AN ABSTRACT OF THE THESIS OF

K. Denise Apperson for the degree of Master of Science in Animal Sciences
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Title: Enzyme Supplementation of Broiler Chicken Diets Containing Whole Flax Seed as a Means to Increase n-3 Fatty Acids in Human Diets

Abstract approved:

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Gita Cherian

Flax seed is a rich source of alpha-linolenic acid (ALA). Feeding broiler birds flax seed can increase n-3 fatty acids (FA) in meat tissues and can increase human intake of n-3 FA. However, non-starch polysaccharides (NSP) in flax seed decrease digestibility of lipids and proteins and have a negative impact on bird performance. Addition of carbohydrase enzymes to flax-based broiler diets can decrease the effects of NSP. Two experiments were conducted to investigate the effect of flax seed and carbohydrase enzyme on bird performance, tissue FA composition, meat lipid quality, lipid metabolism, and foregut morphology.

In Experiment 1, 120 four-day-old broiler chicks were assigned to one of three treatments: Control (corn-soybean meal), Flax (Control adjusted for 15%

flax), and Flax+E (Flax+0.05% enzyme). Birds received the diets for 35 days and tissues (breast and thigh muscle, liver, heart, abdominal fat pad) were collected. Compared to Control, flax-fed birds had higher body weight ($p<0.037$), average daily gain (ADG) ($p<0.034$), and lower feed:gain ($p=0.01$). In breast and thigh muscle from Flax 15+E, there was an increase in ALA ($p<0.001$) and total n-3 FA ($p<0.001$). Over 7.9- and 24-fold increases in long chain (LC) n-3 FA were observed in thigh and breast tissue of Flax+E birds compared to Control ($p<0.001$). Total n-3 FA were higher in the liver and heart of Flax+E relative to Flax and Control ($p<0.002$) along with a decrease in saturated FA ($p=0.001$). Total lipids were lowest in heart ($p=0.022$) and adipose tissue of Flax ($p=0.034$). No difference was observed in total lipids or lipid oxidation products measured as thiobarbituric acid reactive substances (TBARS) in liver, breast, and thigh tissues.

Experiment 2 evaluated the interaction of flax (high, low) and enzyme supplementation (with, without). A total of 112, five-day-old broiler chicks were assigned to one of four treatments: Flax 10 (corn-soybean meal basal diet adjusted for 10% flax), Flax 15 (basal diet adjusted for 15% flax), Flax 10+E (Flax 10+0.05% enzyme), and Flax 15+E (Flax 15+0.05% enzyme). Birds received the diets for 40 days and tissues (breast and thigh muscle, liver, heart, abdominal fat pad) were collected. All parameters were analyzed with two-way ANOVA. Dietary flax level and enzyme did not affect body weight, feed consumption, or ADG during the starter phase. However, final body weight and overall weight gain and ADG were highest in high flax-fed birds ($p=0.005$). No effect of diet on relative organ weights was observed. High flax led to a decrease in total lipids in thigh,

breast, and liver tissues ($p<0.05$) while enzyme supplementation led to a decrease in total lipids in heart tissue ($p=0.002$). Total lipids were a poor predictor of lipid oxidation. TBARS were increased in liver tissue with high flax ($p=0.018$) and decreased in breast tissue with high flax ($p=0.031$). There was no difference in TBARS results in thigh tissue. In thigh and breast muscle, there was no effect of flax level or enzyme on relative percentage of total n-3 FA, LC n-3 FA, or monounsaturated FA. In heart and liver tissues, flax level and enzyme supplementation led to an increase in oleic acid (18:1) and palmitic acid (16:0) ($p<0.04$) and a decrease in arachidonic acid ($p<0.04$). There was a trend for a decrease in DM of excreta with enzyme addition. Addition of enzyme led to large increases in villi height, villi width, and crypt depth in the jejunum in the starter phase ($p<0.04$).

It is concluded that addition of carbohydrase enzymes increases the availability of ALA and higher levels of flax have a larger impact on hepatic and cardiac tissues than muscle tissues. Feeding broilers diets containing whole flax seed with carbohydrase enzymes can provide the consumer with a poultry meat product that is enriched in LC n-3 FA without depressing growth performance of the birds.

We estimate the cost of feeding a broiler bird 15% whole flax seed and 0.05% carbohydrase enzyme mixture for a standard 42-day growth period to be $0.50 per pound of processed meat; some if not most of these costs would be offset by a reduction in corn and other feed ingredients. A serving (100 g) of skinless breast from this bird could provide 18% of the recommended daily intake of LC n-3 PUFA. A serving (100 g) of skinless thigh could provide 25% of the

recommended daily intake of LC n-3 PUFA and would meet international food labeling requirements for labeling as an “enriched” consumer product.

Enzyme Supplementation of Broiler Chicken Diets Containing Whole Flax Seed as a Means to Increase n-3 Fatty Acids in Human Diets

by

K. Denise Apperson

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

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K. Denise Apperson, Author

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This thesis is dedicated to Harry Houdini. I miss you terribly, old man.

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ABBREVIATIONS

AA	arachidonic acid, 20:4 n-6
ADG	average daily gain
AFP	abdominal fat pad
ALA	alpha-linolenic acid, 18:3 n-3
ALS	amyotrophic lateral sclerosis
AMEn	apparent metabolizable energy, nitrogen-adjusted
ANF	anti-nutritional factors
BW	body weight
BWG	body weight gain
CD	villi crypt depth
C-SBM	corn-soybean meal
CLA	conjugated linoleic acid
CP	crude protein
CVD	cardiovascular disease
DHA	docosahexaenoic acid, 22:6 n-3
DM	dry matter
DPA	docosapentaenoic acid, 22:5 n-3
DRI	Dietary Reference Intake
EFA	essential fatty acid
EPA	eicosapentaenoic acid, 20:5 n-3
FA	fatty acid
GE	gross energy
GI	gastrointestinal
GIT	gastrointestinal tract
HDL	high density lipoprotein
IBD	inflammatory bowel disease
LA	linoleic acid, 18:2 n-6
LC	long chain
LC PUFA	long-chain polyunsaturated fatty acid (> 18 carbons)

LDL	low density lipoprotein
LNA	alternate abbreviation for linolenic acid in some publications
MDA	malondialdehyde
ME	metabolizable energy
MUFA	monounsaturated fatty acid
NSP	non-starch polysaccharide
PL	phospholipid
PUFA	polyunsaturated fatty acid
RBC	red blood cell
SBM	soybean meal
SDS	Sudden Death Syndrome
SEM	standard error of the mean
SFA	saturated fatty acid
SGLT1	sodium-glucose transporter 1
TAG	triacylglycerol
TBARS	thiobarbituric acid-reactive substance
TFA	total fatty acids
TL	total lipids
TME	total metabolizable energy
TMEn	total metabolizable energy, nitrogen-adjusted
VH	villi height
VW	villi width
VH:CD	villi height:crypt depth ratio

1 Introduction

High levels of saturated and n-6 fatty acids that characterize Western diets are directly associated with increased incidence of cardiovascular disease, obesity, and many inflammatory conditions. The health-promoting effects of n-3 fatty acids for reducing incidence and delaying onset of cardiovascular disease and other inflammatory disorders are well documented in both animal and human studies. Health agencies recommend increased consumption of n-3 fatty acids, particularly long-chain n-3 polyunsaturated fatty acids, to improve health. Marine sources of n-3 fatty acids are limited in Western diets due to cost, sustainability, and other concerns. Poultry meat products are thus reasonable targets for n-3 fatty acid enrichment. Flax seed is the richest non-marine source of n-3 fatty acids. The seed is comprised of 50% oil and about half of that is the essential fatty acid alpha-linolenic acid, the precursor to the metabolically active long-chain n-3 fatty acids. However, flax seed possesses several anti-nutritional factors including insoluble and soluble non-starch polysaccharides. These molecules can decrease digestibility of nutrients and energy availability in diets. Exogenous enzymes are widely used in monogastric diets to enhance digestibility and decrease the anti-nutritive effects of non-starch polysaccharides.

The efficacy of carbohydrase enzymes in combination with whole flax seed in enriching poultry meat tissues with n-3 fatty acids is not well characterized. We propose that whole flax seed and a mixture of carbohydrase

enzymes in broiler diets would increase n-3 fatty acids in consumable meat tissues without reducing bird growth performance or meat quality. To test this, we conducted two feeding trials with broiler birds. The first feeding trial examined the efficacy of a mixture of carbohydrase enzymes in a diet with 15% whole flax seed compared to a standard corn-soybean meal diet. The second trial examined the effect of the enzyme mixture applied to two dietary levels of whole flax seed (10% and 15%).

Chapters 2 through 6 of this thesis reflect the primary themes incorporated into the title, Enzyme Supplementation of Broiler Chicken Diets Containing Whole Flax Seed as a Means to Increase n-3 Fatty Acids in Human Diets.

Chapter 2 establishes the molecular structure, nomenclature, and general metabolism of fatty acids, including n-3 and n-6 fatty acid synthesis pathways. Lipid peroxidation and its effect on food quality are also discussed.

Chapter 3 is focused on the role of n-3 fatty acids in human health. Other topics covered in the chapter include the n-6:n-3 fatty acid ratio and the efficiency of conversion of alpha-linolenic acid to long-chain polyunsaturated fatty acids.

Chapter 4 discusses sources of fatty acids in poultry diets. The physical structure and chemical composition of flax seed and the anti-nutritive factors in flax seed are described.

Chapter 5 considers the use and effectiveness of exogenous enzymes in poultry diets to increase digestibility and decrease anti-nutritive factors.

Chapter 6 reviews prior poultry feedings trials employing diets containing a source of n-3 FA that were conducted to examine performance or to increase n-3 fatty acids in poultry meat.

Results of Experiment 1, entitled Effect of Whole Flax Seed and Enzyme Addition on Production Performance and Tissue Fatty Acids in Broiler Chickens, are presented in Chapter 7. Results of Experiment 2, entitled Effect of Dietary Levels of Whole Flax Seed With and Without Enzyme Supplementation on Production Performance, Gastrointestinal Morphology, and Tissue Fatty Acids in Broiler Chickens, are presented in Chapter 8.

Chapter 9 concludes with a discussion of costs associated with the use of whole flax seed and enzyme supplementation in broiler diets to enrich n-3 fatty acids in poultry meat, as well as the potential for the meat tissues evaluated in our study to be labeled as enriched consumer products.

2 Fats and Fatty Acids: Structure and Metabolism

Lipids, or fats, are the most energy-dense nutrient available, providing 9 kcal of energy per gram compared to 4 kcal/g for proteins and most carbohydrates. Metabolism of fat supplies most of the daily energy needs for monogastric animals such as humans and poultry. Fatty acids, components of lipids, have important roles in other metabolic functions besides energy, including immune system response and neural and vision functions in humans (Chapter 3).

A fatty acid (FA) is comprised of a hydrocarbon chain (acyl group) with a methyl group (CH_3) at one end and a carboxyl group (COOH) at the other. Fatty acids with 12 or fewer carbons are not typically observed in poultry. In this thesis, FA with more than 18 carbons will be referred to as LC (long chain) fatty acids. The degree of saturation of a FA is determined by the number of double bonds between carbons in the chain backbone. Fatty acids with no double bonds are saturated (SFA), fatty acids with one double bond are monounsaturated (MUFA), and FA with more than one double bond are polyunsaturated (PUFA). Increased saturation of a FA is accompanied by the potential for increased lipid peroxidation since the C-C double bonds are the preferred sites of attack for oxygen radicals (Section 2.1). The degree of saturation, the length of the chain, and the position of double bonds relative to the ends of the chain determine physical properties of the fatty acid such as melting temperature as well as its biological function.

Fatty acids have both systemic and common names. They are also described with a shorthand notation that identifies the number of carbons in the chain, the number of double bonds, and the position of the first double bond as counted from the methyl end of the chain. For example, the SFA with 16 carbons and no double bonds has the common name palmitic acid. It can also be described by the notation 16:0. The 18-carbon PUFA known as linoleic acid, with double bonds at positions 9 and 12 relative to the carboxyl end, is described by the notation 18:2 n-6 (the double bond at position 12 relative to the carboxyl end is at position 6 relative to the methyl end of the acyl chain). The two groups of PUFA that are of particular interest in animal nutrition are n-6 FA with the most distal double carbon bond at position 6 relative to the methyl end, and n-3 FA with the most distal double bond at position 3 relative to the methyl end. Linoleic acid is in the n-6 group. The naming system using omega-3 and omega-6 groups will not be used here.

There are two main classes of lipids in animals: triacylglycerols (TAG) and phospholipids (PL). TAG have three fatty acids, often different, attached by their carboxyl ends to a three-carbon glycerol backbone. Triacylglycerols constitute the majority of the FA in the diet and tissues of monogastrics. Once consumed, TAG are deconstructed in the small intestine, mixed with bile salts to form micelles, and absorbed across the intestinal walls. They are then reconstructed with carrier lipoproteins into chylomicrons and transported to

the liver. The liver sends FA out to tissues for energy use or to fat depots for storage in adipocytes.

Phospholipids are found in membranes of cells and intracellular organelles where they play a role in membrane fluidity and cell signaling. Membrane PL have a saturated FA at the first position on the glycerol backbone, a long-chain polyunsaturated FA (LC PUFA) at the middle position, and a phosphate group at the third position. Enzymes acting at these specific positions continuously "remodel" the FA in PL with the LC PUFA being more susceptible to remodeling. The ability for enzymes to quickly extract the LC PUFA from PL is important because these FA are precursors to biologically active eicosanoids.

With respect to poultry meat tissue, thigh meat typically has higher total lipid (TL) content than breast meat, and a larger proportion of the lipids are in the form of TAG. Breast meat contains a larger proportion of its lipids in the form of PL (Gonzalez-Esquerra and Leeson, 2001). This is the result of the different types of muscle fibers in the two tissues. On a FA percentage basis, breast meat can appear to contain more PUFA than thigh meat because PUFA are preferentially stored in PL. However, on a TL percentage basis, thigh meat contains more PUFA.

Monogastrics such as humans and poultry lack desaturase enzymes that can add double bonds at positions farther than ten carbons from the carboxyl group. Thus these organisms are not able to synthesize linoleic acid (LA, 18:2

n-6) and alpha-linolenic acid (ALA, 18:3 n-3) so these essential fatty acids (EFA) must be consumed through the diet. Plants and bacteria can synthesize ALA from LA using the Δ-15 desaturase enzyme; animals lack this enzyme as well. Main dietary sources of LA include grain, vegetable oil (corn oil, safflower oil), and animal tissue, while sources of ALA include marine products (salmon, sardines, algae, fish meal), oil seeds and their oils, and leafy green vegetables. Although more than 50% of the total fat in leafy greens is ALA, the total fat is less than 2% on a dry matter (DM) basis so vegetable sources cannot provide a substantial amount of n-3 FA to the human diet (Poureslami and Batal, 2012).

In monogastrics, LA and ALA are elongated (increasing the carbon backbone length) and desaturated (double bonds added) by elongase and desaturase enzymes to produce 20- and 22-carbon LC PUFA (Figure 1). The LC PUFA are precursors to bioactive molecules called eicosanoids that are involved in immune system processes. Eicosanoids derived from n-3 precursors promote resolution of inflammatory immune responses while those derived from n-6 precursors are associated with both pro- and anti-inflammatory responses (Chapter 3).

Sprecher (2000) and Calder (2013) provide detailed descriptions of the pathways involved in n-3 and n-6 FA metabolism (Figure 1). Lipids are metabolized in all cells but the liver is the primary site for lipid metabolism in chickens. Adipose tissue and mammary glands are also important sites of lipid metabolism in mammals. There is no interconversion of intermediate products

between the n-3 and n-6 pathways even though they use many of the same enzymes and metabolic processes take place in the same cellular organelles. Desaturase enzymes are specific for the position of the double bond in their substrates. In the n-3 pathway, the 20-carbon product is eicosapentaenoic acid (EPA, 20:5 n-3) while in the n-6 pathway it is arachidonic acid (AA, 20:4 n-6). The Δ-6 desaturase enzyme, involved in the first step and in the final step in both pathways (Figure 1), is rate limiting since there is competition between both pathways for the same enzyme. High levels of ALA (18:3 n-3) can inhibit the n-6 pathway because the Δ-6 desaturase has a greater affinity for this FA (Barcelo-Coblijn and Murphy, 2009). An increase in dietary LC PUFA, that is, an increase in the end products of each pathway, will inhibit the initial elongation of LA and ALA (Burdge and Calder, 2005).

Elongase enzymes that add a two-carbon unit to the FA chain operate in structures in the endoplasmic reticulum called microsomes. In contrast to desaturation, elongation is not a specific process because many different saturated and unsaturated FA can be used as substrates by the same enzyme.

Of particular note, the 22:4 n-6 and 22:5 n-3 FA intermediates are not simply desaturated to make the final metabolites. The process is more complex, involving elongation then desaturation in microsomes followed by one cycle of β-oxidation in peroxisomes, a different cellular structure (Figure 1). Thus complete FA metabolic pathways require enzymes in more than one cellular compartment and shuttling of FA products between them. The FA

shuttles and elongase and desaturase enzymes all represent possible points of regulation for lipid metabolism. Longer chain PUFA (22-carbon and 24-carbon) appear to be readily recycled via β-oxidation in peroxisomes (Figure 1). PUFA with fewer than 22 carbons are typically only recycled when the diet does not contain sufficient amounts of the EFA.

Of particular interest to this study are the LC n-3 PUFA: DHA, docosahexaenoic acid (22:6 n-3); DPA, docosapentaenoic acid (22:5 n-3); and EPA, eicosapentaenoic acid (20:5 n-3). EPA is an important precursor to DPA and DHA as well as to biologically active eicosanoids and resolvins (discussed in Chapter 3). DPA has no known metabolic role in humans except as a precursor to DHA (Brenna and Carlson, 2014). DHA can be consumed preformed or it can be made from DPA but at a metabolic cost. The multi-step process of synthesizing DHA from DPA is thought to be an important regulatory check on the amount of DHA that is made.

Mammalian brains and central nervous systems are rich in DHA (Barcelo-Coblijn and Murphy, 2009), comprising about 15% of the total FA in these tissues. DHA is primarily found in PL in neuronal membranes (Arab-Tehrany et al., 2012). Humans accumulate DHA in their brains during gestation to about two years of age (Brenna and Carlson, 2014). The retina of the eye is also rich in DHA. DHA is not made in these tissues but in the liver.

DHA concentrations in the brain appear to be constant within and across species, suggesting that DHA is a limiting factor for the size of the adult animal

brain (Brenna and Carlson, 2014). DHA levels in heart and brain tissue remain relatively constant even if dietary ALA is increased (Barcelo-Coblijn and Murphy, 2009) although DHA in liver, muscle tissue, and adipose tissue will typically increase (Brenna and Carlson, 2014). DHA may be a critical nutrient for mammalian infant development. Clinical trials of infant humans and primates fed formula or breast milk lacking preformed DHA or containing only the ALA precursor showed decreased visual response and depressed cognitive development (Brenna and Carlson, 2014).

Monounsaturated fatty acids (MUFA) are formed from saturated 16-, 18-, and 20-carbon FA by the action of Δ-9 desaturase, also called stearoyl-CoA desaturase. Oleic acid (18:1 n-9) is the most common MUFA in tissues of animals and can represent up to 50% of the fatty acids in adipose tissue in the form of triacylglycerols. Oleic acid is also a common component of membrane phospholipids. The n-9 class of fatty acids does not produce eicosanoid or bioactive precursors and these FA are not considered essential nutrients.

High dietary levels of n-3 and n-6 PUFA will suppress Δ-5, Δ-6, and Δ-9 desaturases (Nakamura and Nara, 2004, Ntambi and Miyazaki, 2004). However, reflecting the diverse roles of MUFA in the body, Δ-9 desaturase is upregulated by high carbohydrate diets, insulin, cholesterol, and estrogen, and downregulated by leptin and thyroid hormones (Ntambi and Miyazaki, 2004). Expression of Δ-9 desaturase can affect insulin sensitivity, metabolic rate,

obesity, and may also be involved in chronic inflammatory diseases (Ntambi and Miyazaki, 2004).

High dietary levels of MUFA may interfere with elongation of ALA and LA (Broadhurst, 1997), possibly because of competition for elongase enzymes.

In poultry, high dietary levels of LC n-3 PUFA, but not LC n-6 PUFA, suppress MUFA synthesis (Gonzalez-Ortiz et al., 2013). High dietary levels of SFA increase MUFA synthesis while suppressing synthesis of LC PUFA (Ajuyah et al., 1991).

2.1 Lipid Peroxidation of Long-Chain Polyunsaturated Fatty Acids

Long-chain polyunsaturated fatty acids (LC PUFA) are more susceptible to lipid peroxidation than saturated FA. Lipid peroxidation can create undesirable changes in foods (Arab-Tehrany et al., 2012), including:

- nutritional: degradation of EFA and fat-soluble vitamins, decrease in caloric content;
- sensory: off flavor and odor, color change;
- commercial: shortened shelf life, decreased consumer acceptability, food safety.

Lipid peroxidation is not the same process as β-oxidation in which enzymes located in peroxisomes pull two-carbon units off the end of a FA.

Lipid oxidation proceeds in three stages:

- initiation: enzymes attack LC PUFA at C-C double bonds, creating free radicals and peroxides;
- propagation: autoxidation occurs between the products of the initiation step and additional LC PUFA;
- termination: free-radical scavengers interrupt and eventually stop the autoxidation reactions.

The products of lipid peroxidation have been linked to aging, mutagenesis, and carcinogenesis, and the free radicals produced during oxidation may be involved in the development of atherosclerosis (Arab-Tehrany et al., 2012).

Aldehydes and ketones produced during the autoxidation phase are associated with flavors and odors described as “off” or “fishy.” Although similar, the fishy odor of harvested fish is the result of bacterial-mediated oxidation of trimethyloxide to trimethylamine (Lin, 1994, Kolanowski et al., 2007). Fish oil intended for human consumption is deodorized to remove the trimethylamines (Lin, 1994). Fish products, including fish oil, contain high amounts of LC PUFA and are also subject to lipid peroxidation.

Some studies have reported that meat and eggs from poultry fed fish oil or fish meal can have “fishy” flavors and odors. The off odors are not likely due to the consumption of trimethylamines because those would have been removed during processing. Gut bacteria in poultry, humans, and other animals naturally produce a small amount of trimethylamine but there are

other bacterial enzymes that break it down. Some people and some strains of poultry lack the genes for these particular enzymes and will smell fishy even if they do not consume any marine products. In humans, this rare disease is called trimethylaminuria.

Similar problems have been reported when poultry are fed flax seed. For example, Jiang et al. (1992) reported that when layers were fed 15% whole flax seed, about 1/3 of the eggs were rejected for poor sensory qualities. Choline is known to be a precursor to trimethylamine but flax seed has less choline than other oil seeds (Jiang et al., 1992). Betti et al. (2009d) reported that meat tissue from birds fed 17% whole ground flax seed for more than 20 days were not acceptable to a consumer taste panel; these problems were not observed in meat tissues from birds fed 10% whole ground flax seed or when the high flax level was fed for a shorter duration.

The most probable cause of the reported fishy flavor and odor in poultry meat and eggs from chickens fed fish or flax products is lipid peroxidation. Lax oversight during feed processing and storage, and improper handling and storage of eggs and processed meat cuts can contribute to increased peroxidation.

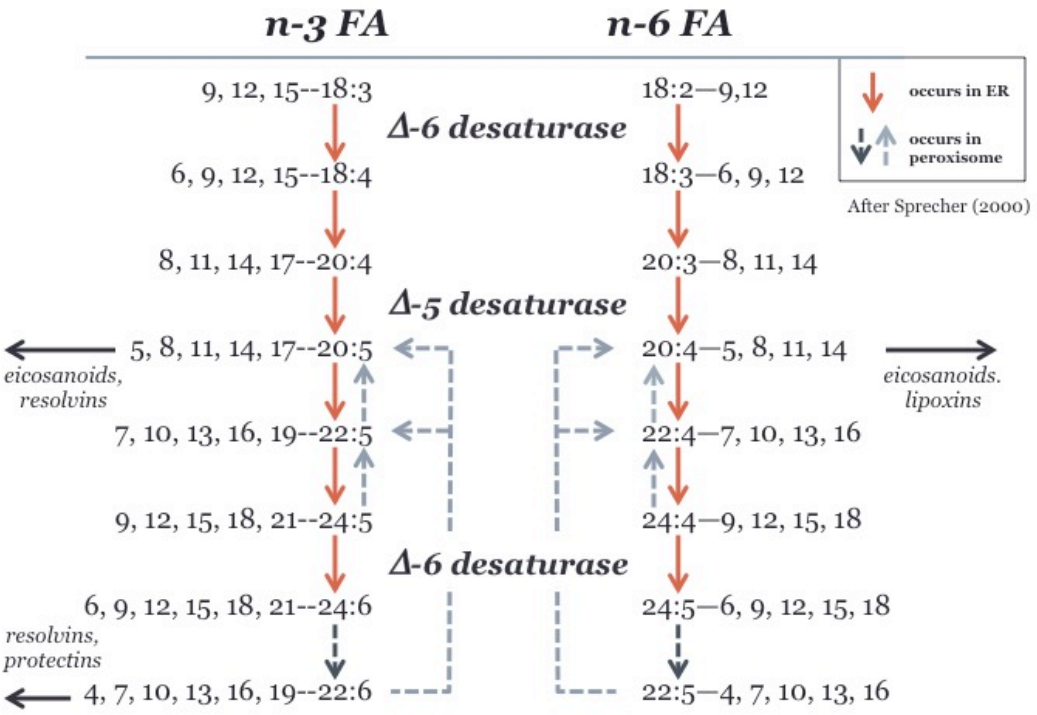


Figure 1. Elongation and desaturation pathways for n-3 and n-6 fatty acids. The two classes of fatty acid compete for the same desaturase enzymes at several points. Most lipid metabolism occurs in the endoplasmic reticulum although formation of the final long-chain metabolites takes place in the peroxisome. After Sprecher (2000). FA, fatty acid; ER, endoplasmic reticulum.

3 Human Health and n-3 Fatty Acids

3.1 Inflammatory Disease and n-3 Fatty Acids

Cardiovascular disease (CVD) is a major cause of death in industrialized countries. According to the American Heart Association 2014 annual report, while the total number of deaths attributed to CVD in the US declined between 2000 and 2010, in 2010 CVD still accounted for 1 in 3 deaths (Go et al., 2014).

It is now established through clinical trials that n-3 fatty acids may decrease the effects or delay the onset of inflammatory disorders and diseases such as CVD (Table 1). Consumption of LC n-3 PUFA may be associated with an array of cardioprotective effects in humans (Flock et al., 2013). These include:

- increased HDL in blood plasma,
- decreased triacylglycerols in blood plasma,
- decreased blood pressure,
- decreased arachidonic acid in blood plasma,
- decreased platelet aggregation.

The list of proposed disease targets for n-3 FA in Table 1 is perhaps overly optimistic, as not all of these outcomes have been tested in clinical trials. However, one example is the longitudinal analysis conducted by Fitzgerald et al. (2014) of long-term dietary and health patterns of more than 1 million participants participating in half a dozen different clinical trials. Their objective was to examine the effects that dietary n-3 PUFA have on

amyotrophic lateral sclerosis (ALS), a progressive neurodegenerative disease with no known cure or treatment. Symptoms of ALS include oxidative stress and inflammation. The authors concluded that increased consumption of either ALA or LC n-3 PUFA, the latter mainly from marine sources, could prevent or delay the onset of ALS.

Most of the potential health benefits of n-3 FA come from LC n-3 PUFA, particularly EPA (20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3). DHA has been associated with decreased incidence of CVD and blood pressure, improvement in cognitive function, and decreased symptoms of dementia, while EPA has been associated with decreased incidence of sudden coronary events, improvements in cognitive function, and decreased inflammation associated with CVD, edema, and erythema (Arab-Tehrany et al., 2012).

Not surprisingly, since LC n-3 PUFA can influence a wide variety of diseases and conditions, there are multiple mechanisms of action proposed for them (Wahle et al., 2003, Deckelbaum et al., 2006, Calder, 2013) (Figure 2). They may:

- act as ligands for cell surface receptors, directly initiating biochemical cascades,
- change composition of cell membrane PL and cell behavior,
- influence immune response via the bioactive eicosanoids,
- influence general processes such as oxidative stress.

The LC PUFA also play a role in gene regulation by mediating transcription factors for genes involved in lipid biochemistry at both tissue-specific and organism scales (Palmquist, 2009). In particular, high levels of n-3 PUFA may drive the movement of FA from storage to oxidation, changing the location and nature of fat depots in the organism (Clarke, 2001). High levels of n-3 FA upregulate genes involved in lipid β-oxidation and downregulate genes that code for lipid synthesis as well as affect insulin control of those genes (Clarke, 2001, Wahle et al., 2003). Thus dietary n-3 FA may play a role in amelioration of metabolic diseases such as diabetes.

The n-3 FA are now understood to play an active role in regulation of resolution of inflammatory immune response. The initiation of an acute inflammatory response also triggers the formation of chemical mediators that ultimately dismantle that same inflammatory cascade (Buckley et al., 2014). The pro-resolution lipid mediators are fundamentally different from anti-inflammatory mediators. The former include lipoxins, resolvins, and protectins. Arachidonic acid (AA, 20:4 n-6) is the precursor for lipoxins; EPA (20:5 n-3) is the precursor for E-series resolvins; DHA (22:6 n-3) is the precursor for D-series resolvins, protectins, and maresins (macrophage mediators). Conversion of these FA into lipid mediators takes place in leukocytes that are drawn to sites of inflammation by AA-derived, pro-inflammatory eicosanoids called prostaglandins. Thus an inflammation response is necessary for initiation of the resolution of that inflammation.

Because pro-resolution lipid mediators are derived from EFA or LC PUFA that are directly consumed, there is a component of nutritional regulation to the resolution response. As noted by Buckley et al. (2014), this helps explain the positive effect of dietary n-3 FA on so many different inflammatory conditions and diseases. Studies with poultry suggest that dietary n-3 FA play the same role in birds (Cook, 2012), an observation that has implications for decreased incidence of inflammatory metabolic disorders in poultry poultry (Section 4.1.1).

Table 1. Proposed health benefits of dietary n-3 fatty acids. This class of fatty acids can decrease incidence and mortality associated with a variety of diseases and conditions that have an inflammatory component. After Calder (2013).

Physiological Role	Target
decrease blood pressure	hypertension, CVD
regulate platelet aggregation	thrombosis, CVD
regulate blood clotting	thrombosis, CVD
decrease plasma TAG concentration	CVD, high cholesterol
modulate FA and TAG metabolism	weight gain, obesity
regulate vascular function	CVD
regulate cardiac rhythm	CVD
regulate heart rate	CVD
decrease inflammation	inflammatory disease (arthritis, IBD, psoriasis, lupus, asthma, dermatitis, etc.), CVD
influence bone mass	osteoporosis
modulate insulin sensitivity	Type 2 diabetes
slow rate of tumor cell growth	hormone-influenced cancers (breast, prostate)
component in visual signaling (rhodopsin)	visual development, especially in pre-term infants
component of brain and neurological tissue	cognitive processes, learning, and behavior in infants and young children
delay onset of neurological disease	ALS, possibly aging

TAG, triacylglycerols; CVD, cardiovascular disease; IBD, irritable bowel disease; ALS, amyotrophic lateral sclerosis.

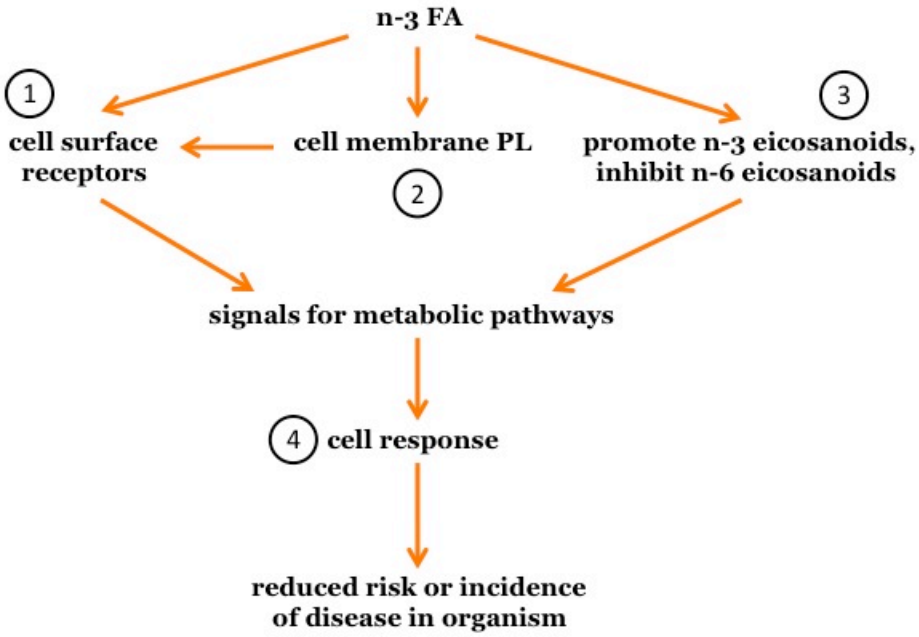


Figure 2. Proposed mechanisms of action for long-chain n-3 polyunsaturated fatty acids. Because this class of fatty acid can influence a wide variety of diseases and conditions, they have multiple mechanisms of action. These include: (1) ligands for cell surface receptors; (2) composition of cell membranes; (3) influence concentrations of other ligands such as hormones through action of eicosanoids; and (4) influence cell response to oxidative stress. After Calder (2013). FA, fatty acid; PL, phospholipid.

3.2 Sources of Fat in Human Diets

Why is the prevalence of chronic inflammatory disorders and disease so high in industrialized countries? Our diets are partly to blame. We consume high levels of SFA and that has been directly linked to increased total cholesterol and low-density lipoprotein (LDL) in blood, increased obesity, and increased risk of CVD and stroke (Sanders, 2013). Principal sources of SFA (mostly 16:0 and 18:0) in western diets include fatty red meat, poultry skin, lard (pork fat), tallow (beef or sheep fat), baked goods, cream, butter, cheese, palm oil, and coconut oil. Corn oil is comprised of 30% linoleic acid (LA, 18:2 n-6), the n-6 FA precursor. High consumption of corn oil contributes to our relatively low consumption of n-3 FA. However, corn oil is not an important source of SFA, which comprise only about 16% of the total lipids in that oil.

A second risk factor is underconsumption of n-3 PUFA, or rather overconsumption of n-6 FA relative to n-3 FA. One measure of this is the n-6:n-3 FA ratio. Barcelo-Coblijn and Murphy (2009) note that, because alpha-linolenic acid (ALA, 18:3 n-3) has a stronger affinity for Δ-6 desaturase (Figure 1) than LA (18:2 n-6), dietary levels of LA up to ten times higher than ALA may be required for suppression of the n-3 FA pathway.

