ABSTRACT

BROWN, CHRISTEN LEANN. Production of Value-Added Pork by Enrichment with Omega-3 Fatty Acids. (Under the direction of Eric van Heugten and Jack Odle.)

This study evaluated the impact of dietary docosahexaenoic acid (DHA) and α-linolenic acid (ALA) on enrichment of n-3 fatty acids in pork when supplemented for the last 4 or 8 weeks of the finisher period. Diets consisted of a corn-soybean meal based control and the control supplemented with either 1.5% or 3.0% DHA-Gold (Novus International, St. Louis MO), or with 1.04% flax seed oil (Jedwards International, Quincy, MA). The oils were substituted for a saturated fat source (Fat Pack 100, Milk Specialties, Dundee, IL) so that energy density remained constant. The 1.5% and 3.0% DHA-Gold diets were formulated to contain 0.27 and 0.54% DHA respectively, whereas the flax oil diet contained 0.54% ALA. Pigs (n=40, 67.7 kg ± 1.1 kg) were housed individually and given ad libitum access to feed and water. Pigs were slaughtered at 115.6 kg ± 5.4 kg BW and a 2.5 cm thick loin chop, belly, and ham samples were collected from each pig. Chops were trimmed into retail cuts and all samples were subsequently ground to obtain a homogeneous 1 g sample. Fat was extracted and fatty acid concentrations were determined using liquid-gas chromatography. The fat supplements did not affect daily weight gain (0.90 ± 0.15 kg), feed intake (2.77 ± 0.65 kg) or feed efficiency (G/F = 0.36 ± 0.06) during the last 4 weeks (P > 0.05), but did alter pork loin fatty acid composition in a time-dependant manner (diet & duration effects, P < 0.05). Target enrichment of 32 mg/100 g in loin would require consumption of 0.12% DHA for 8 weeks or 0.23% DHA for 4 weeks. In ham consumption of 0.20% DHA for 8 weeks or 0.40% DHA for 4 weeks and in belly tissue consumption of
0.056% DHA for 8 weeks or 0.12% DHA for 4 weeks is required for target enrichment. Backfat tissue enrichment was achieved in both 4 weeks and 8 weeks. We conclude that pork tissue content of n-3 fatty acids can be markedly enriched in as little as four weeks of supplementation during the late finisher phase. Furthermore, DHA was enriched more efficiently than ALA, and ALA supplementation did not alter DHA, suggesting limited elongation/desaturation.
Production of Value-Added Pork by Enrichment with Omega-3 Fatty Acids

by
Christen Leann Brown

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APPROVED BY:

__________________________  ___________________________
Dr. Eric van Heugten      Dr. Jack Odle
Chair of Advisory Committee  Co-chair of Advisory Committee

__________________________  ___________________________
Dr. Jonathan Allen      Dr. Lin Xi
BIOGRAPHY

Christen Leann Brown was born May 11, 1987 in Salisbury, NC. She graduated from East Rowan High School in 2005. She received a Bachelor of Science degree in Nutrition and Animal Science from North Carolina State University in 2009. In the Fall of 2009, she re-enrolled at North Carolina State University to obtain Master of Science degrees in Nutrition and Animal Science.
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CHAPTER I

REVIEW OF LITERATURE

*Description and metabolism of fatty acids*

Essential fatty acids have only recently been considered for supplementation in animal nutrition due to the current interest in enrichment of animal products for human nutraceuticals. Sunflower, cottonseed and soybean oil can contain 53-56% of linoleic acid while rapeseed and linseed oils can contain 11-57% α-linolenic acid (Kamal-Eldin et al., 1997). Omega-3 polyunsaturated fatty acids, such as EPA and DHA, are predominately found in fish and can range from 0.02-1.83 g/3-oz serving (Kris-Etherton et al., 2003).

Chemical composition of triglycerides is a parameter that can influence digestibility of fats. Fatty acids are the simplest lipids and have a general form of R-COOH, where R designates a hydrocarbon chain. Such lipids have many biological functions ranging from membrane components, intracellular storage molecules and thermal insulation and padding. Fatty acids can differ in length of their hydrocarbon tail, degree of unsaturation, and positions of double bonds. Fatty acids with a double bond are considered unsaturated and those without double bonds are saturated. Three fatty acids attach to a glycerol backbone and form a triglyceride. The majority of unsaturated fatty acids are attached at the two position of the glycerol backbone in most fish (Carlier et al., 1991). Positions one and three on the glycerol backbone are different due to their ability to more readily interact with enzymes (Horton et al., 2002). Availability of polyunsaturated fatty acids determines their overall utilization and
thus regulates digestion and absorption. Triglycerides make up 96% of human dietary lipids which approximates 80-120 grams per day (Carlier et al., 1991). The abundance of certain fatty acids depends on the type of organism, type of organ and food source. Oleate (18:1), palmitate (16:0), and stearate (18:0) are the most abundant fatty acids in animals. Animals require certain fatty acids that cannot be synthesized (Hortan, 2002). Linoleic and α-linolenic acid are essential fatty acids and must be included in the diet. α-linolenic acid has the potential to be elongated into EPA or DHA.

