DIETARY FACTORS AFFECTING TISSUE PROFILES OF LONG CHAIN POLYUNSATURATED FATTY ACIDS IN CATTLE

by

CHRISTIAN A. ALVARADO-GILIS

B.S., Universidad Austral de Chile, 2004
M.S., Universidad Austral de Chile, 2006

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Animal Sciences and Industry
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2014

Abstract

The main goal of this dissertation was to evaluate different methods to protect polyunsaturated fatty acids (PUFA) against biohydrogenation by ruminal microorganisms. The first chapter is a review of literature pertaining to fat and fatty acid metabolism by ruminants and why these fats are relevant in human nutrition. The second chapter discusses effects of supplementing high concentrations of dietary copper to feedlot cattle to assess impact on PUFA profiles in tissues. Two levels of copper (10 or 100 mg/kg) were supplemented to diets with or without flaxseed during the finishing period for beef heifers. Added copper did not affect performance ($P > 0.15$). Final body weights were similar for cattle fed with or without flaxseed ($P > 0.05$), but cattle fed diets with flaxseed consumed less feed ($P < 0.05$), and therefore were more efficient ($P < 0.01$). Carcass traits were unaffected by treatment. Feeding elevated levels of copper did not appreciably alter proportions of PUFA in plasma, but plasma concentrations of omega-3 fatty acids were greater for heifers fed flaxseed ($P < 0.05$). Chapter 3 describes the evaluation of 3 novel methods to protect PUFA from microbial biohydrogenation activity within the rumen, including a) coextrusion of flaxseed with molasses; b) mixing with soybean meal followed by induction of a non-enzymatic browning reaction; and c) encapsulation of ground flaxseed within a matrix consisting of dolomitic lime hydrate (L-Flaxseed). The resulting products were evaluated using *in vitro* methods to estimate resistance to biohydrogenation or in 12- to 14-d feeding studies in which plasma concentrations of α-linolenic acid (ALA) were measured. Our processing strategies a) and b) did not improve efficiency of omega-3 fatty acid utilization ($P > 0.1$). The *in situ* study of L-flaxseed revealed a 2-fold increase in resistance of ALA to ruminal biohydrogenation, and the concentration in plasma after 14 d on feed was more than 4 times that observed in cattle fed ground flaxseed, suggesting the dolomitic lime hydrate

was effective as a protective matrix. Chapter 4 evaluated performance, carcass traits, and meat quality of finishing beef heifers in response to feeding diets containing L-Flaxseed. Animals were blocked by weight, randomly assigned to individual pens, and pens to 6 dietary treatments: Control (high concentrate finishing diet), ground flaxseed fed at 3 or 6% of diet DM, L-Flaxseed fed at 2, 4, or 6%. Concentration of ALA in meat increased linearly in response to the level of flaxseed fed ($P < 0.05$); Moreover, transfer of dietary ALA to tissues increased by 47% when flaxseed was encapsulated within the dolomitic lime matrix. Cattle that were fed diets with 4 or 6% L-Flaxseed consumed less feed than other treatments ($P < 0.05$), which adversely affected feedlot performance and carcass traits.

DIETARY FACTORS AFFECTING TISSUE PROFILES OF LONG CHAIN POLYUNSATURATED FATTY ACIDS IN CATTLE

by

CHRISTIAN A. ALVARADO-GILIS

B.S., Universidad Austral de Chile, 2004
M.S., Universidad Austral de Chile, 2006

A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Animal Sciences and Industry
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2014

Approved by:

Major Professor
Dr. J.S. Drouillard

Copyright

Abstract

The main goal of this dissertation was to evaluate different methods to protect polyunsaturated fatty acids (PUFA) against biohydrogenation by ruminal microorganisms. The first chapter is a review of literature pertaining to fat and fatty acid metabolism by ruminants and why these fats are relevant in human nutrition. The second chapter discusses effects of supplementing high concentrations of dietary copper to feedlot cattle to assess impact on PUFA profiles in tissues. Two levels of copper (10 or 100 mg/kg) were supplemented to diets with or without flaxseed during the finishing period for beef heifers. Added copper did not affect performance ($P > 0.15$). Final body weights were similar for cattle fed with or without flaxseed ($P > 0.05$), but cattle fed diets with flaxseed consumed less feed ($P < 0.05$), and therefore were more efficient ($P < 0.01$). Carcass traits were unaffected by treatment. Feeding elevated levels of copper did not appreciably alter proportions of PUFA in plasma, but plasma concentrations of omega-3 fatty acids were greater for heifers fed flaxseed ($P < 0.05$). Chapter 3 describes the evaluation of 3 novel methods to protect PUFA from microbial biohydrogenation activity within the rumen, including a) coextrusion of flaxseed with molasses; b) mixing with soybean meal followed by induction of a non-enzymatic browning reaction; and c) encapsulation of ground flaxseed within a matrix consisting of dolomitic lime hydrate (L-Flaxseed). The resulting products were evaluated using *in vitro* methods to estimate resistance to biohydrogenation or in 12- to 14-d feeding studies in which plasma concentrations of α-linolenic acid (ALA) were measured. Our processing strategies a) and b) did not improve efficiency of omega-3 fatty acid utilization ($P > 0.1$). The *in situ* study of L-flaxseed revealed a 2-fold increase in resistance of ALA to ruminal biohydrogenation, and the concentration in plasma after 14 d on feed was more than 4 times that observed in cattle fed ground flaxseed, suggesting the dolomitic lime hydrate

