. It used 0.5 M sodium methoxide in methanol in a 50°C water bath for 10 minutes followed by 5% methanolic HCl in an 80°C water bath for 10 minutes. FA concentration was determined using methyl tridecenoate and nonadecanoic acid (Nu-Chek Prep, Inc., Elysian, MN) as internal standards. The resulting FA methyl esters were extracted in heptane and quantified using an Agilent 6890 gas chromatograph (Agilent Technologies, Palo Alto, CA) equipped with a fused-silica capillary column (SP2560; 100 m x 25 mm i.d. with 0.2-μm film thickness; Supelco, Inc., Bellefonte, PA) and a flame ionization detector (Elkin et al., 2015). The oven temperature program used was 70°C for 4 min, increased at a rate of 8 °C/min to 110 °C, then increased at a rate of 5 °C/min to 170°C for 10 min, and finally increased at a rate of 4 °C/min to 215 °C for 23 min. Inlet and detector temperatures were 250°C. Hydrogen was the carrier gas. Peaks were identified based on purified standards (GLC780, GLC461, GLC 566, and pure 22:1 n-9 [Nu-Chek Prep]; and pure 20:4 n-3 [Caymen Chemical Company, Ann Arbor, MI]). Stearidonic acid (SDA; 18:4 n-3) was identified based on liver FA from chickens fed high-SDA soybean oil in previous experiments Elkin et al. (2015). An equal weight standard (GLC461; Nu-Chek Prep) was used to calculate recovery factors.

Statistics

Data were analyzed using JMP Pro 13.2 (SAS Institute, Cary, NC) using the "Fit Model" procedure. Egg data was analyzed using a 2x2 factorial design with the fixed effect of day, treatment, and treatment by day interaction. Liver and adipose FA data were analyzed with models that included the effect of diet treatment. Treatment means were separated within day 28 by a LSD separation. Data points outside of ± 3 Studentized residuals were considered outliers and removed from the analysis. This rarely occurred for more than one value per variable.

Chapter 4

RESULTS

Yolk Fatty Acid Composition

Yolk samples were collected on two different dates, before initiation of treatments and after 28 days on experimental diets from a set of 50 chickens divided into 5 dietary groups. The yolks were evaluated for FA composition changes between diet groups. The diet affected yolk FA concentration for C14:0, C14:1*cis*-9, C16:0, C16:1*cis*-9, C18:0, C18:1*cis*-11, C18:1*cis*-9, C18:2 n-6, C18:3 n-6, C18:3 n-3, C20:1*cis*-11, C20:2n-6, C20:3n-6, C20:4n-3, C20:4 n-6, C20:5 n-3, C22:1*cis*-13, C22:4 n-6, C22:5 n-3, and C22:6 n-3 (Table 2). The n-3 FA including: C18:3n-3, C20:4n-3, C20:5 n-3, C22:5 n-3, and C22:6 n-3 generally were highest in the control group which had 4% FLAX, 0% HOSO, and 0% HLSO. In the control group, C18:3 n-3 comprised 8.08%, C20:4 n-3 comprised 0.061%, C20:5 n-3 comprised 0.256%, C22:5 n-3 comprised 0.366, and C22:6 n-3 comprised 1.92% of the total yolk FA; this amount decreased as HOSO and HLSO levels increased in the diet. The levels of C18:1 *cis*-9 in the yolks laid by the control diet hens were 39.3% of total yolk FA; this amount had a 12.7% increase as HOSO in the diet was added and a 16.0% decrease as HLSO in the diet was added. The levels of LA (C18:2n-6) in the yolks laid by the control diet hens were 9.66% of total yolk FA; this amount had a 14.9% increase as HOSO in the diet was added, and had a 106% increase as HLSO in the diet was added.

