THE PENNSYLVANIA STATE UNIVERSITY
SCHREYER HONORS COLLEGE

DEPARTMENT OF ANIMAL SCIENCES

EFFECT OF HIGH OLEIC SOYBEAN OIL ON OMEGA-3 FATTY ACID ELONGATION AND INCORPORTATION INTO EGG YOLKS

ALEXANDRA NICOLE KUKOROWSKI
SPRING 2018

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

Reviewed and approved* by the following

Dr. Kevin Harvatine
Associate Professor of Nutritional Physiology
Thesis Supervisor

Dr. Troy Ott
Professor of Reproductive Physiology
Honors Advisor

*Signatures are on file in the Schreyer Honors College.

ABSTRACT

Production of nutritionally enriched eggs is beneficial to egg producers as it increases the value of their product and is important to consumers because they help meet specific nutrient requirements. Omega-3 fatty acid (FA) enriched eggs have proven to be a functional food that is desired by consumers. Very long chain omega-3 (VLC n-3) FA have been identified as protective against diseases such as diabetes mellitus, cardiovascular disease, and cancer, making their presence in food valuable to the consumer (Yashodhara et al., 2009). This research investigates the effects of high dietary oleic acid on VLC n-3 FA elongation and incorporation into the egg yolk.

The effects of dietary high oleic soybean oil (HOSO) and high α-linolenic flaxseed oil (FLAX) were investigated at four different supplementary levels (0%, 1%, 2%, 4%) in a split plot design with four groups of nine white Leghorn chickens. Each feeding period lasted 21 days and egg samples were collected on the final day of the feeding period. The egg yolks and liver tissue samples were directly methylated in a dual methylation procedure and analyzed for FA content by gas chromatography.

Oils had little to no effect on egg production including the number of eggs produced and egg composition such as albumin, shell, and yolk weights. Oleic acid was not affected by FLAX and the highest incorporation occurred at 4% HOSO. There was an interaction between the oils for α-linolenic acid (ALA) incorporation, with FLAX increasing and HOSO decreasing at high levels of ALA. There also was an interaction of oils for incorporation of eicosapentaenoic acid (EPA) into egg yolks. EPA increased with increasing FLAX, but decreased with increasing HOSO. There was no interaction of oils for docosahexaenoic acid (DHA) incorporation, but overall FLAX increased and HOSO decreased DHA deposition.

There are various critical points in FA absorption and incorporation into eggs that may be modified by oleic acid and ALA levels and their interaction. High dietary FLAX allowed for liver elongation of ALA to increase levels of EPA and DHA in egg yolks. Increasing HOSO increased oleic acid in the yolks, but the oleic acid appears to have competed with the n-3 FA for incorporation into the eggs yolks at one or more critical points of absorption and incorporation.

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LIST OF ABBREVIAITONS

FA	Fatty acid
n-3	Omega-3
VLC	Very long chain
HOSO	High oleic soybean oil
FLAX	High α-linolenic flaxseed oil
ALA	α-linolenic acid
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
n-6	Omega-6
PUFA	Polyunsaturated fatty acids
AA	Arachidonic acid
n-9	Omega-9
HDL	High density lipoprotein
LDL	Low density lipoprotein
LC	Long chain
VLDL	Very low density lipoproteins

ACKNOWLEDGEMNETS

I would like to thank all those who made my time at Penn State University enjoyable and enriching. Thank you to Dr. Nüket Acar; Dr. Acar is one of the main reasons I chose to attend Penn State as she opened my eyes to the wonderful opportunities and support provided by the university that would allow me to follow my dreams. Additional thanks goes to Dr. Robert Van Saun and Dr. Lester Griel, both whom greatly aided me obtaining admission to the University of Pennsylvania School of Veterinary Medicine while pushing me to become a more competent and knowledgeable student.

Immense gratitude goes to Dr. Kevin Harvatine for allowing me become part of his lab. I am beyond thankful for the vast wealth of knowledge and support that Dr. Harvatine has shared with me. Further thanks goes to Michel Baldin and Jackie Ying for aiding me in understanding and proficiency of lab techniques and procedures.

Final thanks goes to my Mom and Dad for pushing me to be a better person and student every day of my life.

Chapter 1

INTRODUCTION

Omega-3 FA, such as EPA, and DHA, have been shown to possess biological characteristics and activities that are important in human health and function. High levels of n-3 FA consumption is characteristic of the Mediterranean Diet, which has been shown to possess may benefits for cardiovascular, cognitive, and overall health. Although fish has been shown to be an efficient and effective source of omega-3 FA, meeting n-3 requirements is still of concern in the human population as there is worry about potentially harmful side effects related to fish consumption (Gil & Gil, 2015). In addition, fish consumption is below optimal health levels as it is an expensive source of protein, may not be regionally available, and not palatable to all. These and other factors lead to 65% of the United States population not eating fish on a regular basis (Surai & Sparks, 2001). Furthermore, sustainable harvesting of fish is a concern in many parts of the world making high omega-3, cold-water fish a limited natural resource. Chicken eggs could be a source of n-3 FA, but more research is necessary to determine the limitations and advantages of this functional food as well as the optimal hen diet to produce n-3 FA rich eggs.

In laying hens the dietary effects of high oleic acid on VLC n-3 FA is not known. The hope is that feeding high oleic soybean oil and high alpha-linolenic acid (18:3 n-3) flaxseed oil will allow productions of eggs containing high oleic acid, high very LC n-3 FA, and low omega-6 (n-6) FA. An increase in dietary oleic acid is expected to produce a decrease in n-6 FA concentration, while not drastically effecting the n-3 FA concentration. A decrease in the n-6 FA concentration would be beneficial to consumers as n-6 FA contain more pro-inflammatory factors compared to n-3 FA, which are considered anti-inflammatory.

Chapter 2

LITERATURE REVIEW

Importance of FA

Fatty acids are the principle component of lipid stores, in the form of triglycerides, and cell membranes, in the form of phospholipids. Fatty acids normally provide 30% of the energy intake of humans with the remaining 70% originating from carbohydrates and proteins. Although FA are important energy sources, overconsumption of FA will be stored in adipose tissue which can result in obesity (Tvrzicka, Kremmyda, Stankova, & Zak, 2011).