was effective as a protective matrix. Chapter 4 evaluated performance, carcass traits, and meat quality of finishing beef heifers in response to feeding diets containing L-Flaxseed. Animals were blocked by weight, randomly assigned to individual pens, and pens to 6 dietary treatments: Control (high concentrate finishing diet), ground flaxseed fed at 3 or 6% of diet DM, L-Flaxseed fed at 2, 4, or 6%. Concentration of ALA in meat increased linearly in response to the level of flaxseed fed ($P < 0.05$); Moreover, transfer of dietary ALA to tissues increased by 47% when flaxseed was encapsulated within the dolomitic lime matrix. Cattle that were fed diets with 4 or 6% L-Flaxseed consumed less feed than other treatments ($P < 0.05$), which adversely affected feedlot performance and carcass traits.

Table of Contents

List of Figures

List of Tables

Chapter 1 - Literature review

INTRODUCTION

Animals provide a range of foods for human consumption, including meat and milk products. Animal-derived foods historically have been viewed as excellent sources of protein (essential amino acids) and highly bioavailable minerals and vitamins. In recent years, total fat consumption, and more specifically the consumption of certain fatty acids, has been recognized as having an important role in human nutrition. Fats are concentrated sources of energy that can be useful under conditions of high energy demand, but also can have deleterious consequences when consumed in excess due to their association with obesity, cardiovascular disease, and other chronic health issues. Linoleic and α-linolenic acid (LA and ALA) are two fatty acids that are regarded as essential components of the human diet, as they cannot be synthesized *de novo.* Furthermore, in addition to their energy contribution or structural function some fatty acids (i.e., conjugated linoleic acid and omega-3 fatty acids) are recognized for their important roles as bioactive molecules.

The objectives of this literature review are to describe the fatty acid metabolism in the rumen, address the importance of fatty acids in human nutrition, and provide some alternatives to improve meat quality in terms of its fatty acid composition.

Development of adipose tissue

Adipose tissue is found in all mammals and can be classified into two types: white and brown adipose tissue. The pathway for brown adipose tissue production (mitochondrial protein conductance) allows new born mammals to generate heat without the production of ATP (Lawrence and Fowler, 2002), which is regulated by uncoupling protein 1. White adipose tissue

is considered a protective and long term energy storage depot. Energy intake in excess of requirements results in production of fatty acids, which are stored as triglycerides in adipose tissue. As mammals age, fat tissue develops, starting with visceral fat production followed by subcutaneous fat, with later development of inter- and intramuscular fat (Hausman et al., 2009). The same priority order for nutrient partitioning is observed (Du and Dodson, 2011). Intramuscular fat is deposited within muscular tissue; it is a key determinant of meat quality and aids in palatability, juiciness, and tenderness (Hocquette et al., 2010). In addition, the amount of intramuscular fat defines the marbling score of meat and resulting USDA quality grade.

Adipocytes are derived from undifferentiated cells as they develop; the process involves hyperplasia and hypertrophy, which collectively are known as adipogenesis. In cattle, adipogenesis starts early in the fetal stage where a high proportion of this differentiation occurs. As the animal ages, capacity to produce new adipocytes diminishes. In general, postnatal fat deposition is principally due to hypertrophy (Annison, 1993).