Liver Fatty Acid Composition

The liver FA composition was investigated because the liver is where the majority of lipoproteins are converted to VLDL and $VLDL_y$ lipoproteins, the main lipoproteins deposited in eggs, are made and it is the main site of omega-3 elongation and desaturation. The dietary treatment had an effect on liver FA concentration of C14:1*cis*-9, C16:0, C16:1*cis*-9, C18:0, C18:1*cis*-11, C18:1*cis*-9, C18:2 n-6, C18:3 n-6,

C18:3 n-3 & C20:1*cis*-11, C20:0, C20:2n-6, C20:3 n-3, C20:3n-6, C20:4n-3, C20:4 n-6, C20:5 n-3, C22:0, C22:4 n-6, C22:5 n-3, C 24:0, and C24:1 n-9 (Table 3). The n-3 FA, including C18:3 n-3, C20:3 n-3, C20:4 n-3, C20:5 n-3, and C22:5 n-3, generally had the highest level of fatty acid in the control group, which had 4% FLAX, 0% HOSO and 0% HLSO. In the control group, C18:4 n-3 comprised 6.27%, C20:3 n-3 comprised 0.158%, C20:4 n-3 comprised 0.044%, C20:5 n-3 comprised 0.540%, C22:5 n-3 comprised 0.431% of the total liver FA; this amount decreased as HOSO and HLSO levels in the diet increased. The levels of C18:1 *cis*-9 in the liver of the control diet hens were 34.4% of total yolk FA; this amount had a 15.1% increase as HOSO in the diet was added and a 20.1% decrease as HLSO in the diet was added. The levels of LA (C18:2n-6) in the liver of the control diet hens were 10.6% of total yolk FA; this amount had a 8.5% increase as HOSO in the diet was added, and had a 90.6% increase as HLSO in the diet was added.

Adipose Fatty Acid Composition

The level of FA in adipose was investigated because this is the main location of deposition of dietary FA that are not utilized in yolk production or oxidized. There was an effect of dietary treatment on adipose FA concentration for C 14:0, C14:1*cis*-9, C15:0, C16:0, C16:1*cis*-9, C17:0, C17:1*cis*-10, C18:0, C18:1*cis*-11, C18:1*cis*-9, C18:2 n-6, C18:3 n-6, C18:4 n-3, C20:3 n-3, C20:4 n-3, and C24:0 (Table 4). The n-3 FA, including C18:4 n-3 and C20:3 n-3, C20:4 n-3, generally had the highest level of fatty acid in the control group, which had 4% FLAX, 0% HOSO and 0% HLSO. In the control group, C18:4 n-3 comprised 0.096%, C20:3 n-3 comprised 0.033%, and C20:4 n-3 comprised 0.0087% of total adipose FA; this amount decreased as HOSO and HLSO levels in the diet increased. The levels of C18:1 *cis*-9 in the adipose of the control diet hens were 36.3% of total yolk FA; this amount had a 11.3% increase as HOSO in the diet was added and a 6.9% decrease as HLSO in the diet was added. The levels of LA (C18:2n-6) in the adipose of the control diet hens were 24.5% of total yolk FA; this amount had a 5.7% increase as 2%

HOSO was added to the diet and no change at 4% HOSO, and had a 25.7% increase as HLSO in the diet was added.

Table 2. Egg yolk fatty acid concentrations based on diet compositions of high oleic safflower oil (HOSO) and high linoleic safflower oil (HLSO)