In general, fats are insoluble in water and due to the fact the body is more than 70% water, amphipaths are needed. These are bile acids or phospholipids that contain both hydrophobic and hydrophilic domains that help bring water and lipid together. Beginning in the mouth, lingual lipase from the serous glands at the base of the tongue or gastric lipase secreted by the lingual glands in the stomach initiates triglyceride hydrolysis (Moreau et al., 1988). Shorter fatty acids tend to locate at the carbon one and three positions for more water solubility and more rapid energy sources. This occurs at a low pH in the lumen of the stomach and in absence of bile salts. Lingual or gastric lipases cleave triglycerides and can yield partial glycerides and free fatty acids (Olsen et al., 1998). Protein digestion by pepsin and HCL can aid fat droplet liberation in the stomach and small intestine. The small intestine is the major site of lipid digestion and involves the formation of coarse emulsion to reduce particle size, enzymatic digestion, and mixed micelle formation. Cholecystokinin (CCK) is produced by I-cells in the duodenum and jejunem in response to amino acids and long chain fatty acids (LCFA) (Hamosh, 1990). This causes the contraction of the gall bladder and
increases enzyme synthesis in the pancreas. The intestinal lumen phase uses pancreatic lipase to digest lipids and occurs at the end of the duodenum and the jejunum. The emulsion phase may include pancreatic juices, bile, and triglycerides resulting in increased surface area for enzymatic digestion. Pancreatic bicarbonates work to increase the intestinal pH, which allows optimal enzyme activity (Carlier et al., 1991). Bile salts are powerful emulsifying agents and cause the diameter of the emulsion droplet to decrease to 2000 A. This is essential for diffusion of lipid digestion products into intestinal mucosal cells. This is a limiting step for long chain fatty acid absorption (Hofmann, 1976). Bile salts activate pancreatic cholesterol ester hydrolase to completely hydrolyze cholesterol esters into free fatty acids and free cholesterol. Hydrolysis of fat yields fatty acids, free cholesterol and monoglycerides (Carlier et al., 1991). Reabsorption of bile acids can occur in the distal lumen (95%) by active transport. This is termed “entero-hepatic recirculation”. Fatty acids can be transported into the portal blood, back to the liver. Pancreatic lipase is responsible for the bulk of lipid digestion (Horton et al., 2002). It has a sterospecificity for sn-1 and 3 fatty acids, with sn-3 having the highest preference and is inhibited by bile acids unless co-lipase is present. Short and medium chain fatty acids are hydrolyzed faster than long chain fatty acids (Hamosh, 1990). Also, cholesterol esterase hydrolyzes fatty acid esters. Mixed micelles have a low oil:amphipath ratio and contain products of enzymatic digestion. Micelles also facilitate diffusion through the “unstirred water layer” and penetration through this layer is rate limiting. This is a critical step in dietary fatty acid absorption (Hamosh, 1990). The small size of micelles allows for access between the microvilli. Lipid digestion products are absorbed at
a more rapid rate due to an increase in aqueous concentration gradients by micellar solubilization (Wilson et al., 1971). Mixed micelles dissociate at a low pH before lipid absorption, leaving monomers to be taken up by the enterocytes (Carlier et al., 1991). This low pH is due to the mucus along the luminal side of the enterocyte membrane (Shiau, 1990). Long-chain fatty acid uptake is hypothesized as an energy dependent process. An increase in lipophilic properties of fatty acids can result in more passive permeation. Also, an increase in chain length and a decrease in unsaturation may result in increasing passive permeation (Carlier et al., 1991). At low concentrations, polyunsaturated fatty acids may use facilitate diffusion without requiring energy (Chow et al., 1978a, b). Chain length of fatty acids is the main determinant for fatty acid absorption. Short and medium chain fatty acids (10 carbons are fewer) are more water-soluble and diffuse across the villus tip into the portal vein. Long-chain fatty acids flow through the rough endoplasmic reticulum, the smooth endoplasmic reticulum and finally to the golgi for triglyceride synthesis. From there, triglycerides are packaged with apoprotiens into chylomicrons that cannot enter the bloodstream. Triglycerides are absorbed into the lymphatic system as chylomicrons. However, poultry can absorb triglycerides directly into the bloodstream. They absorb into the portal blood. The main pathway for triglyceride resynthesis is through the 2-monoglyceride pathway (Clark et al., 1960). The Golgi is essential for the glycosylation of the lipoprotein particles, which is needed to transport lipoproteins by secretory vesicles to the plasma membrane for exocytosis from the enterocyte (Sabesin, 1976). In the intestine, lipoproteins migrate toward intracellular gaps to allow direct communication with the lamina propia.
Integration of polyunsaturated fatty acids into lipoprotein is influenced by the quantity of lipids given and the degree of unsaturation of the lipids (Carlier et al., 1991). Polyunsaturated fatty acids (PUFA) can also be used for peroxisome or mitochondria beta oxidation (Palmquist, 2009).

Mattos and Palmquist (1978) found that 76% of linoleic acid is taken up by the mammary gland by use of lipoprotein lipase. However, Brumby et al. (1972) reported mammary lipoprotein lipase hydrolyzes less long-chain PUFA due to their primary transport to phospholipids (Rymer et al., 2003). Conversions of unsaturated fatty acids to highly unsaturated fatty acids are due to desaturases and chain elongation enzymes (Sprecher, 2000). Linolenic acid has a greater affinity for the delta-6 desaturase enzyme than linoleic; however, linoleic acid is in greater concentrations in most swine feed and therefore yields a higher conversion of linoleic acid into omega-6 PUFAs (Palmquist, 2009). This desaturase enzyme is found to be a rate-limiting step and high intake of omega-6 PUFA is known to be a limiting factor that down regulates the conversion of linolenic acid into EPA and DHA (Palmquist, 2009; Vessby et al., 2002). Chain elongation is also inhibited more by PUFA than other fatty acids (Palmquist, 2009). Therefore, increased concentrations of essential fatty acids can inhibit linoleic acid conversion to linolenic acid and their PUFA derivatives (Holman, 1986). The conversion of linolenic acid is down-regulated by an increase in product availability and is unaffected by an increase in substrate availability (Burdge et al., 2003). Stearidonic acid is a component of fish oil and can be found in high concentrations in plants, which can bypass the initial rate-limiting reaction involving the delta-6 desaturase
enzyme. Stearidonic acid is an omega-3 fatty acid 18 carbons long with only cis double bonds and is converted to EPA with 17 to 30% efficiency in humans (Whelan, 2009). Cats, among other animals, have a dietary requirement for arachidonic acid and have low activity of the desaturase enzyme in the liver when deprived of long-chain PUFA. DHA is not synthesized in the liver, however DPA is synthesized in the liver and transported to the brain; there DPA can be converted to DHA (Pawlosky et al., 1994). Omega-6 and omega-3 PUFA are eicosanoid precursors, along with prostanoids and other oxygenated derivatives. DHA is not a precursor of eicosanoids, but is a precursor to docosanoids (Palmquist, 2009). In dogs supplemented with flaxseed, linolenic acid (C18:3n3) was converted to EPA and DPA (C18:4n3) by the action of delta 6 desaturase on linolenic acid; however there was no change in plasma DHA levels (Bauer et al., 1998). α-Linolenic acid has many fates in humans including structural incorporation, transport, storage pools, β-oxidation, and carbon chain elongation (Burdge, 2004). In humans, β-oxidation with these substrates will yield a source of energy (Delany et al., 2000; Bretillon et al., 2001). Oxidation of α-linolenic acid is greater in men than in women (Burdge et al., 2002; Burdge et al., 2003). Studies carried out in human adults showed low conversions of α-linolenic acid to EPA and DPA, demonstrating limited initial steps in the pathway involving desaturase enzymes (Emken et al., 1994; Vermunt et al., 2000; Burdge et al., 2002; Burdge et al, 2002; Burdge et al., 2003). However, in men and women DHA concentrations did not vary due to α-linolenic acid levels (Dyerberg et al., 1980).
Enrichment of Pork