Fatty acids are carboxylic acids with carbon chains varying in length from two to thirty-six carbons and can be found in saturated, unsaturated, and polyunsaturated structures. Saturated FA are characterized by all single bonds between the carbons. Unsaturated FA have at least one carbon-carbon double bond in the carbon chain with polyunsaturated fatty acids (PUFA) containing two or more double bonds in the carbon chain with a methylene between the double bonds, known as a pentidiene configuration. Fatty acids are synthesized in the cytoplasm of cells from two carbon precursors with help from acetyl-CoA carboxylase, fatty acid sy an acyl protein nthase, carrier, and NADPH. Fatty acids are degraded in the mitochondria by a process known as β-oxidation, which produces a release of energy. Peroxisomal oxidation of FA also occurs, but the energy yield is less efficient. Esters of FA that are conjugated with organic alcohols such as cholesterol, glycerol, and sphingosine are also termed lipids. Lipoproteins composed of cholesterol esters, phospholipids, and triacylglycerols circulate in the cardiovascular system. Fatty acids that are not esterified are found bound to plasma albumin (Tvrzicka et al., 2011).

Short chain saturated FA include acetic acid (2:0), propionic acid (3:0), and butyric acid (4:0). In the liver, acetic and propionic acids are oxidized or transformed into FA and glucose, respectively. Medium and long chain saturated FA, including lauric (12:0), myristic (14:0), palmitic (16:0), and stearic (18:0) acids, are characterized by their anterogenic and thrombogenic abilities. They are found in plant sources such as coconut oil, palm kernel oil, cocoa butter, and shea butter and in animal milk and tissue products. High levels of medium chain saturated FA will increase LDL cholesterol and are correlated with coronary heart disease. Very LC saturated FA include arachidonic (20:0), behenic (22:0), lignoceric (24:0), cerotic (26:0), montanic (28:0), and melissic (30:0) acids, which are in low concentrations in most animal and plant products. Some specific inherited metabolic diseases can result in large accumulations of these compounds (Tvrzicka et al., 2011).

Desaturation of FA will produce carbon-carbon double bonds from single bonds in the carbon chain. Humans lack Δ12- and Δ15-desaturases, therefore linoleic (LA, 18:2n-6) and α-linolenic (ALA, 18:3n-3) acids are classified as essential. Further desaturation and elongation of LA and ALA will produce eicosapentaenoic (EPA, 20:5n-3), docosahexaenoic (DHA, 22:6n-3), and arachidonic (AA, 20:4n-6) acids. These conversions are classified as conditionally essential as they are possible, but not efficient in humans and many animals. Desaturation of palmitic acid (16:0) yields palmitoleic acid (16:1n-7) and desaturation of stearic acid (18:0) yields oleic acid (18:1n-9). Further elongation of palmitoleic and oleic acid will yield omega-9 (n-9) FA with chain lengths of twenty to twenty-four carbons (Tvrzicka et al., 2011).

Desaturation can produce FA with either *cis* or *trans* double bonds. *Cis* orientation describes a FA with the two hydrogens of the double bond on the same side of the molecule producing a bend in the structure. Oleic acid (18:1n-9c) is characterized as antiatherogenic and

antithrombic and produces an increase in high density lipoprotein (HDL) and a decrease in low density lipoprotein (LDL) cholesterol. Oleic acid is the primary FA in olive and canola oil. Other *cis* FA include *cis*-vaccenic (18:1n-7c) and palmitoleic (16:1n-7c). Trans FA are characterized as having the hydrogens of the double bond on opposite sides of the molecule, which does not produce a bend and keeps the FA structure closer to that of a saturated FA. *Trans* FA include elaidic (18:1n-9*t*) and vaccenic (18:1n-7*t*) acids. Generally producing an increase in LDL and decrease in HDL cholesterol, *trans* FA from partially hydrogenated fat are found in hardenings and shortenings such as margarine and butter (Tvrzicka et al., 2011).

Fatty Acids and the Mediterranean Diet

The Mediterranean Diet, which has been cited as characteristically high in unsaturated FA including long chain (LC) n-3, EPA, and DHA, has gained notoriety for its human health benefits. These benefits include cardiovascular effects, such as decreased serum cholesterol, decreased blood pressure, and decreased coronary mortality, when compared to those consuming diets typical of Northern European countries and the United States (Huhn, Kharabian Masouleh, Stumvoll, Villringer, & Witte, 2015). Long chain n-3 PUFA have also been shown to be important in every stage of human development, from fetal growth to senescence. In addition to being a prevalent cell membrane component, LC n-3 PUFA have also been associated with relief of depression, bipolar disorder and Crohn's disease. Long chain n-3 PUFA are not one trick ponies as they have been linked to positive benefits in widely varying biological processes. Additionally, DHA has shown an important role in fetal development as an improved DHA status in the mother will positively affect cognition in offspring. DHA also is involved in visual acuity of the retina. Another important unsaturated FA, EPA, is a precursor for anti-

inflammatory mediators associated with combat of inflammatory diseases (Gil & Gil, 2015). Since the aforementioned FA are involved with important processes in human health, the production of a FA fortified chicken egg would show beneficial health advantages for human consumption.

FA Profile Manipulation in Chicken Eggs

One of the first studies to investigate the effect of dietary fat on the composition of egg yolks was performed in the 1930s. The investigation was able to conclude that the addition of unsaturated oils to the diet of the hens would produce a change in the fat composition of egg yolks. Specifically, the study specifically found that by the addition of fat to the diet through hempseed increased iodine value, indicating more unsaturated fatty acids of the egg yolk (Cruickshank, 1934).

Previous investigations into the manipulation of chicken egg FA profiles have been completed with the goal of producing a functional food with increased n-3 PUFA levels. Changes and enhancements to the diets of hens have yielded alternation of egg FA profiles. One study produced a five-fold increase of egg yolk n-3 PUFA content by supplementing a commercial chicken diet with either cod liver oil, canola oil, or linseed oil. Another study produced a thirty-fold increase in linolenic acid and a four-fold increase in DHA with dietary flaxseed feeding. Increases in egg yolk n-3 PUFA have also been produced with dietary fish oil or flaxseed supplementation (Lewis, Seburg, & Flanagan, 2000). A previous investigation into the effects of flaxseed supplementation yielded an increase in alpha-linolenic acid content of egg yolk, a decrease in the n-6 FA arachidonic acid, and an increase of n-3 FA compared to n-6 FA with increasing flaxseed supplementation (Imran et al., 2015).