Fatty Acid, %FA			[1]HOSO		[2]HLSO			[3]*P-values*		
	Day 0	[4]Control	2%	4%	2%	4%	SE	Day	Diet*Day	Diet
C 14:0	0.352	0.299^{a}	0.262^{b}	0.254^{b}	0.289^{a}	0.259^{b}	0.0096	<.0001	<.0001	0.036
C14:1cis-9	0.068	0.050^{a}	[5]ND^{b}	ND^{b}	ND^{b}	ND^{b}	0.0036	<.0001	<.0001	<.0001
C15:0	0.054	0.042	0.045	0.042	0.043	0.043	0.0022	<.0001	0.1825	0.201
C16:0	25.6	23.3^{a}	22.0^{bc}	21.4^{c}	22.7^{ab}	22.3^{b}	0.279	<.0001	<.0001	0.044
C16:1cis-9	2.545	2.43^{ab}	1.86^{c}	1.53^{d}	1.89^{c}	1.42^{d}	0.092	<.0001	<.0001	<.0001
C17:0	0.208	0.142^{d}	0.158^{cd}	0.161^{c}	0.166^{bc}	0.179^{ab}	0.0065	<.0001	<.0001	0.242
C18:0	9.00	9.93^{a}	9.08^{b}	8.60^{c}	9.77^{a}	10.1^{a}	0.158	<.0001	<.0001	0.0018
C18:1cis-11	1.73	1.41^{a}	1.23^{b}	1.12^{c}	1.22^{b}	1.06^{c}	0.034	<.0001	<.0001	<.0001
C18:1cis-9	39.2	39.3^{c}	42.0^{b}	44.3^{a}	35.4^{d}	33.0^{e}	0.485	0.034	<.0001	<.0001
C18:2n6	15.0	9.66^{d}	11.0^{c}	11.1^{c}	16.0^{b}	19.9^{a}	0.394	<.0001	<.0001	<.0001
C18:3n6	0.109	0.030^{d}	0.053^{c}	0.056^{c}	0.080^{b}	0.089^{ab}	0.0060	<.0001	<.0001	0.0061
C18:3n3	0.365	8.08^{a}	6.73^{b}	5.64^{c}	6.98^{b}	5.97^{c}	0.195	<.0001	<.0001	<.0001
C18:4n3	0.045	0.019^{c}	0.029^{b}	0.030^{b}	0.032^{ab}	0.038^{ab}	0.0036	<.0001	0.016	0.065
C20:0	ND	ND	ND	ND	ND	ND	—	—	—	—
C20:1cis-11	0.250	0.162^{c}	0.183^{b}	0.205^{a}	0.156^{c}	0.170^{bc}	0.0062	<.0001	<.0001	0.0031
C20:2n6	0.172	0.071^{e}	0.081^{e}	0.080^{e}	0.117^{d}	0.170^{abc}	0.0058	<.0001	<.0001	<.0001
C20:3n6	0.208	0.153^{d}	0.165^{d}	0.164^{d}	0.200^{bc}	0.201^{abc}	0.0061	<.0001	<.0001	0.015
C20:4n3	ND	0.061^{a}	0.051^{b}	0.041^{c}	0.048^{b}	0.034^{d}	0.0017	<.0001	<.0001	<.0001
C20:4n6	2.09	0.687^{e}	0.841^{d}	0.928^{c}	1.03^{b}	1.295^{a}	0.028	<.0001	<.0001	<.0001
C20:5n3	ND	0.256^{a}	0.168^{b}	0.139^{c}	0.145^{c}	0.091^{d}	0.0058	<.0001	<.0001	<.0001
C22:1cis-13	ND	0.156^{a}	0.119^{bc}	0.100^{d}	0.128^{b}	0.116^{c}	0.0042	<.0001	<.0001	<.0001
C22:4n6	ND	0.030^{bc}	0.014^{d}	0.029^{c}	0.037^{ab}	0.043^{a}	0.0029	<.0001	<.0001	<.0001
C22:5n3	0.072	0.366^{a}	0.305^{b}	0.277^{b}	0.269^{b}	0.204^{c}	0.013	<.0001	<.0001	<.0001
C22:5n6	0.543	ND	ND	ND	ND	ND	—	<.0001	—	—
C22:6n3	0.709	1.92^{a}	1.97^{a}	1.95^{a}	1.72^{b}	1.58^{b}	0.052	<.0001	0.0057	<.0001
C24:1n9	0.148	ND	ND	ND	ND	ND	—	<.0001	—	—

[1]HOSO contained an additional 2 or 4% high oleic safflower oil.
[2]HLSO contained an additional 2 or 4% high linoleic safflower oil.
[3]*P-value* is significant for $p<0.05$
[4]Control and all other treatments contained 4% flaxseed oil.
[5]ND is below the level of detection (< 0.005%)