Current Western diets have n-6:n-3 FA ratios of 20 or higher, suggesting that we are effectively suppressing synthesis of LC n-3 FA. Ancestral human diets are thought to have had n-6:n-3 ratios of between 1 and 3 (Simopoulos, 2000). The increase in the ratio is the result of the introduction of new food

types such as dairy products, refined sugars, and refined vegetable oils that were only possible after the establishment of agriculture, animal husbandry, storage mechanisms, and much later, commercial-scale processing techniques (Cordain et al., 2005). In short, humans now consume far more n-6 FA than needed for optimal health (Palmquist, 2009).

Barcelo-Coblijn and Murphy (2009) noted that the highest accumulations of DHA in brain tissue and plasma are not found in humans consuming diets containing the highest possible amount of ALA but when the n-6:n-3 FA ratio is around 3 to 4. Since LA is also an EFA, a healthy diet cannot eliminate it entirely.

3.3 Recommendations for Consumption of Long-Chain n-3 Polyunsaturated Fatty Acids

There are clinically defined health benefits associated with the increased consumption of EPA (20:5 n-3) and DHA (22:6 n-3). There are existing recommendations for daily intakes of EPA and DHA and total n-3 FA that have been issued by a variety of health organizations. The WHO recommends daily intake of 300-500 mg of EPA and DHA and 800-1000 mg of ALA. Other recommendations are summarized in Flock et al. (2013). Based on the results of numerous clinical trials, Flock et al. (2013) believe that 250 mg/d for EPA and DHA should be viewed as a minimum target. The American Heart Association recommends 500 mg/d of EPA and DHA, increasing up to 1 g/d if

an individual already has a chronic inflammatory disease, and up to 2-4 g/d if an individual specifically needs to lower blood TAG levels.

Because mammalian fetuses receive large amounts of DHA from their mothers and because clinical trials have emphasized the importance of DHA in fetal and early infant cognitive and visual development, pregnant women are given different consumption recommendations. The European Food and Safety Agency recommends that pregnant women should consume more than 250 mg/d of EPA and DHA plus an additional 100-200 mg/d of DHA.

According to the World Health Organization, daily consumption of EPA and DHA in the US is around 100 mg; daily consumption of total n-3 FA is 1.6 g (mostly ALA). By all estimates, our current intake of n-3 FA is not sufficient for optimum health.

3.4 Converting Alpha-Linolenic Acid to Long-Chain Polyunsaturated Fatty Acids

Does consumption of the n-3 precursor ALA (18:3 n-3) provide the same health benefits to humans as direct consumption of the LC n-3 PUFA? As Burdge and Calder (2005) note, this depends on the efficiency of conversion of ALA to the LC PUFA. They reviewed nutritional trials conducted on adult humans. Some of the ALA consumed, around 20% for women and 33% for men, undergoes β-oxidation and it appears to be the preferred energy source for humans, a somewhat surprising result because it is an EFA. This rate of β-oxidation is twice that of palmitic (16:0), stearic (18:0), and oleic (18:1 n-9)

acids. The difference in rates of β-oxidation with sex may be the result of the role of estrogen in FA metabolism. For example, in mammals, LC PUFA are synthesized in maternal livers then transmitted to fetuses through the placenta. This process is regulated by estrogen changes associated with pregnancy. The amount of ALA that undergoes β-oxidation in humans appears to be stable and not affected by changes in ALA or LC n-3 PUFA intake. Some of the carbons obtained from β-oxidation of ALA are recycled to make new FA, mainly SFA and MUFA; however, most of these go to PL in the liver, not to TAG. The authors suggest that β-oxidation of ALA may thus be a regulatory step for conversion of ALA to LC n-3 PUFA. As described in Chapter 2, the 22- and 24-carbon FA are synthesized in the peroxisome. The authors suggest that this permits independent regulation of DHA synthesis from the rest of the n-3 FA pathway. Levels of EPA can be increased in plasma and cell lipids by long term intake of ALA, and if the level of dietary LA is decreased at the same time, even more EPA is synthesized, providing additional support for lowering the n-6:n-3 FA ratios of our diets.

Burdge and Calder (2005) estimate that the efficiency of conversion of ALA to EPA is about 0.2%, ALA to DPA about 0.13%, and ALA to DHA about 0.05%. About 60% of the EPA that is synthesized is converted to DPA (docosapentaenoic acid, 22:5 n-3), and about 40% of the DPA that is synthesized is converted to DHA. They maintain that in healthy adults, even these low conversion levels are sufficient to supply the necessary amounts of

LC n-3 PUFA. Anderson and Ma (2009) note that some studies estimate the efficiency of conversion of ALA to EPA at between 5 and 10%, and that of ALA to DHA between 2 and 5%. In most studies, the efficiency of conversion in humans is based on concentrations of the LC n-3 PUFA in blood. Since lipid metabolism is known to be tissue dependent and LC n-3 PUFA are preferentially stored as PL in cell and organelle membranes, rates of conversion determined from concentrations of LC PUFA in the blood may be too low (Anderson and Ma, 2009, Barcelo-Coblijn and Murphy, 2009). Studies of non-human primates, rats, and mice suggest that less than 1% of dietary ALA is converted to DHA in the brain but this may simply reflect what the brain requires and may not represent an accurate measure of the potential rate of conversion itself (Barcelo-Coblijn and Murphy, 2009).

Welch et al. (2010) conducted a large population study of fish eaters, meat-eaters (no fish), and vegans and vegetarians, recording both n-3 FA intake and the resulting n-3 FA profile of participants' blood serum. Fish eaters directly consumed high levels of ALA, DHA, and EPA. Meat-eaters also consumed these n-3 PUFA but in lower quantities. The primary source of n-3 FA for vegans and vegetarians was enriched fat spreads and dairy products. The authors admit that they were surprised to discover that the differences of the circulating blood concentrations of ALA and the LC n-3 PUFA in the three groups were smaller than the intake differences between the groups,

suggesting that the conversion rate was much higher than usually estimated and not well represented by the levels of the FA in blood.

Nonetheless, it is generally presumed, but by no means proven, that consumption of ALA may not provide the same health benefits as consumption of LC n-3 PUFA.

3.5 Increasing Long-Chain n-3 Polyunsaturated Fatty Acids in Human Diets

We can increase LC n-3 PUFA in our diets by consuming more foods rich in them. These include marine sources, supplements, and enriched consumer products. The best dietary sources of LC n-3 PUFA are marine products. Lean fish store lipids in their liver (e.g., cod) while oily fish store lipids in their muscle (e.g., tuna, sardine, salmon). The amount of EPA and DHA in fish varies with factors such as species, diet, water temperature, and season. Nonetheless, EPA and DHA usually comprise around 30% of the total lipids in fish oil (Calder, 2013). Due to culture, cost, concerns over sustainability, concerns over environmental contamination, and seasonal availability, Western diets contain limited amounts of oily fish and other marine products. Supplements such as fish oil capsules are associated with the same problems. They also represent a quasi-pharmaceutical solution that some consumers may not readily embrace.

There is increasing interest in the development of value-added poultry products, particularly meat and eggs containing increased levels of n-3 FA.

Eggs enriched with n-3 FA are now a common sight on grocery store shelves (Cherian, 2012). Numerous studies have demonstrated that adding n-3 fats to poultry diets will enrich meat tissues as well (Chapter 6).

Enriched poultry meat products have significant market potential. According to Chemnitz and Becheva (2014), poultry meat represented 34% of global meat production in 2013, and poultry meat production is expected to increase by 25% between 2010 and 2020. Most of the increase in consumption will take place in developing countries as rates of meat consumption in the US and Europe have been stagnant for several years. There are several factors behind this rapid growth: poultry provide a low-cost source of protein compared to other production animals despite projected increases in the cost of feed; chickens efficiently convert feed to meat and eggs; they can be intensively raised in facilities with relatively small footprints; and there are few religious prohibitions on eating chicken products. Chemnitz and Becheva (2014) report that poultry meat consumption in the US in 2012 was 45 kg per capita. Between 2010 and 2012, US poultry meat companies produced 19.2 billion kg of meat. Tyson Foods alone sold more than 33 billion dollars worth of broiler meat products to the global market between 2011 and 2013. The market for poultry meat products is well established and growing. Offering the consumer an enriched poultry meat product will not require them to change existing dietary habits.

4 Sources of n-3 Fatty Acids in Poultry Diets

4.1 Fats in Poultry Diets

Fats supply poultry with energy and essential fatty acids, and provide improved absorption of fat-soluble vitamins, pigments, and other supplements. However, other than a minimum level of the EFA linoleic acid (18:2 n-6), there is no specific amount of fat specified for commercial poultry diets. Often the amount of fat used is determined by a least-cost analysis that seeks to provide optimum protein and energy at the lowest cost. Cheap fat sources provide inexpensive calories.

Fats in poultry diets can come from cereal grains such as corn; oil seed products such as whole or ground seed or oil from canola, soybean, or sunflower; animal sources such as tallow and lard; and restaurant grease blends (Table 2). Fats in poultry diets can range from unsaturated FA in oil from oil seeds to highly saturated animal fats. Blended animal/vegetable fat sources are common. There may be little attempt on the part of a commercial facility to feed a specific balance of unsaturated to saturated fats despite the documented improvements in bird health and performance when fewer saturated fats are included (Section 4.1.2).

Excessive fat consumption can lead to metabolic disorders in poultry, including fatty liver syndrome in layers and erratic ovulation and defective egg syndrome in broiler breeders (Leeson and Summers, 2001). These disorders are the result of nutrient imbalance.

4.1.1 Ascites in Broilers

Ascites, accumulation of fluid in the peritoneal cavity, is a symptom of metabolic dysfunction in poultry associated with around 5% of the mortality in the broiler bird industry (Baghbanzadeh and Decuypere, 2008). It is caused by an imbalance between oxygen requirements and oxygen supply (Kalmar et al., 2013). High growth rates of modern poultry strains are associated with increased oxygen demand, a diminished cardiovascular capacity relative to the increased muscle mass, and a high metabolic rate (Julian, 2005, Baghbanzadeh and Decuypere, 2008, Kalmar et al., 2013). Avian lungs are parabronchial, not alveolar, and are relatively rigid with a fixed volume. While rapid growth has been a deliberately selected trait, increased cardiovascular capacity has not been similarly selected because no genetically useful markers have been identified (Baghbanzadeh and Decuypere, 2008, Kalmar et al., 2013).

Symptoms of metabolic dysfunction in poultry include pulmonary hypertension and accumulation of fluid in the peritoneal cavity ("water belly"). The pulmonary vessels can't expand to accommodate the increased blood flow; the result is increased workload on the right ventricle that leads to right ventricle hypertrophy and eventually failure (Julian, 2005). Liver pathologies can also cause ascites (Julian, 2005, Kalmar et al., 2013).

Sudden rupture of the right ventricle might be a cause of broiler Sudden Death Syndrome (SDS) (Baghbanzadeh and Decuypere, 2008). As Julian

(2005) notes, SDS is the cause of up to 4% of the mortalities in the commercial industry. It can occur at any time in the bird's life. It is common for birds to appear healthy up to the point of death.

Management practices that decrease growth rate, such as feed restriction and increased duration of dark periods, have the largest effect on reducing incidence of metabolic dysfunction. Dietary intervention may also be useful, specifically a decrease of unsaturated FA and an increase in n-3 FA in poultry diets (Baghbanzadeh and Decuypere, 2008, Kalmar et al., 2013). While necropsies of birds that died from SDS are often inconclusive, Cherian (2007) notes that some analyses show increases in cardiac LC n-6 FA, increases in serum total lipids, and decreases in cardiac EPA and total n-3 FA. Thus broiler diets that include more n-3 FA may provide the same beneficial health outcomes to the birds as those obtained by humans who consume more n-3 FA (Cherian, 2007).

Results from a simulated high altitude broiler feeding trial conducted by Bond et al. (1996) provide support for this idea. Birds fed 10% flax oil (rich in ALA) in conditions simulating 2200 m altitude had decreased mortality and incidence of ascites compared to birds fed 10% animal/vegetable oil blend (rich in SFA and MUFA) in the same conditions. Birds fed flax oil in conditions simulating 1500 m altitude had decreased hypertension relative to the other group.

4.1.2 Digestion of Fat in the Poultry Gastrointestinal Tract

Tancharoenrat et al. (2014) conducted two experiments with broilers to determine the site of fat digestion in the chicken gastrointestinal tract (GIT). They fed one group of broilers a standard corn-soybean meal (C-SBM) diet with 5% soybean oil that is rich in n-6 PUFA (LA, 18:2 n-6) and MUFA (oleic acid, 18:1 n-9), or tallow that is rich in SFA (palmitic acid, 16:0 and stearic acid, 18:0). A second group of broilers were fed a special fat-free diet for two days to determine the amount of endogenous fat loss at the terminal ileum. They found that calculated fat digestibility was negative for the duodenum, implying net passage of FA through the duodenum. Bile ducts enter the poultry GIT at the distal duodenum and are recycled with digesta into the gizzard. They confirmed that unsaturated FA are more completely digested than saturated FA, and that the digestibility of all FA types was improved in the soybean oil diet compared to the tallow diet. The authors suggest that while unsaturated FA spontaneously form micelles, saturated FA require a higher concentration of bile acids in order to properly emulsify. Chickens digest more than 75% of total fat in the jejunum and the remaining 20% or so in the upper ileum. There is little fat digestion in the lower ileum. Less than 2% of the total fat consumed was undigested at the end of the ileum; less than half of this was from dietary sources. The rest comes mainly from bile acids with contributions from mucin, cholesterol, epithelial cells, and microbial FA.

4.1.3 Reducing Fat Deposition in Poultry

The primary fat depots in poultry are the abdominal fat pad (AFP) and the skin (Ferrini et al., 2008). There are other minor fat depots including intramuscular fat in meat tissues. The AFP can represent about 15% of total body lipids. Since most of it is separated from meat during processing, it contributes nothing to meat quality. Therefore a decrease in AFP volume has the potential to decrease processing time and waste (Gonzalez-Ortiz et al., 2013).

In poultry, as in other monogastric animals, the composition of dietary fat affects the composition and amount of fat deposited in the body. In poultry, the liver is the primary site of lipid metabolism.

The review by Esteve-Garcia (2012) concludes that poultry diets high in PUFA result in improved feed:gain and less fat deposition than diets containing SFA or MUFA from tallow or vegetable oil sources. He notes that other species, including mice, rats, and humans, also have decreased body fat when they consume diets high in PUFA. The combination of the amount of PUFA and the n-6:n-3 FA ratio may influence fat deposition more than the presence of dietary PUFA alone (Qi et al., 2010). In other words, there is an optimum ratio of n-6 and n-3 FA that leads to a decrease in adipose tissue.

Smink et al. (2010) conducted a feeding trial to test whether vegetable oils that are high in SFA would have the same effect on fat deposition as animal sources of SFA. They compared whole-body crude fat measurements on

broiler birds that received diets containing 4-8% sunflower oil or palm oil. Sunflower oil is comprised largely of the PUFA linoleic acid (18:2 n-6) while palm oil is comprised of SFA and MUFA (Table 2). Fat deposition increased in birds receiving palm oil to levels similar to those observed in birds fed saturated animal fat, indicating that the source (animal or vegetable) of the dietary SFA doesn't affect this result. Birds receiving the sunflower oil diets with high levels of n-6 PUFA had increased MUFA synthesis as well as increased β-oxidation.

Crespo and Esteve-Garcia (2001) compared FA composition of tissues from broilers fed diets containing 6 or 10% tallow (SFA and MUFA), olive oil (MUFA, mostly 18:1 n-9), sunflower oil (PUFA, 18:2 n-6), or flax oil (PUFA, 18:3 n-3). Both of the PUFA-rich diets resulted in improved feed:gain and statistically decreased the size of the AFP compared to the diets containing SFA. Enrichment of PUFA in muscle and adipose tissue occurred at the expense of SFA and MUFA. The authors concluded that poultry diets rich in either class of PUFA change the site of adipose storage to other depots besides just the AFP and alter lipid metabolism by increasing β-oxidation.

Ferrini et al. (2008) reached a similar conclusion. They fed broilers diets containing 10% tallow, sunflower oil, or flax oil. Birds fed diets high in PUFA had decreased AFP and skin weights relative to birds fed diets high in SFA; the decrease in AFP was as much as 25%. They concluded that changes in fat deposition were not related to differences in total FA availability or apparent

metabolizable energy (AME) of diets with different fat sources. As long as broiler diets contained sufficient amounts of unsaturated fatty acids, changes in AFP weight were driven by overall changes in fat metabolism.

Gonzalez-Ortiz et al. (2013) fed broilers diets containing 10% tallow or 10% of a mixture of fish oil (LC n-3 PUFA) and flax oil (ALA, 18:3 n-3). Paradoxically, the fish oil plus flax oil treatment led to an increase in SFA in tissues relative to SFA intake from that diet, and a possible inhibition of synthesis of oleic acid (18:1 n-9). Consumption of high levels of preformed LC n-3 PUFA may have inhibited both the conversion of ALA to long-chain metabolites and MUFA synthesis.

In summary, fat deposition is increased in birds receiving diets high in SFA regardless of the source of dietary fats. Increasing dietary levels of PUFA alter lipid metabolism of the birds, resulting in increased β-oxidation and decreased adipose tissue storage. High levels of LC n-3 PUFA in particular may depress MUFA synthesis (Chapter 2). The metabolic response of birds to consumption of preformed LC n-3 PUFA appears to be different from their response to consumption of LA or ALA.

Table 2. General fatty acid composition of common fat sources used in poultry diets. Values shown are % of total lipids. After Cherian (2007).

Fat Source	SFA	MUFA	n-6 PUFA	n-3 PUFA
canola oil	7	61	22	10
flax seed oil	10	18	17	55
soybean oil	15	23	54	8
corn oil	13	29	57	1
safflower oil	10	14	76	tr
sunflower oil	12	16	71	1
palm oil	51	39	10	tr
cottonseed oil	27	19	54	tr
beef tallow	48	49	2	1
restaurant grease	21	52	23	3
fish oil	17	42	11	26
Menhaden fish oil	30	25	2	40

tr, trace.

4.2 Marine Sources of n-3 Fatty Acids

Fish meal and fish oil can be added to poultry diets as a source of LC n-3 PUFA. Feeding LC n-3 PUFA to poultry will increase the amount of LC n-3 PUFA in their tissues in a relatively linear fashion. Poureslami et al. (2010) note that a much smaller amount of fish oil in poultry diets results in a much larger increase of LC n-3 PUFA in tissues compared to diets containing flax oil.

Direct consumption of LC n-3 PUFA may result in undesirable changes in gut morphology of broilers. Aziza et al. (2014) fed 10% camelina meal that contains high levels of ALA (18:3 n-3), 3.2% fish oil, or a mixture of the two fat sources to broilers and examined differences in intestinal morphology. Absorption of nutrients depends in part on mucosal surface area in the jejunum and duodenum of the small intestine. Thus villi height and crypt depth can be used as measures of the ability of the GI tract to effectively absorb nutrients. They found that birds fed diets containing only fish oil had the smallest villi heights and smaller crypt depths. Birds fed fish oil or the fish oil-camelina meal mixture had decreased feed intake and depressed weight gain relative to birds fed only camelina meal. They raised the question of whether fish oil negatively affected palatability of the diet, and whether decreased feed intake or perhaps the form of the FA caused the morphological changes in the GI tract.

In the aquaculture industry, there is considerable interest in replacing fish oil with vegetable sources of n-3 FA. In contrast to the poultry industry,

this is mainly driven by issues of cost rather than by any observed decrease in performance. Turchini and Francis (2009) conducted whole-body FA analysis of rainbow trout that were fed diets that included either 22% fish oil or flax oil. (While this is the published value, it seems extraordinarily high. The actual value is probably a more reasonable 2.2%.) Both dietary treatments also contained 7% fish meal. They found that most of the EPA in the fish oil diet was oxidized although some was converted to DHA and some was deposited in muscle tissue. Some of the ALA in flax oil was also oxidized; even less was converted to LC n-3 PUFA and the levels of the LC n-3 PUFA never reached those of trout fed diets containing fish oil. Most of the ALA in the flax oil was deposited in muscle tissue. Based on their results, authors were not particularly supportive of replacing fish oil with flax oil.

Mateos et al. (2011) observed similar results in a feeding trial of cultured abalone but their interpretation was more positive. Diets contained 1.5% oil comprised of fish oil, flax oil, or combinations of the two oils. As with the trout study, the flax oil diet did not produce the same level of LC n-3 PUFA in the abalone meat tissue as the fish oil diet. Nonetheless, the authors concluded that their study provided good evidence that abalone could successfully convert ALA to LC n-3 PUFA and that flax oil was an effective and cheaper alternative to fish oil.

In summary, at commercial scales, it is not economically feasible or sustainable to feed fish oil or meal to poultry or marine animals. The same

concerns associated with direct human consumption of marine products are still present: sustainability, cost, potential environmental contamination. High levels of fish oil in poultry diets increase the likelihood of lipid peroxidation of the feed (Chapter 3), an issue that was not addressed by either the trout or abalone study.

4.3 Vegetable Sources of n-3 Fatty Acids

There are several terrestrial plants with high amounts of the n-3 FA ALA (Table 3). Oil seeds such as flax and canola are often used in poultry diets as a source of n-3 FA. Flax seed is the richest terrestrial source of n-3 FA. The whole seed contains 40-50% oil and half of that is alpha-linolenic acid (ALA, 18:3 n-3). Whole flax seed also supplies 24% CP and 3900 Kcal/kg ME. Although not shown in Table 3, walnuts contain about the same amount of ALA as canola seed. Walnuts contain high amounts of anti-nutritive tannins and are not used in poultry diets.

The nutrients contained in oil seeds are protected (encapsulated) within the seed coat by an array of soluble and insoluble non-starch polysaccharides. Monogastrics lack the enzymes to digest and break down these molecules. If the seeds or feed containing them are processed in a manner that physically disrupts the seed coat (grinding, pelleting), energy utilization from oil seeds is significantly improved (Chapter 6). Adding enzymes to poultry diets that specifically target non-starch polysaccharides is another method to improve

energy utilization from whole oil seeds; this is discussed in greater detail in Chapters 5 and 6.

Addition of large amounts of vegetable oils to poultry diets can make feed mixing difficult. It also significantly increases the likelihood of lipid peroxidation of the feed. As with fish oil, feeding flax oil to poultry is not economically feasible at commercial scales.

The addition of whole flax seed to poultry diets decreases opportunity for lipid oxidation of the feed. Mixing whole flax seed into the feed does not require additional management steps. Some care must be taken with respect to feed storage but the risk of lipid peroxidation of LC PUFA in the feed is greatly decreased. In the next section, we will consider the composition of flax seed and its use in poultry feed.

Table 3. Dietary sources of n-3 fatty acids. Oil seeds such as flax and canola are often used in poultry diets as a source of n-3 fatty acids. Values shown are % of total lipids. After Cherian (2012).

Common Name	Scientific Name	Linolenic Acid	LC n-3 PUFA	Total n-3 FA
Plant Oils				
Flax	*Linum usitatissimum*	53	0	53
Canola	*Brassica napus*	11	0	11
Camelina	*Camelina sativa*	38	0	38
Chia	*Salvia hispanica*	30	0	30
Oil Seeds and Meals				
Flax seed	*Linum usitatissimum*	22	0	22
Canola seed	*Brassica napus*	5	0	5
Chia seed	*Salvia hispanica*	15	0	15
Camelina meal	*Camelina sativa*	30	0	30
Perilla	*Perilla frutescens*	55	0	55
Marine Products				
Marine algae		0	11.5	11.5
Fish oil		1.5	30	31.5
Fish meal		1.3	8.4	9.7

LC PUFA, long-chain polyunsaturated fatty acid.

4.4 Flax Seed Structure and Composition

4.4.1 Flax Seed Structure

Flax seeds are small, flattened ovals (2-3 mm wide, 3-5 mm long); seeds can sometimes be beaked at one end. An outermost cuticle makes the seeds appear polished and smooth. Cultivars can range in color from yellow to dark brown. In addition to the hard seed coat, the seed is composed of a layer of endosperm cells, the embryo, and two cotyledons (embryonic leaves); 75% of the oil in flax seed is located in the cotyledons.

The seed coat is composed several distinct cell layers (Freeman, 1995). Mucilage cells are located directly beneath the cuticle. Mucilage is a type of water-soluble non-starch polysaccharide. Mucilage cells, polygonal in shape, can quickly absorb large amounts of water, and when hydrated, expand outwards to split the cuticle. Inner mucilage cell walls resist distortion inwards. Electron microscopy has been used to visualize individual mucilage cells (Bhatty, 1995, Freeman, 1995) (Figure 3). Beneath the mucilage cells, the next layer in the seed coat is comprised of lignin fiber cells that are elongated parallel to the long axis of the seed. Beneath the layer of lignin fiber cells is a thin layer containing many small cells that are elongated at right angles to the lignin fiber cells. The final layer of cells in the seed coat is comprised of pigment cells containing tannins that give flax seed its color.

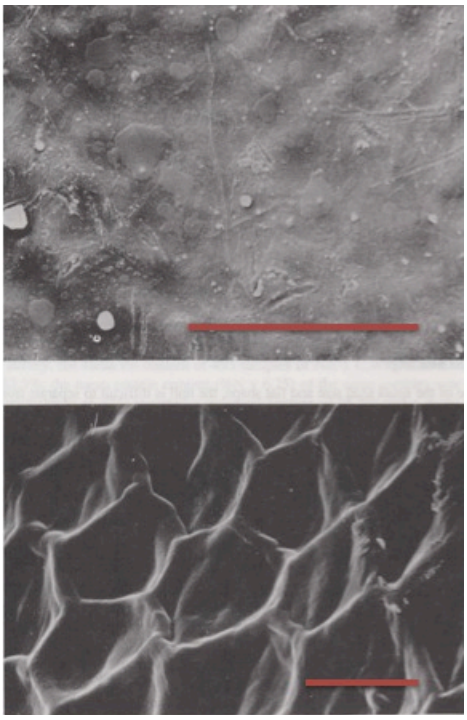


Figure 3. Flaxseed mucilage. SEM photomicrographs of flax seed with unhydrated mucilage (upper photo) and flax seed with mucilage removed by boiling in an alkaline solution (lower photo). Scale bars are approximately 5 micrometers. After Bhatty (1995).

4.4.2 Flax Seed Composition

Flax seeds contain on average 41% oil, although this can be as high as 60% depending on cultivar, season, and geographic origin (Bean and Leeson, 2002a). Based on proximate nutrient analysis, flax seeds are also comprised of 20 to 30% protein and around 30% nitrogen-free extract, corresponding to the carbohydrate fraction.

The composition of oil in flax seed is shown in Table 4. In general, the breakdown is about 70% PUFA, 20% MUFA, and 10% SFA. Most of the PUFA fraction is comprised of ALA (18:3 n-3). Flax seeds contain many times more ALA than other plant sources (Bloedon and Szapary, 2004) (Table 3). An increase in ALA in flax seed is accompanied by a decrease in oleic acid (18:1 n-9) relative to the FA composition of corn and canola oils (Table 4) (Bhatty, 1995).

In contrast, corn oil delivers about 30% PUFA (nearly all of which is LA, 18:2 n-6), 50% MUFA (oleic acid, 18:1 n-9), and 20% SFA. Canola oil delivers about 36% PUFA (two-thirds of this is LA), 56% MUFA (oleic acid), and 6% SFA (Table 4).

Flax seed is considered a functional food, a food that has nutritional benefits beyond its component nutrient content, because it contains ALA, soluble fiber, and lignans, all of which may contribute to a decrease of CVD risk factors (Oomah, 2001, Bloedon and Szapary, 2004). Lignans, water-soluble phenolic compounds, are a class of phytoestrogens. They are common

in fiber-rich plants such as cereals and grains and are particularly abundant in many oil seeds. Precursor lignans in flaxseed are modified by intestinal bacteria to form the bioactive secondary lignans enterodiol and enterolactone (Bloedon and Szapary, 2004). Flaxseed lignans are hydroxyl scavengers and may act as antioxidants to free radicals (Touré and Xu, 2010). The antioxidant activities of flaxseed lignans have been positively correlated to a decrease in symptoms of high cholesterol, CVD, and diabetes (Touré and Xu, 2010).

Table 4. Comparison of principal fatty acids in corn, canola, and flax oil. Values shown are % of total lipids. Flax oil data are summarized from Bhatty (1995).

FA	Corn	Canola	Flax
16:0	13	4	5
18:0	3	2	4
18:1 n-9	52	56	17-25
18:2 n-6	31	26	15
18:3 n-3	1	10	48-56
Total SFA	16	6	5
Total MUFA	52	56	17-25
Total PUFA	32	36	63-71
unsat:sat	5	16	19
n-6:n-3	31	3	< 1

4.4.3 Metabolizable Energy in Flax Seed

As noted above, canola seed can also be a source of n-3 FA in poultry diets. Both canola seed and flax seed provide protein and energy. However, there are large differences in the energy provided by whole seed, oil, and meal (meal is comprised of defatted seeds that are then ground). Lee et al. (1995) conducted precision feeding assays in roosters to compare the energy content of canola seed, oil, and meal to flax seed, oil, and meal. Meal has more protein on a DM% (dry matter percent) basis but it delivers much less gross energy because of the removal of the fat (Table 5a). Oil alone delivers more dietary AMEn (nitrogen-adjusted approximate metabolizable energy) than meal+oil, and whole seeds deliver more dietary AMEn than meal alone (Table 5b). The authors speculate that the differences are due to fiber content and mucilage.

The energy value of canola seed is higher than flax seed. Shen et al. (2004) determined via precision feeding assays that pelleting, autoclaving, or roasting whole flax seed then grinding it could significantly increase TMEn (nitrogen-adjusted total metabolizable energy) to levels similar to that of canola seed or soybean, and improve digestibility over untreated flax seed. Bean and Leeson (2002b), Shen and Chavez (2003), and Shen et al. (2005) observed similar results by heat treating or pelleting whole flax seed.

Table 5. Energy contained in flax seed and canola seed. (a) Analyzed crude protein, fat (ether extract), and GE (gross energy) in flax seed and meal compared to canola seed and meal. (b) Analyzed AMEn and TMEn of flax seed, meal, oil, and meal+oil compared to canola seed, meal, oil, and meal+oil. Energy values in kcal/g. After Lee et al. (1995).

A

	flax seed	flax meal	canola seed	canola meal
CP %	22	31	20	35
fat %	37	5	38	4
GE (kcal/g)	6.5	4.5	6.8	4.4

B

	flax meal	canola meal	flax oil	canola oil
AMEn	2.07	1.98	8.10	8.25
TMEn	2.07	2.09	8.28	8.46
	flax seed	canola seed	flax meal+oil	canola meal+oil
AMEn	3.75	4.46	4.91	5.63
TMEn	3.75	4.56	5.07	5.61

4.5 Anti-Nutritional Factors in Flax Seed: Trypsin Inhibitors and Cyanogenic Glycosides

Flax seed contains anti-nutritive components such as trypsin inhibitors, cyanogenic glycosides, and non-starch polysaccharides; the latter will be discussed in the next section.

The level of trypsin inhibitors in flax seed is less than that in soybeans and canola meal and they probably have a negligible effect on growth performance (Bhatty, 1995).

Cyanogenic glycosides are precursors to HCN (hydrocyanic acid). HCN inhibits cytochrome oxidase, an enzyme used in cell respiration cascades. More than 1000 species of plants produce HCN as a protective mechanism (Feng et al., 2003). The amount of HCN that can be produced from raw feed-grade flax seed is 377 mg/kg; raw human-food-grade flax seed produces 139 mg/kg. In contrast, raw cassava can produce 2450 mg/kg of HCN (Feng et al., 2003). Cyanogenic glycosides and the glycosidase enzymes that convert them to HCN are normally separated in the intact seed but when the seed is ground or digested, the reaction can proceed. The high levels of cyanogenic glycosides in raw cassava make it toxic to animals and humans. In contrast, a human would have to consume 1 kg of ground flax seed to incur toxic levels of cyanide (Touré and Xu, 2010). Ruminants can detoxify HCN in the liver but excess HCN in monogastrics can lead to an enlarged thyroid gland and other disorders.

Heat applied to feed during pelleting appears to substantially decrease the activity of glycosidases as well as evaporate any HCN that has already formed (Feng et al., 2003). It is not clear what levels of these compounds can be tolerated by poultry but the relatively low levels of cyanogenic glycosides in flax seed may not significantly affect growth performance.

4.6 Anti-Nutritional Factors in Flax Seed: Non-Starch Polysaccharides

4.6.1 Insoluble Non-Starch Polysaccharides

Among the anti-nutritional factors (ANF) in flax seed are the non-starch polysaccharides (NSP). These occur in two groups: water-soluble and insoluble. Cellulose and lignin are insoluble while arabinoxylan, β-glucan, and oligo- and polysaccharides containing the sugars arabinose, galactose, rhamnose, and mannanose are typically water-soluble. Cellulose is composed of β-1, 4-linked glucose units (Figure 4). Monogastrics lack digestive enzymes to break β-linked sugars but some species of gut bacteria can utilize shorter oligosaccharide NSP with these links.

Insoluble non-starch polysaccharides, or fiber, are present in all green plant cells but cereal grains, legumes, and oil seeds contain relatively high amounts (Table 6). Cereal grains and oil seeds have a high proportion of insoluble NSP out of total NSP. For example, barley and rye contain around one-third to one-half insoluble NSP out of total NSP, respectively. Legumes

have a high proportion of NSP per DM% and most of that is insoluble (Table 6).

The insoluble NSP fraction is usually considered a poultry diet diluent. Most monogastrics lack significant amounts of fermenting bacteria and are not able to effectively utilize large amounts of fiber, although a minimal amount has been shown to be an important factor in overall gut health for poultry (Choct, 2009, Bao and Choct, 2010). However, genetic sequencing studies show that in chickens, the microbial community of the ileum is dominated by *Lactobacilli*, fermenting facultative anaerobes, while the microbial community of the cecum is dominated by *Clostridium*, obligate anaerobes (Lu et al., 2003). It is possible that chickens can effectively utilize more dietary fiber than commonly assumed.

Effects of fiber in poultry diets include decreased digesta transit time, improved gizzard function and gastroduodenal reflux, and possibly an improvement in starch digestibility, although results of *in vitro* studies are conflicting on this latter point (Hetland et al., 2004). Diets high in fiber can cause birds to eat more. It has also been proposed that fiber stimulates increased secretion of bile acids that may in turn more completely emulsify lipids (Hetland et al., 2004), especially SFA.

4.6.2 Soluble Non-Starch Polysaccharides

Bhatty (1995) recognized that mucilage present in oil seeds and cereal grains has a negative affect on performance. Mucilage is a type of water-

soluble NSP. In flax seed, it is located outside the seed coat and may represent about 5-8% of flax seed by weight (Bhatty, 1995, Rebolé et al., 2002).