Fatty Acid Incorporation into Egg Yolk

De novo lipogenesis does not occur in the ovary, therefore all the lipids, including FA, present in egg yolk must come from diet or synthesis in the liver with subsequent transport from the liver to the ovary (Hermier, 1997). Very low density lipoproteins (VLDL) are triglyceride rich particles and the VLDLy subclass is the lipoproteins targeted for incorporation into the egg yolk by receptor mediated endocytosis (Walzem, Hansen, Williams, & Hamilton, 1999). Very low density lipoproteins are synthesized and secreted by the liver with assembly starting at the endoplasmic reticulum and ending at the Golgi apparatus. Triglycerides from the liver are preferentially packaged into VLDL and cholesterol from peripheral tissues is preferential for HDL. Overall, lipoprotein metabolism is effected by synthesis in liver and uptake from vasculature for storage in adipose. De novo hepatic FA synthesis is dependent upon the level of carbohydrates in the diet to provide the acetyl-CoA precursor for fatty acid synthesis and regulation by metabolic hormones. The liver is also capable of elongating dietary FA to change their length and saturation, whereas hepatic oxidation of FA breaks down FA (Hermier, 1997).

Nutritional and Health Benefits of Eggs

Eggs are one of the most prevalent animal product consumed by humans. This fact can be attributed to the efficiency and renewable qualities of egg production as well as the multitude of culinary ways in which eggs can be prepared for consumption. Largely composed of lipids and protein and low in carbohydrates, eggs are an excellent source of highly bioavailable protein with a nearly ideal amino acid profile for humans (Surai & Sparks, 2001). In addition, egg proteins have been shown to have larger satiety effects than other protein sources. Consumption of eggs for breakfast, instead of a high carbohydrate meal, has been shown to reduce caloric

intake and contribute to longer term weight reduction (McNamara, 2015). Eggs are also a good source of vitamins and minerals and are an excellent source of choline, which is important in fetal and neonatal brain development and has an association of decreasing breast cancer occurrence and mortality. Insufficient choline intake during pregnancy has been linked to occurrences of spina bifida. In addition, it has been shown that nine out of ten Americans are choline deficient (McNamara, 2015). Observational studies have shown that the vitamin A from egg consumption is preventative for two vitamin A deficiencies, night blindness and xerophthalmia. Eggs also contribute important minerals to the diet, including iodine, and eggs consumption contributes to maintaining normal levels. Eggs are high in selenium which is important in immune function, has antioxidant properties, and is a factor in some epigenetic mechanisms. Egg yolk consumption also benefits both zinc and iron absorption in situations where absorption of one or the other could be limited. Due to their high vitamin and mineral status, eggs can be key contibutors to a nutritious diet in developing countries (Iannotti, Lutter, Bunn, & Stewart, 2014).

Antioxidants are also available in eggs. There are a multitude of antioxidant compounds in the yolk including: ovotransferrin, which has a superoxide scavenging ability, ovomucin which inhibits hydrogen peroxide stress in the embryonic kidney, lysozyme, which decreases reactive oxygen species activity, and cytastin, which acts in cellular antioxidant pathways (Nimalaratne & Wu, 2015)

Chapter 3

MATERIALS AND METHODS

Birds and Experimental Diets

A total of 45 white leghorn hens were used in the study with eight hens per treatment group and nine hens serving as controls. The hens used in the study were selected based on their egg production and egg weights before the start of the experiment. The hens were individually housed in battery cages throughout the duration of the study. The experimental group of 36 hens were fed a diet that was low in linoleic acid and nutritionally balanced with de-germinated corn meal, high oleic soybean meal, and no corn distillers grains before the start of the experiment. The study consisted of four distinct time periods of twenty-one days each. The split plot design (Table 1) of the experiment consisted of four levels (0%, 1%, 2%, 4%) of high oleic soybean oil (HOSO; Perdue Agribusiness) and four levels (0%, 1%, 2%, 4%) of high omega-3 oil [high ALA flaxseed oil (FLAX) containing 65% ALA; Polar Food Inc.]. Period 1 started with four groups of eight hens each fed the four distinct levels of HOSO and 0% FLAX. Dietary FLAX was increased to 1% in Period 2, 2% in Period 3, and 4% in Period 4. HOSO levels did not change within group throughout the experiment.

Feed intake and egg weights were taken each day of the experiment. Egg samples were collected on the last day (day 21) of each of the four periods. These egg samples were analyzed for shell, albumin, and yolk weights. Further investigation into the yolk composition was also completed. At the end of the study, the hens were euthanized and samples of adipose, muscle, and liver were taken. These samples further analyzed for FA content.

Table 1. Experimental design testing interaction of diet supplementation with high oleic soybean oil (HOSO) and a high n-3 flaxseed oil (FLAX).

Dietary Sequence	Period 1 21 d	Period 2 21 d	Period 3 21 d	Period 4 21 d
1	0% HOSO + 0% FLAX	0% HOSO + 1% FLAX	0% HOSO + 2% FLAX	0% HOSO + 4% FLAX
2	1% HOSO + 0% FLAX	1% HOSO + 1% FLAX	1% HOSO + 2% FLAX	1% HOSO + 4% FLAX
3	2% HOSO + 0% FLAX	2% HOSO + 1% FLAX	2% HOSO + 2% FLAX	2% HOSO + 4% FLAX
4	4% HOSO + 0% FLAX	4% HOSO + 1% FLAX	4% HOSO + 2% FLAX	4% HOSO + 4% FLAX

Fatty Acid Analysis

Egg yolk, liver, and muscle samples were directly methylated in a dual methylation procedure using 0.5 M sodium methoxide in methanol at 50°C for 10 min followed by 5% methanolic HCl at 80°C for 10 min (Kramer et al., 1997). Methyl tridecenoate and nonadecanoate acid was used as internal standards (NuChek Prep Inc., Elysian, MN). Fatty acid methyl esters were quantified as described above with a modified temperature program (Initial 70°C, increased 8°C/min to 110°C, increased 4°C/min to 165°C for 20 min, increased 2.5°C/min to 215°C, and held for 20 min). Inlet and detector temperatures were 250°C.