Table 3. Liver fatty acid concentrations based on different diet compositions of high oleic safflower oil (HOSO) and high linoleic safflower oil (HLSO)

	[1]Control	[2]HOSO		[3]HLSO			
		2%	4%	2%	4%	SEM	[4]*P-value*
Total FA%	6.17	7.00	6.73	6.08	5.63	0.77	0.55
FA profile, % FA							
C14:0	0.293	0.287	0.261	0.293	0.267	0.020	0.559
C14:1cis-9	0.045^{a}	0.021^{bc}	0.0067^{cd}	0.025^{ab}	[5]ND^{d}	0.0074	0.0008
C15:0	0.045	0.045	0.033	0.041	0.044	0.0078	0.656
C16:0	22.5^{a}	22.1^{ab}	21.0^{b}	23.0^{a}	21.1^{b}	0.492	0.0078
C16:1cis-9	2.14^{a}	1.70^{b}	1.34^{c}	1.76^{b}	1.14^{c}	0.135	<.0001
C17:0	0.151	0.162	0.167	0.155	0.172	0.010	0.450
C18:0	12.9^{ab}	12.4^{b}	11.9^{b}	12.8^{ab}	13.6^{a}	0.400	0.0087
C18:1cis-11	1.34^{a}	1.28^{ab}	1.23^{ab}	1.17^{b}	0.980^{c}	0.046	<.0001
C18:1cis-9	34.4^{bc}	36.7^{b}	39.6^{a}	31.3^{c}	27.5^{d}	1.19	<.0001
C18:2 n6	10.6^{c}	11.3^{c}	11.5^{c}	14.9^{b}	20.2^{a}	0.654	<.0001
C18:3 n6	0.063^{ab}	0.050^{b}	0.021^{c}	0.068^{ab}	0.074^{b}	0.0010	0.0004
C18:3n3 & 20:1cis-11	6.27^{a}	5.27^{ab}	4.11^{c}	5.53^{ab}	5.13^{b}	0.423	0.0058
C18:4n3	0.049	0.044	0.024	0.052	0.049	0.0084	0.056
C20:0	0.057^{c}	0.061^{c}	0.059^{c}	0.080^{b}	0.096^{a}	0.0066	<.0001
C20:2n6	0.146^{b}	0.133^{b}	0.154^{b}	0.174^{b}	0.258^{a}	0.018	<.0001
C20:3n3	0.158^{a}	0.114^{b}	0.105^{b}	0.111^{b}	0.106^{b}	0.011	0.0083
C20:3n6	0.589	0.512	0.544	0.567	0.641	0.062	0.439
C20:4n3	0.044^{a}	0.0080^{b}	ND^{c}	ND^{c}	ND^{c}	0.0028	<.0001
C20:4n6	2.05^{b}	2.20^{b}	2.42^{b}	2.61^{b}	3.35^{a}	0.287	0.0038
C20:5n3	0.540^{a}	0.371^{b}	0.319^{bc}	0.280^{c}	0.173^{d}	0.037	<.0001
C22:0	0.066^{c}	0.061^{c}	0.061^{c}	0.103^{b}	0.123^{a}	0.0081	<.0001
C22:1cis-13	ND	ND	ND	ND	ND	—	—
C22:2n6	ND	ND	ND	ND	ND	—	—
C22:4n6	0.066^{ab}	0.040^{b}	0.034^{b}	0.072^{a}	0.084^{a}	0.013	0.0056
C22:5n3	0.431^{a}	0.368^{ab}	0.310^{b}	0.326^{ab}	0.214^{c}	0.040	0.0016
C22:5n6	ND	ND	ND	ND	ND	—	—
C22:6n3	3.02	3.04	3.18	2.90	2.65	0.307	0.619
C24:0	0.030^{b}	0.0080^{cd}	ND^{d}	0.017^{bc}	0.046^{a}	0.0062	<.0001
C24:1n9	ND^{b}	ND^{b}	0.014^{b}	0.058^{a}	0.074^{a}	0.0083	<.0001
Others	2.07^{a}	1.54^{bc}	1.44^{bc}	1.37^{c}	1.60^{b}	0.082	<.0001