Cui et al. (1994) describe the two primary components of flaxseed mucilage. The acidic fraction is comprised of pectin-like polysaccharides containing a backbone of rhamnose and galacturonic acid with side chains of galactose or arabinose (Figure 5). The side chains are attached to the backbone with β-(1,4) links but sugars in the backbone have different linkages. The neutral fraction composed of very long chains of xylose joined by β-1, 4-linkages with side chains of arabinose or galactose units (Figure 6). The neutral fraction has a much higher viscosity than the acidic fraction. Bhatty (1995) notes that as the xylose fraction increases, the viscosity of flax mucilage increases.

High levels of the soluble NSP mucilage in poultry diets are associated with:

- increased digesta viscosity,
- decreased digesta mixing,
- decreased efficacy of digestive enzymes,
- decreased digestibility of all nutrients but particularly fat,
- delayed transit of digesta through the intestinal tract and thus a greater opportunity for pathogenic bacterial overgrowth,
- and ultimately, decreased growth performance.

Jia and Slominski (2010) suggested that that viscous digesta decreases fat digestibility by impairing lipid emulsification and micelle formation. Incomplete emulsification of lipids may lead to lipid coatings on other nutrients and decreased digestibility (Hetland et al., 2004).

Rebolé et al. (2002) extracted mucilage from flax seeds by boiling them, then dried and ground the extract. Using standard C-SBM diets, they added the ground mucilage at levels of 4 to 16%; they estimated that adding 16% mucilage was equivalent to adding 20% whole seed to the diet. They observed that increasing levels of mucilage resulted in increased digesta viscosity, decreased fat digestibility, and decreased dietary AMEn. The decrease in fat digestibility was smaller than observed when birds are fed whole flax seed so the authors concluded that mucilage was not the only anti-nutritive component of flax seeds affecting digestibility.

Alzueta et al. (2003) fed broilers 8 and 16% ground whole flax seed or 16% demucilaged flax seed that had been boiled then ground. Birds receiving the demucilaged flax diets had improved weight gain and fat digestibility and decreased jejunal digesta viscosity relative to the ground flax seed diets. There was no difference in feed:gain ratio from the demucilaged flax diet relative to control.

Smits et al. (1998) fed broilers a diet containing 1% carboxymethylcellulose, a non-fermenting, water-soluble, high viscosity NSP. A nutritionally adequate, semi-purified corn and soybean protein mixture was

used as the basal diet. After 11 days, they observed large increases in *Clostridia*, *Lactobacillus*, *Bacteroides*, yeast, and mold in the duodenum and jejunum but not the ileum. The birds receiving the viscous NSP had increased water intake but their feed intake and weight gain was not different from the control.

Leeson and Caston (2004) examined the effect that dehulling whole flax seed had on energy utilization in layers. Dehulling is a mechanical process that removes both mucilage and the insoluble fiber of the seed coat. They fed 65-week old layers 10% whole flax seed and whole flax seed mixed with dehulled flax seed in a 1:1 or 1:9 ratio. They observed that dietary AMEn increased with the amount of dehulled flax seed present, and that eggs from layers fed dehulled flax seed contained more n-3 FA than layers fed whole flax seed. They also noted that excreta from layers fed 9% dehulled flax seed was more "consistent." High levels of mucilage in poultry diets are associated with sticky excreta (Hetland et al., 2004) (also see Chapter 8), so we can interpret their comment to mean that smaller amounts of mucilage in the diet led to less sticky excreta.

It is clear that high levels of soluble and insoluble NSP present in flax seed, as well as in other oil seeds, legumes, and cereal grains, can negatively affect performance. Pelleting or grinding may affect total energy availability but do not directly affect NSP. Therefore if we wish to use whole flax seed as a source of n-3 FA in poultry diets, we must consider additional tools that can

decrease or eliminate the anti-nutritive effects of NSP. Exogenous enzymes have been used in the monogastric feed industry for decades. The addition of enzymes designed to specifically target NSP in oil seeds like flax offers a viable solution to the problem.

Table 6. Non-starch polysaccharides (NSP) in (a) cereal grains and (b) legumes. Values are % DM. After Choct (2006).

A

Cereal	Cellulose	Arabino-xylose	Glucanose	Mannose	Galactose	Total NSP
Wheat						
soluble	-	1.8	0.4	tr	0.2	2.4
insoluble	2.0	6.3	0.4	tr	0.1	9.0
Barley						
soluble	-	0.8	3.6	tr	0.1	4.5
insoluble	3.9	7.1	0.7	0.2	0.1	12.2
Rye						
soluble	-	3.4	0.9	0.1	0.1	4.6
insoluble	1.5	5.5	1.1	0.2	0.2	8.6
Corn						
soluble	-	0.1	tr	tr	tr	0.1
insoluble	2.0	5.1	-	0.2	0.6	8.0
Sorghum						
soluble	-	0.1	0.1	tr	tr	0.2
insoluble	2.2	2.0	0.1	0.1	0.1	4.6

tr, trace.

B

Legume	Cellulose	Rhamnose	Arabinose	Mannose	Galactose	Total NSP
Soybean						
soluble	-	0.1	0.5	0.2	0.6	2.7
insoluble	4.4	0.2	2.4	0.7	3.9	16.5
Chickpea						
soluble	-	0.1	0.21	0.1	0.3	0.9
insoluble	-	0.1	3.8	0.4	0.7	14.6
Field pea						
soluble	-	tr	0.2	0.1	0.1	0.5
insoluble	-	-	5.7	-	0.8	15.9

tr, trace.

Figure 4. Cellulose molecule. Cellulase enzyme can break the β-(1,4) links between the glucose units.

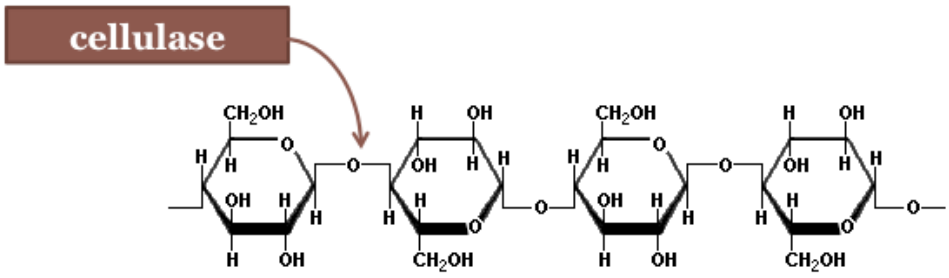


Figure 5. Acidic fraction of mucilage in flax seed. Rhamnose (R) and galacturonic acid (GU) comprise the backbone. Side chains of arabinose or galactose are attached to the backbone with β-(1,4) links. Galactanase enzyme can break the bonds between galactose side chain sugar units.

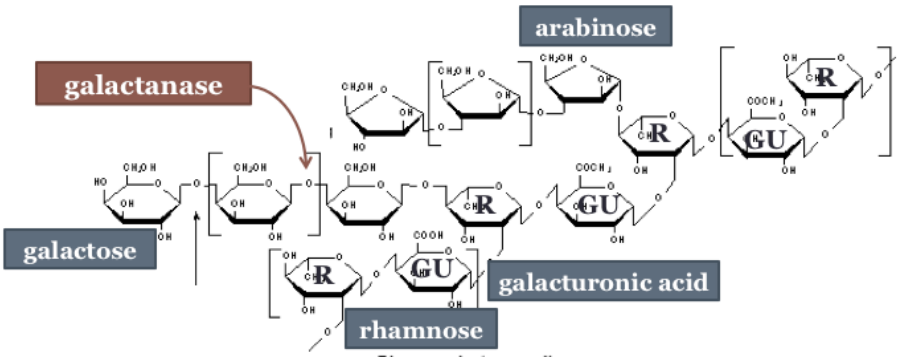
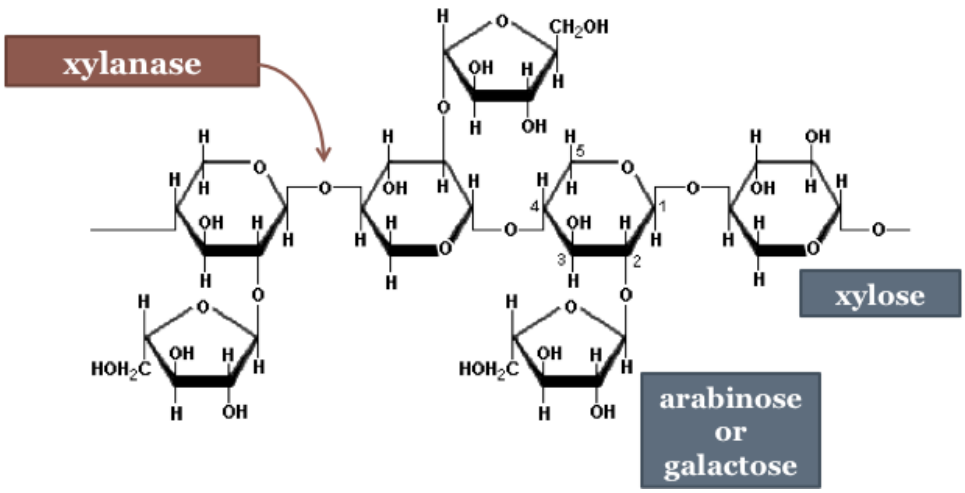


Figure 6. Neutral fraction of mucilage in flax seed. Long chains of xylose units joined by β-(1,4) links form the backbone. Xylanase enzyme can break the bonds between the backbone sugar units. Side chains of arabinose or galactose are also present.



5 Exogenous Enzyme Technologies

5.1 Benefits of Exogenous Enzymes in Animal Diets

Exogenous enzymes added to animal diets are supplemental digestive enzymes that are not produced naturally in the gut or that are not produced at the desired time or in the desired quantity. According to Leeson and Summers (2001), there are several driving forces behind the use of exogenous enzymes in poultry diets: (1) to decrease anti-nutritive effects of feed ingredients; (2) to boost low levels of endogenous enzymes and improve digestibility of standard feed ingredients at different life stages; (3) to decrease the volume of undigested nutrients and waste; (4) to support the inclusion of new protein, carbohydrate, or lipid sources in feed.

The use of exogenous enzymes in monogastric animal feeds is widespread, and they have been used in the poultry industry since the 1980s. The two types of exogenous enzymes commonly added to commercial poultry diets are carbohydrases that target complex, non-starch carbohydrates, and phytases that target phytate, a molecule used by plants to store phosphorus. Although digestion of protein and fat in the chicken involves enzymes such as protease and lipase, improving the digestibility of these nutrient classes is generally not approached through exogenous enzyme treatments but through feed ingredient management and, in the case of some proteins, heat treatment during pelleting. The use of exogenous proteases is increasing but experimental results are not always consistent (Kaczmarek et al., 2014).

Consideration of the use of exogenous phytase, proteases, and lipases in poultry diets is beyond the scope of this thesis.

Benefits that can be obtained from using exogenous enzymes in poultry diets include improved performance, feed conversion (feed:gain ratio), and animal health (gut health); the ability to incorporate lower cost or locally available feed ingredients; and decrease in environmental impact by reducing the type and volume of undigested nutrients (Choct, 2006). Of interest to this study, the use of exogenous enzymes can also help create enriched poultry products.

It has been observed that diets containing feed ingredients with low digestibilities, diets with low AME, or nutritionally deficient diets have the largest response to exogenous enzymes (Bedford, 2000, Cowieson and Adeola, 2005, Bao et al., 2013). But positive performance results can also be observed in nutritionally adequate diets that are supplemented with exogenous enzymes. This led Bao et al. (2013) to suggest that other factors such as gut physiology, microbiology, and immunology also contribute. Age (life stage), metabolic condition, and feed processing can also play a role in the effectiveness of exogenous enzymes.

5.2 Carbohydrases and Non-Starch Polysaccharides

Non-starch polysaccharides (NSP) are the principal targets of exogenous carbohydrase enzymes. According to Choct (2006), the addition of the carbohydrase enzyme β-glucanase was widely adopted for poultry more than

thirty years ago after it was shown to improve the digestibility of barley. Similarly, xylanase was quickly adopted for diets containing wheat and rye.

Chickens lack enzymes to break the β-(1,4) bonds between sugars in the non-starch polysaccharides present in wheat, barley, rye, and the outer coat of oil seeds. These grains and oil seeds have considerable value in poultry diets as alternatives to corn and as sources of protein and fat. However, they possess anti-nutritive effects associated with the indigestible polysaccharides. Their digestibility is improved with the addition of exogenous carbohydrases.

For example, cellulase targets the β-(1,4) bonds between glucose units that comprise the insoluble NSP cellulose (Chapter 4, Figure 4). Galactanase targets the β-(1,4) links between galactose units in side chains of the acidic fraction of mucilage (Chapter 4, Figure 5). Xylanase targets the β-(1,4) links between xylose units that form the backbone of the neutral fraction of mucilage (Chapter 4, Figure 6).

Water-soluble NSP or mucilage is associated with a number of negative effects. Mucilage increases the viscosity of digesta, which in turn leads to increased digesta transit time and increased potential for harmful bacterial overgrowths. Mucilage decreases digesta mixing and overall nutrient digestibility, preventing nutrients from reaching absorption sites in the small intestine.

Although the objective of their study was to either de-hull or de-mucilage whole flax seed in order to better study the protein content, Wanasundara and

Shahidi (1997) made some interesting observations about the relationship between carbohydrase enzymes and flax seed mucilage. They compared the results of three types of treatments: whole seeds soaked in water then agitated, whole seeds soaked in water plus one of three carbohydrase mixtures then agitated, and whole seeds soaked in sodium bicarbonate solution. They found that they extracted less mucilage from the enzyme-soaked seeds, and this mucilage had a lower viscosity than the mucilage from seeds soaked in water only. They also observed that soaking seeds in carbohydrase enzyme or the bicarbonate solution left the seed walls intact but appeared to facilitate removal of seed contents as they extracted the largest amount of protein from these seeds.

Carbohydrases improve digestibility in poultry diets with high amounts of NSP through three possible modes of action. The first is decrease of digesta viscosity by depolymerization of soluble NSP (Carré, 2004, Cowieson and Adeola, 2005). The second is hydrolysis of the sugar bonds in insoluble NSP present in cell walls of the seed coat (Meng et al., 2005, Jia et al., 2008, Jia, 2009, Jia and Slominski, 2010). The third is the hydrolysis of sugar bonds in one or both types of NSP and the formation of shorter oligosaccharides that are more readily utilized by gut bacteria (Bao et al., 2013). It is difficult to design an *in vivo* experiment that definitively distinguishes between these mechanisms. Chesson (1993) suggested that disruption of the cell walls of the seed coat and resulting increase in availability of nutrients in the seed might

contribute more to performance improvements than decreasing the digesta viscosity.

Jia et al. (2008) note that including flax in poultry diets can negatively affect growth performance although results are conflicting. When depressed performance is observed, it is generally attributed to decreased energy utilization. These authors also claim that insufficient breakdown (chemical or mechanical) of insoluble NSP in cell walls is the most important factor in depressed growth performance.

Both canola and flax seeds are small so commercial grinding processes may not be effective. Grinding the seeds with carrier ingredients such as peas or cereal grains can help but the seeds may still not be effectively ruptured. Grinding also increases the opportunity for lipid oxidation of oils during feed storage. Thus there are clear disadvantages associated with adding ground seed to diets. A better solution is to use whole seeds in poultry diets, but this requires the addition of exogenous enzymes to help break apart the insoluble NSP in seed cell walls.

Multi-carbohydrase mixtures appear to be the most effective at both reducing digesta viscosity and degrading cell wall structures (Jia and Slominski, 2010, Slominski, 2011). Although the two terms have become more interchangeable, Chesson (1993) points out that disruption of cell walls is not the same thing as degradation of cell walls. Disruption requires much smaller amounts of enzyme to expose the seed contents. Total degradation, that is,

breaking down oligosaccharide sugars to monosaccharide sugars, may not be useful since monogastrics lack the enzymes and membrane transporters to utilize the unusual sugars in oil seed NSP.

Carbohydrase enzymes may also have a positive effect on performance of poultry fed standard corn-soybean meal (C-SBM) diets that lack significant anti-nutritive components, although results are not consistent between studies (Cowieson, 2010). However, Slominski (2011) suggests that large amounts of carbohydrase enzymes must be added to standard, nutritionally balanced corn-soy diets to have sustained, significant effects on performance.

There are other benefits to the use of carbohydrases. Limited feeding trial data cited by Bao and Choct (2010) suggest that carbohydrase enzymes that specifically target insoluble NSP may alter the microbial gut community by stimulating an increase in lactobacilli with a resulting decrease in gut pH. Both of these factors can stimulate a gut immune response that could remove or inhibit pathogenic bacteria. Bedford (2000) suggests that increasing the rate of digestion by decreasing digesta viscosity could move the location of digestion to a more proximal position in the GI tract above the lower parts of the gut that have the larger bacterial populations, thus reducing the opportunity for pathogenic bacterial overgrowths.

Improvements in NSP and fat digestibility that come with the addition of carbohydrase mixtures can also improve protein digestibility. Cowieson and Bedford (2009) describe the effects of carbohydrase supplementation on

digestibilities of individual amino acids. For corn and wheat diets, Cowieson (2010) notes that digestibility of cysteine in particular may be improved when exogenous carbohydrases are present.

5.3 Feed Processing and Enzymes

Problems associated with the use of exogenous enzymes include survival of the enzyme and retention of activity at gut pH, and retention of enzyme activity following feed processing such as pelleting. In general, pelleting of broiler diets can improve weight gain, decrease feed intake, and improve feed:gain compared to mash diets (Amerah et al., 2011). Since the heat applied during pelleting can affect enzymes, the topic is briefly considered here.

Poultry feed can be subjected to processes including grinding, mixing, conditioning of mash using steam, and extrusion and pelleting (the process of subjecting conditioned mash to temperature and pressure). Amerah et al. (2011) review these processes and discuss their effects on exogenous enzymes. Heat is used to decrease the presence of bacteria such as *Salmonella* and destroy trypsin inhibitors, but heat along with pressure and steam can denature proteins, drive the Maillard browning reaction leading to a loss of lysine, solubilize fiber resulting in an increase in digesta viscosity, and degrade vitamins and exogenous enzymes. As Amerah et al. (2011) point out, it is ironic that the high temperatures designed to eliminate bacterial contaminants may increase the possibility of bacterial infection as a result of increased digesta viscosity and increased transit time in the gut. While heat may degrade

exogenous enzymes, results from *in vitro* studies that measured greatly decreased enzyme activity in processed feed were followed by companion *in vivo* studies that indicated that the remaining enzyme was still effective. It is possible that the heat breaks down NSP, although this has been disproven in the case of flax seed (Chapter 6). Amerah et al. (2011) recommend moderate heating to 85 C; temperatures above that appear to result in excessive gelatinization of starch components.

Steam conditioning has the greatest negative effect on enzyme activity. One solution has been to attempt to genetically engineer thermally stable enzymes. Here, a balance has to be struck such that the enzyme can survive the higher temperatures of processing but is maximally active at lower internal body temperatures. Another solution has been to encapsulate enzymes in a chemical coat that can survive processing temperatures then release the enzymes in the digestive tract of the animal early enough and completely enough for them to be effective.

5.4 Enzymes and Newly Hatched Chicks

Chickens are precocial and omnivorous. Newly hatched chicks undergo a dramatic shift in primary nutrient source from yolk-sac lipids to cereal grain starches (Noy and Uni, 2010). There has been considerable research on the physical and immunological development of the newly hatched chick GI tract. For example, Bar-Shira and Friedman (2006) concluded that during the first week post-hatch, the exposure of the chick gut to feed and bacteria stimulate a

variety of pro-inflammatory metabolic pathways, providing the chick with a fully developed immune response in about a week. Maternal antibodies provide protection during the developmental period. Uni et al. (2003) describe the rapid development of critical SGLT1 (Na-glucose transporters) in villi brush borders in the small intestine in the developing embryo and newly hatched chicks. These transporters are specifically required for the chick to digest its post-hatch diet.

It has been proposed by a number of research groups that enhancing nutrient digestibility in newly hatched chicks with enzyme supplementation could result in performance benefits as a result of either a redirection of metabolic activities towards growth since the need for endogenous enzymes was decreased, or by supplying enzymes that the chick does not yet make in sufficient quantities. For example, Olukosi et al. (2007) conducted feeding trials to determine whether supplementing diets with a combination of xylanase, amylase, and protease, either alone or in combination with phytase, could affect performance of newly hatched chicks. Unfortunately, their experimental corn-SBM diets were designed to be low in ME and P so the effect of phytase alone on performance was just as great as that of phytase and the enzyme mixture together since phosphorus is such an important nutrient. Despite their inconclusive results, they suggest that enzyme supplementation may be beneficial to chicks up to a week in age when they are fed standard corn-SBM diets.

Noy and Sklan (2002) conducted several feeding trials to specifically examine nutrient utilization and the interaction of variable levels of fat, protein, and cellulose (fiber) in newly hatched chicks that were fed the experimental diets for the first seven days. While they observed different utilization and interaction patterns in the young chicks, especially when compared to older broilers, none of the effects displayed in the first seven days persisted past 18 days. They conclude that as long as limiting amino acids are provided to newly hatched chicks, early dietary composition does not have a long-term effect.

The positive effects of enzyme supplementation on growth performance of broilers have been well documented (Chapter 6) but it would seem that the value of enzymes that specifically target newly hatched chicks is less clear.

6 Enriching Poultry Meat and Eggs with n-3 Fatty Acids

In this thesis, I review the results of 54 poultry feeding trials with an emphasis on trials that incorporated flax seed or flax oil into the dietary treatments, and three other feeding trials conducted on swine, trout, and cultured abalone. A majority of the studies are reviewed in this chapter. A metadata analysis of the papers is listed below:

- Feeding trial references (1990-2014): 57
- Studies incorporating a dietary fat source rich in n-3 FA: 43
- Studies incorporating carbohydrase enzymes: 14
- Studies incorporating some form of flax plus carbohydrase enzymes: 4
- Studies incorporating flax oil: 12
- Studies incorporating flax meal: 4
- Studies incorporating whole flax seed: 10
- Studies incorporating ground whole flax seed: 17

A single study may have tested more than one form of flax seed. Outcomes generally fall into three groups: performance, digestibility and energy utilization, and enrichment of tissues (meat, yolk).

Growth performance results from poultry feeding trials with flax diets (whole seed, ground whole seed, meal, oil) are mixed. When comparing the results of different trials, it is important to consider the form of the flax product used, the level of flax in the diet, duration of the dietary treatment,

and additional processing performed on the diet. In general, birds fed flax have lower BW, higher feed intake, and poor feed:gain compared to control birds. However, flax diets generally result in higher levels of total n-3 FA and lower levels of total n-6 FA, including AA, and MUFA in metabolic tissues (heart, liver) and consumable tissues (meat, yolk). Birds fed flax meal plus flax oil generally perform better than birds fed ground whole seed. Birds fed ground whole seed tend to perform better than birds fed whole seed. Heat-treating or pelleting whole flax seed will improve fat digestibility and energy in the diet but generally does not affect performance because heat has little effect on the NSP present in flax. The use of carbohydrase enzymes can improve the performance of flax-fed birds and increase the level of total n-3 FA relative to flax-fed birds that do not receive the enzymes. While *in vitro* tests of the carbohydrase mixtures result in decreased digesta viscosity, this is not a consistent result when the enzymes are used in feeding trials.

6.1 Dietary n-3 Fatty Acids and Growth Performance

Ajuyah et al. (1993) conducted a feeding trial with broilers using a C-SBM diet with 15% whole flax seed. Birds fed the whole flax seed diets had poor feed:gain and significantly decreased live weights relative to control. The FA composition of the diets had a relatively small influence on the PL profile of breast and thigh tissues.

Rodríguez et al. (2001) fed broilers ground whole flax seed at levels of 8, 12, and 16%. With increasing levels of flax, they observed decreased protein

digestibility, fat digestibility, and dietary AMEn, and increased digesta viscosity. However, even at the high flax level, feed intake was not different between treatments. This is not consistent with the observation made in other studies that birds fed higher levels of flax seed have a higher feed intake.

Ortiz et al. (2001) fed 4-week-old broilers a specialized fat-free diet containing 8, 12, 16, and 24% ground whole flax seed. They observed that increasing flax levels were associated with decreased total fat digestibility.

Bean and Leeson (2003) conducted a long-term trial with layers. Test diets for layers were ramped up to 10% whole flax seed starting in week 28; the trial concluded at week 60. Body weights of flax-fed hens were lower than control. Although flax-fed hens produced more eggs, the yolk weight was lower than control. Most studies in which layers are fed a diet containing flax note increased egg production.

Cherian and Hayat (2009) conducted another long-term feeding trial with layers to examine liver histopathological characteristics. Layers were fed 10% whole flax seed from week 32 to 64. The flax diet decreased hepatic and plasma fat content, hepatic TAG, and the total number of fat vacuoles in liver relative to the C-SBM control.

Lee et al. (1991) conducted two feeding trials comparing the performance of birds fed ground whole canola or flax seeds that were added to the diet in raw or heat-treated form to that of birds fed canola or flax meal plus oil or tallow. The diets delivered 10% or 20% canola or flax treatment. All diets

contained 3% or 8% fish meal. Heat-treating flax seeds gave no improvement in performance. When flax seeds were mixed with canola oil, birds still had depressed BW and poor feed:gain. Flax meal plus flax oil resulted in better performance than ground whole seed. Notably, for birds fed 10% and 20% canola treatments, BW and feed efficiency were not different from the SBM control. The authors concluded that flax seeds in poultry diets, even when ground, depress energy utilization because the nutrients in the seed are not fully available to digestive enzymes. They confirmed that the anti-nutritive components in flax were not sensitive to heat.

Ajuyah et al. (1991) conducted a feeding trial with broilers comparing raw flax seed, heat-treated flax seed, flax meal plus flax oil, and flax meal plus tallow to diets containing canola seed with similar treatments. The flax and canola diets contained 10% of those components and all diets contained 8% fish meal as well. Flax meal plus flax oil resulted in more LA, ALA, and total n-3 FA in meat tissues relative to the other flax treatments. Mixing tallow with flax meal decreased the amount of LC n-3 PUFA in meat tissues. Flax diets produced more total n-3 FA, mainly ALA and EPA, in meat tissues relative to canola diets, consistent with the fact that flax seed contains several times more ALA than canola seed (Table 4). Even though meal plus oil mixtures deliver less energy than whole seed (Table 5), the nutrients are more accessible to digestion by the bird and thus a higher level of n-3 FA enrichment can be reached. Although not discussed in the paper, the LC n-3 PUFA in the 8% fish

meal included in the diets would have suppressed elongation of ALA in the flax. It is more likely that the ALA was directed to β-oxidation while the LC n-3 PUFA in the fish meal were stored in meat tissues.

Lopes et al. (2013) conducted a feeding trial to compare the performance of broilers fed between 3 and 5% soybean oil or flax oil. They observed no difference in body weight, feed intake, body weight gain, feed:gain, carcass traits such as meat yield, or proximate nutritional composition of meat tissues (e.g., crude protein, crude fat). The authors noted that while soybean oil contains mostly LA while flax oil contains mostly ALA, the total amount of PUFA in the oils is about the same (Table 2). Studies comparing performance of broilers fed flax oil to those fed other vegetable oils or tallow that are higher in SFA and MUFA do observe large differences in performance and tissue composition.

Baeza et al. (2013) conducted a feeding trial with fast-growth and slow-growth strains of broilers. The fast-growth birds received 1.3% flax oil or 5% extruded flax seed in their diet while the slow-growth birds were fed 1.3% flax oil or 6.5% extruded flax seed. The authors found that the flax diets had no effect on growth performance, meat yield, meat processing, meat cooking, or meat sensory parameters. Consistent with other studies that suggest that β-oxidation increases when poultry diets contain high levels of n-3 FA, carcass fat was decreased in fast-growth birds receiving the flax diets. Interestingly, the authors noted that the fast-growth birds incorporated more n-3 FA into

meat tissue than slow-growth birds even though n-3 FA levels in the slow-growth diets were higher.

6.2 Optimum n-6:n-3 Fatty Acid Ratio in Poultry Diets

Qi et al. (2010) conducted feeding trials to investigate the optimal dietary ratio of n-6 to n-3 FA for enriching poultry meat tissue with n-3 FA. They used diets containing 3% oil comprised of mixtures of corn oil and flax oil to create n-6:n-3 ratios ranging from 30 to 2.5. These values reflect the amounts of LA and ALA in the diets because the vegetable oils used as the dietary fat sources contain very low amounts of LC PUFA. They found that decreasing the n-6:n-3 ratio led to decreased mortality (although this was not statistically significant), improved feed:gain, and increased fat pad thickness. With decreasing n-6:n-3 ratios, Qi et al. (2010) noted an increase in deposition of ALA and a significant increase (3.5%) in deposition of EPA (20:5 n-3) and DPA (22:5 n-3) in breast meat; a n-6:n-3 ratio of 10 was optimal for this particular result. Because flax oil is comprised mainly of ALA (18:3 n-3), this suggests a relatively efficient conversion of this n-3 FA precursor to its LC PUFA products in poultry. Poureslami et al. (2010) estimated the rate of conversion of ALA to EPA and DHA in poultry was between 1 and 3%, higher than the conversion rate estimated for humans, although that may be greatly underestimated (Section 3.4).

Kartikasari et al. (2012) fed broilers flax oil, canola oil, and macadamia nut oil to determine an optimum n-6:n-3 ratio for enriching meat tissue in LC

n-3 PUFA. The amount of LA (18:2 n-6) in each diet was held constant and the three oils were adjusted to deliver n-6:n-3 ratios ranging from 10.5 to 0.6; the lowest ratio was only achieved by including 7.5% flax oil in the diet. They found that decreasing the ratio led to larger increases of LC n-3 PUFA in the meat: 8.9% of total FA in breast tissue and 5.6% of total FA in thigh tissue were LC n-3 PUFA in birds receiving 7.5% flax oil. Decreasing the ratio did not affect total lipids because the increase in LC n-3 PUFA was offset by a decrease in MUFA in all three tissues. This is consistent with other studies in which high levels of LC n-3 PUFA suppressed the Δ-9 desaturase used in MUFA synthesis. Liver tissue showed a large increase in EPA (20:5 n-3) while breast and thigh tissue had large increases in DPA (22:5 n-3). The authors suggest that chickens have limited capacity to convert DPA to DHA.

Puthpongsiriporn and Scheideler (2005) conducted a feeding trial with layers to determine if n-3 FA could improve the immune response to vaccination in newly hatched birds, and if they could determine the optimum n-6:n-3 ratio that gave the best response. Layers were fed diets in three stages from week 0 (newly hatched) to week 16. Diets contained variable amounts of corn oil and ground flax seed (less than 5% of diet) to give n-6:n-3 ratios of 17, 8, 4, and 2. They applied a vaccination schedule similar to that used in commercial layer operations. Blood serum was tested for antibody production against Newcastle disease virus, infectious bronchitis virus, and infectious bursal disease virus. At weeks 8 and 16, samples of spleen and thymus tissue

were collected, and at week 16, samples of bursus of Fabricius tissue were collected. There was a positive effect on antibody production with decreasing ratio but the effect was small and only observed in older birds and at the lowest ratios (4 and 2). By week 16, at the lowest n-6:n-3 ratio, both the spleen and thymus had increased levels of ALA, EPA, and DHA, and decreased levels of AA. While it does not appear that n-3 FA have a larger effect on the immune response in younger birds, they do have a positive effect in older birds.

Pilevar et al. (2011) conducted a similar study in layers using a diet with 3% soybean oil and additional fish oil to create dietary n-6:n-3 ratios of 10, 6, and 2. The birds were given an extraordinarily complex vaccination protocol as well as two sheep red blood cell (RBC) challenges. The results of their study were inconclusive. There were differences in antibody titers but the differences were not consistent in direction (increase or decrease), with time (age of bird), for specific antibodies, or with n-6:n-3 ratio.

6.3 Enrichment of n-3 Fatty Acids in Meat Tissue

Zuidhof et al. (2009), Betti et al. (2009a), and Betti et al. (2009d) conducted a broiler feeding trial to determine the optimal duration needed to enrich meat tissues to 300 mg total n-3 FA/100 g when whole ground flax seed was the dietary fat source. There were two levels of flax, 10% and 17%, and 8 durations of flax treatment. They observed negative effects on performance at high flax levels after only 8 days of flax treatment; performance further decreased when the flax treatment was longer. Flax treatments depressed feed

intake but the authors describe the results as “erratic” and not clearly related to flax level or duration. Feed:gain in particular became poor when flax treatment was longer than 12 days, and was poorer in high flax relative to low flax treatments. Meat yields and thus probably protein digestibility were similarly affected. However, the authors were able to enrich breast meat to the desired level in 12 days with the high flax treatment and 24 days with the low flax treatment. The total n-3 PUFA in breast tissue after 35 d of flax treatment were ALA (356.9-434.9 mg/100 g), EPA (12.1-14.1 mg/100 g), DPA (22.4-25.9 mg/100 g), and DHA (9.9-12.2 mg/100 g). DPA was the most abundant LC n-3 PUFA in both PL and TAG fractions of both breast and thigh tissue.

Jia et al. (2010) examined the effect of pelleting broiler diets containing 12% ground whole flax seed. They found that the flax diets had no effect on total FA in breast, leg, wing, or skin tissues relative to control. However, the flax treatment led to increased ALA in all tissues, and an increase in EPA in meat and skin tissues and DHA in breast tissue. They attribute the difference to the fact that PL are more numerous in breast tissue while TAG are the dominant lipid form in adipose and thigh tissue. The increase in n-3 FA came at the expense of n-6 FA, SFA, and MUFA.

Turner et al. (2014) fed whole flax seed extruded with peas (flax levels 5% and 10%) to pigs that had been blocked by sex and initial BW. Their objective was to enrich three cuts of meat to 300 mg total n-3 FA per 100 g meat. Pork meat cuts typically contain both lean meat and adipose tissue that have very

different FA profiles. A sex difference was present throughout the 11-week trial because male pigs add more adipose tissue than females. SFA and MUFA in the meat tissues did not differ between the flax diets but were significantly lower than control. They observed an increase in LA (18:2 n-6) in the meat cuts at the high flax level. We believe that this is probably correlated with adipose tissue deposition since LA is preferentially stored when diets contain large amounts of n-3 FA (Chapter 7). The amount of ALA and LC n-3 PUFA increased with flax level but varied with cut type as a function of the amount of adipose tissue. All three meat cuts reached desired enrichment levels. This study makes an interesting comparison to poultry feeding trials because of the differences in TL and adipose tissue content of meat cuts. It also demonstrates that pigs are able to effectively synthesize LC n-3 PUFA from dietary ALA.