Statistical Analysis

Data was analyzed as a split plot design in JMP (version 12; SAS Institute, Cary, NC). The model included the fixed effect of level of HOSO, FLAX, and their interaction. Data points with Studentized residuals outside of ± 3.0 were considered outliers and excluded from analysis. A protected LSD was used for mean separation.

Chapter 4

RESULTS

This experiment used varying levels of dietary supplemental FLAX and HOSO to test FA incorporation into egg yolks through dietary manipulation. Despite the widely-varied FA concentration and profile of the experimental diets, the birds perform well on all treatments (Table 2). Egg production ranged from 84.13% to 87.30% of the days for the varying levels of HOSO. Egg production showed a small decrease at the highest level of FLAX, where egg production dropped to 82.94% of the days; this was a 3.44 to 5.02 percentage unit decrease from the other levels of FLAX. Egg weight did not change over the different diets and ranged from 61.27 to 62.12 g with the varying HOSO levels and 61.08 to 62.85 g with the varying FLAX levels. Feed intake was from 98.1 g/day to 99.9 g/day at the different HOSO levels and from 97.8 g/day to 11.5 g/day on the different FLAX levels. Only two variations were seen in egg composition with the changing diets (Table 3). There was an increase in the proportion of albumin at 2% and 4% HOSO with albumin levels of 65.0% and 65.3% of the egg, respectively. A decrease in the shell as a proportion of the egg was observed at 4% HOSO with 8.41% of the egg being shell, a 0.17 to 0.38 percentage unit decrease from the other levels of HOSO.

The amount of fatty acid in 144 egg yolks was determined by gas chromatography with flame ionization detections with dual internal standards (13:0 and 19:0). The fatty acid content was measured in units of mg/yolk (Table 4). HOSO and FLAX were shown to have an interaction on the amount of FA incorporated into the egg yolk for C18:3 n-3, C20:5 n-3, C22:5 n-6, and total VLC n-3. For C18:3 n-3, the largest incorporation was 474 mg/yolk at 4% FLAX and 0% HOSO and the smallest amount was 15.2 mg/yolk at 0% FLAX and 2% HOSO. The

highest amount of C20:5 n-3 was 11.8 mg/yolk incorporated at 4% FLAX and 1% HOSO; the lowest amount was 0.17 mg/yolk incorporated at 0% FLAX and 4% HOSO. Although C22:5 n-3 and C22:6 n-3 did not show interactions between HOSO and FLAX, the highest amount was incorporated when feeding higher FLAX and lower HOSO. Specifically, C22:6 n-3 incorporation was the highest, 99.8 mg/yolk, at 2% FLAX and lowest, 38.3 mg/yolk, at 0% FLAX. The total VLC n-3 FA showed an interaction between HOSO and FLAX with the highest levels of n-3 VLC FA incorporated at 2% FLAX and 0% HOSO (133.2 mg/yolk) and the lowest level of incorporation was at 0% FLAX and 0% HOSO (40.7 mg/yolk).

The highest level of incorporation for C22:5 n-6 (21.05 mg/yolk) was seen at 0% HOSO and 0% FLAX. No C22:5 n-6 was incorporated at 2% and 4% FLAX with any level of HOSO. An interaction between HOSO and FLAX was not observed for C18:2 n-6; more of the FA was incorporated at high levels of FLAX and low levels of HOSO. No interaction was seen for C20:4 n-6, with the highest incorporation (89.8 mg/yolk) at 0% FLAX and the lowest incorporation (42.7 mg/yolk) was at 4% FLAX.

For C16:0, a gradual decrease in incorporation was seen with increasing amount of HOSO and increasing amount of FLAX, although an interaction between the two was not observed. Increasing levels of HOSO was correlated with a decrease in the amount of C16:1 per egg yolk and decreasing levels of FLAX showed an increase in C16:1. For C18:0, increasing levels of HOSO were correlated with a decrease in the FA and increasing levels of FLAX were correlated with an increase. Increasing HOSO and decreasing FLAX corresponded with an increase in C18:1 c-9, despite the fact that there was not a significant interaction between HOSO and FLAX. At 0% HOSO 1996 mg/yolk of C18:1 c-9 was incorporated and this increased to 2297 mg/yolk at 4% HOSO.

Liver FA profile was invested because fatty acids incorporated into eggs originate from the liver and the liver is also the site of elongation and desaturation. For liver tissue, variation in FA level with 4% FLAX and varying levels of HOSO, 0%, 1%, 2%, and 4%, was seen for C16:0, C16:1 cis-9, C17:0, C18:1 cis-9, C18:3 n-3, C18:4 n-3, C20:1 cis-11, C20:5 n-3, and C22:5 n-3 (Table 5). In general, the n-3 FA, C18:3 n-3, C18:4 n-3, C20:5 n-3, and C22:5 n-3, had the highest level of fatty acid in the liver tissue with 0% HOSO. At 0% HOSO, C18:3 n-3 composed 6.83% of the liver FA; this value decreased as supplementary HOSO was added to the diet. C18:4 n-3 follows the same pattern with 0.04% of the liver FA at 0% HOSO. C20:5 n-3 composed 0.42% of liver FA at 4% HOSO and increased at lower levels of HOSO. C18:2 c-9 composed 38.77% of the liver FA at 4% HOSO, this value decreased for the other three HOSO treatment levels ranging from 31.35% to 34.16% liver FA.

Table 2. Production parameters of hens fed varying supplemental amounts of high oleic soybean oil (HOSO) and high n-3 flaxseed oil (FLAX).