[1]Control and all other treatments contained 4% flaxseed oil.
[2]HOSO contained an additional 2 or 4% high oleic safflower oil.
[3]HLSO contained an additional 2 or 4% high linoleic safflower oil.
[4]*P-value* is significant for $p<0.05$
[5]ND is below the level of detection (< 0.005%)

Table 4. Adipose fatty acid concentrations based on different diet compositions of high oleic safflower oil (HOSO) and high linoleic safflower oil (HLSO)

	[1]Control	[2]HOSO		[3]HLSO			
		2%	4%	2%	4%	SEM	[4]*P-value*
Total FA %	87.3[a]	88.0[a]	88.7[a]	87.4[a]	84.5[b]	0.955	0.024
FA profile %FA							
C14:0	0.759[a]	0.687[b]	0.651[bc]	0.675[bc]	0.629[c]	0.018	<.0001
C14:1cis-9	0.101[a]	0.085[bc]	0.091[ab]	0.092[ab]	0.080[c]	0.0041	0.0091
C15:0	0.133[a]	0.124[b]	0.120[b]	0.123[b]	0.116[b]	0.0031	0.0054
C16:0	17.5[a]	15.8[bc]	15.6[bc]	16.2[b]	15.4[c]	0.287	<.0001
C16:1cis-9	2.61[a]	2.15[b]	2.43[ab]	2.43[ab]	2.09[b]	0.126	0.022
C17:0	0.238[a]	0.225[ab]	0.214[bc]	0.219[bc]	0.209[c]	0.0054	0.0030
C17:1cis-10	0.138[a]	0.126[ab]	0.133[a]	0.126[ab]	0.116[b]	0.0046	0.0105
C18:0	5.55[a]	5.21[ab]	4.79[c]	5.26[ab]	4.95[bc]	0.138	0.0026
C18:1cis-11	1.93[a]	1.79[b]	1.78[b]	1.75[b]	1.63[c]	0.037	<.0001
C18:1cis-9	36.3[c]	38.2[b]	40.4[a]	34.3[d]	33.8[d]	0.475	<.0001
C18:2 n6	24.5[d]	25.9[c]	24.5[d]	27.5[b]	30.8[a]	0.492	<.0001
C18:3 n6	0.167[a]	0.120[bc]	0.115[bc]	0.153[ab]	0.100[c]	0.015	0.015
C18:3n3 & 20:1cis-11	7.79	7.41	7.12	8.79	7.84	0.476	0.119
C18:4n3	0.096[a]	0.064[b]	0.066[b]	0.085[a]	0.061[b]	0.0066	0.0012
C20:0	0.109	0.116	0.110	0.123	0.125	0.0080	0.4525
C20:2n6	0.044	0.047	0.041	0.048	0.049	0.0022	0.072
C20:3n3	0.033[ab]	0.029[bc]	0.026[c]	0.033[a]	0.028[c]	0.0016	0.0077
C20:3n6	0.029	0.028	0.025	0.028	0.024	0.0020	0.283
C20:4n3	0.0087[a]	[5]ND[b]	ND[b]	ND[b]	ND[b]	0.0012	<.0001
C20:4n6	0.025	0.032	0.030	0.032	0.033	0.0025	0.183
C24:0	ND[c]	0.0077[b]	0.011[ab]	0.011[ab]	0.012[a]	0.0014	<.0001
Others	1.88[ab]	1.79[bc]	1.73[c]	1.91[a]	1.90[a]	0.037	0.0018

[1]Control and all other treatments contained 4% flaxseed oil.

[2]HOSO contained an additional 2 or 4% high oleic safflower oil.