There are limited sources of omega-3 fatty acid rich feedstuffs (Givens et al., 2000). Soybean and canola oil both contain large quantities of linolenic acid and high amounts of linoleic acid is found in soybean oil. EPA and DHA are limited to fish oil (Givens et al., 2006). Growing pigs require 2% of dietary energy as linoleic acid for optimal growth and energy utilization and to decrease chances of deficiencies (Stahly, 1984). There is a maximum intake level for linoleic acid to avoid problems with meat quality; concentrations over 150 mg/g subcutaneous fat results in excessively soft back fat and a reduced shelf life for meat products (Wood et al., 1999). This is to be avoided considering industry practice has been to produce very lean pigs. In lean pigs, the majority of fatty acids is found in the membrane phospholipids and is more unsaturated than the storage triacylglycerols (Wood et al., 1999). However, muscle containing less than 5% fat was less favorable in eating quality than muscle containing 9% fat in pork loin (Stahly, 1984). The feeding of linseed for short periods prior to slaughter and feeding increased levels of vitamin E may increase omega-3 fatty acids in pork without inducing unfavorable flavors (Wood et al., 1999). There is evidence that supplementing up to 10% ground linseed into the diet can increase linolenic acid in muscle by 3-fold, resulting in an increase by 10-fold in adipose tissue. EPA and DPA may be increased 3-fold, but no change in DHA content was observed. Supplemental fish or marine algae oil has the ability to increase EPA and DHA content of pork significantly (Palmquist, 2009).
**Uses & importance of DHA**

Most essential fatty acids can be found in phospholipids as may be expected due to amphiphilic properties of the molecules (Palmquist, 2009). Many long-chain PUFAs in animal tissues are found in the esterified form and are typically located in the sn-2 position of the phospholipids (Christie, 2007). Polyunsaturated fatty acids are concentrated in the inner cell membrane layer (Pike, 2003). DHA is an affecter of the outer membrane composition and signaling (Li et al., 2005). Polyunsaturated fatty acids have important structural roles in cell membranes and are involved in the synthesis of lipid mediators which play key roles in cardiovascular and inflammatory disease (Carlier et al., 1991). Omega-3 fatty acids play a role in membrane maintenance, specifically with the nervous system, which is essential for life processes (Bourre, 1989). Polyunsaturated fatty acids are key to the fluidity of cell membranes. The greatest change occurs with unsaturation during the transition temperature and is caused by the first introduction of a double bond into a saturated fatty acid (Hulbert et al., 1999). Through diet modification of fatty acids, membrane phospholipid composition can be altered impacting functions via receptors, transport pathways and enzymes (Goodnight, 1982). Long-chain polyunsaturated fatty acids are precursors that are involved in cardiovascular and inflammatory diseases. Elmadfa et al., (2009) stated dietary recommendations of total PUFA should be 3% of energy intake per day to prevent deficiencies and 6% to prevent chronic diseases. Saturated fatty acids were also recommended at 6-7% of calories, while mono-unsaturated fatty acids were 12-14% of calories (Simopoulos, 1989). Recommendations were formed and based on a 2600 kcal/day
intake, due to the interaction between omega-3 and omega-6 polyunsaturated fatty acids. Increased weight and growth could be obtained through supplementation of fatty acids, including but not limited to linoleic (LN) and linolenic (ALA) acids (Burr et al., 1930; Burr et al., 1932). There has been recent work done to examine essential fatty acids and their role in gene expression. This involves multiple processes including transcription factors that are essential for genes controlling systematic and tissue-specific homeostasis (Palmquist, 2009). Omega-3 PUFAs and their derivatives regulate fuel partitioning and directing of fatty acids from storage to oxidation (Clarke et al., 2001; Wahle et al., 2003). Therefore omega-3 fatty acids may be used for modifying weight in livestock and prevent obesity. DHA is inversely related to body size. Smaller animals contain a larger quantity of DHA in membranes, have greater protein leakage, and a greater metabolic rate per unit of body mass. Cold environment dwelling animals contain great amounts of DHA in their membranes (Hulbert et al., 1999). Humans contain less membrane DHA and lower conversion rates of linolenic acid to DHA, compared to laboratory animals. DHA concentrations are greater in human infants rather than adults (Brookes et al., 1998).