Genetic regulation of fatty acid synthesis in beef

At a genetic level, studies have proposed that peroxisome proliferator-activated receptor (PPAR) γ and CCAAT-enhancer-binding proteins (C/EBP) have important roles in control of adipogenesis, and their expressions direct this process from multipotent cells. PPARγ is a hormone receptor located in the nuclear membrane, and four isoforms of this receptor have been identified. The PPARγ2 is directly related to adipogenesis by forming a heterodimer with retinoic X receptor (RXR), which then binds a specific regulatory region of target genes to initiate transcription (Hausman et al., 2009). The target genes are those involved in transport and metabolism of fatty acids as: lipoprotein lipase (LPL, EC 3.1.1.34), fatty acid transport protein, oxidized LDL receptor, phosphoenolpyruvate carboxykinase (PEPCK, EC 4.1.1.32), GLUT4 and

others. C/EBP is another important transcription factor that regulates adipogenesis; its β and δ isoforms are expressed early and temporally in the adipogenesis process, while the α isoform is expressed later. It has been observed that the C/EBPα induces adipogenesis in fibroblasts and aids in differentiation of mature adipocytes (Brun and Spielgelman, 1997), but it cannot operate efficiently without PPARγ. An additional important regulator of adipogenesis is a sterol responsive element-binding protein-1c (SREBP-1c), which increases expression of PPARα and its transcriptional activity. This interaction enhances expression of genes that code for proteins such as fatty acid synthase (FAS, EC2.3.1.85) and fatty acid binding protein, leading to growth of adipocytes and accumulation of fatty acids.

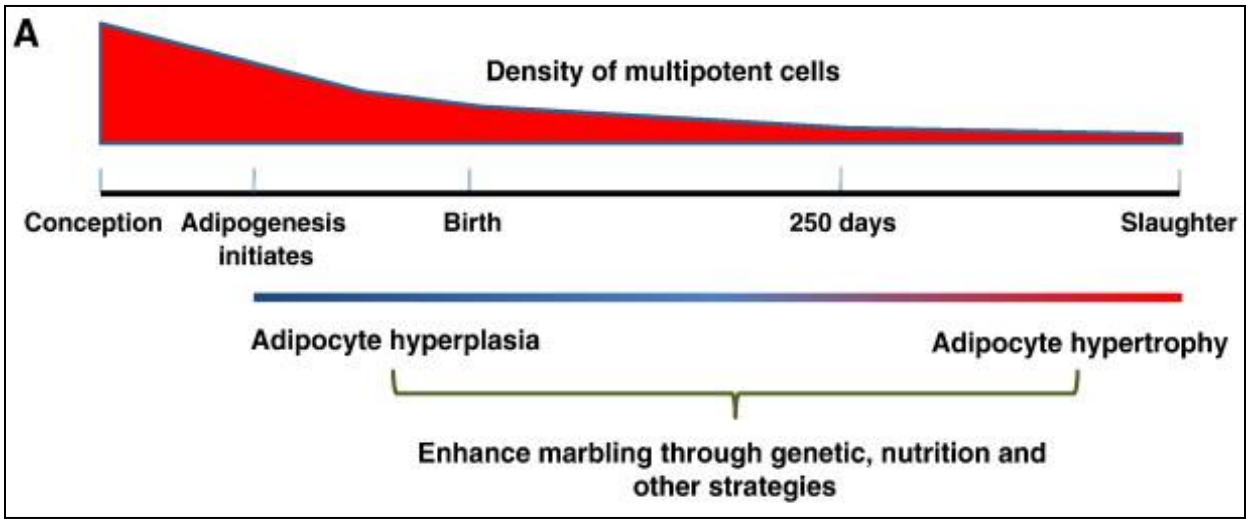
Manipulation of marbling and fatty acid profile in cattle

The density of multipotent cells decreases as animals age, making it more effective to improve marbling by manipulation of the animal's metabolism in early life stages (i.e., as a fetus) through pre-weaning. Efforts to affect marbling after 250 days of age has minimal effect on hyperplasia (Du and Dodson, 2011; Figure 1.1).

In animals more than 250 days of age most fat deposition is due to increases in adipocyte size by accumulation of long chain fatty acid (LCFA) as triglycerides. Sources of LCFA include direct absorption from the diet and *de novo* synthesis. The main precursor for *de novo* synthesis of LCFA is acetyl-CoA. In non-ruminants, acetate is produced from glucose via pyruvate inside mitochondria; the acetate is then transported to the cytosol. In ruminants, however, proteins that participate in this pathway (i.e., ATP citrate lyase and GLUT4) have low activity (Anisson, 1993) due to volatile fatty acids (VFA) being end product of rumen fermentation. Acetate is absorbed directly by the ruminal epithelium and then transported to adipose tissue and activated

in the cytosol. Acetate is used as a precursor for acetyl-CoA (Bergen and Mersmann, 2005) via the reaction catalyzed by the enzyme acetyl-CoA synthetase.

Figure 1.1: Development of intramuscular fat by hyperplasia and hypertrophy in early and late stages of life, respectively (Du and Dodson, 2011).