6.4 Layer Diets and Fatty Acid Composition of Yolk and Hatchlings

Cherian and Sim (1991) conducted a study with layers to examine the relationship between diets of hens and the FA composition of yolks, embryos, and newly hatched chicks. Using a wheat-SBM basal diet, some birds received 8% or 16% ground whole flax seed while others were fed 16% ground whole canola seed. The authors observed increases in n-3 FA (mostly ALA) in egg yolks as early as day 15 in birds receiving the canola and flax diets. Some DHA was also present, and levels of AA were decreased relative to control. In plasma of newly hatched chicks, the only LC n-3 PUFA in control chicks was DHA

while chicks from canola and flax eggs had DHA, DPA, and EPA in their blood plasma at levels higher than that measured in the yolks.

The presence of LC n-3 PUFA in yolks indicates that layers can effectively convert dietary ALA to the LC products and deposit them in yolks. The presence of even more LC n-3 PUFA in chick plasma suggests that the chick is also converting ALA present in the yolk. And the fact that brains of embryo and newly hatched chicks had the same amounts of DHA suggests that this is occurring when the embryo is developing. In mammals, fetal brains acquire DHA from the maternal liver through the placenta. This study provides evidence that chickens appear to be able to synthesize the LC n-3 PUFA needed for brain development.

Increasing PUFA in the flax diets came at the expense of MUFA; this was not observed in the canola diet with its larger n-6:n-3 ratio. It has been suggested that diets rich in PUFA can inhibit Δ-9 desaturase, the enzyme used in MUFA synthesis pathways. It would thus appear that either larger amounts of n-3 PUFA or a lower n-6:n-3 ratio are necessary to inhibit Δ-9 desaturase activity, not simply an elevation of all PUFA classes.

Cherian (2007) described another set of experiments in which layers were fed variable amounts of fish oil and sunflower oil. As a result of the increased LC n-3 PUFA in egg yolks, the sole source of FA for the developing embryo, chicks hatched from those eggs had high levels of these FA in their heart and liver up to 42 days post-hatch.

Cherian (2008) examined the effect of the age of broiler breeder hens and dietary n-3 FA on hatching egg quality and yolk FA composition. Broiler breeders were fed diets with 1.75% fish oil and 1.75% tallow or 3.5% fish oil from weeks 26-62. Birds receiving the high n-3 FA diet had decreased egg weight, yolk weight, shell weight, and yolk color. However, the yolks of their eggs had higher levels of total n-3 FA and DHA. Older hens produced heavier eggs after week 38 in both treatments. In general, older hens also produced eggs with decreased shell weight and shell thickness. One of the more interesting findings of this study was that younger hens incorporate less DHA into their eggs. The hens incorporated the most DHA into the eggs in week 38. In the low n-3 FA treatment group, hens older than 38 weeks also added less DHA to their eggs as they aged. In both high and low n-3 FA treatment groups, hens added more AA into their eggs as they aged. The author suggested that this indicated a decreased capacity of older hens to synthesize LC n-3 PUFA. However, since both diets directly supplied LC n-3 PUFA and very little of the n-3 FA precursor ALA, it was not necessary for the hens to synthesize DHA. It is more likely that the hens had a decreased capacity to transport LC n-3 PUFA into the eggs. It is also possible that as the hens aged, their own requirements for LC n-3 PUFA increased.

Chen et al. (2014) conducted a feeding trial with 28-week-old geese to examine the effect of n-3 FA on egg production and quality and on meat quality of hatchlings from eggs enriched in n-3 FA. Flax meal was added to the

diets of laying geese at 5, 10, and 15% levels. Birds receiving flax meal diets had increased egg production and increased feed efficiency. Eggs from birds fed the higher levels of flax meal had more ALA, DPA, total n-3 PUFA, and less AA than the controls. One-day-old goslings from geese fed higher levels of flax had higher levels of ALA and total n-3 PUFA and lower levels of AA and SFA in thigh meat relative to controls.

Leeson et al. (2000) conducted a feeding trial with layers with the objective of determining if the performance of smaller birds was affected by a flax seed diet more than performance of larger birds. Other studies have noted that feed intake generally increases when poultry are fed flax seed diets. The authors fed 18-week-old layers diets with 10% or 20% ground whole flax seed. High flax-treatment birds produced fewer eggs, smaller eggs, and as observed in other studies, had the highest feed intakes. However, initial BW had no effect on egg production. Birds fed flax had lower BW than control birds but initial weight class was not a factor. The authors conclude that poor performance with high flax levels is not related to initial BW.

Aziza et al. (2013) examined egg quality and yolk FA in layers fed 10% camelina or flax meal. Birds receiving the flax diet produced larger eggs with thinner shells, and ALA digestibility was decreased. Birds receiving the camelina diet had higher amounts of ALA and total n-3 PUFA in yolks relative to the other diets, and LA digestibility was decreased.

6.5 Carbohydrase Enzymes and Flax Seed

Slominski et al. (2006) examined energy utilization from broiler diets containing flax seed and mixtures of carbohydrases through *in vitro* study of enzyme mixtures, a precision feeding assay with roosters, and a broiler feeding trial. For the *in vitro* trial, the authors tested a variety of enzymes on ground flax seed that had been defatted in hexane. They were specifically looking for a decrease in total NSP and individual sugars such as xylose, rhamnose, and mannanose. Combinations of enzymes were better at depolymerization of the NSP than individual enzymes.

In the precision feeding assay with adult roosters, the authors tested the three enzyme mixtures that were most effective *in vitro*. They applied the enzyme mixtures at a 0.1% level to whole flax seed that had been hammer milled then ground. In the broiler feeding trial, the authors added 15% ground whole flax seed to a C-SBM base diet and an enzyme mixture containing cellulase, pectinase, xylanase, and glucanase at levels of 0.02, 0.01, and 0.05% of the diet.

In the precision feeding assay, addition of the enzyme mixtures increased the TMEn of ground whole flax seed to levels near that of canola and soybean. There was no difference in the outcomes between the three enzyme mixtures tested. The increase in energy availability created by addition of the enzymes was accompanied by an increase in fat and NSP digestibilities. The authors interpret this as evidence that the enzyme mixtures were depolymerizing

insoluble NSP in the flax seed cell wall, creating better access to the seed contents. In the broiler feeding trial, the control diet with 15% flax and no added enzymes had poor feed:gain, poor ileal fat digestibility, and low dietary AMEn. At the highest level (0.05%) of enzyme, feed:gain improved and apparent total tract digestibility of DM, fat, and NSP increased. They did not observe a significant decrease in digesta viscosity with enzyme addition.

Jia and Slominski (2010) conducted three feeding trials on broilers to examine whether particle size, addition of exogenous enzymes, addition of bile salts, and pelleting of diet could decrease the amount of NSP in flax seed. The results of prior work showed that some mixtures of carbohydrases could hydrolyze insoluble NSP in cell walls but were not measurably reducing digesta viscosity (Slominski et al., 2006). Therefore this study investigated the effect of other potential mechanisms on digesta viscosity.

The authors began with *in vitro* tests of individual carbohydrases and mixtures of enzymes on mucilage extracted from whole flax seeds. The enzyme mixture that produced the largest *in vitro* decrease in mucilage viscosity and the enzyme mixture used in Slominski et al. (2006) were tested in a feeding trial incorporating a C-SBM diet with 15% ground whole flax seed. The enzymes were applied at 0.02 or 0.05%. A second feeding trial was conducted with a new enzyme mixture made from the combination of the two mixtures used in the first trial. This new mixture contained pectinase, cellulase, xylanase, glucanase, mannanase, and galactosidase. In the second trial, 15%

whole flax seed was coarsely or finely ground, the enzyme mixture was applied at 0.05%, and bile salts were added at 0.05%. In the third feeding trial, 15% flax seed was included in the diet as whole seed, coarsely ground seed, or finely ground seed with the same enzyme mixture used in the second trial. All the diets were pelleted. A finely ground flax diet was left in mash form for comparison.

As noted in previous experiments by this group, mixtures of carbohydrases performed better *in vitro* than individual enzymes. The combination of cellulase and pectinase in particular decreased mucilage viscosity from 10.0 to 2.6 mPa s. In the first feeding trial, both enzyme mixtures (the one that decreased viscosity by the largest amount *in vitro* and the one that was thought to depolymerize insoluble NSP *in vivo*) improved feed efficiency and increased fat and NSP digestibility. There was no difference in growth performance between birds receiving the different enzyme mixtures. Digesta viscosity was decreased only when the enzymes were applied at the higher level. Results were similar for the second and third trials. Bile salts had no effect on performance and flax particle size had no effect on feed intake or BWG. However, birds receiving the mash diets had lower feed intake and lower BWG than birds receiving the pelleted diets; addition of enzyme had no effect on this outcome.

Because there was no performance difference between the two enzyme mixtures in the first feeding trial, the authors couldn't tell if other observed

changes were due to decreased digesta viscosity or to hydrolysis of cell wall NSP. However, they believed that the results of the second and third trials suggested that breakdown of the cell wall is more important, citing increased ileal digestibility of NSP, increased total fat digestibility, and improved feed:gain as evidence that endogenous digestive processes had increased access to cell contents. Although breakdown of NSP in seed coats is important, additional efforts to decrease flax particle size produced no additional gains. Pelleting offers tangible improvements in performance mainly by reducing feed intake and not through any direct effect on NSP. Bile salts offered little improvement because they are more critical for emulsification of saturated FA and the experimental diets contained mostly unsaturated FA.

Jia et al. (2008) tested the effectiveness of carbohydrase mixtures on flax seed and canola seed in layer diets. They added 15% whole canola or flax seed that had been ground with cereal grain then mixed with the rest of the diet. A third diet contained a commercial mixture of ground and extruded peas and whole flax seed, supplying flax at 7.5% of the diet. Diets were pelleted then crumbled. The authors state that no intact seeds were found after pelleting. An enzyme mixture containing pectinase, cellulase, xylanase, glucanase, mannanase, galactanase, and amylase was added to the diets. Relative to the canola diet, birds fed the 15% flax diet had higher feed intake, lower BW, poor feed:gain, lower egg production, and produced eggs with the lowest specific gravity and poor shell quality. The addition of the enzyme mixture significantly

improved all of these parameters for birds receiving the flax diet but generally had no effect on the canola or peas-flax diets. The addition of the enzyme did not decrease jejunal digesta viscosity in flax treatments: the digesta viscosity of flax-fed birds was several times higher than that of canola-fed birds. Addition of the enzyme improved NSP digestibility of the flax diet but had no effect on fat digestibility of any of the treatments.

The authors concluded that insoluble NSP was the most important factor in the observed performance for the flax and peas-flax diets. The increased level of insoluble NSP led to decreased energy utilization so the birds increased their feed intake to compensate. As a result of improved nutrient availability with the addition of the enzyme mixture, n-3 FA deposition, particularly DHA, was increased in yolks from birds fed flax and peas-flax diets.

The decreased nutrient digestibility caused by the presence of the mucilage negatively affected calcium absorption from the diet. As a result, the birds produced eggs with lower specific gravity. The addition of the enzyme mixture did not measurably decrease digesta viscosity although egg quality parameters did improve.

6.6 Carbohydrase Enzymes and Other Feed Ingredients

In an early trial testing the effect of enzymes on other poultry feed ingredients besides wheat, barley, or rye, Slominski and Campbell (1990) evaluated the effect of enzymes on digestibility and hydrolysis of NSP in canola meal. Canola seed contains about 20% NSP of which 2-4% is water-soluble.

Around 5% of the insoluble fraction is cellulose. The authors conducted three experiments. They conducted an *in vitro* test of a "multi-activity complex of cell-wall degrading enzymes" (the composition was not specified in the paper). They applied the enzyme mixture at 1 and 3% to commercial canola meal. In the second experiment, they fed 20-week old layers (10 caecotomized, 10 intact) a semi-purified diet containing 40% commercial canola meal. In the third experiment, they fed two groups of layers the same semi-purified diet with 40% commercial canola meal and 1% of the enzyme mixture. In the two feeding trials, they tested excreta for NSP and individual sugars. In addition to glucose, other sugars in the NSP of canola seed include arabinose, xylose, galactose, and uronic acid. The digestibility of the semi-purified diet was similar in the caecotomized and intact birds, suggesting that insoluble NSP are not digested in the ceca. The enzyme applied at 1% level hydrolyzed all the relevant sugars *in vitro*, and significantly improved digestion of insoluble NSP.

Meng et al. (2006) conducted a set of companion studies on canola seed similar to those of Slominski et al. (2006) (see previous section). They did not perform *in vitro* tests of enzyme, and the broiler feeding trial also included canola meal plus canola oil as one of the dietary treatments. Experimental parameters and performance and digestibility results were similar to those of Slominski et al. (2006). Because performance of birds fed ground canola seed did not reach the performance levels of meal+oil-fed birds, the authors

conclude that using whole ground canola seed in poultry diets requires the use of enzyme supplementation to achieve better energy and nutrient use.

Adeola et al. (2010) investigated energy utilization and digestibility of a C-SBM broiler diet containing corn distillers grains and a mixture of xylanase and amylase. The birds received the diet for 7 days. The authors found that addition of the carbohydrases improved ileal digestibility and metabolizable energy of the diets.

Igbasan et al. (1997) examined the effect of carbohydrases on peas in broiler diets. Peas contain 25-55% water-soluble pectic polysaccharides that were known to produce a viscous solution *in vitro*. The authors used a purified corn-pea-casein diet in the ratio 45:45:6 that was designed to have low ME and low CP. They tested four levels of pectinase and a mixture of pectinase and galactosidase. The addition of enzymes to C-SBM diets that have low ME and low CP has been successful in improving performance and nutritive value of the diet but in this case, the use of enzymes to improve the nutritive value of peas was not successful. The authors concluded that the pectic polysaccharides present in peas do not have detrimental, anti-nutritive effects.

Kocher et al. (2002) examined the effect of carbohydrases on SBM in broiler diets. Soybean meal contains about 20% NSP, with a water-soluble fraction of about 3%. The authors applied a mixture of glucanase, cellulase, and pectinase, added as a powder to a basal C-SBM diet that was then pelleted, and galactanase in a liquid form that was sprayed on the pelleted diet. The

enzymes were applied at the manufacturer's recommended level and a level five times that (0.4 and 2% for the enzyme mixture; 0.94 and 4.7% for the galactanase). Addition of the enzymes had no effect on growth, feed intake, feed conversion, excreta moisture content, or digesta viscosity. Both enzymes decreased the amount of insoluble NSP in ileum digesta. Neither enzyme was effective at the lower level on most outcomes. Upon analysis, the authors concluded that the enzyme mixture was removing sugars such as galactose, rhamnose, and arabinose from polysaccharides, effectively cleaving the long chains. Galactanase pulled individual galactose units off the polysaccharides that were apparently subsequently used by cecal bacteria for fermentation. Nonetheless, NSP present in SBM do not represent substantial anti-nutritive elements and do not require the addition of carbohydrase enzymes.

Meng et al. (2004) examined the addition of a mixture of carbohydrases (0.04%) or lipase (0.02%) to broiler diets containing 5% tallow or canola oil. The carbohydrase mixture contained xylanase, glucanase, and cellulase. The enzymes and oils were added to four separate dietary treatments containing either wheat, SBM, canola meal, or peas that were designed to be nutritionally suboptimal. The type of fat had no effect on BW although birds receiving the tallow diet had poor feed:gain. Addition of the carbohydrase mixture improved BWG and feed:gain and decreased insoluble NSP in the small intestine for both fat treatments while lipase had no effect on performance with treatment. That broilers would have sufficient lipase is not surprising since their only

source of energy as embryos is lipids in the yolk. Other studies have indicated that carbohydrase enzymes nearly always improve performance when birds are fed nutritionally deficient diets.

Meng et al. (2005) conducted additional studies of carbohydrases added to broiler diets combining wheat, SBM, canola meal, and peas. They first added enzymes to the individual feed ingredients *in vitro*. Enzyme mixtures decreased NSP more effectively than single enzymes. The authors tested four enzyme mixtures on a diet containing all four ingredients (the diet was not nutritionally deficient in this feeding trial). All of the enzyme mixtures improved BWG, feed conversion, dietary AMEn, apparent ileal digestibility of starch and protein, and apparent total tract digestibility of NSP. The combination of wheat, canola meal, and peas in a single diet increased the amount of NSP enough that the enzymes were able to make a difference in performance and digestibilities.

7 Experiment 1: Effect of Whole Flax Seed and Enzyme Addition on Production Performance and Tissue Fatty Acids in Broiler Chickens

7.1 Introduction

Review of prior poultry feeding trials (Chapter 6) reveals that no experiments have been conducted to determine how the combination of whole flax seed and a mixture of carbohydrase enzymes affects the incorporation of n-3 FA into consumable tissues, meat quality, and lipid metabolism in the bird. We propose that the combination of a mixture of carbohydrase enzymes and whole flax seed will increase n-3 FA in consumable meat tissues without reducing growth performance.

We devised two tests of this hypothesis. The objective of Experiment 1 was to determine the efficacy of a carbohydrase enzyme mixture in a diet with whole flax seed relative to a control corn-soybean meal diet on growth performance, lipid metabolism, and fatty acid composition of tissues in broiler birds. Experiment 2 is discussed in Chapter 8.

7.2 Materials and Methods

7.2.1 Birds and Dietary Treatments

One hundred and twenty day-old Ross x Ross broiler chicks were obtained from a commercial hatchery. The chicks were randomly placed in 12 floor pens, 10 birds per pen. From days 1 to 3, the chicks were fed a

commercial starter diet. Throughout the trial, birds were allowed free access to water and feed. Starting on day 4, chicks received one of three dietary treatments: Control, Flax, or Flax+E. Four pens were assigned to each treatment. A lighting program of 23L:1D was used for the entire experiment. The chicks were not vaccinated. The birds were housed under standard conditions of temperature, humidity, and ventilation. All protocols were approved by Oregon State University's Institutional Animal Care and Use Committee to ensure adherence to Animal Care Guidelines.

The basal corn-soybean meal diet (Control) was adjusted for Flax (15% whole flax seed) and Flax+E (15% whole flax seed plus 0.05% enzyme mixture as-fed). Diets were formulated to be isonitrogenous and isocaloric. Starter diets (days 4-20) had 23% CP and 3200 Kcal/kg ME, while grower diets (days 21-35) had 22% CP and 3300 Kcal/kg ME. Diets were mixed at the Oregon State University Poultry Center and provided as mash. Composition and nutrient analysis for the diets are shown in Tables 7 and 8.

The enzyme mixture used in both experiments is Omegazyme (Canadian Bio-Systems, Calgary). The composition of the enzyme mixture is shown in Table 9. As discussed in Chapters 4-6, effective depolymerization of the complex NSP molecules in flax seed requires a mixture of enzymes with a variety of targets.

On days 4, 20, and 35, birds and feed were weighed and feed consumption was recorded for each pen. Average bird weights and feed:gain ratio were calculated.

7.2.2 Tissue Collection

On day 36, two birds from each pen (8 birds per treatment) were randomly selected, weighed then euthanized with CO2 gas. The following tissues were collected and weighed: liver, heart, spleen, gizzard, abdominal fat pad (AFP), and GI tract. Collection of AFP tissue included fat surrounding the gizzard, bursa of Fabricius, and cloaca. Gizzards were emptied and rinsed before weighing. Tissue samples from each bird (heart, liver, AFP, right pectoralis major without skin, and right biceps femoris without skin) were collected and stored at -20 C until analysis.

7.2.3 Total Lipid and Fatty Acid Analysis

Total lipids were extracted from approximately 2 g of feed samples and tissues according to the method of Folch et al. (1957) using a 2:1 solution of chloroform and methanol. Visible fat was removed from the meat tissue samples before lipid extraction. Total lipid content was determined gravimetrically. Fatty acid methyl esters were prepared with boron trifluoride methanol as the methylating agent using methods reported earlier (Cherian et al., 2002). Fatty acid analysis was performed with an HP 6890 gas chromatograph (Hewlett-Packard Co., Wilmington, DE) equipped with an autosampler, flame ionization detector, and SP-2330 fused silica capillary

column. Samples in hexane (1 μL) were injected with helium as a carrier gas into the column programmed for ramped oven temperatures. Initial oven temperature was set at 150 C, held for 1.5 min, then ramped at 15 C/min to 190 C and held for 20 min, then ramped again at 30 C/min to 230 C and held for 3 min. Inlet and detector temperatures were both 250 C. Fatty acid methyl esters were identified by comparison with retention times of authentic standards (Nuchek Prep, Elysian, MN). Peak areas and percentages were calculated using Hewlett-Packard ChemStation software (Agilent Technologies Inc., Wilmington, DE). Fatty acid values are reported as percentages.

7.2.4 Thiobarbituric Acid Reactive Substances

Lipid peroxidation was measured as thiobarbituric acid-reactive substances (TBARS) expressed in malondialdehyde equivalents. Samples were prepared using methods described by Cherian et al. (2002). Briefly, 2 g of tissue (liver, breast, thigh) were minced then mixed with 18 mL of 3.86% perchloric acid and butylated hydroxytoluene (50 μL in 4.5% ethanol), after which the samples were homogenized. The homogenate was filtered and duplicate samples of the filtrate were mixed with 20 mM TBA in distilled water and incubated in a boiling water bath for 60 minutes. Absorbance was determined at 531 nm. Results of duplicate samples were averaged. TBARS values are expressed as milligrams of malondialdehyde per g of tissue.

7.2.5 Statistical Analyses

The effects of the dietary treatments on tissue fatty acids and bird performance were analyzed by one-way ANOVA using SAS (version 9.2). Each pen was considered an experimental unit. Significant differences between treatment means were analyzed by Duncan's multiple-range test when $p < 0.05$ (Steel and Torrie, 1980). For $0.05 < p < 0.10$, results are discussed if means suggested a trend. Computations were done using the General Linear Models procedure of SAS. Least square means and pooled SEM are reported.

Table 7. Experiment 1: starter diet composition and calculated nutrient analysis (as-fed basis).

Ingredients (%)	Control [1]	Flax	Flax+E
Corn, ground	47.0	41.0	41.0
Soybean meal (47% CP)	37.0	32.0	31.6
Wheat middlings	10.0	8.8	8.8
Corn oil	3.5	-	-
Canola oil	-	1.0	1.0
Limestone, ground	1.6	1.6	1.6
DL-Methionine	0.35	0.4	0.4
Salt	0.3	0.3	0.3
Dicalcium phosphate	0.2	0.2	0.2
Vitamin-mineral premix [2]	0.2	0.2	0.2
Flax seed	-	15.0	15.0
Enzyme	-	-	0.05
Calculated Nutrient Analyses			
ME (Kcal/kg)	3200	3200	
Ca (%)	0.90	0.90	
Available P (%)	0.52	0.52	
Lysine (%)	1.10	1.10	
Methionine + Cysteine (%)	0.87	0.87	
CP (%)	23.0	23.0	

[1] Control, Flax, Flax+E represent corn-soybean meal basal diet (Control), and basal diet with flax seed 15% (Flax) or flax seed 15% plus enzyme (Flax+E).

[2] Supplied per lb feed: Vitamin A1, 740,000 IU; Vitamin D3, 440,000 IU; Vitamin E, 1,200 IU; Vitamin B12, 1.6 mg; riboflavin, 800 mg; pantothenic acid, 1000 mg; niacin, 6,000 mg; menadione, 135 mg; choline, 50,000 mg; thiamine, 275 mg; folic acid, 45 mg; pyridoxine, 180 mg; manganese, 2.5%; zinc, 2.0%; selenium, 120 ppm; copper, 2,000 ppm; iodine 1,145 ppm; iron 1.8%.

Table 8. Experiment 1: grower diet composition and calculated nutrient analysis (as-fed basis).

Ingredients (%)	Control [1]	Flax	Flax+E
Corn, ground	50.0	41.8	41.8
Soybean meal (47% CP)	32.2	27.6	27.6
Wheat middlings	11.8	11.4	11.4
Corn oil	3.2	-	-
Canola oil	-	1.2	1.2
Limestone, ground	1.9	1.9	1.9
DL-Methionine	0.4	0.4	0.4
Salt	0.3	0.3	0.3
Dicalcium phosphate	0.2	0.3	0.3
Vitamin-mineral premix [2]	0.2	0.2	0.2
Flax seed	-	15.0	15.0
Enzyme	-	-	0.05
Calculated Nutrient Analyses			
ME (Kcal/kg)	3300	3300	
Ca (%)	0.94	0.94	
Available P (%)	0.50	0.50	
Lysine (%)	1.11	1.11	
Methionine + Cysteine (%)	0.90	0.90	
CP (%)	22.0	22.0	

[1] Control, Flax, Flax+E represent corn-soybean meal basal diet (Control), and basal diet with flax seed 15% (Flax) or flax seed 15% plus enzyme (Flax+E).

[2] Supplied per lb feed: Vitamin A1, 740,000 IU; Vitamin D3, 440,000 IU; Vitamin E, 1,200 IU; Vitamin B12, 1.6 mg; riboflavin, 800 mg; pantothenic acid, 1000 mg; niacin, 6,000 mg; menadione, 135 mg; choline, 50,000 mg; thiamine, 275 mg; folic acid, 45 mg; pyridoxine, 180 mg; manganese, 2.5%; zinc, 2.0%; selenium, 120 ppm; copper, 2,000 ppm; iodine 1,145 ppm; iron 1.8%.

Table 9. Composition of the enzyme mixture used in dietary treatments Flax+E in Experiment 1 and Flax 10+E and Flax 15+E in Experiment 2 (Chapter 8). The enzyme mixture was included at a level of 0.05% as-fed.

Enzyme	Activity (U/g)
cellulase	2800
xylanase	1000
glucanase	600
mannanase	400
galactanase	50
amylase	1000
protease	200

7.3 Results

7.3.1 Growth Performance

Growth performance and feed consumption results are shown in Table 10. Flax-fed birds had significantly higher final BW (Figure 7), overall weight gain, and higher overall daily gain than control-fed birds. Flax-fed birds consumed significantly more feed in the starter phase. There were no differences with dietary treatment in feed consumption and feed:gain ratio in the grower phase.

Effect of diet on organ weights is shown in Table 11. On day 35, weights of heart tissue and GIT expressed as a percent of live BW varied significantly with diet. Relative weights of heart tissue significantly decreased in flax-fed birds relative to Control with no difference between Flax and Flax+E treatments. Relative weights of GIT significantly increased in Flax-fed birds relative to Control and Flax+E treatments. There was no difference in live BW of the birds selected from each treatment for tissue collection. No differences with dietary treatment were observed in the relative weights of liver, gizzard, spleen, and AFP.

There was a 22.5% mortality rate for birds in the Control pens. Deaths occurred in all four pens throughout the study. A necropsy of one of the birds did not reveal a cause of death (Oregon State University Veterinary Pathology Laboratory).

7.3.2 Total Lipids

A significant effect of diet on total lipids of heart and AFP was observed (Table 12). Total lipids in heart tissue were significantly higher in Flax+E-fed birds (2.53%) relative to Flax (2.21%). Total lipids in AFP were the same for Control and Flax+E treatments (64.85 and 66.37%, respectively) but were significantly decreased in Flax-fed birds (53.79%). Total lipids in breast tissue ranged from 0.87 to 1.22% while total lipids in thigh tissue ranged from 1.79 to 2.5%. Although not significant, lowest values of total lipids in thigh tissues were in Flax+E birds (Table 12).

7.3.3 Tissue TBARS

TBARS values did not change significantly with dietary treatment for liver, breast, or thigh tissues (Table 13). However, as shown in Figure 8, oxidation in breast tissue from the Flax+E treatment is numerically larger (53%) than in Control, and oxidation in thigh tissue from the Control treatment is numerically larger (82%) than in Flax+E.

7.3.4 Fatty Acid Profiles of Diets and Tissues

The FA composition of the starter and grower diets is shown in Table 14. The control diets provided 1.7 times more palmitic acid (16:0) than the flax diets. All diets provided about the same amount of stearic acid (18:0) and oleic acid (18:1 n-9). With n-6:n-3 ratios less than 1, flax diets provided between 12 and 15 times more linolenic acid (18:3 n-3) and half the linoleic acid (18:2 n-6)

than the control diets (Figure 9). Both starter and grower flax diets provided between 24-28% linoleic acid.

In liver tissue (Table 15), there was no difference in 16:0 between treatments despite differences in 16:0 in the diets. There was a significant decrease in total SFA in liver tissue in Flax+E-fed birds relative to Flax- and Control-fed birds. There was a significant increase in ALA in Flax+E-fed birds relative to both Control and Flax treatments as well as in total n-3 FA (Figure 10). There was no difference in LA despite significant differences in this FA in the diets. There was a trend of decreasing total n-6 FA in flax-fed birds relative to Control (Figure 10) mainly due to a decrease in concentration of LC metabolites such as arachidonic acid (20:4 n-6). Most of the increase in total n-3 FA in Flax+E birds came from a significant increase in LC n-3 PUFA. DHA (22:6 n-3) in Flax+E-fed birds increased by 3.87% relative to Control while EPA (20:5 n-3) and DPA (22:5 n-3) each increased 1.29% relative to Control (Figure 11).

The FA profile of heart tissue is shown in Table 16. There is a significant decrease in total SFA in Flax+E-fed birds relative to Flax- and Control-fed birds. We observed 16-carbon non-oxidative metabolites of ethanol (fatty acid ethyl esters) in heart tissue from all three treatments. These are not artifacts of the methylation process but are instead produced naturally (Lange, 1982). ALA and total n-3 FA are both significantly higher in Flax+E-fed birds relative to the Control treatment (Figure 12). There was a significant decrease in total n-6

FA in Flax+E relative to Flax and Control treatments although LA was highest in Flax birds (Figure 12). As observed in liver tissue, flax diets resulted in decreases in concentration of AA in heart tissue relative to Control. Despite an increase in LC n-3 FA in the flax-fed birds (Figure 13), total PUFA were lower because of the decrease in n-6 FA metabolites. More than half of the increase in total n-3 FA came from LC n-3 PUFA instead of ALA. In heart tissue from Flax+E-fed birds, EPA (20:5 n-3) showed the largest increase at 1.1% while DPA increased 0.99% and DHA increased 0.19% relative to control (Figure 13).

In adipose tissue (Table 17), there was no difference in 16:0 or in total SFA between treatments. There was a significant increase of ALA (18:3 n-3) in Flax+E birds relative to control, and a significant decrease of LA in flax-fed birds relative to Control (Figure 14). Although total n-6 FA significantly decreased in flax-fed birds, there was no difference in amounts of AA (20:4 n-6) with dietary treatment. Total n-3 FA significantly increased in adipose tissue of Flax+E birds. No LC n-3 PUFA were detected in adipose tissue of Control birds but there were minor amounts of LC n-3 PUFA in AFP of flax-fed birds. There was an increase in MUFA (mainly 18:1) in the flax treatments relative to Control. Total lipids were lowest in the AFP of Flax birds.

In thigh tissue (Table 18), there was no difference in individual SFA, total SFA, individual MUFA, or total MUFA between treatments. ALA increased more than four-fold in flax-fed birds relative to Control (Figure 15). The increase in ALA accounted for half of total n-3 FA in the flax-fed birds. The

amount of total n-3 FA in thigh samples from Flax+E-fed birds was significantly higher (8.1%) relative to Control (1.4%). There was a decrease in LA and total n-6 FA in flax-fed birds relative to Control (Figure 15). Flax+E-fed birds had a significant increase of LC-n-3 PUFA relative to Flax- and Control-fed birds (Figure 16). EPA increased slightly to 0.7% and DPA increased to 1.6% in thigh tissue from the Flax+E treatment relative to Control. DHA increased in both flax treatments relative to control. There was no difference in total lipid content of thigh tissue among the dietary treatments.

In breast tissue (Table 19), there was no difference in 16:0, 18:0, total SFA, or total MUFA with dietary treatment. There was a greater than five-fold increase in ALA in Flax+E-fed birds relative to Control (Figure 17) and, similar to thigh tissue, a significant increase in total n-3 FA in Flax+E-fed birds (7.8%) relative to Flax (5.6%) and Control (0.8%). More than half of the increase in total n-3 FA was from LC n-3 PUFA. In Flax+E-fed birds, DPA (22:5 n-3) increased 2.25% and DHA (22:6 n-3) increased to 1.7% relative to Control (Figure 18). There was a similar trend for increased EPA in Flax+E birds. There was a decrease in LA and total n-6 FA in thigh tissue from flax-fed birds (Figure 17). Although there was no difference in total PUFA between treatments, breast tissue from flax-fed birds had a decrease in total LC n-6 PUFA and an increase in total LC n-3 PUFA relative to Control (Figure 18).

Table 10. Experiment 1: effect of whole flax seed with and without enzyme supplementation relative to corn-soybean meal control on production performance of broiler birds.

	Dietary Treatment [1]			Pooled SEM	*P*-value
	Control	Flax	Flax+E		
Starter (4-20 days)					
Initial BW at d 4 (g)	97.25	95.00	93.25	1.24	0.128
Weight gain (g)	465.06 [b]	507.70 [ab]	533.50 [a]	15.41	0.034
ADG (g/d)	31.00 [b]	33.85 [ab]	35.57 [a]	1.03	0.034
Feed consumption (g)	805.56 [b]	986.95 [a]	1022.50 [a]	35.88	0.004
Feed:gain	1.74	1.95	1.92	0.06	0.070
Grower (21-35 days)					
BW at d 21 (g)	562.31 [b]	602.70 [ab]	626.75 [a]	14.72	0.037
Weight gain (g)	391.10 [b]	732.10 [a]	679.50 [a]	76.40	0.024
ADG (g/d)	26.07 [b]	48.80 [a]	45.30 [a]	5.09	0.024
Feed consumption (g)	2436.50	2522.20	2773.80	147.31	0.292
Feed:gain	8.88	3.55	4.11	1.99	0.170
Overall (4-35 days)					
Final BW at d 35 (g)	953.40 [b]	1334.80 [a]	1306.20 [a]	72.29	0.008
Weight gain (g)	856.10 [b]	1239.80 [a]	1213.00 [a]	72.11	0.008
ADG (g/d)	27.62 [b]	39.99 [a]	39.13 [a]	2.33	0.008
Feed consumption (g)	3242.00 [b]	3509.20 [ab]	3796.30 [a]	137.34	0.055
Feed:gain	3.86 [a]	2.87 [b]	3.13 [b]	0.18	0.010
Overall Mortality (%)	22.5	2.5	2.5		

[1] Control, Flax, Flax+E represent corn-soybean meal basal diet (Control), and basal diet with 15% whole flax seed (Flax) or 15% whole flax seed plus enzyme (Flax+E).

[a-c] Means within a row with no common superscript differ when $p < 0.05$. n = 4. Data are normalized to per-bird basis.

BW, body weight; ADG, average daily gain.