Linoleic acid (C18:2, n-6) and alpha linolenic acid (C18:3, n-3) are 2 of the main essential fatty acids due to the fact they cannot be synthesized by mammals. However, they can be converted into n-6 and n-3 polyunsaturated long chain fatty acids with aid of desaturases and elongases. Arachondic (C20:4, n-6), eicosapentaenoic (EPA) (C20:5, n-3) and docosahexaenoic acid (C20:6, n-6) are conditionally essential fatty acids used in a broad spectrum of important physiological roles of the body (Carlier et al., 1991). Arachondic acid
and EPA are precursors to prostaglandins I2 and I3 along with thromboxane A2 and A3, through cyclo-oxygenases and phospholipases (Fischer et al., 1983). Prostaglandin endoperoxide H synthase-1 and prostaglandin endoperoxide H synthase-2 are the key enzymes that are involved in the first committed step in prostanoid synthesis from fatty acids. Synthase-1 is always in the tissue, while synthase-2 is induced by tumors and growth factors (Palmquist, 2009).

Prostanoids are involved with the inflammatory response and occur at low levels in tissues. Platelet aggregation in plasma is inhibited by EPA (Palmquist, 2009). EPA is a competitive inhibitor of arachindonic acid (Higgs et al., 1986). Omega-3 and omega-6 fatty acids compete for enzymes, which can result in omega-3 fatty acids inhibition of arachidonic acid synthesis from linoleic acid; yielding decreased levels of thromboxane A2 (Holman, 1964). Eicosanoids formed from omega-3 PUFAs have opposite effects of those from omega-6 PUFAs. PUFAs regulate the inflammatory response and processes through the balance of the omega-3 and omega-6 ratio (Palmquist, 2009). Long-chain omega-3 fatty acids, at high levels, will decrease the production of inflammatory eicosanoids, cytokines, and adhesion molecules. They will also produce anti-inflammatory mediators known as resolvins (Palmquist, 2009).

EPA and DHA affect cytokines and growth factors in a way that is responsible for the antiatherosclerotic action, by reducing proinflammatory cytokines, tumor necrosis factor-α and mRNA concentrations of proatherosclerotic growth factors (Schacky, 2007).
Therefore, increasing EPA or DHA increases these fatty acids in inflammatory phospholipids (Palmquist, 2009). However, there is no anti-inflammatory effect from linolenic acid. Intake including omega-3 PUFA preserves immune function better than standard consumption, lacking PUFAs (Calder, 2006a). Fish and fish oils which are rich in long-chain omega-3 PUFA can be cardioprotective in many pathways, including effects on arrhythmias, endothelial function, and thrombosis (Roche et al., 2000).

Another benefit of dietary omega-3 fatty acids is attributed to the reduction in plasma triacylglycerol concentrations in hypertriglyceridemic subjects (Anil, 2007). The “n-3 index” is a new marker for tissue omega-3 content and is a detector of risk factors such as cardiovascular disease (CVD). This reflects the sum of EPA and DHA in erythrocyte membranes (Harris et al., 2008). Omega-3 fatty acids, mainly EPA and DHA, inhibit carcinogenesis (Palmquist, 2009). Improvement in clinical, biological and the quality of life were seen with administration of at least 1.5 g/d of EPA and DHA in patients with advanced cancer (Colomer et al., 2007). Mechanisms by which omega-3 fatty acids alter carcinogenic processes include suppression of fatty acid derived eicosanoids, influences on transcription factor activity, gene expression, signal transduction pathways, changes in estrogen metabolism, alterations in free radical production and mechanisms involved in insulin sensitivity and membrane fluidity (Larsson, 2004).

Linolenic acid conversion to DHA is increased in women due to the action of estrogen (Williams et al., 2006). A deficiency in omega-3 PUFA has been shown to alter the dopaminergic and serotonergic neurotransmitter systems (McNamara, 2006).
There are strong links between PUFA, brain function, and behavior (Chalon, 2006). Omega-3 fatty acids also have potential benefits in depression and bipolar disorder. It is recommended by the American Heart Association’s dietary guidelines to intake 1 g/d of EPA + DHA (McCann et al., 2005). These guidelines are supported by findings that link low levels of DHA to maternal postpartum depression (McCann et al., 2005). Also, DHA was found at decreased levels in the brain and plasma of patients with dementia (Schaefer et al., 2006). The recommendations necessary to prevent deficiency symptoms is 0.6 to 1.2% of energy for linolenic acid, up to 10% of this can be from EPA or DHA (Gebauer et al., 2006).

DHA accumulates in a developing brain as an important aid in normal neural and visual functions. An estimated 67 mg/d of DHA is acquired by the fetus during the third trimester of gestation (Innis et al., 2003). Omega-3 and omega-6 fatty acids may continue to alter brain function by changes in neuronal membrane fluidity, membrane-bound enzymes, the amount and affinity of receptors, effects on the functioning of neuronal membrane ionic channels, and the production of neurotransmitters and brain peptides (Palmquist, 2009). Supplementation in term infants with daily doses of 100 mg DHA and 200 mg arachindonic acid can improve visual development (Carlson, 2009). The Perinatal Lipid Intake Working Group stated “dietary fat intake in pregnancy and lactation should be as recommended for the general population; pregnant and lactating women should aim to achieve an intake of at least 200 mg DHA/d.” They also stated women of child-bearing age should consume one or two portions of seafood weekly. Long-chain PUFA regulate reproductive hormones that affect ovarian follicle development, generation and regression of the corpus luteum, and
implantation or maintenance of the embryo (Moore et al., 2006). Eicosanoids, from long-chain PUFA metabolism, are messengers in reproductive signaling (Webb et al., 2004).