[3]HLSO contained an additional 2 or 4% high linoleic safflower oil.

[4]*P-value* is significant for $p<0.05$

[5]ND is below the level of detection (< 0.005%)

Chapter 5

DISCUSSION

The experiment was performed to analyze the effects of oleic acid (18:1n-9) and linoleic acid (18:2n-6) on the conversion of ALA (18:3n-3) to VLC n-3 FA and deposition in chicken egg yolks. The experiment showed that both oleic acid and LA had negative effects on the degree of deposition of ALA in yolks, although oleic acid had a smaller but still statistically significant reduction in deposition of ALA and VLC n-3 FA.

In chickens, there are different metabolic destinations of FA after absorption in the intestine and processing in the liver. FA can be deposited in yolks as $VLDL_y$, deposited in adipose tissue or broken down further through β oxidation of FA and transported between the liver and other tissues.

One FA used in the experiment is oleic acid (18:1n-9), and its deposition in the yolk increased as HOSO levels were increased. The oleic acid in the yolk also decreased as dietary HLSO in the diet increased. This same pattern was found in the liver and adipose samples suggesting that the levels of FA in tissues has a correlation with the levels and composition of FA provided in the diet. It also suggests that linoleic acid interferes with the processing of oleic acid and transfer into the yolks. The other oil used in the experiment is linoleic acid (LA 18:2n-6), and its and its deposition in the yolk increased as HLSO levels were increased and as dietary HOSO in the diet increased. The same pattern was present in liver and adipose suggesting that the levels of FA in tissues has a correlation with the levels and composition of FA provided in the diet. This suggests that Oleic acid has a positive effect on deposition of LA in yolks which makes sense as oleic acid is a precursor to n-6 FA (Oliveira et al., 2010). When comparing the magnitude of the percent increase of LA and oleic acid in the yolks by addition of HLSO and HOSO respectively, it is clear that the introduction of LA to the diet has a greater effect on LA deposition than dietary oleic acid does on deposition of yolk oleic acid. This deposition pattern was also present in the

liver and adipose samples suggesting that dietary levels of LA and oleic acid have different efficiencies of deposition.

Omega-3 FA were found in yolks at the highest levels in the control group with 4% FLAX, 0% HOSO and 0% HLSO. LC n-3 FA like C18:3n-3, C20:4n-3, and C20:5n-3 had a negative correlation between level of HOSO in the diet or HLSO in the diet and deposition of FA in the yolk. The samples with higher levels of incorporation in the yolk were at the lower concentrations of the HLSO and HOSO. C22:5n3 also had a negative correlation between level of HLSO in diet and deposition in the yolk. The higher level of deposition occurred at the lower HLSO concentration. There was a decrease in C22:5n-3 deposition with the addition of HOSO, but there was not a significant difference between the deposition in the yolk at the different concentrations of HOSO. The dietary level of HOSO did not have a significant impact on the deposition of C22:6n-3 in the yolk, but there was a slight increase in levels of this FA in the yolk. Addition of HLSO, on the other hand, led to a significant decrease in C22:6n-3 in the yolk. There was not a significant difference between the deposition of this FA in the yolk at the different concentrations of HLSO. The concentration of various n-3 FAs were also higher in the liver when HLSO and HOSO were not supplemented in the diet. This suggests that the composition of FA in the diet, amount of LA and oleic acid negatively affect the deposition and processing of n-3 FA in the liver and negatively affects further deposition in yolks. This suggests that oleic acid and n-3 acid have similar metabolic functions, but they are slightly different. LA is a direct competitor for the same pathways as ALA and as a result has a greater effect on reduction of ALA deposition in yolks and most likely competes with ALA during the metabolic processes in the liver. Oleic acid is not a direct competitor and as a result has a less significant impact on ALA deposition in yolks. It most likely competes with ALA during absorption in the intestine or deposition in the yolk.