Egg Production[d], %				HOSO				
Oil Level	HOSO	FLAX	FLAX	0%	1%	2%	4%	SE
0%	87.30	87.96[a]	0%	86.24	91.53	87.30	84.13	2.41
1%	85.58	87.57[a]	1%	91.53	87.30	84.13	87.83	2.41
2%	84.13	86.38[a]	2%	87.30	84.13	87.83	85.19	2.41
4%	87.83	82.94[b]	4%	84.13	87.83	85.19	85.71	2.41
SE	1.20	1.20	SE	2.41	2.41	2.41	2.41	
P-value	0.12	0.02	Int. *P* =	0.715				
Egg Weight[d], g				HOSO				
Oil Level	HOSO	FLAX	FLAX	0%	1%	2%	4%	SE
0%	62.01	61.08	0%	61.32	61.61	61.70	63.39	1.22
1%	61.75	61.35	1%	61.61	61.70	63.39	60.97	1.22
2%	62.12	61.88	2%	61.70	63.39	60.97	61.24	1.22
4%	61.27	62.85	4%	63.39	60.97	61.24	62.17	1.22
SE	0.61	0.61	SE	1.22	1.22	1.22	1.22	
P-value	0.77	0.19	Int. *P* =	0.99				
Feed Intake, g/d				HOSO				
Oil Level	HOSO	FLAX	FLAX	0%	1%	2%	4%	SE
0%	98.08	99.23	0%	96.56	98.92	99.35	97.47	3.18
1%	99.65	100.46	1%	98.92	99.35	97.47	97.23	3.18
2%	99.86	98.32	2%	99.35	97.47	97.23	101.13	3.18
4%	98.19	97.76	4%	97.47	97.23	101.13	98.89	3.18
SE	1.59	1.59	SE	3.18	3.18	3.18	3.18	
P-value	0.79	0.65	Int. *P* =	0.8994				
Feed Efficiency, Egg/Feed				HOSO				
Oil Level	HOSO	FLAX	FLAX	0%	1%	2%	4%	SE
0%	1.82[b]	1.85	0%	1.83	1.76	1.85	1.83	0.06
1%	1.9[a]	1.88	1%	1.76	1.85	1.83	1.82	0.06
2%	1.92[a]	1.85	2%	1.85	1.83	1.82	1.96	0.06
4%	1.83[b]	1.89	4%	1.83	1.82	1.96	1.88	0.06
SE	0.030	0.030	SE	0.06	0.06	0.06	0.06	
P-value	0.0423	0.7815	Int. *P* =	0.57				

Table 3. Composition of eggs from hens fed varying amounts of high oleic soybean oil (HOSO) and high n-3 flaxseed oil (FLAX).

Yolk, % of egg				HOSO				
Oil Level	HOSO	FLAX	FLAX	0%	1%	2%	4%	SE
0%	26.95	26.54	0%	27.16	26.74	27.30	26.61	0.56
1%	27.02	26.65	1%	26.74	27.30	26.61	26.82	0.52
2%	26.23	26.78	2%	27.30	26.61	26.82	26.63	0.52
4%	26.28	26.52	4%	26.61	26.82	26.63	27.04	0.56
SE	0.27	0.27	SE	0.56	0.56	0.52	0.56	
P-Value	0.06	0.89	Int. *P* =	0.8789				
Albumin, % of egg				HOSO				
Oil Level	HOSO	FLAX	FLAX	0%	1%	2%	4%	SE
0%	64.47[b]	64.72	0%	64.05	64.57	64.28	64.97	0.57
1%	64.21[c]	64.68	1%	64.57	64.28	64.97	64.42	0.53
2%	65.00[a]	64.68	2%	64.28	64.97	64.42	64.36	0.53
4%	65.31[a]	64.91	4%	64.97	64.42	64.36	64.29	0.57
SE	0.27	0.00	SE	0.57	0.57	0.53	0.57	
P-Value	0.02	0.92	Int. *P* =	0.9461				
Shell, % of egg				HOSO				
Oil Level	HOSO	FLAX	FLAX	0%	1%	2%	4%	SE
0%	8.58[a]	8.74	0%	8.79	8.70	8.42	8.42	0.18
1%	8.77[a]	8.68	1%	8.70	8.42	8.42	8.76	0.17
2%	8.77[a]	8.54	2%	8.42	8.42	8.76	9.01	0.17
4%	8.41[b]	8.58	4%	8.42	8.76	9.01	8.66	0.18
SE	0.09	0.09	SE	0.18	0.18	0.17	0.18	
P-Value	0.01	0.38	Int. *P* =	0.637				

Table 4. Amount of selected fatty acids per egg yolk of hens feed varying supplemental amounts of high oleic soybean oil (HOSO) and high n-3 flaxseed oil (FLAX).

Fatty acid, mg/yolk

C16:0				HOSO				
Oil Level	HOSO	FLAX	FLAX	0%	1%	2%	4%	SE
0%	1282[a]	1253[a]	0%	1287	1327	1241	1157	37.4
1%	1280[a]	1249[a]	1%	1335	1295	1249	1119	33
2%	1185[b]	1213[a]	2%	1305	1245	1178	1122	33
4%	1103[c]	1134[b]	4%	1200	1251	1073	1012	35
SE	17.31	17.55	SE	37.4	35	33	35	
P-Value	<0.001	<0.001	Int. *P* =	0.52				

C16:1				HOSO				
Oil Level	HOSO	FLAX	FLAX	0%	1%	2%	4%	SE
0%	153[a]	145[a]	0%	161.7	161.46	146.72	110.71	7.15
1%	139[b]	132[b]	1%	165.15	144.07	126.08	93.18	6.31
2%	121[c]	125[b]	2%	163.12	130.81	115.66	91.04	6.31
4%	92[d]	103[c]	4%	122.51	120.6	95.76	72.65	6.69
SE	3.31	3.36	SE	7.15	6.69	6.31	6.69	
P-Value	<0.001	<0.001	Int. *P* =	0.23				

C18:0				HOSO				
Oil Level	HOSO	FLAX	FLAX	0%	1%	2%	4%	SE
0%	454[a]	395[b]	0%	427	427	373	354	16.7
1%	441[a]	406[b]	1%	450	426	394	354	14.8
2%	386[b]	412[b]	2%	458	430	385	375	14.8
4%	366[b]	433[a]	4%	480	480	392	380	15.7
SE	7.75	7.86	SE	16.7	15.7	14.8	15.7	
P-Value	<0.001	<0.01	Int. *P* =	0.78				