Linoleic, linolenic acid, and long-chain PUFA are transported across the placenta in humans by placental fatty acid-building and fatty acid-transfer proteins to maintain fetal development (Koletzko et al., 2007b). Sheep, cows, and pigs have less permeability to fatty acids through placental transfer (Battaglia et al., 1988). However, cats have been shown to have a placenta impermeable to fatty acids (Elphick et al., 1984).

Linoleic acid and arachindonic acid were found in lower proportions in cellular compartments of semen, during collection over the summer (Argov et al., 2007).

Supplementing bovine animals with 250 mg/d of EPA and DHA may improve sperm quality (Brinsko et al., 2005). These positive results with omega-3 fatty acids have resulted in current research focused on animal enrichment.
CHAPTER II:

INTRODUCTION

Throughout history dietary fat has been a controversial public health concern. Most foods that are commonly consumed contain fat. The main form of fat present in food is a triacylglycerol, sometimes called triglyceride (Sanders, 2010). Triglycerides have three fatty acids attached to a glycerol backbone through ester linkages. Dietary fat makes up the primary source of energy at rest and during exercise. They contain 9 kcal/g which is almost twice the energy density of carbohydrate and protein (Manore et al., 2000).

There are both risks and benefits of fat consumption. Fat contains essential fatty acids, such as linoleic acid (C18:2) and α-linolenic (ALA) acid (C18:3) which cannot be synthesized in the body. These can be used as precursors for regulatory compounds found in the body. α-Linolenic acid is classified as an omega-3 fatty acid occurring primarily in plant and vegetable oils (Brouwer et al., 2004 and Patenaude et al., 2009). Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are two omega-3 fatty acids primarily found in fish oils; which are derivatives of α-linolenic acid. Theses conditionally essential fatty acids are derived from essential fatty acid precursors such as (ALA) and are required in the production of prostaglandins, thromboxanes, leukotrienes and prostacyclins; therefore playing an important role in blood clotting, blood pressure, vascular dilation, heart rate and immune response (Manore et al., 2000). Fat-soluble vitamins are also contained in fat which are required for many metabolic processes (Manore et al., 2000). However fat can be a concern
for many; excess fat can lead to obesity, cardiovascular disease, diabetes, and some forms of cancer.

The imperative aspects of omega-3 fatty acids in developmental processes and the beneficial roles in human health have been well examined since the 1990’s. In association with these developments, the American Heart Association increased the recommended level of omega-3 fatty acids in the human diet. Fish oil is a well known available source for omega-3 dietary supplementation. However, there is concern for potential mercury contamination (Francesconi et al., 1992 and Dorea, 2006). This is a great concern for pregnant and lactating mothers. Also, long-term consumption of drugs that prevent obesity, diabetes and coronary heart disease may have side effects on human health (Curb et al., 1985). Other prospects dealing with dairy product enrichment can have potential negative issues for individuals suffering from lactose malabsorption (Simoons, 1978). Pork is one of the most popular constituents in a daily meal and is very susceptible to tissue fatty acid alteration by diet modification. Therefore, elevating the content of omega-3 fatty acids in pork by feed supplementation could have a great potential health benefit to consumers.

**Statement of the Problem**

Omega-3 fatty acids are essential dietary nutrients that have beneficial effects in human diets and the potential to improve growth and development, reduce obesity and coronary heart disease, decrease plasma lipid concentrations, stimulate fat oxidation and cell membrane incorporation. Cellular membranes of the retina, brain and myocardium, are especially
enriched in omega-3 fatty acids; which suggests these fatty acids are important to proper function of the cell (Surette, 2008). Also, long-term use drugs for preventing obesity and coronary heart disease have side effects on human health. Pork supplementation in human diets can serve as a method to increase anti-inflammatory and tissue damage responses (Weylandt et al., 2008). However, limited information is available concerning pork enrichment with long-chain omega-3 fatty acids such as DHA.

**Purpose of the Study**

The purpose of this study was to evaluate how much omega-3 fatty acids are required and the duration required for pork enrichment to reach 32 mg/100 g pork in crossbred finishing pigs. 32 mg/100 g enrichment is required to obtain an omega-3 enrichment label for nutrition products.

**Objectives of the Study**

The primary objective of this study was to determine the time course required for enrichment of 32 mg/100 kg pork in various pork products with omega-3 fatty acids when supplemented in feed at incremental levels. We hypothesized that finisher pig diets supplemented with 0.27, 0.54 DHA or 0.54 linoleic acid will yield pork product enrichment of at least 32 mg/100 g pork.
CHAPTER III

METHODS AND MATERIALS

All experimental procedures were reviewed and accepted by the Institutional Animal Care and Use committee of North Carolina State University. Pain and stress to animals were minimized throughout the duration of the experiment.

Diet Allotment and Experimental Design

A total of 40 cross-bred finishing pigs (BW=67.7 ± 1.1 kg) were obtained from North Carolina State University Swine Educational Unit in the summer of 2009. Pigs were blocked by gender and weight 24 days prior to the experiment and given a common finisher diet while housed at the North Carolina State University Educational Swine Unit. Pigs were randomly allocated within block to 8 treatments. The treatments were arranged according to a 2 x 4 factorial design, with pigs individually housed in pens 1.83 m × 2.4 m allowing ad libitum access to feed and water. Pigs were fed 4 diets varying in omega-3 polyunsaturated fatty acid levels for a period of 4 or 8 weeks prior to slaughter. Diets consisted of a corn-soybean meal control (0% docosahexaenoic acid), control with 1.5% DHA Gold (Martek, Columbia, MD) (0.27% DHA), control with 3.0% DHA Gold (0.54% DHA), and control with 1.04% Flax seed oil (0.54% α-linolenic acid). Initially a basal diet was manufactured and then split to create identical treatments. Diets were enriched with commercially available oils concentrated in DHA (42%) and ALA (52%) to avoid negative pork quality issues.
Table 1: Composition of Experimental Treatments, as fed basis