Chapter 6

CONCLUSIONS

This experiment attempted to assess the degree to which increased dietary linoleic and oleic acid negatively affects the deposition of n-3 FA in egg yolks. It was found that a higher level of dietary oleic acid (18:1n-9), provided through HOSO, had a positive effect on deposition of oleic acid in the yolk, and a higher level of dietary linoleic acid (18:2n-6), provided through HLSO, had a positive effect on deposition of LA in the yolk. Increased dietary LA, provided through HLSO, had a negative effect on deposition of oleic acid in the yolk. In addition, the added oils HOSO and HLSO negatively affected the deposition of LC n-3 FA and VLC n-3 FA in yolks. This supports previous experiments that showed dietary n-6 FA had a negative effect on deposition of n-3 FA in yolks as a result of competition for the Δ-desaturase enzymes. Further research in the desaturase pathways and into FA conversion would help pinpoint the control points in the experiment and would get the research group closer to developing a high n-3 fatty acid egg.

Chapter 7

REFERENCES

Applegate, E. (2000) Introduction: Nutritional and Functional Roles of Eggs in the Diet. *Journal of the College of Nutrition, 19*(2), 495S-498S. doi:10.1080/07315724.2000.10718971

Baucellis, M., Crespo, N., Barroeta, A., López-Ferrer, S., Grashorn, M. (2000). Incorporation of different polyunsaturated Fatty Acids into eggs. *Poultry Science, 79*, 51-59 doi:10.1093/ps/79.1.51

Bujo, H., Hermann, M., Lindstedt, K., Johannes, N., Schneider, W. (1997). Low Density Lipoprotein Receptor Gene Family Members Mediate Yolk Deposition. *J. Nutr, 127*, 801S-804S doi:10.1093/jn/127.5.801S

Elkin, R. (1997). An Overview of Recent Developments in Avian Lipoprotein Metabolism. *J. Nutr., 127*, 793S-794S doi:10.1093/jn/127.5.793S

Elkin, R., Ying Y., Harvatine, K. (2015). Feeding Laying Hens Stearidonic Acid-Enriched Soybean Oil, as Compared to Flaxseed Oil, More Efficiently Enriches Eggs with Very Long-Chain n-3 Polyunsaturated Fatty Acids. *J. Agric. Food Chem., 63*(10), 2789–2797 doi:10.1021/jf505185u

Goldberg, E., Ryland, D., Gibson, R., Aliani, M., House, J. (2013). Designer laying hen diets to improve egg fatty acid profile and maintain sensory quality. *Food Science and Nutrition, 1*(4), 324-335 doi:10.1002/fsn3.47

Hermier, D. (1997). Lipoprotein Metabolism and Fattening in Poultry. *J. Nutr., 127*, 805S-808S doi:10.1093/jn/127.5.805S

Imran, M., Anjum, F. M., Nadeem, M., Ahmad, N., Khan, M. K., Mushtaq, Z., & Hussain, S. (2015). Production of Bio-omega-3 eggs through the supplementation of extruded flaxseed meal in hen diet. *Lipids Health Dis, 14*, 126. doi:10.1186/s12944-015-0127-x

Kassis, N., Gigliotti, J., Beamer, S., Tou, J., Jaczynski, J. (2012) Characterization of lipids and antioxidant capacity of novel neutraceutical egg products developed with omega-3-rich oils. *J Sci Food Agric, 92*, 66-73 doi:10.1002/jsfa.4542

Kremmyda, L., Tvrzicka, E., Stankova, B., Zak, A. (2011). Fatty Acids as Biocompounds: Their Role in Human Metabolism, Health and Disease – a Review. Part 2: Fatty Acid Physiological Roles, and Applications in Human Health and Disease. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub, 155*(3), 195-218. doi: 10.5507/bp.2011.052

Kritchevsky, S., Kritchevsky, D. (2000). Egg Consumption and Coronary Heart Disease: an Epidemiologic Overview. *Journal of the College of Nutrition, 19*(5), 549S-555S. doi: 10.1080/07315724.2000.10718979