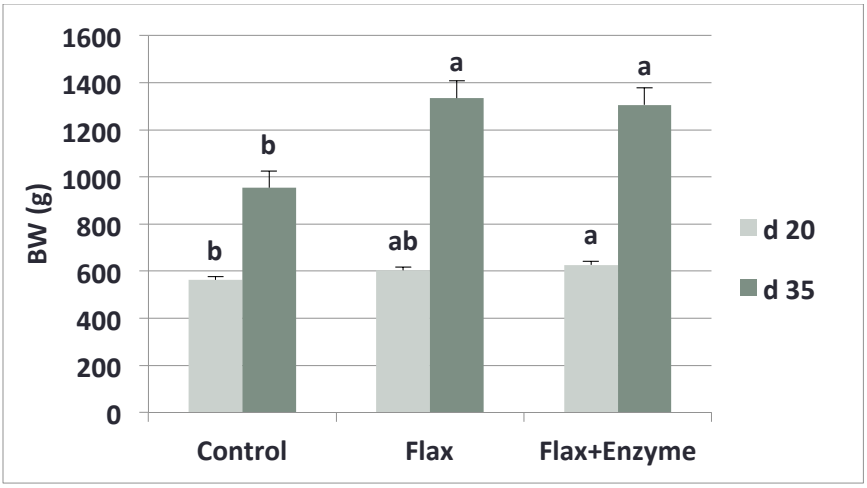


Figure 7. Experiment 1: effect of whole flax seed with and without enzyme supplementation relative to corn-soybean meal control on body weight at day 20 and day 35. Control, Flax, Flax+E represent corn-soybean meal basal diet (Control), and basal diet with 15% whole flax seed (Flax) or 15% whole flax seed plus enzyme (Flax+E). $^{a\text{-}b}$ Means of bars for each measurement day with no common superscript differ when $p < 0.05$. n = 4. SEM shown in Table 10. Data are normalized to per-bird basis.

Table 11. Experiment 1: effect of whole flax seed with and without enzyme supplementation relative to corn-soybean meal control on organ weight on day 35. Values shown are percent of live body weight.

Relative Organ Weight	Dietary Treatment [1]			Pooled SEM	*P*-value
	Control	Flax	Flax+E		
Bird LW (g)	1632.5	1446.3	1498.8	74.17	0.211
Liver	2.63	2.97	2.49	0.17	0.140
Gizzard	1.69	1.72	1.85	0.10	0.495
Heart	0.77 [a]	0.60 [b]	0.59 [b]	0.03	0.001
Spleen	0.14	0.11	0.13	0.01	0.342
AFP	1.04	0.51	1.14	0.25	0.189
GIT	5.85 [b]	7.12 [a]	6.09 [b]	0.25	0.004

[1] Control, Flax, Flax+E represent corn-soybean meal basal diet (Control), and basal diet with 15% whole flax seed (Flax) or 15% whole flax seed plus enzyme (Flax+E).

[a-b] Means within a row with no common superscript differ when $p < 0.05$.

n=8.

Two birds were randomly selected from each pen for sample collection. There were four pens per treatment.

LW, live weight; AFP, abdominal fat pad; GIT, gastrointestinal tract.

Table 12. Experiment 1: effect of whole flax seed with and without enzyme supplementation relative to corn-soybean meal control on tissue total lipids.

Total Lipids (%)	Dietary Treatment [1]			Pooled SEM	*P*-value
	Control	Flax	Flax+E		
Liver	3.96	4.37	4.12	0.34	0.704
Heart	2.35 [ab]	2.21 [b]	2.53 [a]	0.07	0.022
AFP [2]	64.85 [a]	53.79 [b]	66.37 [a]	3.36	0.034
Thigh	2.46	2.50	1.79	0.28	0.158
Breast	1.22	0.87	0.99	0.14	0.210

[1] Control, Flax, Flax+E represent corn-soybean meal basal diet (Control), and basal diet with 15% whole flax seed (Flax) or 15% whole flax seed plus enzyme (Flax+E).

[2] For AFP only, n=6 for Control group; all other tissues and treatments, n = 8.

[a-b] Means within a row with no common superscript differ when $p < 0.05$. Two birds were randomly selected from each pen for sample collection. There were four pens per treatment. AFP, abdominal fat pad.

Table 13. Experiment 1: effect of whole flax seed with and without enzyme supplementation relative to corn-soybean meal control on TBARS assay for lipid peroxidation. Values are mg of MDA per g of tissue.

TBARS	Dietary Treatment [1]			Pooled SEM	*P*-value
	Control	Flax	Flax+E		
Liver	2.21	2.09	2.05	0.15	0.733
Breast	1.93	2.48	3.65	0.62	0.158
Thigh	3.93	3.32	3.22	0.30	0.226

[1] Control, Flax, Flax+E represent corn-soybean meal basal diet (Control), and basal diet with 15% whole flax seed (Flax) or 15% whole flax seed plus enzyme (Flax+E).
Two birds were randomly selected from each pen for sample collection.
$p < 0.05$, n=8. There were four pens per treatment.
TBARS, thiobarbituric acid-reactive substance; MDA, malondialdehyde.

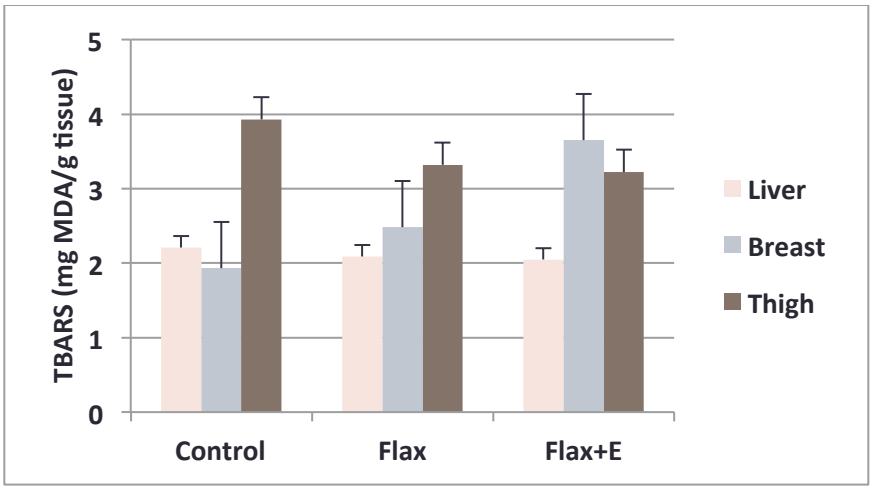


Figure 8. Experiment 1: effect of whole flax seed with and without enzyme supplementation relative to corn-soybean meal control on TBARS for liver, breast, and thigh tissues. Control, Flax, Flax+E represent corn-soybean meal basal diet (Control), and basal diet with 15% whole flax seed (Flax) or 15% whole flax seed plus enzyme (Flax+E). SEM shown in Table 13. n = 8.

Table 14. Experiment 1: fatty acid composition and total lipids in starter and grower diets.

Diet FA (%)	Diet [1]			
	Control Starter	Flax Starter	Control Grower	Flax Grower
16:0	13.56	7.96	12.72	7.09
18:0	2.84	3.06	2.14	2.99
18:1	25.87	23.31	26.56	21.85
18:2 n-6	55.19	28.40	54.99	24.33
18:3 n-3	2.54	37.28	3.59	43.73
Total SFA	16.40	11.02	14.86	10.08
Total MUFA	25.87	23.31	26.56	21.85
Total n-6 FA	55.19	28.40	54.99	24.33
Total n-3 FA	2.54	37.28	3.59	43.73
Total PUFA	57.73	65.68	58.58	68.06
n-6:n-3	21.7	0.8	15.3	0.6
TL (%)	4.94	6.23	3.28	6.12

[1] Control, Flax represent corn-soybean meal basal diet (Control), or basal diet with 15% flax seed (Flax).

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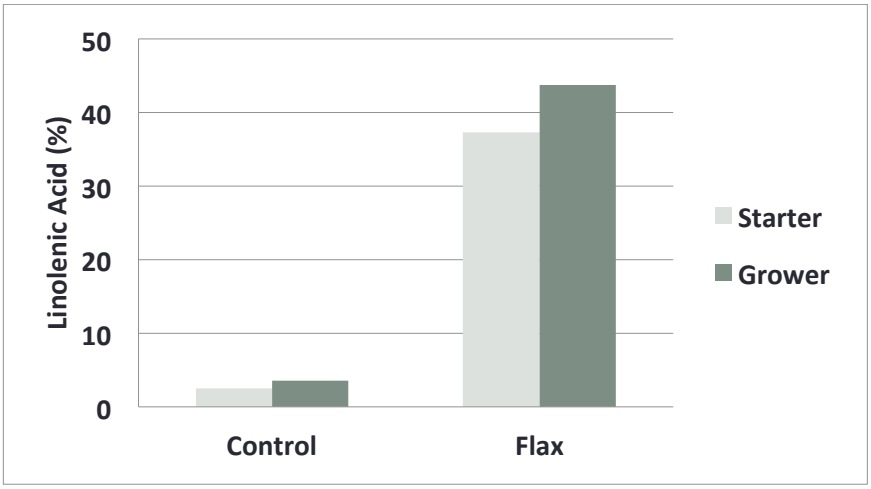

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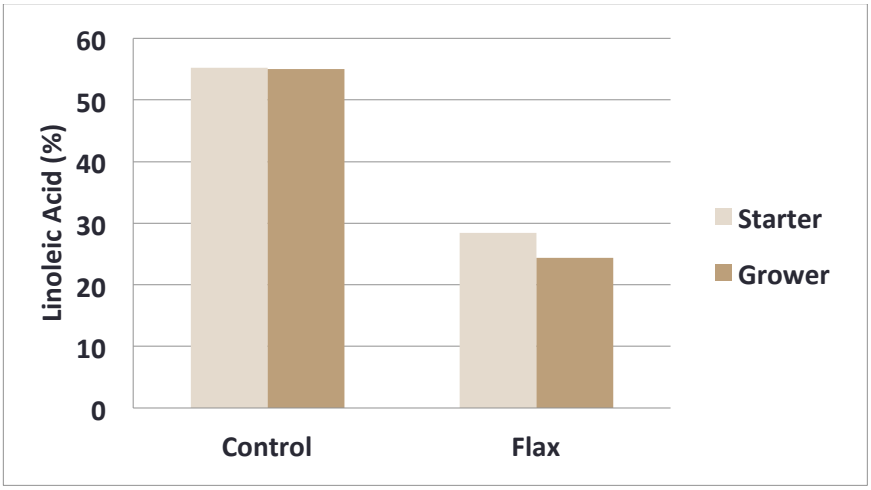

Figure 9. Experiment 1: (a) linolenic acid (18:3 n-3) and (b) linoleic acid (18:2 n-6) in starter and grower diets. Control and Flax represent corn-soybean meal basal diet (Control) and basal diet with 15% whole flax seed (Flax).

Table 15. Experiment 1: effect of whole flax seed with and without enzyme supplementation relative to corn-soybean meal control on liver fatty acids and total lipid content.

Liver FA (%)	Dietary Treatment [1]			Pooled SEM	*P*-Value
	Control	Flax	Flax+E		
16:0	20.89	22.98	19.63	1.50	0.303
16:1	1.39	2.59	2.48	0.77	0.482
18:0	22.06	19.41	17.74	1.43	0.125
18:1	15.60	23.60	23.20	3.15	0.154
18:2 n-6	19.09	16.62	18.00	1.74	0.610
18:3 n-3	0.10 ^{b}	0.79 ^{b}	2.19 ^{a}	0.31	0.001
20:1 n-9	1.57 ^{a}	0.65 ^{b}	0.91 ^{ab}	0.25	0.044
20:3 n-6	0.78	0.82	0.95	0.13	0.657
20:4 n-6	12.55 ^{a}	6.87 ^{b}	7.55 ^{b}	1.42	0.020
20:5 n-3	0.00 ^{c}	0.50 ^{b}	1.29 ^{a}	0.12	<0.001
22:4 n-6	1.46 ^{a}	0.36 ^{b}	0.28 ^{b}	0.16	<0.001
22:5 n-6	1.40 ^{a}	0.14 ^{b}	0.00 ^{b}	0.15	<0.001
22:5 n-3	0.43 ^{b}	0.73 ^{b}	1.29 ^{a}	0.15	0.003
22:6 n-3	1.56 ^{b}	2.69 ^{ab}	3.87 ^{a}	0.57	0.032
Total SFA	43.68 ^{a}	43.38 ^{a}	37.81 ^{b}	0.75	<0.001
Total MUFA	18.57	26.85	26.58	3.79	0.237
Total n-6 FA	35.67	25.06	26.98	3.21	0.066
Total n-3 FA	2.09 ^{b}	4.71 ^{b}	8.64 ^{a}	0.90	0.002
Total PUFA	37.76	29.78	35.61	3.88	0.341
Total LC n-6	16.57 ^{a}	8.39 ^{b}	8.83 ^{b}	1.62	0.003
Total LC n-3	1.99 ^{b}	3.92 ^{b}	6.45 ^{a}	0.77	0.002
n-6:n-3	17.07	5.32	3.12		
TL (%)	3.96	4.37	4.12	0.34	0.704

[1] Control, Flax, Flax+E represent corn-soybean meal basal diet (Control), and basal diet with flax seed 15% (Flax) or flax seed 15% plus enzyme (Flax+E).
$^{a-c}$ Means within a row with no common superscript differ when $p < 0.05$. n=8. Two birds were randomly selected from each pen for sample collection. There were four pens per treatment.
TL, total lipids.

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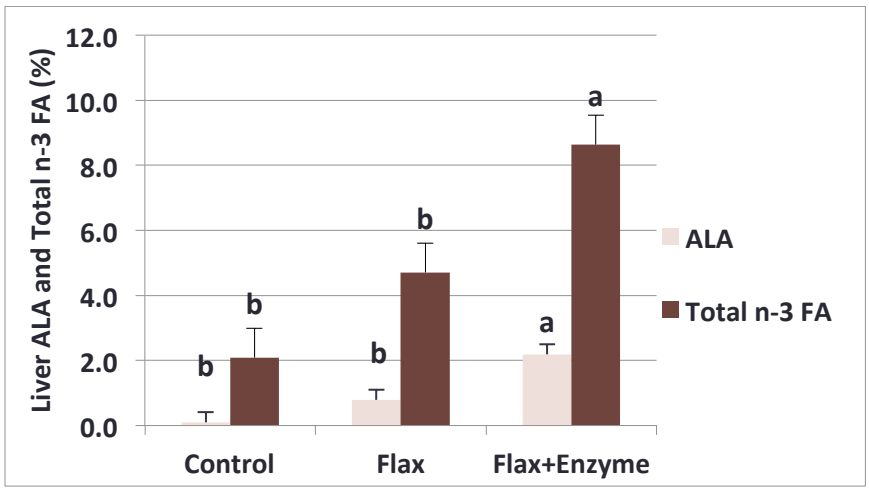

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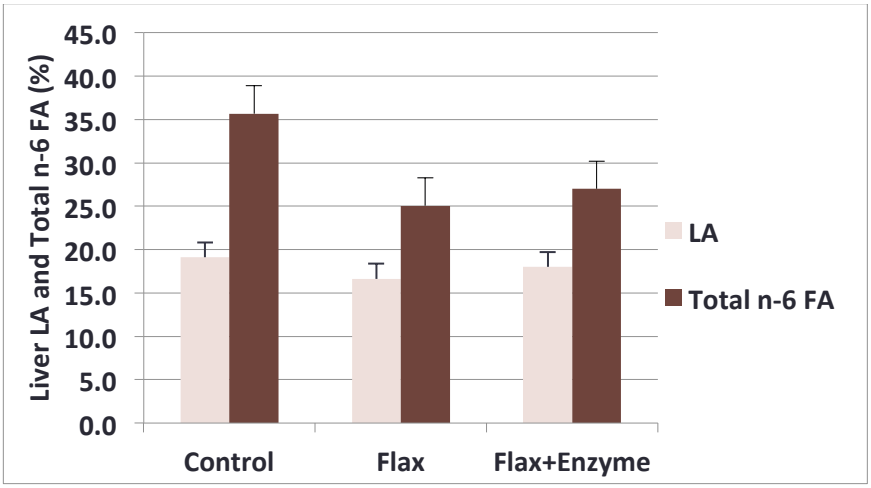

Figure 10. Experiment 1: effect of whole flax seed with and without enzyme supplementation relative to corn-soybean meal control on percent of (a) linolenic acid (ALA, 18:3 n-3) and total n-3 fatty acids and (b) linoleic acid (LA, 18:2 n-6) and total n-6 fatty acids in liver tissue. Control, Flax, and Flax+E represent corn-soybean meal basal diet (Control), and basal diet with 15% whole flax seed (Flax) or 15% whole flax seed plus enzyme (Flax+E). $^{a\text{-}b}$ Means for bars for each fatty acid with no common superscript differ when $p < 0.05$. SEM shown in Table 15. n=8.

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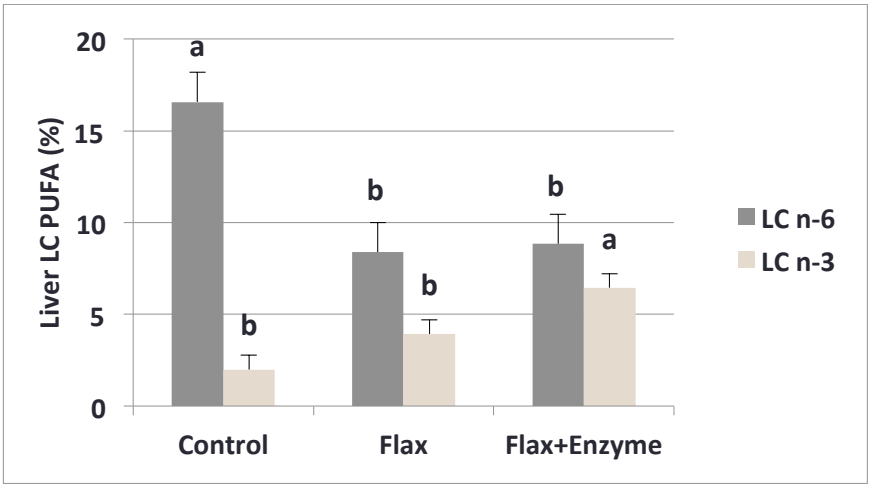

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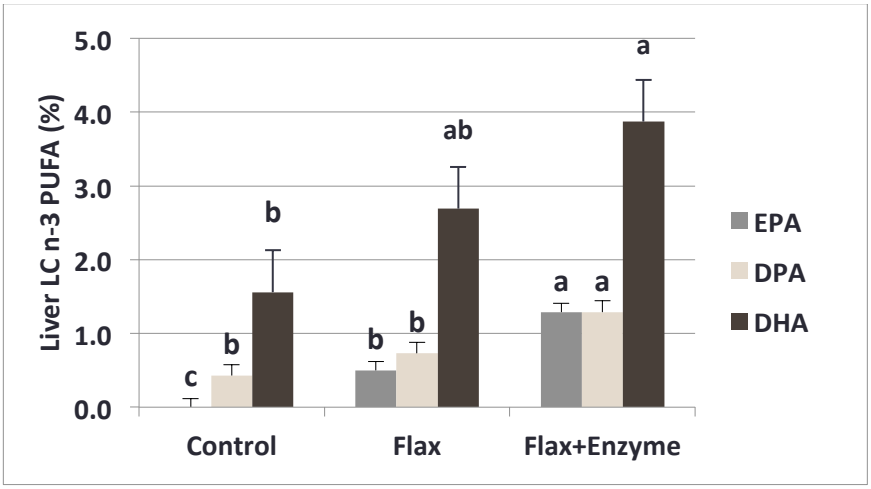

Figure 11. Experiment 1: effect of whole flax seed with and without enzyme supplementation relative to corn-soybean meal control on (a) LC PUFA, and (b) LC n-3 PUFA in liver tissue. Control, Flax, and Flax+E represent corn-soybean meal basal diet (Control), and basal diet with 15% whole flax seed (Flax) or 15% whole flax seed plus enzyme (Flax+E). $^{a-c}$ Means for bars for each fatty acid with no common superscript differ when $p < 0.05$. SEM shown in Table 15. n=8.

Table 16. Experiment 1: effect of whole flax seed with and without enzyme supplementation relative to corn-soybean meal control on heart fatty acids and total lipid content.

Heart FA (%)	Diet [1]			Pooled SEM	*P*-Value
	Control	Flax	Flax+E		
16:0 EE	3.55 [a]	3.11 [b]	2.53 [c]	0.12	<0.001
16:0	17.78	16.80	17.72	0.33	0.089
16:1	0.25 [ab]	0.00 [b]	0.87 [a]	0.21	0.024
17:0	2.16 [a]	1.99 [a]	1.35 [b]	0.15	0.003
18:0	18.91 [b]	21.41 [a]	17.73 [b]	0.50	0.001
18:1	12.02 [b]	13.65 [b]	19.07 [a]	0.67	<0.001
18:2 n-6	23.84 [b]	27.75 [a]	25.79 [ab]	0.97	0.032
18:3 n-3	0.00 [b]	0.42 [b]	1.45 [a]	0.31	0.011
20:3 n-6	0.20	0.22	0.61	0.20	0.296
20:4 n-6	19.14 [a]	13.23 [b]	10.59 [b]	1.09	<0.001
20:5 n-3	0.00 [c]	0.45 [b]	1.12 [a]	0.15	0.001
22:4 n-6	2.15 [a]	0.00 [b]	0.00 [b]	0.09	<0.001
22:5 n-3	0.00 [b]	0.81 [a]	0.99 [a]	0.20	0.005
22:6 n-3	0.00	0.17	0.19	0.06	0.091
Total SFA	42.40 [a]	43.31 [a]	39.32 [b]	0.63	0.001
Total MUFA	12.28 [b]	13.65 [b]	19.93 [a]	0.82	<0.001
Total n-6 FA	45.32 [a]	41.21 [b]	36.98 [c]	0.86	<0.001
Total n-3 FA	0.00 [c]	1.83 [b]	3.76 [a]	0.43	<0.001
Total PUFA	45.32 [a]	43.04 [b]	40.74 [c]	0.76	0.002
Total LC n-6	21.49 [a]	13.45 [b]	11.20 [b]	1.06	<0.001
Total LC n-3	0.00 [b]	1.42 [a]	2.31 [a]	0.34	0.001
n-6:n-3	45.32	22.52	9.83		
TL (%)	2.35 [ab]	2.21 [b]	2.53 [a]	0.07	0.022

[1] Control, Flax, Flax+E represent corn-soybean meal basal diet (Control), and basal diet with flax seed 15% (Flax) or flax seed 15% plus enzyme (Flax+E).

[a-c] Means within a row with no common superscript differ when $p < 0.05$. n=8. Two birds were randomly selected from each pen for sample collection. There were four pens per treatment.

TL, total lipids; 16:0 EE, nonoxidative metabolites of ethanol (fatty acid ethyl esters) produced in the heart.

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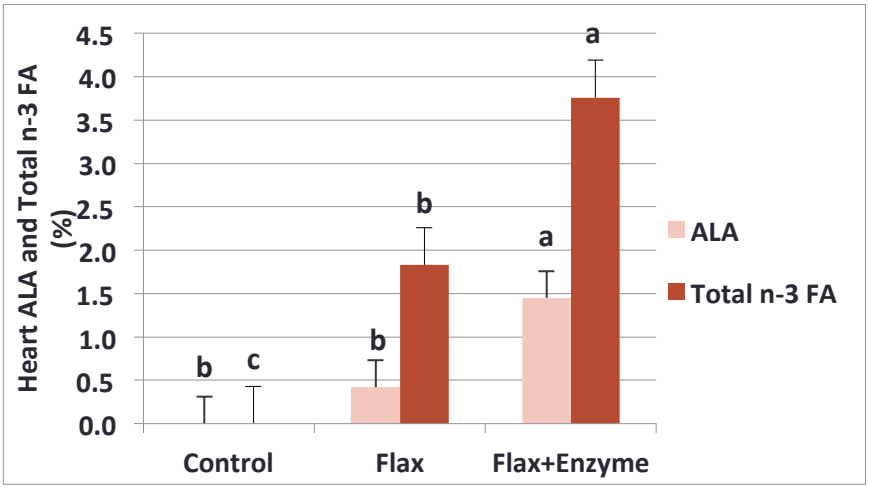

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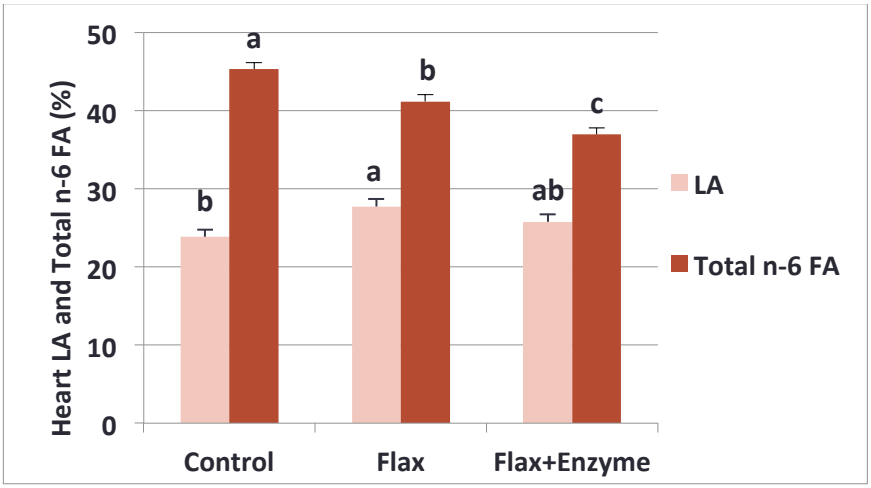

Figure 12. Experiment 1: effect of whole flax seed with and without enzyme supplementation relative to corn-soybean meal control on percent of (a) linolenic acid (ALA, 18:3 n-3) and total n-3 fatty acids and (b) linoleic acid (LA, 18:2 n-6) and total n-6 fatty acids in heart tissue. Control, Flax, and Flax+E represent corn-soybean meal basal diet (Control), and basal diet with 15% whole flax seed (Flax) or 15% whole flax seed plus enzyme (Flax+E). $^{a\text{-}c}$ Means for bars for each fatty acid with no common superscript differ when $p < 0.05$. SEM shown in Table 16. n=8.

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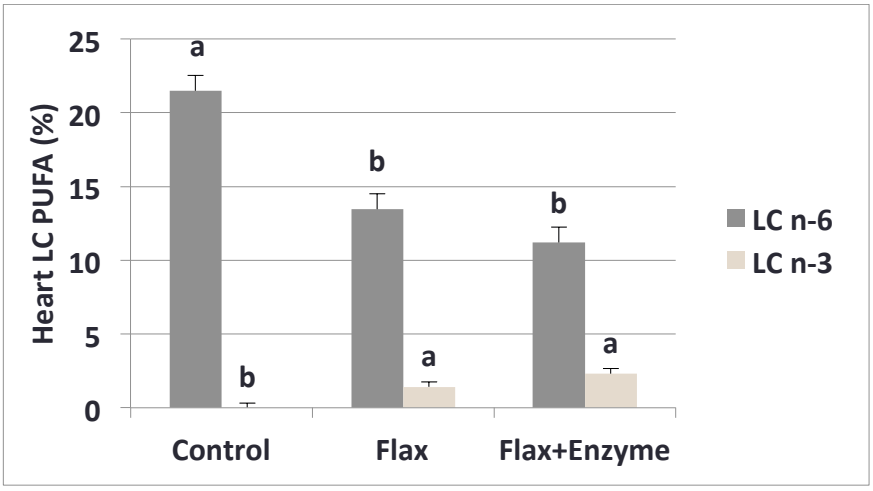

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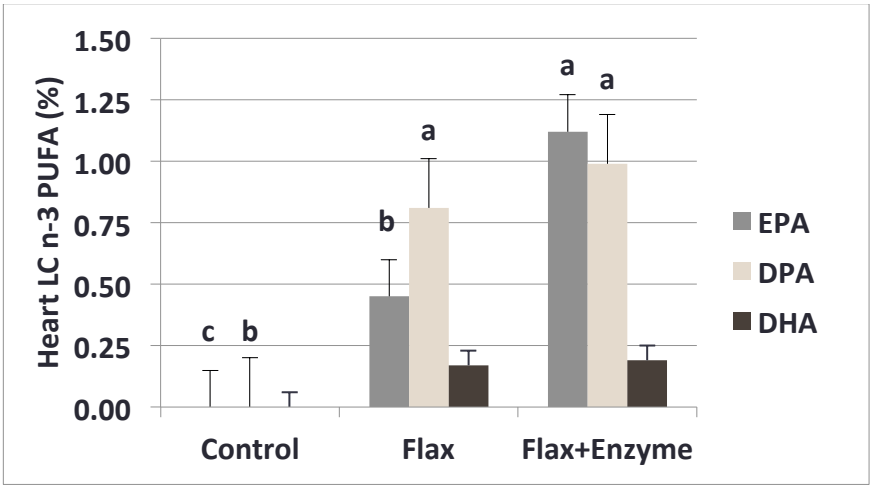

Figure 13. Experiment 1: effect of whole flax seed with and without enzyme supplementation relative to corn-soybean meal control on (a) LC PUFA, and (b) LC n-3 PUFA in heart tissue. Control, Flax, and Flax+E represent corn-soybean meal basal diet (Control), and basal diet with 15% whole flax seed (Flax) or 15% whole flax seed plus enzyme (Flax+E). $^{a-c}$ Means for bars for each fatty acid with no common superscript differ when $p < 0.05$. SEM shown in Table 16. n=8.

Table 17. Experiment 1: effect of whole flax seed with and without enzyme supplementation relative to corn-soybean meal control on abdominal fat pad fatty acids and total lipid content.

AFP FA (%)	Diet [1]			Pooled SEM	*P*-Value
	Control [2]	Flax	Flax+E		
14:0	0.46	0.56	0.56	0.04	0.215
16:0	20.07	18.83	19.38	0.71	0.474
16:1	5.51	4.99	7.71	0.77	0.164
18:0	4.72 ^{ab}	5.59 ^{a}	4.20 ^{b}	0.36	0.040
18:1	34.11 ^{b}	39.27 ^{a}	39.70 ^{a}	1.06	0.002
18:2 n-6	31.93 ^{a}	22.02 ^{b}	18.42 ^{b}	1.93	0.002
18:3 n-6	0.18	0.11	0.11	0.03	0.170
18:3 n-3	1.25 ^{c}	6.53 ^{b}	8.61 ^{a}	0.46	<0.001
20:0	0.42 ^{b}	0.55 ^{a}	0.50 ^{ab}	0.03	0.015
20:1 n-9	0.26 ^{a}	0.15 ^{b}	0.14 ^{b}	0.04	0.055
20:4 n-6	0.49	0.48	0.34	0.07	0.216
20:5 n-3	0.00	0.08	0.07	0.03	0.065
22:5 n-3	0.00 ^{b}	0.12 ^{a}	0.09 ^{a}	0.02	0.004
22:6 n-3	0.00	0.06	0.03	0.02	0.068
Total SFA	25.73	25.63	24.79	0.60	0.496
Total MUFA	40.01 ^{b}	44.55 ^{ab}	47.06 ^{a}	1.71	0.026
Total n-6 FA	32.75 ^{a}	22.72 ^{b}	18.96 ^{b}	1.98	0.001
Total n-3 FA	1.25 ^{c}	6.90 ^{b}	8.99 ^{a}	0.50	<0.001
Total PUFA	34.01	29.61	27.94	2.13	0.140
Total LC n-6	0.64	0.58	0.43	0.07	0.129
Total LC n-3	0.00 ^{b}	0.37 ^{a}	0.37 ^{a}	0.06	0.001
n-6:n-3	26.20	3.29	2.11		
TL (%)	64.85 ^{a}	53.79 ^{b}	66.37 ^{a}	3.36	0.034

[1] Control, Flax, Flax+E represent corn-soybean meal basal diet (Control), or basal diet with flax seed 15% (Flax), or flax seed 15% plus enzyme (Flax+E).

[2] n=6 for Control; all other tissues and treatments, n = 8.

[a-c] Means within a row with no common superscript differ when $p < 0.05$.

Two birds were randomly selected from each pen for sample collection. There were four pens per treatment.

TL, total lipids; AFP, abdominal fat pad.

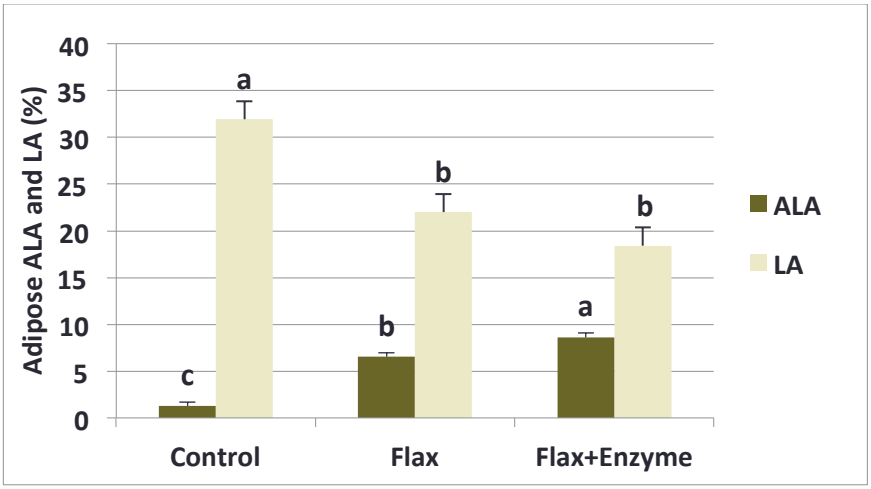

Figure 14. Experiment 1: effect of whole flax seed with and without enzyme supplementation relative to corn-soybean meal control on linolenic acid (ALA, 18:3 n-3) and linoleic acid (LA, 18:2 n-6) in adipose tissue. Control, Flax, and Flax+E represent corn-soybean meal basal diet (Control), and basal diet with 15% whole flax seed (Flax) or 15% whole flax seed plus enzyme (Flax+E). $^{a\text{-}c}$ Means for bars for each fatty acid with no common superscript differ when $p < 0.05$. n=6 for Control, all other treatments, n=8. SEM shown in Table 17.

Table 18. Experiment 1: effect of whole flax seed with and without enzyme supplementation relative to corn-soybean meal control on thigh fatty acids and total lipid content.