<table>
<thead>
<tr>
<th>Item</th>
<th>0</th>
<th>0.27 DHA</th>
<th>0.54 DHA</th>
<th>0.54 ALA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ingredients, %</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corn</td>
<td>77.1</td>
<td>76.4</td>
<td>75.8</td>
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<tr>
<td>Soybean meal (48% CP)</td>
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<td>Limestone</td>
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<tr>
<td>Santoquin(^1)</td>
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<tr>
<td>Vitamin/Mineral premix(^2)</td>
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<td>Sodium Selenite, 0.06%</td>
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<td>Fat Pack(^3)</td>
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<td>DHA Gold(^4)</td>
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<td>Flax Oil</td>
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**Calculated composition**

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<tr>
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<td>P, %</td>
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<tr>
<td>Ca, %</td>
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1,4 Novus International, St. Charles, MO 63304, USA

2 Supplied per kilogram of complete diet: 4113.8 IU of vitamin A; 586.4 IU of vitamin D; 23.5 IU of vitamin E; 0.01 mg of vitamin B12; 2.94 mg of riboflavin; 17.64 mg of niacin; 11.76 mg of d- pantothenic acid; 0.88 mg of folic acid; 0.12 mg of biotin; 110.2mg Zn; 110.2 mg Fe; 11 mg Cu; 26.4 mg Mn; 0.2 mg I; and 0.2 mg Se

3 Advance Fat Pak 100, MSC, P.O. Box 278, Dundee, IL 60118
Animal Management and Sample Collection

Pigs and feeders were weighed at 0, 4, and 8 weeks. All animals were slaughtered (Pine Ridge Processing Plant, Micro, NC) at the same time (115.6 +/- 5.4 kg BW) using electrocution. 2.5 cm loin chops were collected along the back in addition to belly, ham from the thigh, and backfat samples along the back following procedures by Averette et al.(2002). Loin chops were trimmed to retail cuts and ground three times, using a meat grinder, to obtain homogenous samples. Belly and ham samples were also ground the same as loin chops to obtain homogenous samples. Collected samples were frozen immediately and stored at -80°C for fatty acid analysis.

Chemical Analysis

The omega-3 polyunsaturated fatty acid concentration of tissue samples was determined by gas-liquid chromatography (Averette et al., 2002). Percent fat levels of diet composition were determined using the Goldfish method of fat extraction. Diet samples were obtained from every third feed bag and collected during feed production. 5-10 g samples of each diet were weighed into cellulose thimbles. 40 ml of ethyl ether was weighed out and a small amount was poured over the diet sample. The rest was poured into pre-dried and weighed beakers. Beakers and thimbles were attached to the Gold Fish fat extraction system. During the process, all beakers were kept boiling and solvent levels were kept adequate.

After 4 hours, beakers were allowed to cool. Cooled beakers were placed into a steam bath to evaporate solvent. After all solvent was evaporated, beakers were placed in an oven
(105°C-110°C) to dry off moisture. Beakers were cooled in a desiccator. Beakers and fat were weighed and the weight of the empty beaker was subtracted. The grams of fat were then divided by the weight of the sample extracted and multiplied by 100 to obtain % fat (AOAC, 1998).

Fatty acids in tissue samples were extracted and methylated using methods described in Averette et al.; (2002). This process included using 1 gram of tissue with the addition of 20 mL of 1:1 chloroform: methanol reagent and homogenization for 1 minute. The homogenized samples were filtered using Whatman filter paper. Filter paper was squeezed to remove excess liquid and 4 ml of saline was added. The mixture was vortexed and then centrifuged for 10 minutes at 870 g. The lower phase was removed, evaporated under nitrogen and placed in the oven for 2 hours at 104°C. Samples were cooled in dessicator and then weighed. During the methylation procedure, 50-100 mg of the fat sample was combined with 1 ml of reagent 1 (45 g sodium hydroxide, 150 ml methanol, 150 ml distilled water) and placed in a hot water bath (100°C) for 30 minutes. After 30 minutes, tubes were cooled in tap water and 2 ml of reagent 2 (275 ml methanol and 325 ml 6 N HCL) were added. The tubes were placed back into the hot water bath (100°C) for 10 minutes. After 10 minutes, tubes were cooled in tap water and 3 ml of reagent 3 (200 ml methyl-tert-butyl-ether and 200 ml hexane) were added. Tubes were rotated for 25 minutes and the lower phase was removed and discarded. 3 ml of reagent 4 (10.8 g sodium hydroxide and 900 ml distilled water) were added and centrifuged for 10 minutes at 870 g.
The methylated fatty acids were analyzed using a Hewlett Packard 5890 gas chromatograph (Hewlett Packard, Avondale, PA) equipped with a flame ionization detector.

The target of our research was to obtain an enrichment level of at least 32 mg/100 g pork in order to support a commercial label of “omega-3 fatty acid enriched pork.”

**Statistical Analysis**

All statistical analyses were conducted using the GLM procedure of SAS (SAS Inst., Cary, NC) according to a 2 x 4 factorial randomized complete block design. The least square means ± SEM was used for the data expression and the differences between treatments were considered significant when the P-value is ≤ 0.05.
CHAPTER IV

RESULTS

Fat supplements did not affect daily weight gain (0.90 ± 0.15 kg) (P=0.27), feed intake (2.77 ± 0.65 kg) (P=0.61) or gain:feed (0.33 ± 0.07 kg).

The DHA concentration in loin tissue increased linearly over controls, in pigs fed 0.27% DHA and 0.54% DHA (Table 2) (P=<0.0001) and enrichment increased with feeding duration (P=0.034). Also, ALA increased in loin tissue in control, 1.5% DHA and 3.0% DHA diets, but was not found to be statistically significant for phase (P=0.316) or diet (P=0.474). Flax diets showed no enrichment in loin for 4 or 8 weeks in loin tissue.