Lenihan-Geels, G., Bishop, K., Ferguson, L. (2013) Alternative Sources of Omega-3 Fats: Can We Find a Sustainable Substitute for Fish? *Nutrients, 5*, 1301-1315 doi:10.3390/nu5041301

Lewis, N., Seburg, S., Flanagan, N. L. (2000). Enriched Egg as a Source of N-3 Polyunsaturated Fatty Acids for Humans. *Poultry Science, 79*, 971-974. doi:10.1093/ps/79.7.971

McNamara, D. J. (2000). Dietary cholesterol and atherosclerosis. *Biochimica et Biophysica Acta, 1529*, 310-320. doi:10.1016/S1388-1981(00)00156-6

McNamara, D. J. (2015). The Fifty Year Rehabilitation of the Egg. *Nutrients, 7*, 8716-8722. doi:10.3390/nu7105429

Oliviera, D., Baião, N., Cançado, S., Grimaldi, R., Souza, M., Lara, L., Lana, A. (2010). Effects of lipid sources in the diet of laying hens on the fatty acid profiles of egg yolks. *Poultry Science, 89*, 2484-2490 doi:10.3382/ps2009-00522

Pham, A., Lee, J., Shannon, J., Bilyeu, K. (2010). Mutant Alleles of FAD2-1A and FAD2-1B combine to produce soybeans with the high oleic acid seed oil trait. *Plant Biology, 10*(195), doi:10.1186/1471-2229-10-195

Simopoulos, A. P. (1991). Omega-3 fatty acids in health and disease and in growth and development. *Am J Clin Nutr, 54*, 438-463. doi:10.1093/ajcn/54.3.438

Simopoulos, A. P. (2002). The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomed Pharmacother, 56*, 365-379. doi:10.1016/S0753-3322(02)00253-6

Stoddard, T. Voytas, D., Mathis, L., Zhang, F. (2014) Creation of High Oleic Soybean oil via Targeted Mutagenesis of FAD2 gene family. *Plant Biotech,* 1-7

Tvrzicka, E., Kremmyda, L., Stankova, B., Zak, A. (2011). Fatty Acids as Biocompounds: Their Role in Human Metabolism, Health and Disease – a Review. Part 1: Classification, Dietary Sources and - Biological Functions. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub*, *155*(2), 117-130. doi: 10.5507/bp.2011.038

Walzem, R., Hansen, R., Williams, D., Hamilton, R. (1999). Estrogen Induction of $VLDL_y$ Assembly in Egg-Laying Hens. *J. Nutr., 129*, 467S-472S doi:10.1093/jn/129.2.467S

Yannakoupoulos, A. L. (2007). Egg Enrichment in Omega-3 Fatty Acids. In Yannakoupoulos, A. L. Bioactive Egg Compounds (159-168) Berlin, Germany: Springer-Verlag.

ACADEMIC VITA

Academic Vita of Elizabeth Ann Flaherty
eaf5316@psu.edu

Education

B.S. in Veterinary and Biomedical Sciences — Schreyer Honors College
Minor in Spanish — Graduation: May 2018
Pennsylvania State University
University Park, PA

Kansas State University
Matriculation: Fall 2018

Extracurricular Activities

Penn State Karate Club
- Treasurer 2017-2018
- Rokkyu (fifth level student)
- Certified Member of IWKA (Isshin-Ryu World Karate Association)
- Member 2016-2018

Special Olympics Pennsylvania Summer Games
- Volunteer 2017

Atlas THON Special Interest Organization
- Member 2014-2016

Centre County Paws
- Volunteer 2017-Present

Animal Friends Shelter
- Volunteer 2015-2016

Professional Experience

West Liberty Animal Hospital Volunteer 2014-2016
VCA Northview Volunteer 2015-2016
Laurel Highlands Animal Hospital Volunteer 2015
PSERT Foal Watch (Penn state equine research team) 2015
Brown Equine Hospital Volunteer 2009, 2011, 2012