Thigh FA (%)	Diet [1]			Pooled SEM	*P*-Value
	Control	Flax	Flax+E		
14:0	0.53	0.47	0.51	0.08	0.841
15:1	1.60	1.48	1.85	0.22	0.490
16:0	19.98	18.98	19.61	0.66	0.562
16:1	3.97	3.44	3.95	0.49	0.700
17:0	0.66	0.43	0.23	0.17	0.242
18:0	9.30	9.93	9.62	0.64	0.789
18:1	28.38	33.29	32.21	1.46	0.065
18:2 n-6	26.61 [a]	20.59 [b]	19.41 [b]	1.38	0.003
18:3 n-3	0.93 [b]	4.09 [a]	4.66 [a]	0.31	<0.001
20:0	0.28	0.34	0.12	0.09	0.236
20:1	0.57 [a]	0.28 [b]	0.06 [c]	0.07	0.001
20:3 n-6	0.31 [b]	0.35 [b]	0.61 [a]	0.07	0.012
20:4 n-6	4.73	3.31	3.44	0.60	0.201
20:5 n-3	0.00 [c]	0.41 [b]	0.68 [a]	0.08	<0.001
22:4 n-6	1.16 [a]	0.47 [b]	0.19 [b]	0.12	<0.001
22:5 n-6	0.34 [a]	0.00 [b]	0.00 [b]	0.02	0.002
22:5 n-3	0.24 [c]	1.04 [b]	1.58 [a]	0.12	<0.001
22:6 n-3	0.20 [b]	0.95 [a]	1.22 [a]	0.17	0.001
Total SFA	30.75	30.14	30.09	0.55	0.644
Total MUFA	34.51	38.49	38.07	1.74	0.228
Total n-6 FA	33.16 [a]	24.72 [b]	23.65 [b]	1.76	0.002
Total n-3 FA	1.37 [c]	6.49 [b]	8.14 [a]	0.39	<0.001
Total PUFA	34.53	31.20	31.79	1.91	0.438
Total LC n-6	6.55 [a]	4.13 [b]	4.24 [b]	0.72	0.044
Total LC n-3	0.44 [c]	2.40 [b]	3.48 [a]	0.33	<0.001
n-6:n-3	24.20	3.81	2.91		
TL (%)	2.46	2.30	1.79	0.28	0.158

[1] Control, Flax, Flax+E represent corn-soybean meal basal diet (Control), or basal diet with flax seed 15% (Flax), or flax seed 15% plus enzyme (Flax+E).

[a-c] Means within a row with no common superscript differ when $p < 0.05$. n=8. Two birds were randomly selected from each pen for sample collection. There were four pens per treatment. TL, total lipids.

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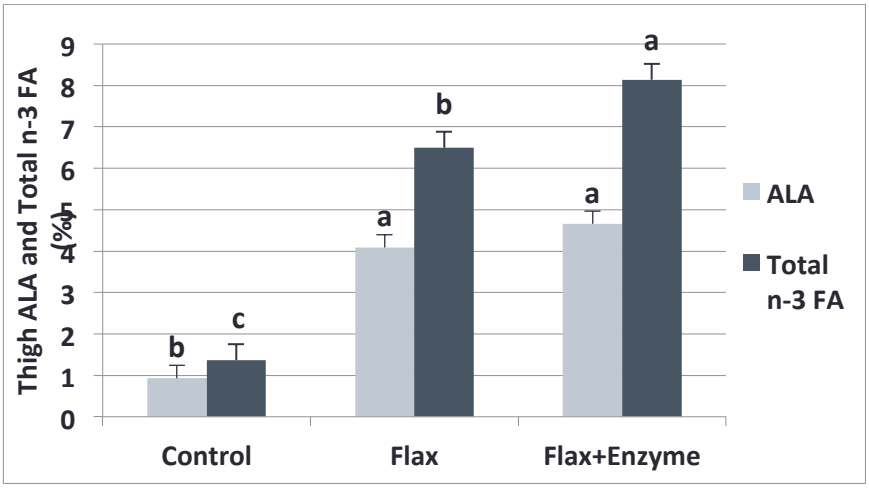

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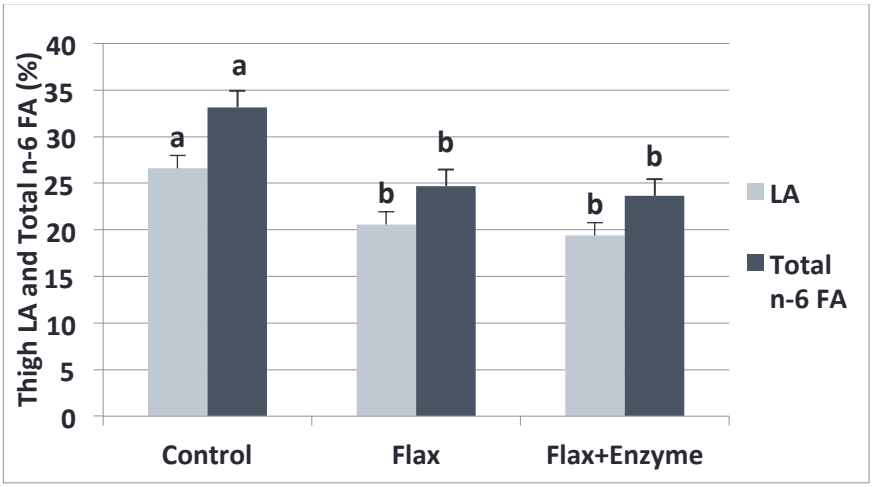

Figure 15. Experiment 1: effect of whole flax seed with and without enzyme supplementation relative to corn-soybean meal control on (a) linolenic acid (ALA, 18:3 n-3) and total n-3 FA, and (b) linoleic acid (LA, 18:2 n-6) and total n-6 FA in thigh tissues. Control, Flax, and Flax+E represent corn-soybean meal basal diet (Control), and basal diet with 15% whole flax seed (Flax) or 15% whole flax seed plus enzyme (Flax+E). $^{a\text{-}c}$ Means for each fatty acid with no common superscript differ when $p < 0.05$. SEM shown in Table 18. n=8.

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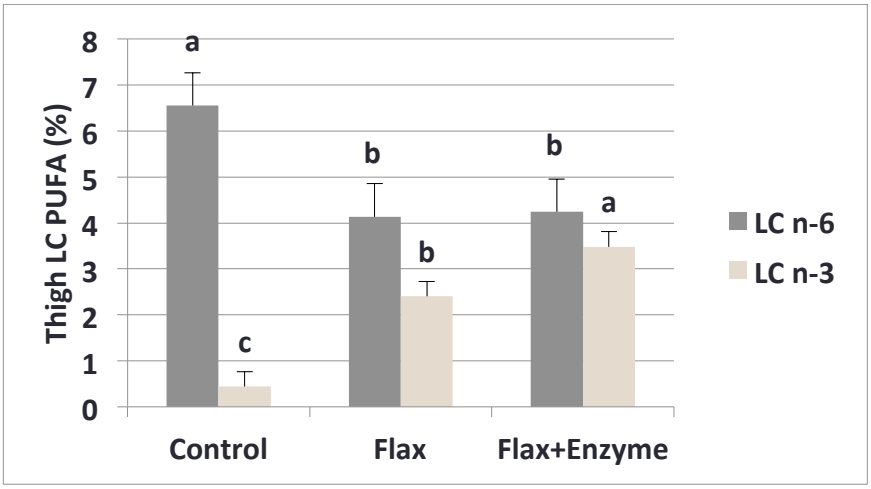

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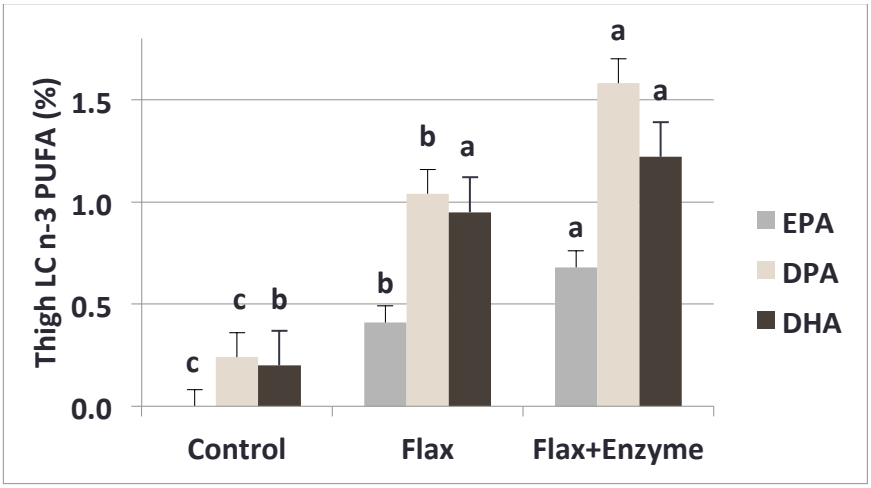

Figure 16. Experiment 1: effect of whole flax seed with and without enzyme supplementation relative to corn-soybean meal control on (a) LC PUFA, and (b) LC n-3 PUFA in thigh tissue. Control, Flax, and Flax+E represent corn-soybean meal basal diet (Control), and basal diet with 15% whole flax seed (Flax) or 15% whole flax seed plus enzyme (Flax+E). [a-c] Means for bars for each fatty acid with no common superscript differ when $p < 0.05$. SEM shown in Table 18. n=8.

Table 19. Experiment 1: effect of whole flax seed with and without enzyme supplementation relative to corn-soybean meal control on breast fatty acids and total lipid content.

Breast FA (%)	Diet [1]			Pooled SEM	*P*-Value
	Control	Flax	Flax+E		
14:0	0.39 [a]	0.59 [a]	0.00 [b]	0.13	0.015
15:1	2.31	2.28	2.44	0.34	0.938
16:0	22.09	21.78	21.77	0.43	0.834
16:1	3.71	3.57	3.57	0.53	0.978
18:0	8.69	10.18	9.71	0.68	0.307
18:1	29.42	32.28	32.52	1.57	0.318
18:2 n-6	24.16 [a]	18.33 [b]	17.86 [b]	0.91	<0.001
18:3 n-3	0.62 [c]	2.13 [b]	3.41 [a]	0.26	<0.001
20:1	0.94 [a]	0.49 [ab]	0.00 [b]	0.17	0.003
20:3 n-6	0.40	0.50	0.42	0.15	0.887
20:4 n-6	5.53	4.14	3.90	0.77	0.292
20:5 n-3	0.00	0.34	0.46	0.13	0.060
22:4 n-6	1.49 [a]	0.03 [b]	0.00 [b]	0.16	<0.001
22:5 n-6	6.00	0.00	0.00	0.04	0.385
22:5 n-3	0.00 [b]	1.83 [a]	2.25 [a]	0.19	<0.001
22:6 n-3	0.18 [b]	1.55 [a]	1.71 [a]	0.21	<0.001
Total SFA	32.54	31.17	31.48	0.51	0.160
Total MUFA	36.38	38.61	38.53	1.75	0.602
Total n-6 FA	31.65 [a]	23.00 [b]	22.18 [b]	1.35	<0.001
Total n-3 FA	0.79 [c]	5.85 [b]	7.82 [a]	0.47	<0.001
Total PUFA	32.45	28.85	30.00	1.54	0.261
Total LC n-6	7.49 [a]	4.66 [b]	4.32 [b]	0.86	0.031
Total LC n-3	0.18 [b]	3.72 [a]	4.41 [a]	0.38	<0.001
n-6:n-3	40.06	3.93	2.84		
TL (%)	1.22	0.87	0.99	0.14	0.210

[1] Control, Flax, Flax+E represent corn-soybean meal basal diet (Control), or basal diet with flax seed 15% (Flax), or flax seed 15% plus enzyme (Flax+E).

[a-c] Means within a row with no common superscript differ when $p < 0.05$. n=8. Two birds were randomly selected from each pen for sample collection. There were four pens per treatment. TL, total lipids.

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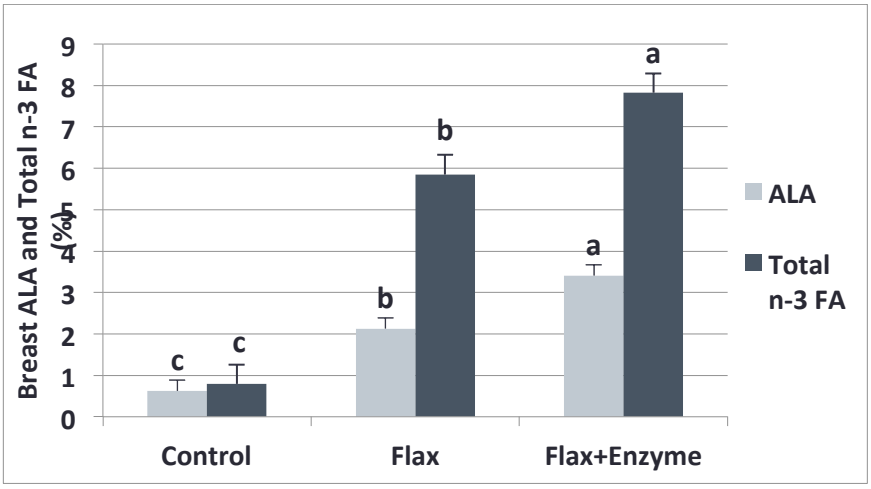

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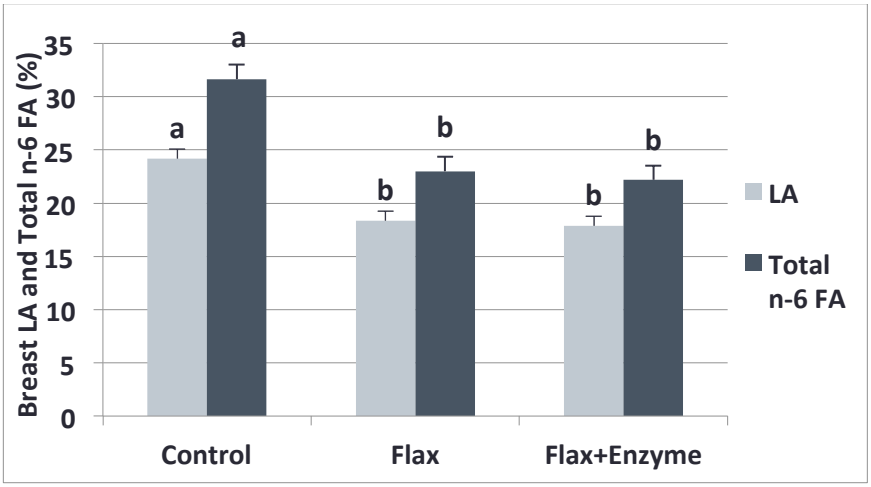

Figure 17. Experiment 1: effect of whole flax seed with and without enzyme supplementation relative to corn-soybean meal control on (a) linolenic acid (ALA, 18:3 n-3) and total n-3 FA, and (b) linoleic acid (LA, 18:2 n-6) and total n-6 FA in breast tissues. Control, Flax, and Flax+E represent corn-soybean meal basal diet (Control), and basal diet with 15% whole flax seed (Flax) or 15% whole flax seed plus enzyme (Flax+E). $^{a-c}$ Means for bars for each fatty acid with no common superscript differ when $p < 0.05$. SEM shown in Table 19. n=8.

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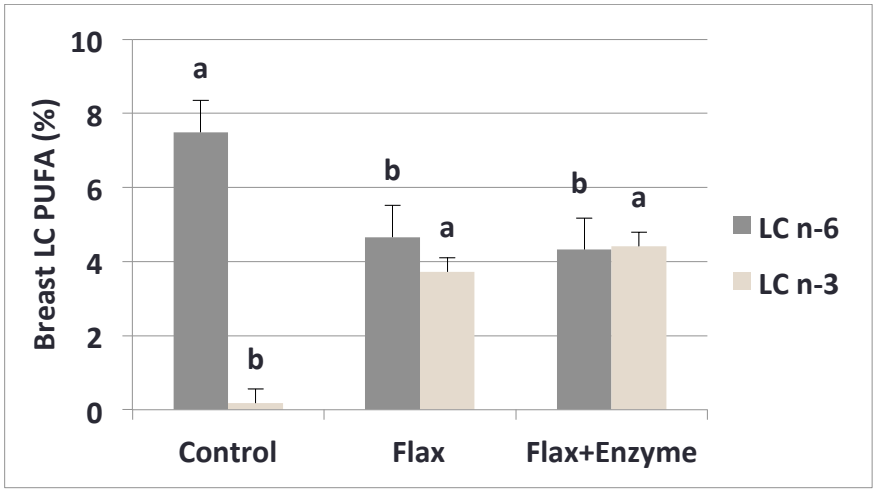

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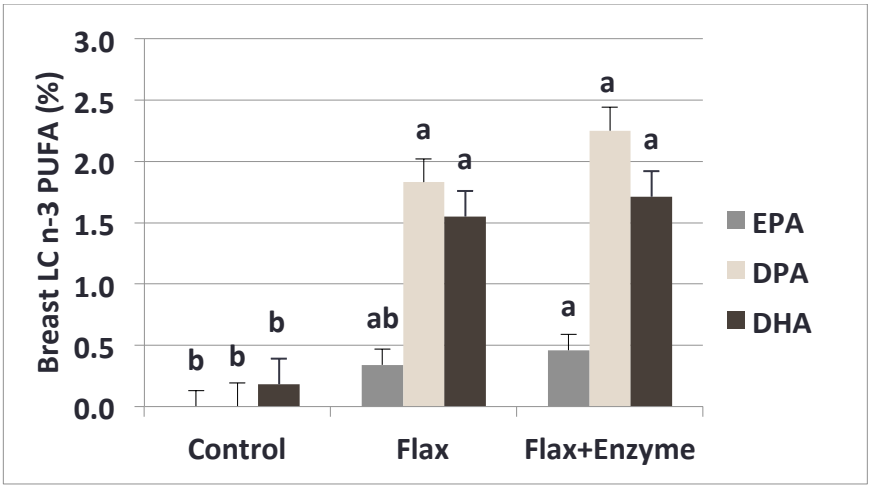


Figure 18. Experiment 1: effect of whole flax seed with and without enzyme supplementation relative to corn-soybean meal control on (a) LC PUFA, and (b) LC n-3 PUFA in breast tissue. Control, Flax, and Flax+E represent corn-soybean meal basal diet (Control), and basal diet with 15% whole flax seed (Flax) or 15% whole flax seed plus enzyme (Flax+E). $^{a\text{-}b}$ Means for bars for each fatty acid with no common superscript differ when $p < 0.05$. SEM shown in Table 19. n=8.

7.4 Discussion

7.4.1 Growth Performance

In this experiment, we did not observe a decrease in body weight in birds receiving flax diets in contrast to results from other flax feeding trials. Higher feed intakes of the flax-fed birds were associated with larger weight gain. One possible difference is that many trials incorporate ground whole flax seed while we mixed whole flax seed directly into the diets. Grinding the seeds without additional treatment such as pelleting, heat treatment, or enzyme addition may have exposed the birds in those trials to more of the ANF in flax seeds. Grinding may have also increased lipid oxidation in the feed before it was consumed, introducing less beneficial oxidation products into the guts of the birds.

As discussed in Chapter 5, it has been proposed that exogenous enzymes might provide a larger benefit to newly hatched chicks as they transition from a lipid to a carbohydrate diet while their immune system and digestive tracts are developing. In the grower phase and overall growth period, there was no difference in performance (final BW, weight gain, average daily gain) between the Flax and Flax+E treatments. Our results suggest that even if such benefits are initially present, they do not persist.

As discussed in the following sections, flax treatments had a larger effect on FA composition of tissues than on growth performance.

7.4.2 Metabolic Tissues (Liver and Heart)

Hearts from birds fed flax diets were smaller than hearts from Control birds. One of the Control birds selected for tissue collection on day 35 had typical symptoms of metabolic dysfunction: fluid accumulation in the peritoneal cavity, a nodular, pale-colored liver, and a visibly enlarged right ventricle of the heart. We observed other birds in the Control pens with chests enlarged from fluid accumulation. Therefore, we suggest that the increased n-3 FA in the flax diets may have decreased inflammation associated with right ventricular hypertrophy (Julian, 2005) and that the smaller hearts of the flax-fed birds were possibly healthier hearts. This is in line with suggestions that poultry receiving diets rich in n-3 FA obtain some of the same health benefits as humans.

In liver and heart tissue of Flax+E-fed birds, ALA was significantly increased relative to the other treatments, suggesting that one of the alternative fates for dietary ALA is storage in the metabolic tissues. In both heart and liver tissue, most of the increase in total n-3 FA came through increases in LC n-3 PUFA, and significant increases of EPA, DPA, and DHA are observed in Flax+E-fed birds. DHA is preferentially accumulated in the liver, while EPA and DPA are preferentially accumulated in the heart. This suggests that a mixture of carbohydrases added to poultry diets containing whole flax seed results in the availability of more ALA to the birds by degradation of

insoluble NSP in flax seed cell walls. This result also suggests that broiler birds can effectively convert ALA to the LC metabolites.

Total n-6 FA and AA in particular decreased in all tissues from the flax diets indicating that increased dietary ALA depressed n-6 FA metabolism. There is no evidence that the addition of the enzyme led to a greater decrease in concentration of total n-6 FA than the flax treatment without the enzyme. It is possible that the amount of ALA that the birds were able to obtain from the whole flax seed without enzyme was sufficient to depress n-6 FA metabolism, and that additional dietary ALA does not depress it further. Because AA-derived eicosanoids play a critical role in initiating an inflammatory immune response (Buckley et al., 2014), the n-6 FA pathways cannot be shut down entirely.

7.4.3 Adipose Tissue

Total lipids in adipose tissue were lowest in birds receiving the Flax diet and highest in Control and Flax+E. Although not statistically significant, the weight of the AFP as a percent of live body weight was also lowest in birds receiving the Flax diet. The control and flax diets delivered about the same amount of total PUFA and MUFA with only small differences in total SFA (Table 14). Most of the PUFA in the control diet was LA while most of the PUFA in the flax diet was ALA. This is reflected in the fatty acid composition of the adipose tissue. Control birds accumulated more LA in adipose tissue while Flax+E birds accumulated more ALA (Table 17). Although previous studies

have suggested that poultry diets high in PUFA can decrease abdominal fat pad weight and alter patterns of fat deposition compared to diets high in SFA or MUFA (Crespo and Esteve-Garcia, 2001, Ferrini et al., 2008, Esteve-Garcia, 2012), our experimental results do not address this.

Adipose tissue in poultry is not a metabolic tissue but a primary site for the storage of TAG. AA and other LC PUFA are typically stored in PL in the membranes of organelles involved in lipid metabolism and in cell membranes. Thus it is not surprising that there was no difference in the levels of AA with dietary treatment and that LC PUFA levels in AFP were very low.

More ALA and total n-3 FA was stored in adipose tissue from flax treatments than from Control, but the amount of total n-3 FA stored was several times lower than dietary intake. This suggests that high levels of dietary LA are preferentially stored in adipose tissue while high levels of dietary ALA have other fates such as β-oxidation and LC FA synthesis. It also indicates systemic suppression of n-6 FA metabolism in the flax treatments. This result provides additional evidence that carbohydrase enzymes in poultry diets containing whole flax seed can effectively degrade insoluble NSP in flax seed cells walls, exposing lipids in the seed to digestive enzymes and resulting in an increase in the amount of ALA available to the birds, and a subsequent increase in n-3 FA metabolism.

7.4.4 Consumable Meat Tissues (Thigh and Breast)

Total lipid contents in the consumable tissues were 1 to 1.2% for breast and 1.8 to 2.5% for thigh with no significant effect from dietary treatment. However, trends in TL for breast and thigh tissue suggest that Flax+E-fed birds had slightly leaner muscle tissue than Control birds. This might be due to changes in lipid metabolism caused by the increase in dietary n-3 FA and a decreased n-6:n-3 ratio. There may have been less FA directed towards intramuscular fat depots in meat tissue from flax-fed birds.

Total n-3 FA in thigh and breast tissue from Flax+E-fed birds were higher than Control, representing 8.1% and 7.8% of total lipids, respectively. About half of that increase in both tissues is due to enrichment of LC n-3 PUFA with highest levels reached in Flax+E-fed birds. Relative to the dietary levels of LA and ALA, there was little accumulation of ALA in the meat tissues of flax-fed birds, with values between 4.1 and 4.7% in thigh tissue and between 2.1 and 3.4% in breast tissue, suggesting that most of the dietary ALA was directed to β-oxidation with a small fraction directed to LC FA synthesis. The amount of LC n-3 PUFA in meat tissue was significantly increased by the addition of the enzymes.

The increase in LC n-3 PUFA in both metabolic and consumable meat tissues of Flax+E-fed birds provides evidence that the carbohydrase mixture is effectively degrading insoluble NSP of flax seed cell walls and that the subsequent increase in ALA stimulates n-3 FA metabolism with increased

conversion of ALA to the LC n-3 PUFA. This is also evidence that poultry can effectively convert ALA to the LC metabolites, and that feeding poultry diets rich in ALA can provide the consumer with a healthy product enriched in LC n-3 PUFA.

The TBARS assay is used to quantify the concentration of oxidation products (specifically, malondialdehyde) and can be used as a proxy measure for the relative amount of LC PUFA present in tissue. There are no significant differences in TBARS assays with dietary treatment in the three tissues tested (liver, thigh, breast). Liver tissue has the lowest values of the three, thigh the highest; these results do not correlate with total lipid values of those tissues. In the tissues, there are significant increases in LC n-3 PUFA and significant decreases in LC n-6 PUFA from the flax diets relative to control. However, the total PUFA in each tissue does not vary with dietary treatment; this is probably the best explanation for the TBARS results.

7.5 Conclusion

We conclude that the combination of whole flax seed plus carbohydrase enzymes led to the degradation of NSP in flax seed and increased the availability of ALA and other nutrients. Higher n-3 FA levels in the diet with the addition of enzyme to whole flax seed produced heavier but leaner birds. There was a larger effect of increased levels of n-3 FA on composition of tissues than on performance. There was a suppression of n-6 FA metabolic pathways with a significant reduction of synthesis of LC n-6 PUFA. In breast and thigh

meat from Flax+E birds, more than 50% of the increase in total n-3 FA was due to increases in LC n-3 PUFA (EPA and DHA). Whole flax seed plus carbohydrase enzymes is a viable tool for increasing n-3 FA in poultry meat tissue without reducing growth.

8 Experiment 2: Effect of Dietary Levels of Whole Flax Seed With and Without Enzyme Supplementation on Gastrointestinal Morphology, Production Performance, and Tissue Fatty Acids in Broiler Chickens

8.1 Introduction

Review of prior poultry feeding trials (Chapter 6) reveals that no experiments have been conducted to determine how the combination of whole flax seed and a mixture of carbohydrase enzymes affects the incorporation of n-3 FA into consumable tissues, meat quality, and lipid metabolism in the bird. Our hypothesis is that the combination of carbohydrase enzymes and whole flax seed will increase n-3 FA in consumable meat tissues without reducing growth performance. Our previous experiment showed that enzyme addition to flax-based broiler diets altered lipid metabolism and led to a significant increase in the amount of n-3 FA in tissues relative to control (Chapter 7).

The objective of experiment 2 was to evaluate the effect of flax level (high, low) and enzyme (with, without) on production performance, gastrointestinal morphology, water content of digesta and excreta, and fatty acid composition of tissues in broiler chickens.

8.2 Materials and Methods

8.2.1 Birds and Dietary Treatments

One hundred and twelve day-old Ross x Ross broiler chicks were obtained from a commercial hatchery. The chicks were randomly placed in 16 floor pens, 7 birds per pen. From days 1 to 4, the chicks were fed a commercial starter diet. Throughout the trial, birds were allowed free access to water and feed. On day 5, the chicks were weighed and assigned to one of four dietary treatments: Flax 10, Flax 10+E, Flax 15, Flax 15+E. There were four pens per treatment. A lighting program of 23L:1D was used for the entire experiment. The chicks were not vaccinated. The birds were housed under standard conditions of temperature, humidity, and ventilation. All protocols were approved by Oregon State University's Institutional Animal Care and Use Committee to ensure adherence to Animal Care Guidelines.

The basal corn-soybean meal diet used in Experiment 1 was adjusted for Flax 10 (10% whole flax seed as-fed) and Flax 15 (15% whole flax seed as-fed). Treatments Flax 10+E and Flax 15+E included 0.05% as-fed enzyme mixture. Diets were formulated to be isonitrogenous and isocaloric. Starter diets (days 5-22) had 23% CP and 3225 Kcal/kg ME, while grower diets (days 23-40) had 21% CP and 3220 Kcal/kg ME. Diets were provided as mash. Composition and nutrient analysis for the diets are shown in Tables 20 and 21.

The enzyme mixture used in both experiments is Omegazyme (Canadian Bio-Systems, Calgary). The composition of the enzyme mixture is shown in

Table 9 (Chapter 7). As discussed in Chapters 4-6, effective depolymerization of the complex NSP molecules in flax seed requires a mixture of enzymes with a variety of targets.

On day 23, feed consumption per pen was recorded. On day 42, birds and feed were weighed and feed consumption per pen was recorded. Average bird weight by pen and feed:gain ratio were calculated.

8.2.2 Sample Collection

On day 21, excreta was collected from each pen and stored at -20 C until analysis. On day 22, one bird from each pen (4 birds per treatment) was randomly selected, weighed then euthanized with CO2 gas. Total gut weight, including the liver, pancreas, spleen, and small intestine, was recorded for each bird. The gastrointestinal tract (GIT) was separated into duodenum (gizzard to end of duodenal loop), jejunum (end of duodenal loop to Meckel's diverticulum), and ileum (Meckel's diverticulum to cecal junction). Digesta was removed from the duodenum and jejunum of each bird and collected into tubes (one tube per bird). One- to two-centimeter samples taken from the middle of the duodenal loop and jejunum from each bird were placed in buffered formalin solution for morphometric analysis.

On day 43, two birds from each pen (8 birds per treatment) were randomly selected, weighed then euthanized with CO2 gas. For the Flax 15 diet, only six birds were selected because one of the pens had no birds remaining by day 43. Tissue weights for liver, heart, spleen, gizzard, AFP, and

GIT were recorded for each bird. Gizzards were not emptied before weighing. Tissue samples from each bird (heart, liver, AFP, skinless right pectoralis major, and skinless right biceps femoris) were collected and stored at -20 C until analysis.

8.2.3 Total Lipid and Fatty Acid Analysis

Total lipids were extracted from approximately 2 g of feed samples and tissues according to the method of Folch et al. (1957) using a 2:1 solution of chloroform and methanol. Total lipid content was determined gravimetrically. Fatty acid methyl esters were prepared with boron trifluoride methanol as the methylating agent using methods reported earlier (Cherian et al., 2002). Fatty acid analysis was performed with an HP 6890 gas chromatograph (Hewlett-Packard Co., Wilmington, DE) equipped with an autosampler, flame ionization detector, and SP-2330 fused silica capillary column. Samples in hexane (1 μL) were injected with helium as a carrier gas into the column programmed for ramped oven temperatures. Initial oven temperature was set at 150 C, held for 1.5 min, then ramped at 15 C/min to 190 C and held for 20 min, then ramped again at 30 C/min to 230 C and held for 3 min. Inlet and detector temperatures were both 250 C. Fatty acid methyl esters were identified by comparison with retention times of authentic standards (Nuchek Prep, Elysian, MN). Peak areas and percentages were calculated using Hewlett-Packard ChemStation software (Agilent Technologies Inc., Wilmington, DE). Fatty acid values are reported as percentages.

8.2.4 Thiobarbituric Acid-Reactive Substances

Lipid peroxidation in tissues was measured as thiobarbituric acid-reactive substances (TBARS) expressed in malondialdehyde equivalents as described by (Cherian et al., 2002). Briefly, 2 g of tissues were minced then 18 mL of 3.86% perchloric acid and butylated hydroxytoluene (50 μL in 4.5% ethanol) were added, after which the samples were homogenized. The homogenate was filtered and duplicate samples of the filtrate were mixed with 20 mM TBA in distilled water and incubated in a boiling water bath for 60 minutes. Absorbance was determined at 531 nm. Results of duplicate samples were averaged. TBARS values are expressed as milligrams of malondialdehyde per g of tissue.

8.2.5 Digesta and Excreta Dry Matter

Dry matter content (DM) was determined for digesta and excreta. Duplicate samples for each pen (excreta) and bird (digesta) were weighed, dried in a 110 C oven for 4 hours then weighed again. Results for duplicate samples were averaged.

8.2.6 Gastrointestinal Morphology

Duodenum and jejunum samples were fixed in neutral-buffered formalin and embedded in paraffin. Sections were cut from each block and stained with hematoxylin and eosin (Oregon State University Veterinary Pathology Laboratory). Gastrointestinal morphology was evaluated by examination with light microscopy using LAS X software (version 1.1.0.12420; Leica

Microsystems GmbH) running on a Leica digital microscope model DMI 6000B. Slides were viewed in normal planar light using 5x objective magnification. Multiple measurements of villi height (VH), villi width at the midpoint (VW), and crypt depth (CD) were made on each jejunum and duodenum sample. The VH:CD ratio was calculated. Gastrointestinal morphometric data from individual birds were pooled for each dietary treatment.

8.2.7 Statistical Analyses

Two-way ANOVA was used for all parameters to analyze effects of flax level (low including Flax 10 and Flax 10+E treatments, high including Flax 15 and Flax 15+E treatments) and enzyme (no enzyme including Flax 10 and Flax 15 treatments, with enzyme including Flax 10+E and Flax 15+E treatments). The ANOVA model was based on the equation

$$Y_{ij} = \mu + D_{ij} + E_{ij} + DE_{ij} + e_{ij}$$

where μ is the overall mean; D_{ij} is the effect of flax level; E_{ij} is the effect of enzyme; DE_{ij} is the effect of $_{ij}$th interaction between flax and enzyme; and e_{ij} is the experimental random error. Significant differences among treatment means were analyzed by Tukey's range test when $p < 0.05$. Means and pooled SEM are reported.

Table 20. Experiment 2: starter diet composition and calculated nutrient analysis (as-fed basis).

Ingredients (%)	Flax 10 [1]	Flax 10+E	Flax 15	Flax 15+E
Corn, ground	42.0	42.0	38.0	38.0
Soybean meal (47% CP)	32.0	32.0	32.0	32.0
Wheat middlings	11.2	11.2	10.4	10.4
Canola oil	0.9	0.9	0.8	0.8
Limestone, ground	2.0	2.0	2.0	2.0
Lysine	0.2	0.2	0.2	0.2
DL-Methionine	0.3	0.3	0.3	0.3
Salt	0.4	0.4	0.4	0.4
Dicalcium phosphate	0.5	0.5	0.5	0.5
Vitamin-mineral premix [2]	0.5	0.5	0.5	0.5
Flax seed	10.0	10.0	15.0	15.0
Enzyme	-	0.05	-	0.05
Calculated values				
ME, Kcal/kg	3225		3225	
Ca, %	1.00		1.00	
Available P, %	0.55		0.55	
Lysine, %	1.09		1.09	
Methionine + Cysteine, %	0.81		0.81	
CP, %	23.0		23.0	

[1] Flax 10, Flax 10E, Flax 15, and Flax 15E represent corn-soybean meal diets with whole flax seed at dietary levels of 10% and 15% with and without enzyme.

[2] Supplied per lb feed: Vitamin A-1, 740,000 IU; Vitamin D_3, 440,000 IU; Vitamin E, 1,200 IU; Vitamin B12, 1.6 mg; riboflavin, 800 mg; pantothenic acid, 1000 mg; niacin, 6,000 mg; menadione, 135 mg; choline, 50,000 mg; thiamine, 275 mg; folic acid, 45 mg; pyridoxine, 180 mg; manganese, 2.5%; zinc, 2.0%; selenium, 120 ppm; copper, 2,000 ppm; iodine 1,145 ppm; iron 1.8%.

Table 21. Experiment 2: grower diet composition and calculated nutrient analysis (as-fed basis).