C18:1 cis-9				HOSO				
Oil Level	HOSO	FLAX	FLAX	0%	1%	2%	4%	SE
0%	1996[c]	2201[a]	0%	1960	2239	2257	2348	75.3
1%	2197[b]	2226[ab]	1%	2044	2185	2363	2313	66.4
2%	2236[ab]	2189[ab]	2%	2046	2138	2229	2342	66.4
4%	2297[a]	2111[b]	4%	1936	2227	2095	2184	70.4
SE	34.85	35.34	SE	75.3	70.4	66.4	70.4	
P-Value	<0.001	0.1	Int. *P* =	0.48				

C18:2 n-6				HOSO				
Oil Level	HOSO	FLAX	FLAX	0%	1%	2%	4%	SE
0%	541[a]	420[c]	0%	462	417	399	403	22
1%	528[a]	514[b]	1%	545	524	498	487	19.4
2%	497[b]	531[b]	2%	542	551	535	496	19.4
4%	477[b]	578[a]	4%	616	620	554	524	20.5
SE	10.17	10.31	SE	22	20.5	19.4	20.5	
P-Value	<0.001	<0.001	Int. *P* =	0.63				

C18:3 n-3				HOSO				
Oil Level	HOSO	FLAX	FLAX	0%	1%	2%	4%	SE
0%	232[a]	16[d]	0%	16.55	15.83	15.18	16.48	11.29
1%	205[b]	129[c]	1%	150.94	135.97	124.54	105.22	9.96
2%	180[c]	228[b]	2%	285.39	231.47	215.28	178.49	9.96
4%	150[d]	394[a]	4%	474.42	437.36	364.89	298.77	10.56
SE	5.23	5.3	SE	11.29	10.56	9.96	10.56	
P-Value	<0.001	<0.001	Int. *P* =	<0.001				

C20:4 n-6				HOSO				
Oil Level	HOSO	FLAX	FLAX	0%	1%	2%	4%	SE
0%	58.4	89.8[a]	0%	89.77	91.18	89.77	88.38	3.24
1%	62.7	63.3[b]	1%	59.83	62.45	65.5	65.53	2.86
2%	62.6	51.0[c]	2%	44.32	51.33	53.09	55.14	2.86
4%	62.9	42.7[d]	4%	39.87	45.83	42.18	42.74	3.03
SE	1.5	1.52	SE	3.24	3.03	2.86	3.03	
P-Value	0.1	<0.001	Int. *P* =	0.71				

C20:5 n-3				HOSO				
Oil Level	HOSO	FLAX	FLAX	0%	1%	2%	4%	SE
0%	6.33[a]	0.41[d]	0%	0.48	0.53	0.45	0.17	0.45
1%	5.92[a]	3.90[c]	1%	4.76	4.34	3.81	2.71	0.4
2%	4.9[b]	6.74[b]	2%	8.83	7.04	6.09	5.01	0.4
4%	3.89c	9.99a	4%	11.26	11.76	9.26	7.68	0.43
SE	0.21	0.21	SE	0.45	0.43	0.4	0.43	
P-Value	<0.001	<0.001	Int. *P* =	<0.001				

C22:5 n-6				HOSO				
Oil Level	HOSO	FLAX	FLAX	0%	1%	2%	4%	SE
0%	5.82[a]	18.12[a]	0%	21.05	20.13	16.29	14.99	0.74
1%	5.57[a]	2.04[b]	1%	2.24	2.15	1.75	2.03	0.65
2%	4.51[b]	0[c]	2%	0	0	0	0	0.65
4%	4.25[b]	0[c]	4%	0	0	0	0	0.69
SE	0.34	0.35	SE	0.74	0.69	0.65	0.69	
P-Value	<0.01	<0.001	Int. *P* =	<.0001				

C22:5 n-3				HOSO				
Oil Level	HOSO	FLAX	FLAX	0%	1%	2%	4%	SE
0%	12.10[a]	3.67[d]	0%	3.8	3.69	3.72	3.46	0.87
1%	10.34[b]	9.92[c]	1%	12.26	10.27	9.73	7.41	0.77
2%	9.66[b]	12.51[b]	2%	15.8	12.28	11.75	10.22	0.77
4%	8.20[c]	14.19[a]	4%	16.55	15.1	13.45	11.69	0.81
SE	0.4	0.41	SE	0.87	0.81	0.77	0.81	
P-Value	<0.001	<0.001	Int. *P* =	0.09				

C22:6 n-3				HOSO				
Oil Level	HOSO	FLAX	FLAX	0%	1%	2%	4%	SE
0%	84.23	38.30[c]	0%	36.02	37.34	37.81	42.02	4.14
1%	82.61	92.04[b]	1%	95.57	92.27	93.35	86.98	3.65
2%	79.23	99.83[a]	2%	106.48	98.84	96.88	97.11	3.65
4%	78.98	94.89[ab]	4%	98.83	102.01	88.89	89.82	3.87
SE	1.92	1.94	SE	4.14	3.87	3.65	3.87	
P-Value	0.14	<0.001	Int. *P* =	0.25				

Total VLC n-3				HOSO				
Oil Level	HOSO	FLAX	FLAX	0	1	2	4	SE
0	104.5[a]	42.5[a]	0	40.7	41.8	42	45.7	4.54
1	100.2[ab]	106.6[a]	1	113.8	107.7	107.4	97.4	4.28
2	94.8[bc]	120.7[b]	2	133.2	119.7	116.1	113.6	4.28
4	92.0[c]	121.7[c]	4	130.5	131.4	113.7	111.2	4.54
SE	2.17	2.17	SE	4.28	4.54	4.28	4.54	
P	<0.001	<0.001	Int. P =	0.04				

Table 5. Liver FA concentration of hens fed varying amounts of high oleic soybean oil (HOSO) and high n-3 flaxseed oil (FLAX).