Ham tissue showed a linear increase in DHA enrichment 0.27% DHA and 0.54% DHA diets (P=<0.0001; Table 3) for 4 and 8 weeks. The duration of supplementation in ham enrichment was statistically significant (P=0.004). Flax diets showed highest enrichment of ALA (P=<0.0001) for 4 and 8 weeks, along with DHA enrichment in ham at 8 weeks.

Belly enrichment increased linearly for 0.27% DHA and 0.54% DHA diets (P=<0.0001) for 4 and 8 weeks (P=0.001; Table 4). ALA enrichment in belly showed a similar increase and was highest in the flax diet (P=<0.0001), while phase was not significant (P=0.985). Also, flax treatments showed little DHA enrichment at 8 weeks in the belly.
Backfat enrichment (Table 5) showed a linear increase in DHA enrichment for 0.27% and 0.54% DHA diets (P=0.003) for 4 and 8 weeks. Duration for DHA enrichment in backfat was not statistically significant (P=0.654). ALA enrichment in backfat increased linearly across all diets (P=<0.0001) for 4 and 8 weeks (P=0.833). The flax diet showed little DHA enrichment for 4 or 8 weeks in backfat tissue.

Figure 1 shows target enrichment of 32 mg/100 g in loin would require consumption of 0.12% DHA for 8 weeks or 0.23% DHA for 4 weeks. Also, R²= 0.85 for 8 weeks and R²= 0.87 for 4 weeks indicating DHA enrichment in loin tissue closely fits an increasing linear model.

Figure 2 showed target enrichment of 32 mg/100 g in ham would require consumption of 0.18% DHA for 8 weeks or 0.40% DHA for 4 weeks. DHA enrichment in ham very closely fits a linear model with R²= 1 for 8 weeks and R²= 0.9 for 4 weeks.

Figure 3 showed target enrichment of 32 mg/100 g in belly would require consumption of 0.056% DHA for 8 weeks or 0.12% DHA for 4 weeks. Belly tissue enrichment levels showed R²= 0.88 for 4 and 8 weeks, indicating a similar fit to a linear model as the loin tissue.

Figure 4 showed DHA enrichment increased linearly for 4 or 8 weeks. These enrichment values fit a linear model as closely, having a R²= 0.97 for 4 weeks and R²= 0.98 for 8 weeks.
TISSUE ENRICHMENT

Figure 1: Enrichment of DHA in pork loin tissue following dietary supplementation of DHA for the last 28 or 56 days of the finisher period
Figure 2: Enrichment of DHA in pork ham tissue following dietary supplementation of DHA for the last 28 or 56 days of the finisher period.
Figure 3: Enrichment of DHA in pork belly tissue following dietary supplementation of DHA for the last 28 or 56 days of the finisher period.
Figure 4: Enrichment of DHA in pork backfat tissue following dietary supplementation of DHA for the last 28 or 56 days of the finisher period
Table 2: Fatty acid concentrations (wt%) in pork loin following the feeding of 0.27 or 0.54% DHA, or 0.54% ALA for the last 4 or 8 weeks of the finisher period

<table>
<thead>
<tr>
<th></th>
<th>4wk LSMMeans</th>
<th>8wk LSMMeans</th>
<th>Std Error</th>
<th>Phase</th>
<th>Diet</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>0.27%DHA</td>
<td>0.54%DHA</td>
<td>0.54%ALA</td>
<td>Control</td>
<td>0.27%DHA</td>
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<tr>
<td>C14:0</td>
<td>3.529</td>
<td>1.836</td>
<td>1.545</td>
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<td>1.804</td>
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<td>C16:1</td>
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<td>C18:1t</td>
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<td>0.546</td>
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<td>0.726</td>
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<td>C18:1c</td>
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<td>43.401</td>
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<td>0.423</td>
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<td>0.466</td>
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<td>0.592</td>
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<td>0.600</td>
<td>0.483</td>
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<td>0.053</td>
<td>0.104</td>
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<td>0.405</td>
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<td>0.007</td>
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<td>0.905</td>
<td>0.000</td>
<td>0.000</td>
<td>0.613</td>
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a Omega 6 form of fatty acid
b Omega 3 form of fatty acid
t,c Trans, cis form of fatty acid
Table 3: Fatty acid concentrations (wt%) in pork ham following the feeding of 0.27 or 0.54% DHA, or 0.54% ALA for the last 4 or 8 weeks of the finisher period

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<th>4wk</th>
<th>8wk</th>
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Values are least squares means (LSMeans) of concentrations (wt%) of fatty acids in pork ham following the feeding of 0.27 or 0.54% DHA, or 0.54% ALA for the last 4 or 8 weeks of the finisher period. Standard errors (Std Error) are presented for each comparison. P values for phase (Phase), diet (Diet), and interaction (Interaction) are also provided. Bold values indicate significant differences at the 0.05 level.

- **a** Omega 6 form of fatty acid
- **b** Omega 3 form of fatty acid
- **t,c** Trans, cis form of fatty acid
Table 4: Fatty acid concentrations (wt%) in pork belly following the feeding of 0.27 or 0.54% DHA, or 0.54% ALA for the last 4 or 8 weeks of the finisher period

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<td>0.54%ALA</td>
<td>Std Error</td>
<td>Phase</td>
<td>Diet</td>
<td>Interaction</td>
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<tr>
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a Omega 6 form of fatty acid  
b Omega 3 form of fatty acid  
t,c Trans, cis form of fatty acid
Table 5: Fatty acid concentrations (wt%) in pork backfat following the feeding of 0.27 or 0.54% DHA, or 0.54% ALA for the last 4 or 8 weeks of the finisher period

<table>
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<tr>
<th></th>
<th>4wk LSMeans</th>
<th>8wk LSMeans</th>
<th>Std Error</th>
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<th>Diet</th>
<th>Interaction</th>
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<td>0.107</td>
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<td>1.549</td>
<td>2.433</td>
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<td>0.548</td>
<td>0.437</td>
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a Omega 6 form of fatty acid
b Omega 3 form of fatty acid
t,c Trans, cis form of fatty acid
CHAPTER V

DISCUSSION

Omega-3 fatty acid supplementation can change plasma lipid concentrations, modify cell membrane properties and cell signaling metabolites among other functions mediated by membrane functions (Goodnight, 1982; Philbrick et al., 1987). Monogastric animals are very susceptible to tissue fatty acid alteration through diet modification (Marriott et al., 2002). The purpose of the current research was to determine the time course of enrichment of various pork products with omega-3 fatty acids when fed at incremental levels and, thereby producing a value-added product that will be attractive to health-conscious consumers. This study confirms that pork can be enriched with long chain PUFA through dietary modification and manipulation.