Ingredients (%)	Flax 10 [1]	Flax 10+E	Flax 15	Flax 15+E
Corn, ground	46.8	46.8	42.0	42.0
Soybean meal (47% CP)	25.6	25.6	25.0	25.0
Wheat middlings	13.2	13.2	13.4	13.4
Canola oil	0.7	0.7	0.8	0.8
Limestone, ground	2.0	2.0	2.0	2.0
Lysine	0.2	0.2	0.2	0.2
DL-Methionine	0.3	0.3	0.3	0.3
Salt	0.4	0.4	0.4	0.4
Dicalcium phosphate	0.5	0.5	0.5	0.5
Vitamin-mineral premix [2]	0.5	0.5	0.5	0.5
Flax seed	10.0	10.0	15.0	15.0
Enzyme	-	0.05	-	0.05
Calculated values				
ME, Kcal/kg	3220		3220	
Ca, %	0.98		0.98	
Available P, %	0.55		0.55	
Lysine, %	1.10		1.10	
Methionine + Cysteine, %	0.72		0.72	
CP, %	21.0		21.0	

[1] Flax 10, Flax 10E, Flax 15, and Flax 15E represent corn-soybean meal diets with flax seed at dietary levels of 10% and 15% with and without enzyme.

[2] Supplied per lb feed: Vitamin A-1, 740,000 IU; Vitamin D_3, 440,000 IU; Vitamin E, 1,200 IU; Vitamin B12, 1.6 mg; riboflavin, 800 mg; pantothenic acid, 1000 mg; niacin, 6,000 mg; menadione, 135 mg; choline, 50,000 mg; thiamine, 275 mg; folic acid, 45 mg; pyridoxine, 180 mg; manganese, 2.5%; zinc, 2.0%; selenium, 120 ppm; copper, 2,000 ppm; iodine 1,145 ppm; iron 1.8%.

8.3 Results

8.3.1 Growth Performance

Production performance of birds fed two levels of flax and enzyme is shown in Table 22. There were no differences in body weight at day 22 of growth. A trend in higher weight gain during the starter period was observed for birds fed 10% flax compared to those fed 15% flax (p=0.08). However, feeding a high level of flax led to a significant increase in body weight and ADG during the grower phase. No difference in feed consumption or feed efficiency was observed due to flax level or enzyme supplementation during the starter or grower phase. Overall weight gain and ADG was higher in birds fed a high level of flax compared to those fed a low level.

Mortality rates were 0% for Flax 10, 0% for Flax 10+E, 25% for Flax 15, and 4.2% for Flax 15+E (Table 22). All of the birds in one of the four pens receiving the Flax 15 treatment were dead by the end of the starter period. A necropsy of one of the birds did not provide a conclusive cause of death (Oregon State University Veterinary Pathology Laboratory). The pens used for the Flax 15 birds in Experiment 2 were the same pens used for Control birds in Experiment 1 (Chapter 7).

There was no difference between treatments in live body weight or weight of the GIT collected on day 22 (Table 23). A significant interaction between flax level and enzyme supplementation was observed for gut weight (g) and gut

weight (% of body weight). There was no difference between treatments in organ weights on day 43 (Table 24).

8.3.2 Total Lipids

The effect of flax level and enzyme supplementation on tissue total lipid content is shown in Table 25. A significant increase in lipid content of liver tissue was observed due to high flax. Level of flax did not affect total lipids in heart tissue. However, the addition of enzyme increased total lipids in heart tissue. A significant flax x enzyme interaction was noted for total lipid content of adipose tissue, with a trend for an increase in total lipids in adipose tissue from birds receiving the low flax diets. High dietary flax led to a significant decrease of total lipids in breast and thigh muscle. No effect of enzyme on total lipid content of muscle tissue was observed.

8.3.3 Tissue TBARS

The effect of flax level and enzyme supplementation on tissue TBARS is shown in Table 26. A significant decrease in TBARS in liver tissue was observed due to high flax. No effect of enzyme on liver TBARS was observed. There were no significant differences in TBARS results between dietary treatments for thigh tissues. In breast tissues, oxidation values were significantly higher in high flax relative to low flax but enzyme supplementation led to a significant decrease in breast TBARS.

8.3.4 Dry Matter of Excreta and Digesta

Dietary flax level had no effect on dry matter (DM) content of excreta and digesta (Table 27). However, enzyme supplementation led to a trend of decreased DM in excreta (p=0.09). A significant interaction between flax level and enzyme supplementation on excreta DM was observed.

8.3.5 Gastrointestinal Morphology

Results of the morphometric analysis of the duodenum and jejunum samples collected on day 22 are shown in Table 28. In the duodenum, high flax led to a significant decrease in villi width. Addition of enzyme led to a trend in decrease in crypt depth (p= 0.09) in the duodenum. High flax led to a significant decrease in the villi height:crypt depth ratio, and a significant interaction between flax level and enzyme was observed in the same parameter. In the jejunum, high flax and enzyme supplementation increased villi height, villi width, and crypt depth (Figure 19). The villi height:crypt depth ratio was also increased. A significant interaction between crypt depth and villi height:crypt depth ratio was observed.

8.3.6 Fatty Acid Profiles of Diets and Tissues

Analysis of FAME from feed samples reveals that the Flax 15 starter diet delivered 2.3% more ALA than the Flax 10 starter diet, and the Flax 15 grower diet delivered 7.8% more ALA than the Flax 10 grower diet (Table 29, Figure 20). There was more LA (18:2 n-6) in the 10% flax diets in both starter and grower phases as a result of the additional corn (Table 29). As a result, the n-

6:n-3 ratios of the Flax 15 diets were lower than the Flax 10 diets but this is a technicality since the ratios for all diets were < 1. Both starter and grower Flax 15 diets delivered slightly less SFA and MUFA than the starter and grower Flax 10 diets.

Results of analysis of FAME from liver tissue are shown in Table 30. High flax led to significant increases in palmitic acid (16:0), palmitoleic (16:1) and oleic acid (18:1) while decreasing stearic acid (18:0), arachidonic acid, EPA, DPA and DHA. Overall, feeding high flax led to significant increases in MUFA and n-6:n-3 ratio, and decreases in n-3 FA, total PUFA, LC n-3 FA in liver tissue. Addition of enzyme led to increases in 16:0, 16:1, and 18:1 and decreases in 18:0, 20:4 n-6, DPA, and DHA. Overall, enzyme supplementation led to an increase in total MUFA and decreases in n-6 FA, n-3 FA, total PUFA, LC n-6 and LC n-3 FA. No effect of flax level or enzyme was observed for linoleic acid (18:2 n-6) or alpha-linolenic acid (18:3 n-3) in liver tissue. Total fat content of liver was increased with high flax. No effect of enzyme on liver total fat content was observed.

Results of analysis of FAME from heart tissue are shown in Table 31. High flax led to significant increases in palmitic acid (16:0), palmitoleic (16:1), oleic acid (18:1), and ALA. A significant decrease in stearic acid (18:0), linoleic acid, 20:3 n-6, arachidonic acid, EPA, and DPA was observed in the heart tissue of birds fed 15% flax. Overall, a high level of flax led to an increase in MUFA and decreases in SFA, PUFA, n-6 FA, LC n-6 FA, and LC n-3 FA.

Addition of enzyme led to increases in 16:0, 16:1, 18:1, and ALA and decreases in 18:0, linoleic acid, and arachidonic acid. Overall, enzyme supplementation led to an increase in MUFA along with decreases in n-6 FA, n-3 FA, total PUFA, LC n-6 FA, LC n-3 FA, and n-6:n-3 ratio in heart tissue. It should be noted that a significant interaction between flax level and enzyme supplementation was observed for all FA except EPA, DPA, and total n-3 FA in heart tissue. Total fat content of heart tissue was higher in birds fed the enzyme supplemented diet than those fed diets without enzyme.

Analysis of FA composition of adipose tissue is shown in Table 32. Adipose tissue is composed mainly of palmitic acid (16:0, about 19%), oleic acid (18:1 n-9, about 38%), and LA (18:2 n-6, about 20-21%). The concentration of FA with more than 18 carbons was minimal (<0.1-0.7%). Of all FA measured, only 20:0 varied with treatment, increasing with high flax. Values of the n-6:n-3 ratio showed a trend to increase with high flax ($p=0.06$). The total lipid content of AFP from high flax was decreased relative to low flax.

Fatty acid composition of thigh muscle is shown in Table 33. Feeding high flax diet led to significant increases in stearic, arachidonic acid, and total LC n-6 FA. No effect of flax level was observed on ALA, n-3 FA, or total LC n-3 FA. Enzyme supplementation had no effect on any FA tested in the thigh muscle. High level of flax led to a decrease in total lipid content in thigh muscle. No effect of enzyme supplementation on total lipid content of thigh muscle was observed.

Fatty acid composition of breast muscle is shown in Table 34. Neither dietary level of flax nor enzyme supplementation had an effect on any of the FA measured in breast muscle. A trend for increase in DHA ($p = 0.06$) and LC n-3 FA ($p = 0.07$) was observed in birds fed the high flax diet. High dietary flax led to a significant decrease in total lipids in breast and thigh muscle.

Table 22. Experiment 2: effect of flax seed with and without enzyme supplementation on production performance.

	Dietary Treatment [1]				Pooled SEM	p-values		
	low flax	high flax	no enzyme	with enzyme		flax	enzyme	flax x enzyme
Initial BW (g)	71.79	69.65	75.72	71.07				
Starter (5-22 days)								
BW day 22 (g)	498.75	492.50	487.50	503.75	39.16	0.876	0.686	0.261
WG (g)	428.04	419.11	413.75	433.39	39.68	0.086	0.630	0.279
ADG (g)	25.18	24.65	24.34	25.50	2.33	0.826	0.630	0.279
FC (g)	1086.00	1088.60	1192.90	981.70	124.55	0.983	0.116	0.283
Feed:gain	2.77	2.66	3.11	2.33	0.54	0.843	0.175	0.226
Grower (23-40 days)								
BW day 40 (g)	1470.8 [b]	1613.80 [a]	1511.91	1560.00	38.53	0.004	0.361	0.925
WG (g)	927.08 [b]	1132.38 [a]	1036.19	1056.25	65.68	0.038	0.901	0.649
ADG (g)	57.18 [b]	66.61 [a]	60.95	62.13	3.86	0.038	0.901	0.649
FC (g)	2559.90	2281.40	2433.90	2407.40	376.30	0.474	0.945	0.021
Feed:gain	2.63	2.31	2.71	2.29	0.26	0.277	0.174	0.043
Overall (5-40 days)								
WG (g)	1400.12 [b]	1540.34 [a]	1438.03	1489.64	38.93	0.005	0.323	0.958
ADG (g)	40.03 [b]	44.01 [a]	41.09	42.56	1.11	0.005	0.323	0.958
FC (g)	3645.90	3370.10	3626.80	3389.10	457.56	0.558	0.613	0.029
Feed:gain	2.62	2.40	2.79	2.28	0.28	0.472	0.116	0.060
Mortality (%)	0	0	25.0	4.2				

[1] Dietary treatments low flax (Flax 10 and Flax 10E), high flax (Flax 15 and Flax 15E), no enzyme (Flax 10 and Flax 15), and with enzyme (Flax 10E and Flax 15E) represent corn-soybean meal diets with flax seed at dietary levels of 10% and 15% with and without enzyme at 0.05% as-fed.

[a-c] Means within a row with no common superscript differ when $p<0.05$. n=16 except high flax and no enzyme treatments that included Flax15 where n=14 in grower period and overall.

Data normalized to per-bird basis.

BW, body weight; WG, weight gain; ADG, average daily gain; FC, feed consumption.

Table 23. Experiment 2: effect of flax level and enzyme supplementation on body weight and gut weight on day 22.

	Dietary Treatment [1]				Pooled SEM	p-values		
	low flax	high flax	no enzyme	with enzyme		flax	enzyme	flax X enzyme
BW (g)	498.75	492.50	487.50	503.75	20.18	0.875	0.686	0.261
gut weight (g)	84.59	84.59	83.62	85.57	6.72	0.997	0.804	0.055
gut weight (% BW)	16.90	17.12	17.07	16.95	0.71	0.764	0.858	0.031

[1] Dietary treatments low flax (Flax 10 and Flax 10E), high flax (Flax 15 and Flax 15E), no enzyme (Flax 10 and Flax 15), and with enzyme (Flax 10E and Flax 15E) represent corn-soybean meal diets with flax seed at dietary levels of 10% and 15% with and without enzyme at 0.05% as-fed.

[a-c] Means within a row with no common superscript differ when $p<0.05$. n=8. Samples were collected on day 22 from one bird per pen, the pen was the experimental unit, and there were four pens per treatment.
BW, body weight.

Table 24. Experiment 2: effect of flax level and enzyme supplementation on body weight and organ weight on day 42.

Relative Organ Weight (% BW)	Dietary Treatment [1]				Pooled SEM	p-values		
	low flax	high flax	no enzyme	with enzyme		flax	enzyme	flax X enzyme
Bird BW (kg)	1.64 [b]	1.86 [a]	1.64 [b]	1.84 [a]	0.12	0.007	0.025	0.075
Liver	2.27	2.44	2.38	2.33	0.07	0.179	0.645	0.950
Gizzard	0.10	0.10	0.10	0.10	0.00	0.162	0.477	0.510
Heart	0.02	0.03	0.03	0.02	0.00	0.812	0.798	0.432
Spleen	0.03	0.03	0.03	0.03	0.00	0.533	0.328	0.113
AFP	0.59	0.54	0.59	0.54	0.05	0.615	0.646	0.288
GIT	0.95	1.11	1.14	0.92	0.21	0.605	0.467	0.167

[1] Dietary treatments low flax (Flax 10 and Flax 10E), high flax (Flax 15 and Flax 15E), no enzyme (Flax 10 and Flax 15), and with enzyme (Flax 10E and Flax 15E) represent corn-soybean meal diets with flax seed at dietary levels of 10% and 15% with and without enzyme at 0.05% as-fed.

[a-c] Means within a row with no common superscript differ ($p<0.05$). n=16 except high flax and no enzyme treatments that included Flax15 where n=14.
Two birds were randomly selected from each pen for sample collection on day 42, experimental unit was the pen, and there were four pens per treatment.
BW, body weight. AFP, abdominal fat pad. GIT, gastrointestinal tract.

Table 25. Experiment 2: effect of flax level and enzyme supplementation on total lipids in tissues.

Total Lipids (%)	Dietary Treatment [1]				Pooled SEM	p-values		
	low flax	high flax	no enzyme	with enzyme		flax	enzyme	flax X enzyme
Liver	2.69 [b]	3.15 [a]	2.80	3.00	0.19	0.010	0.333	0.658
Heart	2.32	2.38	2.25 [b]	2.43 [a]	0.07	0.226	0.002	0.435
AFP	62.47	57.45	58.59	61.48	3.23	0.056	0.206	0.035
Thigh	3.63 [a]	1.98 [b]	2.81	2.90	0.66	0.052	0.800	0.759
Breast	1.00 [a]	0.72 [b]	0.92	0.83	0.11	0.006	0.519	0.727

[1] Dietary treatments low flax (Flax 10 and Flax 10E), high flax (Flax 15 and Flax 15E), no enzyme (Flax 10 and Flax 15), and with enzyme (Flax 10E and Flax 15E) represent corn-soybean meal diets with flax seed at dietary levels of 10% and 15% with and without enzyme at 0.05% as-fed.

[a-c] Means within a row with no common superscript differ when $p<0.05$. n=16 except high flax and no enzyme treatments that included Flax15 where n=14.

Two birds were randomly selected from each pen for sample collection on day 42, experimental unit was the pen, and there were four pens per treatment.

AFP, abdominal fat pad.

Table 26. Experiment 2: effect of flax level and enzyme supplementation on TBARS assay for lipid peroxidation.

Tissue (mg MDA/g tissue)	Dietary Treatment [1]				Pooled SEM	p-values		
	low flax	high flax	no enzyme	with enzyme		flax	enzyme	flax X enzyme
Liver	8.92 [a]	7.57 [b]	8.65	7.98	0.59	0.018	0.290	0.562
Thigh	2.69	2.28	2.76	2.28	0.29	0.266	0.226	0.278
Breast	0.35 [b]	0.98 [a]	0.94 [a]	0.42 [b]	0.35	0.031	0.047	0.264

[1] Dietary treatments low flax (Flax 10 and Flax 10E), high flax (Flax 15 and Flax 15E), no enzyme (Flax 10 and Flax 15), and with enzyme (Flax 10E and Flax 15E) represent corn-soybean meal diets with flax seed at dietary levels of 10% and 15% with and without enzyme at 0.05% as-fed.

[a-c] Means within a row with no common superscript differ when $p<0.05$. n=16 except high flax and no enzyme treatments that included Flax15 where n=14.

Two birds were randomly selected from each pen for sample collection, experimental unit was the pen, and there were four pens per treatment.

TBARS, thiobarbituric acid-reactive substance; MDA, malondialdehyde.

Table 27. Experiment 2: effect of flax level and enzyme supplementation on dry matter content of digesta and excreta collected on day 22.

Dry Matter (%)	Dietary Treatment [1]				Pooled SEM	p-values		
	low flax	high flax	no enzyme	with enzyme		flax	enzyme	flax X enzyme
Excreta	27.12	27.54	29.06	25.60	2.55	0.824	0.091	0.018
Digesta	23.46	21.88	22.96	22.38	0.92	0.558	0.827	0.586

[1] Dietary treatments low flax (Flax 10 and Flax 10E), high flax (Flax 15 and Flax 15E), no enzyme (Flax 10 and Flax 15), and with enzyme (Flax 10E and Flax 15E) represent corn-soybean meal diets with flax seed at dietary levels of 10% and 15% with and without enzyme at 0.05% as-fed.

[a-c] Means within a row with no common superscript differ when $p<0.05$. n=8. Excreta samples were collected on day 21 from each pen with four pens per treatment. Digesta contents were collected on day 22 from one bird per pen with four pens per treatment.

Table 28. Experiment 2: effect of flax level and enzyme supplementation on gastrointestinal morphology on day 22.

Villi Morph. (mm)	Dietary Treatment [1]				Pooled SEM	p-values		
	low flax	high flax	no enzyme	with enzyme		flax	enzyme	flax X enzyme
Duodenum								
VH	820.45	777.68	804.71	793.41	79.01	0.157	0.707	<0.0001
VW	102.93 [a]	92.95 [b]	100.34	95.54	5.37	0.001	0.108	0.018
CD	186.84	196.50	198.93	184.41	7.62	0.262	0.093	0.441
VH:CD	4.57 [a]	4.07 [b]	4.22	4.43	0.42	0.011	0.297	<0.0001
Jejunum								
VH	638.61 [b]	689.75 [a]	590.98 [b]	737.38 [a]	64.09	0.049	<0.001	0.344
VW	91.29	93.21	86.54 [b]	97.96 [a]	4.80	0.552	0.001	0.537
CD	167.95 b	194.93 a	173.64 [b]	189.23 [a]	15.48	0.001	0.046	0.006
VH:CD	3.89	3.70	3.53 [b]	4.06 [a]	0.29	0.280	0.003	0.014

[1] Dietary treatments low flax (Flax 10 and Flax 10E), high flax (Flax 15 and Flax 15E), no enzyme (Flax 10 and Flax 15), and with enzyme (Flax 10E and Flax 15E) represent corn-soybean meal diets with flax seed at dietary levels of 10% and 15% with and without enzyme at 0.05% as-fed.

[a-c] Means within a row with no common superscript differ when $p<0.05$. n=56.
One bird was randomly selected from each pen on day 22 for sample collection and there were four pens per treatment.
VH, villi height; VW, villi width; CD, crypt depth; VH:CD, villi height:crypt depth ratio.

Figure 19. Experiment 2: Effect of effect of flax level and enzyme supplementation on villi morphology in the jejunum (a) Flax 10; (b) Flax 15; (c) Flax 15+E. Samples were collected on day 21. In the jejunum, villi height and crypt depth were significantly larger in birds receiving high flax and enzyme-supplemented diets. Dietary treatments low flax (Flax 10 and Flax 10E), high flax (Flax 15 and Flax 15E), no enzyme (Flax 10 and Flax 15), and with enzyme (Flax 10E and Flax 15E) represent corn-soybean meal diets with flax seed at dietary levels of 10% and 15% with and without enzyme at 0.05% as-fed.

A

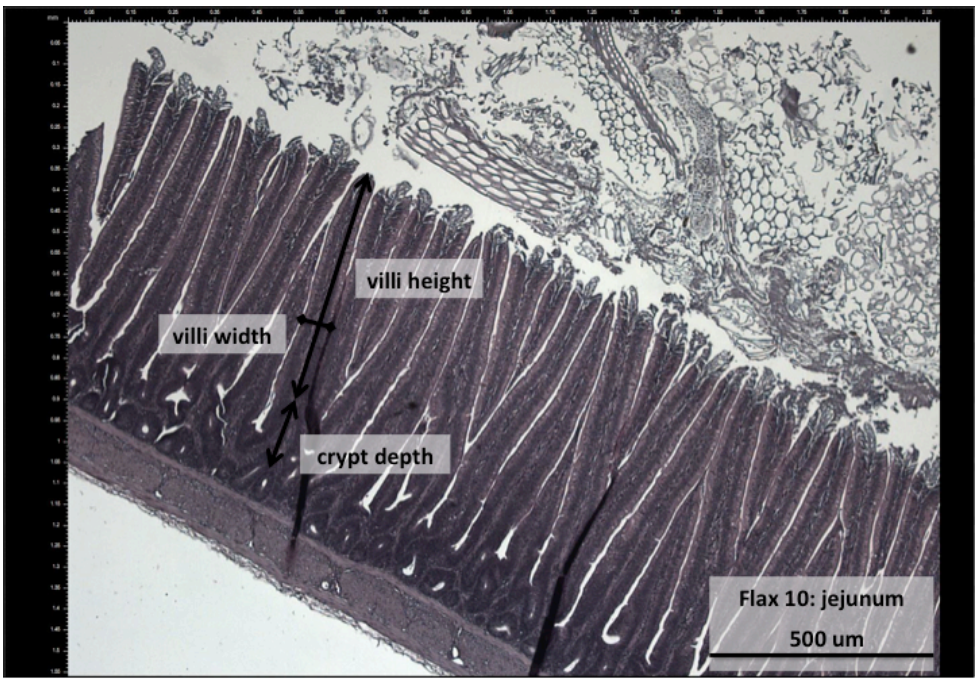


Figure 19. continued.

B

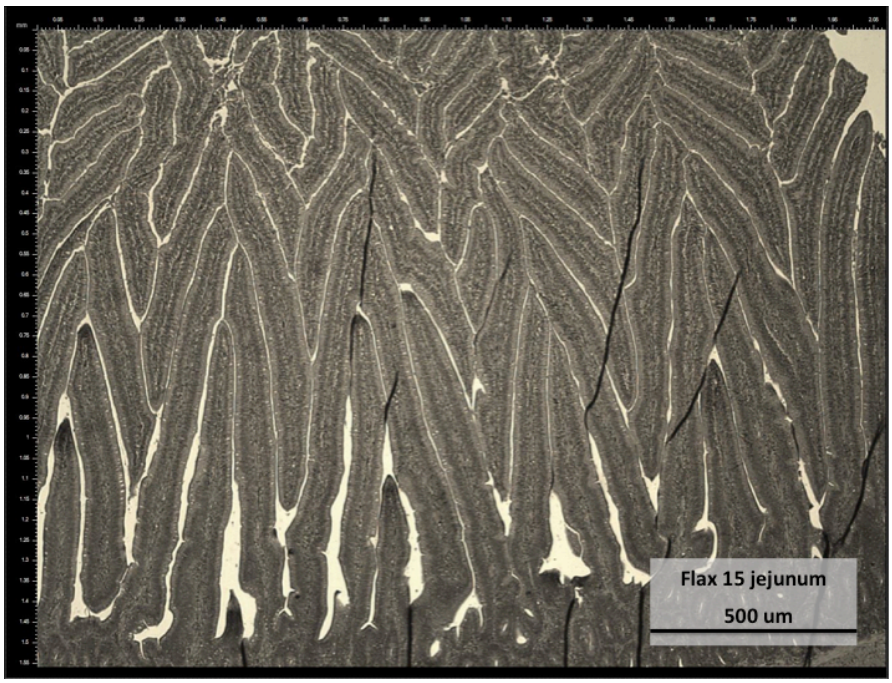


Figure 19. continued.

C

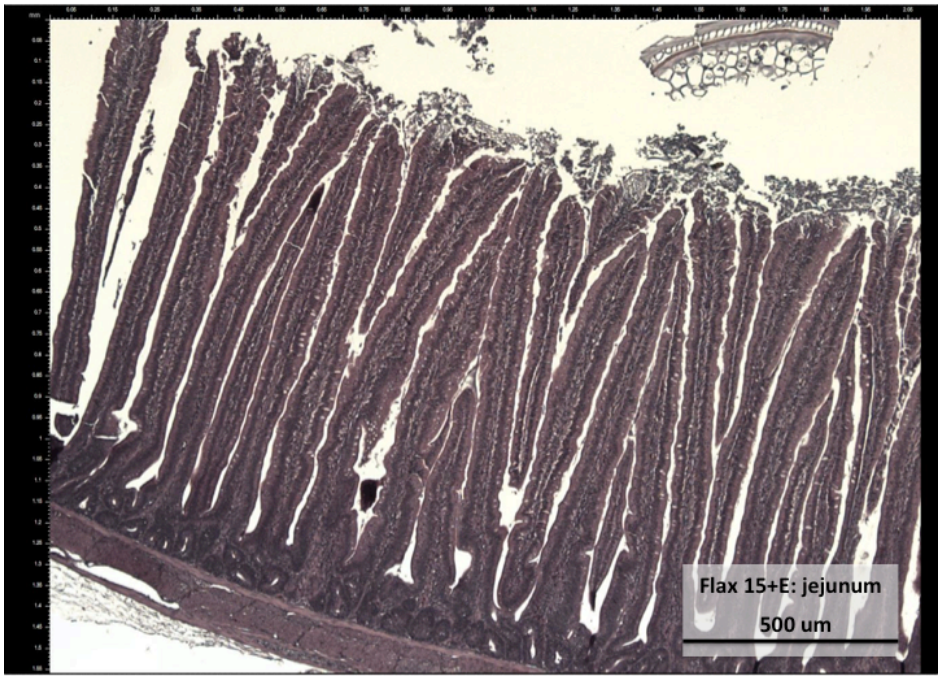


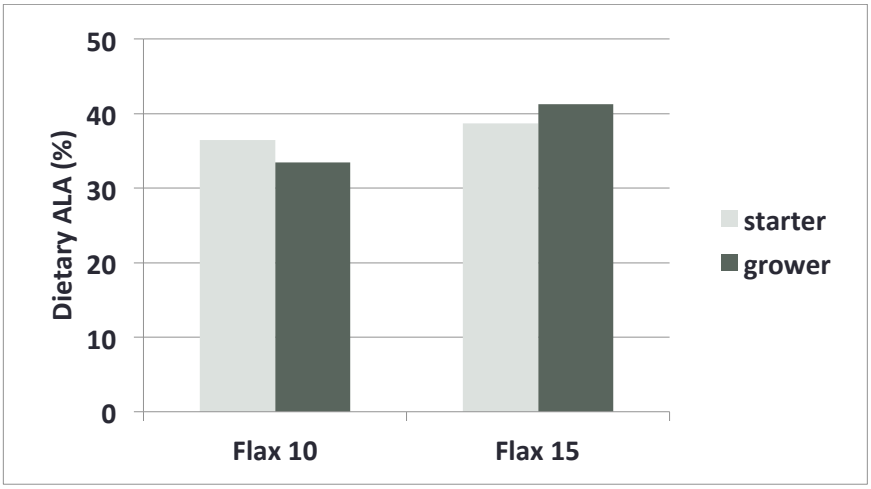
Figure 19. continued.

Table 29. Experiment 2: fatty acid composition and total lipid content of starter and grower diets.

Diet FA (%)	Dietary Treatment [1]			
	Flax 10 Starter	Flax 15 Starter	Flax 10 Grower	Flax 15 Grower
16:0	8.18	7.77	8.47	7.37
18:0	3.37	3.46	3.24	3.49
18:1	24.20	23.87	25.08	22.34
18:2 n-6	27.43	25.89	29.40	24.77
18:3 n-3	36.43	38.68	33.45	41.27
Total SFA	11.94	11.56	12.07	11.16
Total MUFA	24.20	23.87	25.08	22.34
Total n-6 FA	27.43	25.89	29.40	25.22
Total n-3 FA	36.43	38.68	33.45	41.27
Total PUFA	63.86	64.57	62.85	66.49
n-6:n-3	0.75	0.67	0.88	0.61
TL	5.53	5.39	4.50	6.31

[1] Flax 10 and Flax 15 represent corn-soybean meal basal diet with flax seed at dietary levels of 10% and 15%.

A



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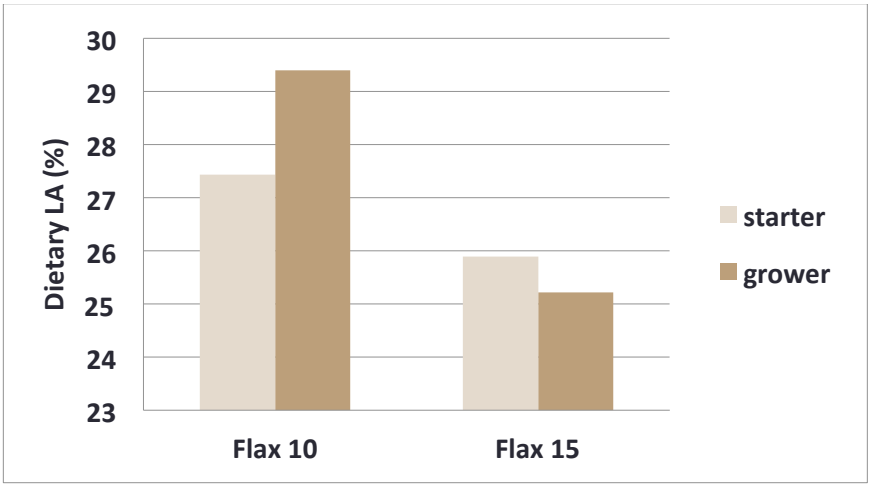

Figure 20. Experiment 2: fatty acid composition of starter and grower diets, (a) alpha-linolenic acid (ALA, 18:3 n-3) and (b) linoleic acid (LA, 18:2 n-6). Flax 10 and Flax 15 represent corn-soybean meal diets with flax seed at levels of 10% and 15%.

Table 30. Experiment 2: effect of dietary levels of flax seed with and without enzyme supplementation on liver fatty acids and total lipid content.

Liver	Dietary Treatment [1]				Pooled SEM	p-values		
	low flax	high flax	no enzyme	with enzyme		flax	enzyme	flax X enzyme
16:0	19.43 [b]	21.45 [a]	19.04 [b]	21.54 [a]	1.22	0.026	0.011	0.880
16:1	1.54 [b]	2.40 [a]	1.27 [b]	2.53 [a]	0.62	0.042	0.006	0.173
18:0	22.75 [a]	20.24 [b]	23.63 [a]	19.78 [b]	1.82	0.017	0.001	0.234
18:1	16.99 [b]	23.57 [a]	16.40 [b]	23.26 [a]	3.78	0.008	0.009	0.234
18:2 n-6	18.93	17.65	18.87	17.86	0.81	0.295	0.447	0.278
18:3 n-3	2.39	1.64	2.08	2.01	0.34	0.090	0.967	0.339
20:3 n-6	0.94	0.97	0.87	1.03	0.07	0.844	0.286	0.574
20:4 n-6	9.22 [a]	7.47 [b]	9.95 [a]	7.05 [b]	1.32	0.048	0.003	0.449
20:5 n-3	2.21	1.26	2.18	1.40	0.47	0.056	0.152	0.956
22:5 n-3	1.58 [a]	0.64 [b]	1.61 [a]	0.72 [b]	0.49	0.001	0.003	0.931
22:6 n-3	3.87 [a]	2.39 [b]	3.92 [a]	2.53 [b]	0.78	0.002	0.004	0.695
Total SFA	42.18	41.75	42.67	41.38	0.66	0.635	0.170	0.269
Total MUFA	18.63 b	26.12 [a]	17.67 [b]	26.02 [a]	4.48	0.009	0.007	0.214
Total n-6 FA	29.15	26.20	29.87 [a]	25.94 [b]	2.05	0.126	0.056	0.265
Total n-3 FA	10.05 [a]	5.92 [b]	9.79 [a]	6.66 [b]	1.98	0.007	0.052	0.864
Total PUFA	39.19 [a]	32.12 [b]	39.66 [a]	32.60 [b]	3.92	0.015	0.022	0.390
Total LC n-6	10.22	8.55	11.00 [a]	8.08 [b]	1.33	0.071	0.004	0.375
Total LC n-3	7.66 [a]	4.29 [b]	7.72 [a]	4.66 [b]	1.65	0.003	0.012	0.872
n-6:n-3	3.38 [b]	5.53 [a]	3.85	4.84	0.91	0.001	0.176	0.772
Total Lipids (%)	2.69 [b]	3.15 [a]	2.80	3.00	0.19	0.010	0.333	0.658

[1] Dietary treatments low flax (Flax 10 and Flax 10E), high flax (Flax 15 and Flax 15E), no enzyme (Flax 10 and Flax 15), and with enzyme (Flax 10E and Flax 15E) represent corn-soybean meal diets with flax seed at dietary levels of 10% and 15% with and without enzyme at 0.05% as-fed.

[a-c] Means within a row with no common superscript differ when $p<0.05$. n=16 except high flax and no enzyme treatments that included Flax15 where n=14.

Table 31. Experiment 2: effect of flax level and enzyme supplementation on heart fatty acids and total lipid content.