Diet	Means					
HOSO	0%	1%	2%	4%		
FLAX	4%	4%	4%	4%	SE	*P*-Value
Total FA, %	4.91	5.81	4.98	6.07	0.52	0.27
FA profile, % FA						
C14:0	0.25	0.27	0.22	0.24	0.01	0.10
C14:1, cis-9	0.03	0.03	0.02	0.01	0.01	0.06
C15:0	0.07	0.06	0.07	0.07	0.00	0.88
C16:0	21.79[a]	21.29[a]	20.21[b]	19.68[b]	0.31	<0.001
C16:1, cis-9	1.84[a]	1.63[a]	1.23[b]	1.1[b]	0.13	<0.001
C17:0	0.19[d]	0.23[c]	0.28[b]	0.34[a]	0.01	<0.001
C18:0	13.09	12.80	13.09	12.10	0.44	0.35
C18:1, cis-9	31.35[b]	34.08[b]	34.16[b]	38.77[a]	1.31	<0.01
C18:1, cis-11	1.18	1.16	1.09	1.11	0.05	0.53
C18:2 n-6	13.41	13.43	13.22	12.52	0.61	0.68
C18:3 n-6	0.07	0.06	0.06	0.06	0.00	0.19
C18:3 n-3	6.83[a]	5.32[b]	5.56[b]	4.46[b]	0.40	<0.01
C18:4 n-3	0.04[a]	0.03[b]	0.02[b]	0.01[b]	0.00	<0.01
C20:0	0.08	0.07	0.08	0.08	0.01	0.57
C20:1, cis-11	0.13[b]	0.13[b]	0.15[a]	0.17[a]	0.01	<0.001
C20:2 n-6	0.18	0.18	0.18	0.17	0.01	0.75
C20:3 n-6	0.68	0.64	0.72	0.67	0.07	0.83
C20:3 n-3	0.00	0.00	0.00	0.00	0.00	0.00
C20:4 n-6	2.69	2.90	3.39	2.92	0.29	0.38
C20:4 n-3	0.05	0.04	0.04	0.03	0.00	0.11
C20:5 n-3	0.62[a]	0.55[a]	0.59[a]	0.42[b]	0.05	0.03
C22:0	0.16	0.15	0.18	0.15	0.01	0.55
C22:1	0.16	0.13	0.14	0.12	0.01	0.10
C22:4	0.08	0.08	0.09	0.08	0.01	0.54
C22:5 n-3	0.55[a]	0.39[b]	0.43[b]	0.35[c]	0.03	<0.001
C22:5 n-6	0.00	0.00	0.00	0.00	0.00	0.41
C22:6 n-3	3.68	3.24	3.77	3.48	0.28	0.56
C24:0	0.05	0.05	0.06	0.05	0.00	0.41
C24:1	0.08	0.08	0.09	0.07	0.01	0.18
Others	0.69[b]	0.97[b]	0.85[a]	0.77[a]	-	-

Chapter 5

DISSCUSSION

This investigation accomplished its intent to investigate the effect of varying dietary HOSO and FLAX levels on FA incorporation into egg yolks. The experimental diets were in part successful because the diets were palatable and energetically suitable for the hens as shown by feed intake and egg production. Previous experimental high n-3 FA diets concur with this result, showing unchanged egg laying patterns (Baeza et al., 2015).

There are different critical control points related to the fate of the dietary FA. These control points include: FA absorption in the intestines, the alternative being excretion in the feces, FA transportation to different tissues such as liver, muscle, and adipose, and FA oxidation, oxidation within tissue, recycling from non-hepatic tissues, elongation and desaturation in the liver, and lipoprotein packaging in the liver (Figure 1).

Oleic acid, C18:1 c-9, in the egg yolk increased with high HOSO and low FLAX. This suggests that the dietary oleic acid was able to out compete other FA at the level of intestinal absorption or at packaging into VLDLy particles destined for the yolk. This finding shows that manipulation of the dietary FA ratios changes the ratio of the FA that are ultimately absorbed, processed in the liver, and packaged in the egg yolk.

Omega-3 FA showed increased prevalence in egg yolks when FLAX was at a higher percentage in the diet. The omega-3 FA increase has also previously been shown in other diets supplemented with flaxseed (Goldberg, Ryland, Aliani, & House, 2016; Lee et al., 2016) . The n-3 FA were outcompeted at one of the various points of regulation when HOSO was also high in the diet. Omega-3 FA such as, ALA (C18:3 n-3) and EPA (C20:5 n-3), showed that incorporation was affected by an interaction between the dietary levels of FLAX and HOSO with

the highest levels near the high FLAX and low HOSO end of the spectrum. Although, C22:5 n-3 and DHA (C22:6 n-3) did not have effects due to an interaction between FLAX and HOSO, their highest level of incorporation was also near the high FLAX and low HOSO end of the spectrum. A four-fold increase in DHA was shown by Lewis in a diet with supplemental flaxseed (N. M. Lewis, 2000). The concentration of various n-3 FA was also higher in the liver when HOSO was not supplemented in the diet. The presence of high n-3 FA in both the egg yolks and the liver when FLAX was high in the diet suggests that the ALA in the FLAX was sufficiently absorbed in the intestines, desaturated and elongated in the liver, packaged in yolk specific LDL, and exported to the ovaries (Figure 2).

Figure 1. Metabolic and excretory pathways of FA from dietary ingestion.