Diet consisting of a control with 0% docosahexaenoic acid, control with 1.5% DHA Gold (Martek, Columbia, MD) (0.27% DHA), control with 3.0% DHA Gold (0.54% DHA), and control with 1.04% flax seed oil (0.54% α-linolenic acid) were chosen based on a preliminary study by Jaturasitha et al. (2009) which fed 0%, 1%, and 3% tuna oil for the early fattening stage or the late fattening stage. They found an increase in omega-3 fatty acids, including EPA and DHA; along with a decrease in omega-6 fatty acids in lean and adipose tissue. Marriott et al. (2002) fed 90 ppm of DHA for 3 weeks and achieved 30% of the desired levels of pork enrichment. The results of our study are supportive to those found by Haak et al., 2008 who fed pigs a 1.2% level of linseed and fish oil for either the whole fattening phase, the first 8 weeks, or 6-9 weeks prior to slaughter.
They found fish oil during both phases resulted in the greatest EPA and DHA enrichment in the logissimus thoracis muscle. DHA concentrations were found to be highest in the 6-9 week group prior to slaughter. In our study belly enrichment of 32 mg/100 g pork was obtained by using the lowest levels of DHA supplementation due to higher levels of lipid in meat composition (Sethi et al., 2007). Belly tissue samples averaged 34.4 % fat, ham tissue samples averaged 10.2 % fat, and loin samples were 12.4 % fat on average. DHA enrichment exceeded \( \alpha \)-linolenic acid enrichment. The conversion using ALA to form DHA or EPA is restricted due to competition with linoleic acid for the delta-6 desaturase enzyme (Dunbar et al., 2002). Meadus et al. (2009) found results supportive to ours by feeding pigs increasing levels of DHA from microalgae Schizochytrium (Martek Biosciences) until they reached approximately 110 kg. They found a significant increase in bacon between the diets and a linear conversion rate between diet levels and the level of DHA in bacon. Similar results were observed when pre and postweaning lambs were fed either a diet containing no inclusion of PUFA or a diet with a 2:1 ratio of soybean to linseed oil. By feeding linseed and soybean oil, results showed an increase in total PUFA content of fat and muscle tissue without adverse effects on growth performance or carcass characteristics (Radunz et al., 2009). Similar results were found by Betti et al. (2009) where broilers receiving either 10% or 17% of flaxseed oil alone for 11.3-26.2 days prior to processing, showed enrichment of 300 mg per 100 g in omega-3 PUFA. These findings could be explained through higher levels of supplementation. In our study flax was fed for 4 or 8 weeks prior to slaughter and no conversion to DHA was found in those treatments.
Huang et al. (2008) evaluated effects on fatty acid composition in pig muscle of feeding diets consisting of 10% linseed for 30, 60, and 90 days prior to slaughter. Through extended treatment durations, they observed a linear increase in omega-3 PUFA, but not including DHA, in the muscle and backfat of the pig. Further findings by Enser et al. (2000) found a 35% increase of DHA in pig muscle and adipose tissue. By supplementing linseed to diets to contain 10 g/kg linoleic acid and 4 g/kg of α-linolenic acid from 25 kg live weight to 95 kg pigs. These results can mainly be explained by using a strategy to decrease dietary linoleic acid and increase α-linolenic acid ratios. Also, they used lower dietary levels of α-linolenic acid, compared to other studies (Riley et al., 1998a,b; Ahn et al., 1996; Cherian and Sim, 1995; Specht-Overholt et al., 1997) where dietary levels of α-linolenic acid ranged from 15-35 g/kg. These showed higher levels of α-linolenic acid, EPA, and DHA levels which may indicate competitive exclusion of DHA from tissue lipids. (Enser et al., 2000). Two other studies conducted by Musella et al. (2009) and Corino et al. (2008) showed results similar to those found in our study, where diets consisting of 5% whole extruded linseed yielded an increase in n-3 PUFA and a decrease from 12 to 3 in the n-6:n-3 ratio in muscle and adipose tissues. In contrast these studies did show a small increase from the control diet and the linseed treatment in DHA. These results could be explained due to the supplement levels being at 5% which is higher than our 0.54% supplementation but not high enough to interfere with the normal α-linolenic acid level in phospholipids. Also, 5% whole extruded linseed oil contained approximately 20 g ALA/100 g fatty acids. Guillevic et al. (2009) also provided results supportive by feeding either linseed oil or sunflower oil prior to
slaughter. Total omega-3 fatty acids, including DHA, were higher in the linseed treatments and lower in the sunflower oil treatments. This can be explained due to linseed or flaxseed oil containing high levels of the omega-3 fatty acid α-linolenic acid and sunflower oil containing high levels of linoleic acid, an omega-6 fatty acid.

In conclusion, pork loin, ham, belly, and backfat content of DHA was enriched during 4 weeks or 8 weeks during the late finisher phase. There was little to no enrichment of DHA from α-linolenic acid treatments and DHA enrichment exceeded α-linolenic acid enrichment.
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