Heart	Dietary Treatment [1]				Pooled SEM	p-values		
	low flax	high flax	no enzyme	with enzyme		flax	enzyme	flax X enzyme
16:0 EE	3.04 [a]	1.82 [b]	3.12 [a]	1.92 [b]	0.74	<0.0001	<0.0001	<0.0001
16:0	16.19 [b]	17.10 [a]	15.53 [b]	17.59 [a]	0.93	0.026	<0.0001	0.026
16:1	1.37 [b]	3.22 [a]	1.16 [b]	3.16 [a]	1.26	<0.0001	<0.0001	<0.0001
17:1	1.64 [a]	1.05 [b]	1.58 [a]	1.19 [b]	0.36	0.001	0.033	0.001
18:0	18.35 [a]	13.25 [b]	18.94 [a]	13.38 [b]	3.50	<0.0001	<0.0001	<0.0001
18:1	15.24 [b]	24.64 [a]	15.31 [b]	23.32 [a]	5.70	<0.0001	<0.0001	<0.0001
18:2 n-6	25.24 [a]	23.94 [b]	26.19 [a]	23.33 [b]	1.37	0.022	<0.0001	0.010
18:3 n-3	1.70 [b]	3.97 [a]	1.55 [b]	3.80 [a]	1.53	<0.0001	<0.0001	<0.0001
20:3 n-6	1.06 [a]	0.68 [b]	1.06 [a]	0.73 [b]	0.22	<0.0001	<0.0001	<0.0001
20:4 n-6	11.84 [a]	7.29 [b]	12.01 [a]	7.74 [b]	2.89	<0.0001	<0.0001	<0.0001
20:5 n-3	1.22 [a]	0.45 [b]	1.06	0.71	0.32	0.001	0.151	0.809
22:4 n-6	0.56	0.41	0.52	0.46	0.13	0.196	0.655	0.014
22:5 n-3	0.91 [a]	0.39 [b]	0.80	0.56	0.22	0.010	0.247	0.852
22:6 n-3	0.24	0.08	0.14	0.20	0.07	0.131	0.538	0.433
Total SFA	38.13 [a]	33.04 [b]	37.92 [a]	33.91 [b]	3.13	<0.0001	<0.0001	<0.0001
Total MUFA	18.69 [b]	29.45 a	18.45 [b]	28.24 [a]	6.53	<0.0001	<0.0001	<0.0001
Total n-6 FA	39.06 [a]	32.61 [b]	40.02 [a]	32.56 [b]	4.49	<0.0001	<0.0001	<0.0001
Total n-3 FA	4.07	4.90	3.55 [b]	5.28 [a]	1.16	0.175	0.009	0.001
Total PUFA	43.13 [a]	37.51 [b]	43.58 [a]	37.84 [b]	3.43	<0.0001	<0.0001	<0.0001
Total LC n-6	13.60 [a]	8.51 [b]	13.74 [a]	9.05 [b]	3.25	<0.0001	<0.0001	<0.0001
Total LC n-3	2.38 [a]	0.93 [b]	2.01	1.48	0.59	0.003	0.298	0.885
n-6:n-3	11.97	13.08	16.50 [a]	8.71 [b]	5.27	0.739	0.025	0.003
Total Lipids (%)	2.32	2.38	2.25 [b]	2.43 [a]	0.07	0.226	0.002	0.435

[1] Dietary treatments low flax (Flax 10 and Flax 10E), high flax (Flax 15 and Flax 15E), no enzyme (Flax 10 and Flax 15), and with enzyme (Flax 10E and Flax 15E) represent corn-soybean meal diets with flax seed at dietary levels of 10% and 15% with and without enzyme at 0.05% as-fed.

[a-c] Means within a row with no common superscript differ when $p<0.05$. n=16 except high flax and no enzyme treatments that included Flax15 where n=14.

16:0 EE, nonoxidative metabolites of ethanol (fatty acid ethyl esters) produced in the heart.

Table 32. Experiment 2: effect of flax level and enzyme supplementation on adipose tissue fatty acids and total lipid content.

AFP	Dietary Treatment [1]				Pooled SEM	p-values		
	low flax	high flax	no enzyme	with enzyme		flax	enzyme	flax X enzyme
14:0	0.55	0.60	0.56	0.59	0.02	0.095	0.224	0.430
16:0	19.40	19.28	19.05	19.60	0.25	0.877	0.458	0.735
16:1	5.81	5.59	5.37	6.01	0.41	0.539	0.069	0.031
17:0	0.32	0.36	0.33	0.34	0.02	0.077	0.700	0.054
18:0	4.96	4.86	5.09	4.76	0.21	0.709	0.230	0.141
18:1	38.39	38.68	38.74	38.34	0.45	0.773	0.679	0.319
18:2 n-6	20.38	21.74	21.02	21.02	0.74	0.135	0.914	0.154
18:3 n-3	8.15	6.67	7.83	7.13	0.63	0.306	0.677	0.946
20:0	0.52 [b]	0.68 [a]	0.57	0.62	0.07	0.0001	0.281	0.487
20:3 n-6	0.11	0.12	0.11	0.12	0.01	0.621	0.717	0.205
20:4 n-6	0.35	0.39	0.38	0.36	0.05	0.228	0.554	0.009
20:5 n-3	0.13	0.09	0.11	0.11	0.02	0.272	0.822	0.572
22:5 n-3	0.11	0.08	0.09	0.10	0.02	0.391	0.773	0.358
Total SFA	25.93	25.87	25.73	26.05	0.16	0.948	0.727	0.789
Total MUFA	44.71	44.81	44.58	44.91	0.73	0.942	0.805	0.178
Total n-6 FA	20.98	22.41	21.65	21.64	0.78	0.125	0.907	0.139
Total n-3 FA	8.41	6.85	8.03	7.37	0.66	0.294	0.709	0.909
Total PUFA	29.39	29.25	29.68	29.02	0.66	0.948	0.754	0.463
Total LC n-6	0.46	0.51	0.49	0.48	0.04	0.158	0.627	0.022
Total LC n-3	0.26	0.17	0.20	0.24	0.05	0.157	0.049	0.258
n-6:n-3	2.96	3.93	3.37	3.45	0.44	0.062	0.980	0.274
Total Lipids (%)	62.47	57.45	58.59	61.48	3.23	0.056	0.206	0.035

[1] Dietary treatments low flax (Flax 10 and Flax 10E), high flax (Flax 15 and Flax 15E), no enzyme (Flax 10 and Flax 15), and with enzyme (Flax 10E and Flax 15E) represent corn-soybean meal diets with flax seed at dietary levels of 10% and 15% with and without enzyme at 0.05% as-fed.

[a-c] Means within a row with no common superscript differ when $p<0.05$. n=16 except high flax and no enzyme treatments that included Flax15 where n=14.

AFP, abdominal fat pad.

Table 33. Experiment 2: effect of flax level and enzyme supplementation on thigh fatty acids and total lipid content.

Thigh	Dietary Treatment [1]				Pooled SEM	p-values		
	low flax	high flax	no enzyme	with enzyme		flax	enzyme	flax X enzyme
14:0	0.41	0.29	0.36	0.35	0.06	0.289	0.950	0.335
15:1	1.14	1.61	1.28	1.43	0.23	0.071	0.668	0.218
16:0	20.35	20.83	20.21	20.90	0.38	0.496	0.344	0.474
16:1	4.50	4.20	4.28	4.44	0.24	0.326	0.584	0.142
18:0	7.48 [b]	8.67 [a]	7.71	8.32	0.60	0.029	0.329	0.137
18:1	35.25	33.56	35.36	33.68	1.41	0.218	0.251	0.051
18:2 n-6	20.19	20.99	20.54	20.58	0.54	0.309	0.980	0.155
18:3 n-3	6.03	4.65	5.80	5.03	0.61	0.228	0.554	0.860
20:4 n-6	2.18 [b]	3.13 [a]	2.54	2.69	0.49	0.023	0.830	0.057
20:5 n-3	0.30	0.13	0.20	0.24	0.08	0.128	0.648	0.546
22:5 n-3	0.82	0.82	0.73	0.90	0.07	0.992	0.398	0.870
22:6 n-3	0.45	0.38	0.37	0.46	0.05	0.679	0.583	0.868
Total SFA	28.52	29.87	28.48	29.74	0.71	0.153	0.219	0.949
Total MUFA	41.08	39.55	41.03	39.79	1.30	0.313	0.454	0.082
Total n-6 FA	22.61	24.35	23.26	23.57	1.07	0.037	0.849	0.036
Total n-3 FA	7.61	5.98	7.09	6.63	0.67	0.260	0.807	0.817
Total PUFA	30.22	30.33	30.36	30.20	0.95	0.954	0.932	0.238
Total LC n-6	2.40 [b]	3.37 [a]	2.71	2.98	0.54	0.028	0.653	0.032
Total LC n-3	1.58	1.33	1.30	1.60	0.17	0.566	0.448	0.759
n-6:n-3	3.76	5.20	4.68	4.23	0.70	0.102	0.519	0.314
Total Lipids (%)	3.63 [a]	1.98 [b]	2.81	2.90	0.66	0.052	0.800	0.759

[1] Dietary treatments low flax (Flax 10 and Flax 10E), high flax (Flax 15 and Flax 15E), no enzyme (Flax 10 and Flax 15), and with enzyme (Flax 10E and Flax 15E) represent corn-soybean meal diets with flax seed at dietary levels of 10% and 15% with and without enzyme at 0.05% as-fed.

[a-c] Means within a row with no common superscript differ when $p<0.05$. n=16 except high flax and no enzyme treatments that included Flax15 where n=14.

Table 34. Experiment 2: effect of flax level and enzyme supplementation on breast fatty acids and total lipid content.

Breast	Dietary Treatment [1]				Pooled SEM	p-values		
	low flax	high flax	no enzyme	with enzyme		flax	enzyme	flax X enzyme
15:1	2.82	2.89	2.95	2.77	0.09	0.824	0.560	0.746
16:0	22.24	21.55	21.67	22.14	0.36	0.279	0.412	0.632
16:1	3.07	2.96	2.73	3.27	0.26	0.771	0.168	0.385
18:0	10.11	10.35	10.35	10.11	0.24	0.651	0.620	0.350
18:1	31.84	30.31	31.24	31.03	1.10	0.223	0.938	0.068
18:2 n-6	18.83	19.21	18.90	19.10	0.58	0.534	0.775	0.025
18:3 n-3	4.26	3.50	4.44	3.43	0.52	0.472	0.364	0.635
20:4 n-6	4.08	4.53	4.41	4.18	0.36	0.367	0.593	0.130
20:5 n-3	0.09	0.33	0.15	0.25	0.15	0.162	0.627	0.106
22:5 n-3	1.43	1.77	1.63	1.55	0.16	0.328	0.773	0.569
22:6 n-3	0.75	1.38	0.95	1.11	0.26	0.057	0.710	0.545
Total SFA	32.43	31.95	32.02	32.37	0.26	0.627	0.701	0.805
Total MUFA	37.74	36.16	36.91	37.07	1.19	0.275	0.850	0.082
Total n-6 FA	22.91	24.20	23.63	23.41	1.09	0.106	0.694	0.004
Total n-3 FA	6.52	6.97	7.17	6.35	0.39	0.754	0.548	0.876
Total PUFA	29.43	31.16	30.79	29.76	1.32	0.339	0.521	0.151
Total LC n-6	4.08	4.99	4.72	4.31	0.56	0.114	0.400	0.087
Total LC n-3	2.27	3.47	2.73	2.92	0.49	0.073	0.872	0.669
n-6:n-3	4.43	4.18	4.38	4.24	0.26	0.778	0.902	0.484
Total Lipids (%)	1.00 [a]	0.72 [b]	0.92	0.83	0.11	0.006	0.519	0.727

[1] Dietary treatments low flax (Flax 10 and Flax 10E), high flax (Flax 15 and Flax 15E), no enzyme (Flax 10 and Flax 15), and with enzyme (Flax 10E and Flax 15E) represent corn-soybean meal diets with flax seed at dietary levels of 10% and 15% with and without enzyme at 0.05% as-fed.

[a-c] Means within a row with no common superscript differ when $p<0.05$. n=16 except high flax and no enzyme treatments that included Flax15 where n=14.

8.4 Discussion

8.4.1 Growth Performance

In this experiment, we examined the effect of two levels of whole flax seed with and without enzyme supplementation on production performance, gastrointestinal morphology, lipid metabolism, meat quality, and FA profile of tissues. Based on the results of our first experiment, we expected that enzyme degradation of NSP in flax seed should be an effective strategy for increasing the amount of n-3 FA available to endogenous digestive and metabolic processes (Chapter 7).

Results obtained on production performance suggest that flax level was more effective in the grower phase than the starter phase. Higher levels of flax led to significantly higher BWG and ADG in the grower phase and overall growth period. Enzyme supplementation did not influence any of the production parameters tested in the grower or starter phase. There were no significant effects on growth performance that were due to enzyme only. Weight gains did not differ with treatment in the starter period. We believe that during the starter period, birds receiving high flax and enzyme supplementation were redirecting energy to enable the large changes in jejunum villi morphology rather than growth.

High flax levels during the starter period did not result in reduced BW or ADG relative to low flax levels. Thus we did not observe depression of performance outcomes due to high dietary flax levels that have been observed

in other studies (see references in Chapter 6). Possible differences between those studies and the one described here include the form of flax seed and inclusion of other ingredients in the feed. The use of ground flax seed or flax seed meal greatly increases the risk of oxidation of the oils in the flax seed and this may have affected palatability of the diet or altered the ME before the feed was consumed. Inclusion of other feed ingredients such as wheat and barley in the basal diets used in other studies may have added to the water-soluble NSP already present in the digesta from flax seed.

On the basis of performance outcomes in Experiment 2, we conclude that a corn-soybean meal poultry diet containing 15% whole flax seed results in higher final BW and higher overall ADG than a diet with 10% whole flax seed with or without enzyme.

8.4.2 TBARS

Lipid oxidation results closely follow those for total PUFA in liver and thigh muscle tissues: decreased PUFA result in decreased lipid oxidation. However, lipid oxidation in breast muscle was sensitive to dietary flax and enzyme supplementation. The increase in sensitivity of breast muscle to lipid oxidation may be due to the lipid profile of the tissue (e.g., increased concentration of phospholipids). Because of the variations in SFA, MUFA, and n-6 FA, particularly LA, in the different tissues tested, TL is not a good predictor of lipid oxidation. We note that in the current study, the TBARS

assays were conducted within one month of sample collection. There was little time for further changes in oxidation state of the tissues.

8.4.3 Metabolic Tissues (Heart and Liver)

Total lipids in liver tissue increased with high flax while total lipids in heart tissue increased with enzyme supplementation. The increase in TL in liver and heart was mainly due to increases in 16:0, 16:1, and 18:1. It was also observed that LC n-3 PUFA were lowest in hepatic and cardiac tissues of birds fed the high level of flax. These results are consistent with other experiments that demonstrated that high levels of LC n-3 FA suppress Δ-9 desaturase and MUFA synthesis. As expected, high levels of dietary ALA also depressed LC n-6 FA metabolism, leading to decreases in LC n-6 FA in both metabolic tissues. Results obtained from consumable tissues (e.g., breast muscle) discussed below indicate that there was a trend for increased LC n-3 FA synthesis with increasing dietary ALA ($p = 0.07$). However, it appears that when dietary ALA is high, LC n-3 FA are not accumulated in metabolic tissues, and LC FA synthesis in these tissues may be depressed. The addition of enzyme also significantly decreased synthesis of LC n-6 and n-3 FA in liver tissue. There was significantly more ALA in heart tissues from birds fed enzyme-supplemented diets. However, high ALA or enzyme supplementation did not result in significant changes in heart DHA. This is consistent with the fact that DHA levels in the heart are not strongly influenced by dietary n-3 FA whereas

levels of DHA in the liver can vary over a wide range in response to dietary changes in n-3 FA (Brenna and Carlson, 2014).

8.4.4 Adipose Tissue

Results from Experiment 2 reveal that flax level and enzyme supplementation have minimal effects on the fat content and FA composition of the adipose tissue of broilers. This is not surprising because in avians, hepatic tissue is the major site of lipogenesis. We observed a trend for decreased fat content in birds fed high flax ($p=0.056$). Similarly, a trend in the increase of the n-6:n-3 ratio was observed in high flax-fed chickens. Lack of any effect on FA composition of the adipose tissue indicates that there may be a saturation effect of FA incorporation into adipose tissue.

8.4.5 Consumable Meat Tissues (Thigh and Breast)

Overall differences in fatty acid composition of breast tissues due to flax level and addition of enzyme were negligible except for a trend in the increase of DHA with high dietary flax. However, thigh tissues accumulated more stearic, arachidonic acid, and total LC n-6 FA with high flax levels. Long-chain PUFA are preferentially stored in membrane PL instead of TAG, and breast tissue is known to contain more PL than TAG. Thus it is not surprising that levels of DHA in breast tissue are 0.75 to 1.38% while levels of DHA in thigh tissue are <0.5%. Overall, breast tissue incorporated relatively more LC n-6 and LC n-3 PUFA than thigh tissue. Nonetheless, because TL values of thigh tissue is over 2.7- to 3.6-fold higher than breast tissue, thigh meat from broiler

birds fed 10% or 15% whole flax seed would provide more LC n-3 PUFA than breast meat.

8.4.6 Excreta DM and Enzyme Treatment

Results of prior feeding trials reviewed in Chapter 6 suggested that carbohydrase enzymes work in poultry diets containing flax seed by degrading insoluble NSP in the seed coat and improving access to oil and other nutrients inside the seed. They may also reduce viscosity of soluble NSP. The tissue FA composition results discussed above provide evidence that the carbohydrase mixture used in the current study is effectively degrading NSP in flax seed and enhancing exposure of the seed contents to digestive enzymes. However, at day 22, the observed effect of flax level and enzyme supplementation on excreta and digesta DM was minimal. Addition of enzyme led to a trend in decreased DM of excreta. A decrease in dry matter implies there is more water in the excreta, that is, the excreta is less viscous. However, we observed a significant flax level x enzyme interaction in excreta, suggesting that the decrease in DM was not observed in excreta from Flax 15+E birds. The amount of enzyme may have been too low to affect the excreta DM from the high level of flax seed. However, we believe that it is more likely that it simply took longer for the same amount of enzyme to significantly change the viscosity of the excreta at the higher flax level. In Experiment 2, we observed sticky excreta on all birds in all treatments starting around day 10 (Figure 21). Excreta and digesta samples were collected on days 21 and 22, respectively. While the amount of

sticky excreta in pens receiving Flax 10+E diets decreased noticeably between days 21-24, it was not until after day 28 that we observed the same change in the pens receiving the Flax 15+E diets. Although we did not collect additional digesta and excreta measurements at that time, this observation supports the idea that more time may be required for the enzyme supplement to reduce digesta and excreta viscosity when broiler birds are fed diets with high flax levels. It should be mentioned that the excreta samples collected were from birds kept in floor pens. Ideally, to accurately measure the impact of diet and enzymes, birds should be kept in metabolic cages. Further studies on the effect of flax level and enzymes and duration of treatment on excreta and digesta DM should be conducted on birds housed in metabolic cages with standardized facilities for excreta collection.

8.4.7 Gastrointestinal Morphology and Enzyme Treatment

Flax level and enzyme supplementation influenced duodenum and jejunum morphology differently. By day 22, high flax reduced villi width and the VH:CD ratio in the duodenum while the opposite effect was observed in the jejunum. The effects of enzyme on villi morphology were more evident in the jejunum than the duodenum. Lipid digestion in chickens occurs in the jejunum and upper ileum (Tancharoenrat et al., 2014). Addition of enzyme resulted in increases in villi height, width, and crypt depth in the jejunum at both flax levels, suggesting that carbohydrase enzymes added to flax-based diets reduced the effect of NSP of whole flax seed in early phases of growth. The

utilization of FA and other nutrients in flax seed appears to be associated with early increases in villi surface area, ultimately leading to alterations in tissue lipid profiles as observed in the current study. Growth performance outcomes discussed above suggest that enzyme addition is also effective during the grower phase. To more clearly define the contribution of enzyme to growth performance, excreta DM, and villi morphology, additional data need to be collected at the end of the grower period.

8.5 Conclusions

We conclude that the combination of whole flax seed plus carbohydrase enzymes led to the degradation of NSP in flax seed and increased the availability of ALA and other nutrients. Higher n-3 FA levels in the diet produced heavier but leaner birds. BWG was not reduced in the starter period due to high flax levels, possibly due to the form of flax used in this study (whole seed). Because of variations in SFA, MUFA, and n-6 FA in the tissues, TL is not a good predictor of lipid oxidation. However, decreased total PUFA is generally associated with decreased lipid oxidation. In the heart and liver tissues, high flax levels resulted in lowest levels of LC n-3 PUFA, increased MUFA, and depressed n-6 FA metabolism. High levels of flax did not result in significant differences in FA profiles of adipose tissue, possibly due to a saturation effect for incorporation of PUFA. There was no difference in FA composition in breast and thigh tissues between treatments. There was trend for reduced DM content of excreta with enzyme treatment, but not for Flax

15+E. It may require more time for the enzymes to act on soluble NSP at the higher flax level. High flax and enzyme addition led to a significant increase in surface area (VH, VW, CD) of jejunal villi in the starter period.

Increasing n-3 FA in broiler diets (high flax level, enzyme supplementation) can result in increased n-3 FA, particularly LC n-3 FA, in all tissue relative to corn-soybean meal controls. The two feeding trials presented here provide clear evidence that broilers can convert ALA to LC metabolites. We do not observe a depression of growth performance from diets with more ALA. There is evidence that enzyme supplementation and high levels of ALA produce large changes in gut morphology, increasing jejunum villi surface area that drive the increased growth in the grower period and the observed FA profiles in the tissues. These studies confirm that the use of carbohydrase enzymes can result in degradation of NSP in whole flax seed and increase availability of ALA and other nutrients to broiler birds without depressing growth performance. The use of whole flax seed plus carbohydrase enzymes can also increase LC n-3 FA in meat tissues.

Figure 21. Example of sticky excreta observed in birds fed diets with whole flax seed.

9 Conclusions

9.1 Cost of Enriching Poultry Meat in n-3 FA

Is it economically feasible to enrich poultry meat products using whole flax seed and a carbohydrase enzyme supplement? The birds in Experiment 2 consumed between 80 to 100 g of feed per day per bird. For a growth period of 42 days, they consumed between 330-420 g/bird of whole flax seed at the 10% level and between 500 and 630 g/bird of whole flax seed at the 15% level. Although cost will depend on factors such as geographic location, an average cost of a 50-lb bag of feed-grade whole flax seed is around $35. Therefore, the total cost of whole flax seed per bird for the complete 42-day growth period will range between $0.52-0.65 for 10% flax level and between $0.78-0.97 for 15% flax level. These costs will be partially offset by a decrease in the amount of corn and soybean meal needed when whole flax seed is added to the diet. In summary, for less than $1/bird, between 10-15% whole flax seed can be added to broiler diets as a source of n-3 FA for enrichment of meat tissue.

The enzyme mixture costs approximately $1/100 g. The enzyme was applied at 0.05% of the diet. The total enzyme consumed per bird for a 42-day growth period is between 1.7 and 2.1 g. The total cost of enzyme mixture per bird would range from $0.017 to $0.021.

If we assume that 1 kg of a 2 kg bird is recoverable, that is, consumable meat tissue, then the additional cost per pound of meat for flax and enzyme would be $0.50 or less.

There should be no additional costs for feed and carcass handling. Whole flax seed and the enzyme, a loose powder, can be mixed directly into the diets without additional handling. The carcasses of broilers fed this diet can be processed in the same manner as other broilers.

The question of whether consumers would purchase enriched broiler meat products that might cost $0.50 more per pound depends on advertising and education. Western consumers are becoming more aware of the benefits of increasing n-3 FA in their diets. Eggs enriched in n-3 FA are now a common sight on grocery store shelves; typical retail prices for enriched eggs are more than $3 higher per dozen than regular eggs. It is likely that the consumer would accept a slightly higher price for a healthier product.

9.2 Creating an Enriched Consumer Product

Average daily consumption of n-3 FA in the US is 1.6 g/d, and EPA and DHA comprise a small fraction of this (100-200 mg/d) (Arab-Tehrany et al., 2012). The WHO recommends that Western consumers increase their daily intake of EPA and DHA to 300-500 mg/d. A reasonable and cost-effective source for this additional dietary LC n-3 PUFA is enriched poultry meat products.

The definition of an n-3 FA-enriched product varies with country. In Canada, poultry meat can be labeled as enriched if it delivers 300 mg total n-3 FA per 100 g serving. For the EU, enriched poultry meat can deliver 300 mg of ALA per 100 g serving or 40 mg of LC n-3 PUFA per 100 g serving. We were

not able to determine USDA specifications for labeling poultry meat as enriched.

Therefore, to conclude this study, we pose two questions: did we increase the n-3 FA in the consumable meat tissues to a level that would meet standards for labeling as “enriched”, and how many mg of n-3 FA would a typical thigh or breast from our experiments deliver to the consumer.

Using TL and FA values from the Control and Flax+E treatments of Experiment 1, the mg of FA per 100 g of skinless thigh and skinless breast meat are shown in Table 35. We will assume a recommended daily intake target of 300 mg of EPA + DHA per day, and an enrichment target of 40 mg of EPA + DHA or 300 mg of total n-3 FA. Thigh meat from chickens fed 15% whole flax seed plus 0.05% carbohydrase enzyme mixture would deliver 34 mg of EPA + DHA per 100 g serving, 11% of the recommended daily intake and 85% of the level required to be labeled enriched. Breast meat would deliver 21.5 mg of EPA and DHA per 100 g serving, 7% of the recommended daily intake and 53% of the enrichment target. Total n-3 FA for breast and thigh from the Flax+E treatment are 77.4 and 145.7, respectively. Although the Flax+E meat tissues from Experiment 1 would make a contribution to daily intake of EPA+DHA, the amounts of total n-3 FA and LC n-3 PUFA are not high enough to classify the breast and thigh meat as enriched.

The small meat tissue samples that we removed for total lipid extracts (approximately 2 g) were from sections of muscle that were trimmed of visible

fat. As a result, the very low TL values that we measured for our samples may not be representative of the poultry meat product that a consumer would purchase in the market. Ideally, a more accurate TL measurement could have been obtained by grinding entire thigh and breast samples with and without skin and using 2 g samples of that for lipid extraction. In lieu of that, we can use the USDA National Nutrient Database (http://ndb.nal.usda.gov/ndb/) to estimate the amount of TL in raw poultry meat cuts with and without skin that are typically available to the consumer. A 100 g serving of raw thigh meat without skin typically has 4% TL, while the same cut with skin has 15% TL. A 100 g serving of raw breast meat without skin typically has 2.6% TL; with skin, TL increase to 9%.

We recalculated the mg of FA in meat tissue using the USDA values for TL with results shown in Tables 36 (breast) and 37 (thigh). With adjusted TL values, skinless breast delivers 203 mg of total n-3 FA per 100 g serving (68% of the enrichment target). However, the same meat cut delivers 56.5 mg of EPA +DHA, 18% of the recommended daily intake target and 141% of the level required for enrichment. The skinless breast would thus qualify for enriched labeling in the EU. With the adjusted TL values, skinless thigh meat would deliver 325.6 mg of total n-3 FA and 48.9 mg of EPA + DHA (25% of recommended daily intake) and would also qualify for enriched labeling in Canada and the EU.

Tables 36 and 37 also show the mg of FA in breast and thigh meat with skin. Poultry skin is a form of adipose tissue. Based on our experimental results, when broilers are fed diets high in ALA, LA is preferentially stored in adipose tissue while ALA is redirected to β-oxidation and LC FA synthesis. LC PUFA are preferentially stored in PL of organelle and cell membranes and are present in very low amounts in adipose tissue. Therefore, the addition of skin to the meat servings adds almost no LC n-3 PUFA and small amounts of ALA. In fact, 100 g of breast meat with skin delivers less LC n-3 PUFA than 100 g of skinless breast meat. While processed meat cuts are commonly packaged without skin, consumers who purchase whole broiler birds are more likely to consume the thigh and breast cuts with skin. Consumers who eat a Flax+E breast with skin will still get 16% of their recommended daily intake of EPA+DHA, and 22% if they eat a thigh with skin.

In Table 38, we show mg of n-3 FA present in skinless thigh tissue from the study conducted by Jia et al. (2010). In Table 39, we show mg of n-3 FA present in skinless breast tissue from the study conducted by Zuidhof et al. (2009) and Betti et al. (2009a). The experimental diet in these studies included ground whole flax seed; neither included a carbohydrase enzyme supplement. Jia and co-workers measured 1.4% TL in the skinless thigh from their flax treatment. The Zuidhof/Betti team did not report TL for meat tissues in their papers. Without the addition of carbohydrase enzymes, flax diets resulted in greatly increased accumulation of ALA and lower accumulations of

LC n-3 PUFA in meat tissues. In the study from the Jia team, 100 g of skinless thigh meat contained 156.8 mg of ALA (Table 38). In comparison, the same amount of skinless thigh meat from our study contained only 83.4 mg of ALA (Table 35). In the study from the Zuidhof/Betti team, 100 g of skinless breast contained as much as 434.9 mg of ALA (Table 39). In comparison, the same amount of skinless breast meat from our study contained only 33.8 mg of ALA (Table 35).

This comparison highlights the efficacy of the carbohydrase enzyme mixture when used with whole flax seed. Based on our experimental results, enzymes degrade insoluble NSP in flax seed cell walls and increase the amount of ALA that is available. The increase in n-3 FA suppresses n-6 FA metabolism and enhances n-3 FA metabolism, redirecting ALA to other metabolic fates. There is less ALA, more LA, and more LC n-3 PUFA in meat tissues as a result. When flax seed is used without enzyme supplements, an increase in total n-3 FA and LC n-3 PUFA can still be obtained in meat tissues but levels of LC n-3 PUFA do not reach desired levels of enrichment (40 mg/100 g tissue).

Table 35. n-3 FA in skinless thigh meat and skinless breast meat from the Experiment 1. Table shows mg of FA in 100 g of skinless thigh and 100 g of skinless breast. Experimental Flax+E treatment was 15% whole flax seed, 0.05% carbohydrase enzyme mixture. Control was standard corn-soybean meal diet. The diets were fed for 35 days.

FA	Diet		% Desired Enrichment
	Control	Flax+E [1]	
mg FA / 100 g skinless thigh			
LA (18:2 n-6)	654.6	347.4	
ALA (18:3 n-3)	22.9	83.4	
EPA (20:5 n-3)	0.0	12.2	
DPA (22:5 n-3)	5.9	28.3	
DHA (22:6 n-3)	4.9	21.8	
EPA + DHA	4.9	34.0 (11%)	85
Total n-3 FA	33.7	145.7	49
TL (g/100 g tissue)	2.5	1.8	
mg FA / 100 g skinless breast			
LA (18:2 n-6)	294.8	176.8	
ALA (18:3 n-3)	7.6	33.8	
EPA (20:5 n-3)	0.0	4.6	
DPA (22:5 n-3)	0.0	22.3	
DHA (22:6 n-3)	2.2	16.9	
EPA + DHA	2.2	21.5 (7%)	53
Total n-3 FA	9.6	77.4	26
TL (g/100 g tissue)	1.2	0.99	

[1] Values in parentheses are percent of a recommended daily intake of 300 mg/d of EPA+DHA. Values in far right column are percent of the desired level of enrichment, 300 mg total n-3 FA or 40 mg LC n-3 PUFA.

Table 36. Calculated n-3 FA in breast meat from Experiment 1 using TL values from the USDA National Nutrition Database. Table shows mg of FA in 100 g of breast with and without skin. Experimental Flax+E treatment was 15% whole flax seed, 0.05% carbohydrase enzyme mixture. Control was standard corn-soybean meal diet. The diets were fed for 35 days.

FA	Diet		% Desired Enrichment
	Control	Flax+E [1]	
mg FA / 100 g skinless breast			
LA (18:2 n-6)	628.2	464.4	
ALA (18:3 n-3)	16.1	88.7	
EPA (20:5 n-3)	0.0	12.0	
DPA (22:5 n-3)	0.0	58.5	
DHA (22:6 n-3)	4.7	44.5	
EPA + DHA	4.7	56.5 (18%)	141
Total n-3 FA	20.5	203.3	68
USDA TL (g/100 g tissue)	2.6	2.6	
mg FA / 100 g breast with skin			
LA (18:2 n-6)	840.5	571.5	
ALA (18:3 n-3)	25.7	158.0	
EPA (20:5 n-3)	0.0	10.8	
DPA (22:5 n-3)	0.0	50.6	
DHA (22:6 n-3)	4.0	38.1	
EPA + DHA	4.0	48.9 (16%)	122
Total n-3 FA	29.5	259.1	86
USDA TL (g/100 g tissue)	9	9	

[1] Values in parentheses are percent of a recommended daily intake of 300 mg/d of EPA+DHA. Values in far right column are percent of the desired level of enrichment, 300 mg total n-3 FA or 40 mg LC n-3 PUFA.

Table 37. Calculated n-3 FA in thigh meat from Experiment 1 using TL values from the USDA National Nutrition Database. Table shows mg of FA in 100 g of thigh with and without skin. Experimental Flax+E treatment was 15% whole flax seed, 0.05% carbohydrase enzyme mixture. Control was standard corn-soybean meal diet. The diets were fed for 35 days.

FA	Diet		% Desired Enrichment
	Control	Flax+E [1]	
mg FA / 100 g skinless thigh			
LA (18:2 n-6)	1064.4	776.4	
ALA (18:3 n-3)	37.2	186.4	
EPA (20:5 n-3)	0.0	27.2	
DPA (22:5 n-3)	9.6	63.2	
DHA (22:6 n-3)	8.0	48.8	
EPA + DHA	8.0	76.0 (25%)	190
Total n-3 FA	54.8	325.6	108
USDA TL (g/100 g tissue)	4	4	
mg FA / 100 g thigh with skin			
LA (18:2 n-6)	1431.6	963.9	
ALA (18:3 n-3)	52.3	300.5	
EPA (20:5 n-3)	0.0	24.3	
DPA (22:5 n-3)	8.2	55.2	
DHA (22:6 n-3)	6.8	42.0	
EPA + DHA	6.8	66.3 (22%)	166
Total n-3 FA	67.2	425.1	142
USDA TL (g/100 g tissue)	15	15	

[1] Values in parentheses are percent of a recommended daily intake of 300 mg/d of EPA+DHA. Values in far right column are percent of the desired level of enrichment, 300 mg total n-3 FA or 40 mg LC n-3 PUFA.

Table 38. n-3 FA in skinless thigh from a feeding trial conducted by Jia et al. (2010). Table shows mg of FA in 100 g of skinless thigh. Experimental treatment was 12% ground whole flax seed. The control was comprised of wheat-barley-soybean meal. Diets were fed for 36 days.

FA	Diet		% Desired Enrichment
	Control	Flax [1]	
mg FA / 100 g skinless thigh			
LA (18:2 n-6)	329.0	251.9	
ALA (18:3 n-3)	56.1	156.8	
EPA (20:5 n-3)	4.2	13.2	
DHA (22:6 n-3)	5.6	10.4	
EPA + DHA	9.8	23.6 (8%)	59
Total n-3 FA	76.5	205.6	68
TFA (g/100 g tissue)	1.5	1.4	

[1] Values in parentheses are percent of a recommended daily intake of 300 mg/d of EPA+DHA. Values in far right column are percent of the desired level of enrichment, 300 mg total n-3 FA or 40 mg LC n-3 PUFA.

Table 39. n-3 FA in skinless breast from feeding trial conducted by Zuidhof et al. (2009) and Betti et al. (2009a). Table shows mg of FA in 100 g of skinless breast. Experimental treatment was 10% or 17% ground whole flax seed fed for 35 days.

mg FA / 100 g skinless breast	
ALA (18:3 n-3)	356.9-434.9
EPA (20:5 n-3)	12.1-14.1
DPA	22.4-25.9
DHA (22:6 n-3)	9.9-12.2
EPA + DHA	22.0-26.3
Total n-3 FA	401.4-487.2

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