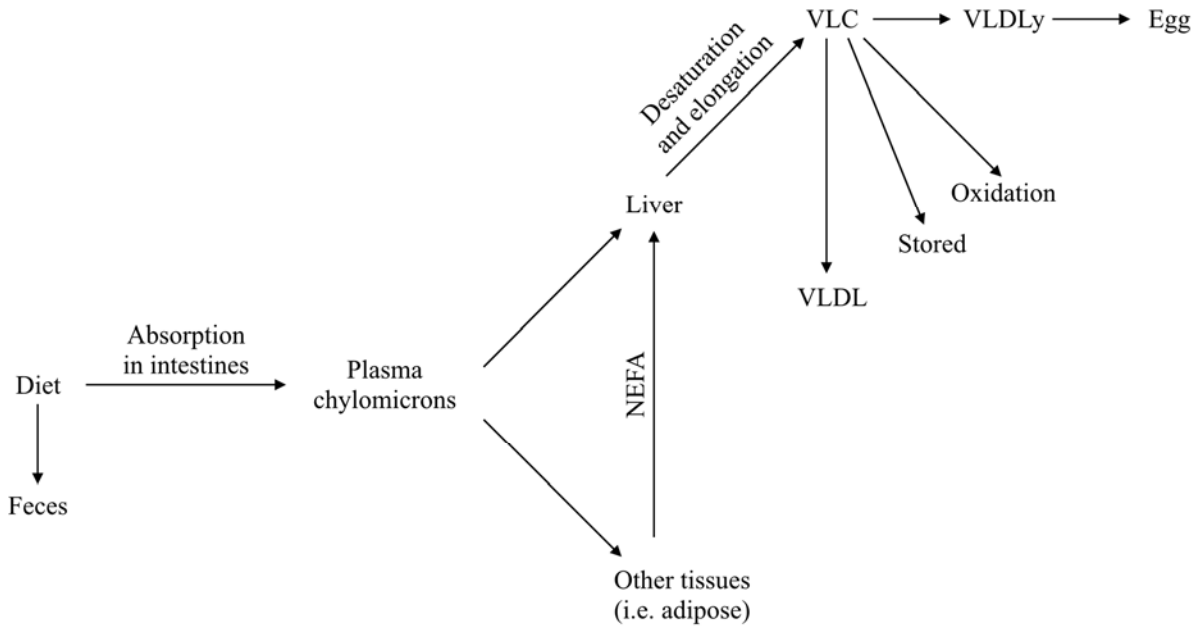
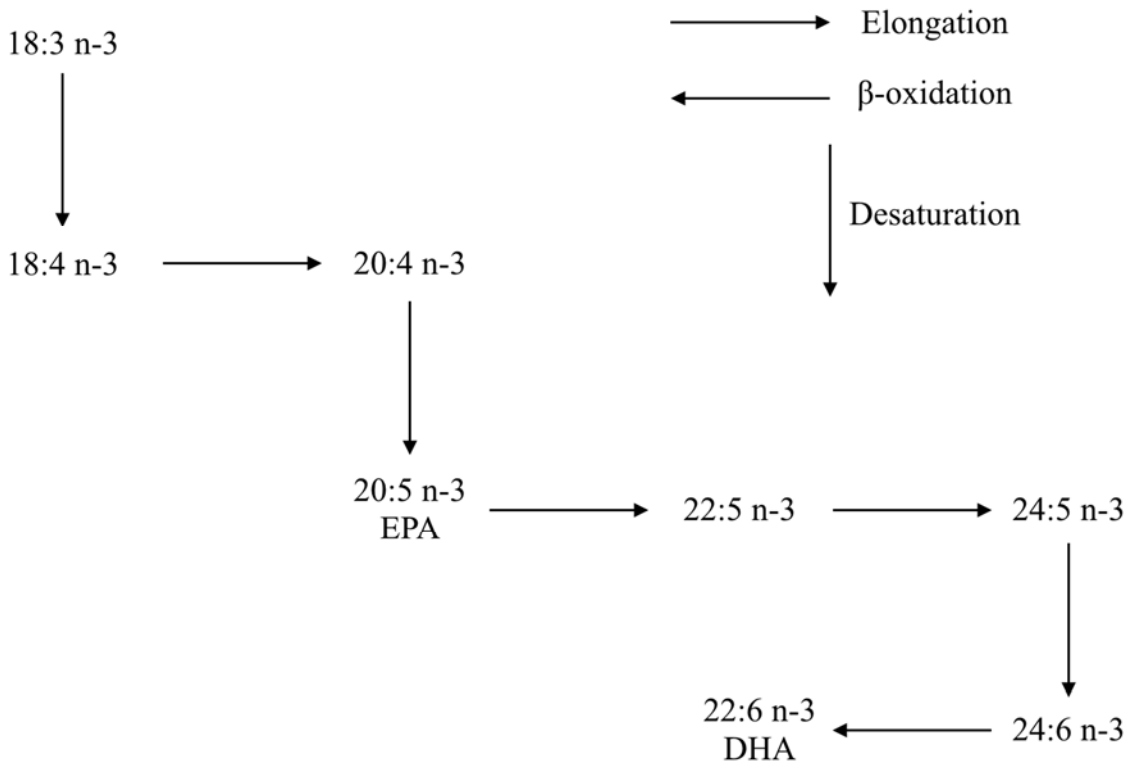


Figure 2. Elongation, desaturation, and β-oxidation of alpha-linoleic acid (Tvrzicka, 2011).



Chapter 6

CONCULSIONS

The goal of altering FA composition of egg yolks by dietary oils was accomplished to produce an egg high in n-3 and n-9 FA while low in n-6 FA. It was also shown that although high HOSO increases the oleic acid content of the yolks, the oleic acid appears to have competed with n-3 FA for incorporation into the egg yolk at one or multiple critical points. Further research analyzing desaturase and elongase gene expression in the liver would allow better understanding of which critical point or points are more controlling of FA deposition into the egg yolk.

Chapter 7

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ACADEMIC VITA

Academic Vita of Alexandra Nicole Kukorowski
akukorowski@gmail.com

Education

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

University of Pennsylvania School of Veterinary Medicine
Matriculation: Fall 2017

Honors and Awards

Pennsylvania State University Scholarships
- Schreyer Honors College Academic Excellence Scholarship
- Provost Award
- Oswald Scholarship
- Kenton D. Rexford Honors Scholarship
- Claire and Charles Miller Memorial Scholarship

Association Memberships and Activities

Pre-Vet Club
- Treasurer: 2015-2017
- Stay Over Chair: 2015-2016
- Stay Over Committee Member: 2014-2015
- Pre-Vet Symposium Committee Member: 2014-2015

Coaly Society
- Outstanding Senior Contest Co-Chair: 2015-2016
- Member: 2015-2017

Penn State Equestrian Team
- Member: 2014-2017

GLOBE Special Living Option
- Resident: 2014-2016

Professional Experience

Easton Veterinary Clinic and Rehabilitation Center, Technician Assistant: 2015-Present
Equine Practice of Dr. T. R. Corkran, Intern: 2010-2015