The second LCFA source comes from dietary fat. Ruminant diets frequently have low fat content (2 to 4%), and dietary fats predominantly consist of polyunsaturated fatty acids (PUFA) from triglycerides and phospholipids. Abundant fatty acids found in cattle diets are C18:1, C18:2 *n-6*, and C18:3 *n-3* (OA, LA, and ALA, respectively). Depending on the diet, these fatty acids may constitute 50 to 60% or more of total dietary fat (Harfoot and Hazlewood, 1997). The fatty acid profile of fat that reaches the small intestine often is very different, however, due to extensive biohydrogenation of polyunsaturated lipids by ruminal microorganisms.

Ruminal metabolism of dietary fat

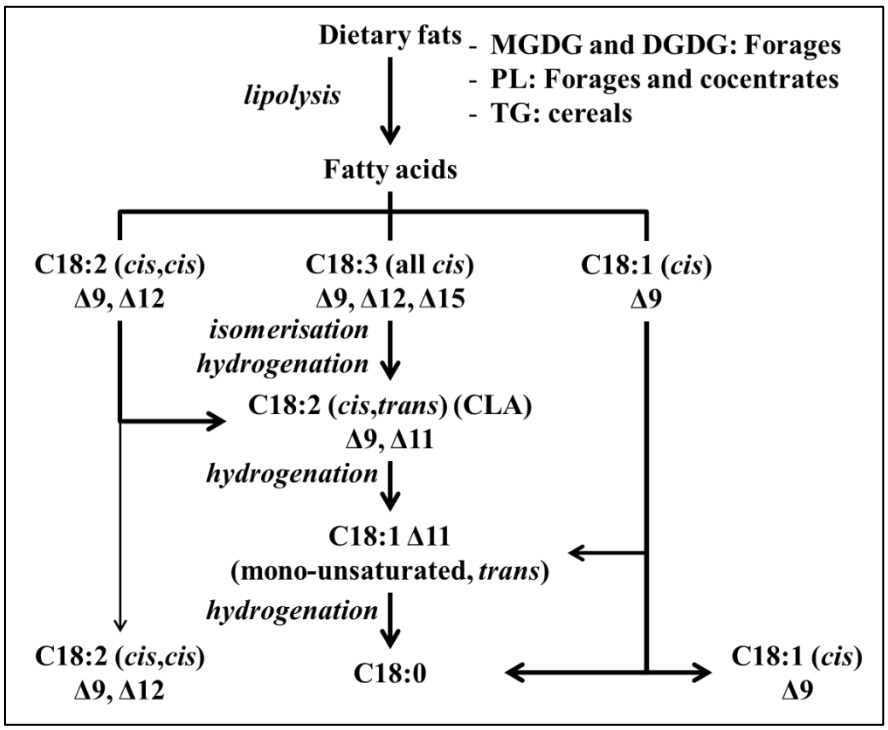
Dietary fats are hydrolyzed in the rumen by microbial lipases, and their constituents, LCFA and glycerol, are released (Jenkins, 1993). Glycerol is rapidly fermented to yield VFA.

Unsaturated fatty acids (UFA) may flow directly to the small intestine where they are available for absorption. Most, however, are saturated to varying degrees by ruminal microorganisms in a process known as biohydrogenation (Jenkins et al., 2008).

Polyunsaturated fatty acids are toxic to ruminal bacteria (Maczulak et al., 1981), and biohydrogenation is thus a protective mechanism to decrease their toxicity. Maia et al. (2007) reported that at PUFA concentrations of 50 μg/mL growth of cellulolytic bacteria was totally inhibited. Relative toxicity of PUFA was ranked as: C20:5 *n-3*>C22:6 *n-3*>C18:3 *n-3*>C18:2. Figure 1.2 shows the biohydrogenation of OA, LA, and ALA. The first step in this process is isomerization of the *cis*-12 double bond to *trans*-11, then the reduction by microbial reductase of the *cis*-9 double bond (Jenkins, 1993; Geay et al., 2001). About 90% of PUFA can be hydrogenated in the rumen and the free fatty acids that flow to the small intestine can be more than 75 to 80% saturated (Harfoot and Hazlewood, 1997). This process could be considered favorable overall, as increases in PUFA could adversely affect fiber degradation. Additionally, some desired intermediate compounds with trans-double bond configurations are produced (i.e., conjugated linoleic acid, CLA). The biohydrogenation process constitutes a hydrogen sink, effectively maintaining a healthy rumen environment (Jenkins, 1993; Harfoot and Hazlewood, 1997; Bauman et al., 2011).

Dietary fats that flow to the small intestine, principally nonesterified long-chain fatty acids (NEFA) created in the rumen by rumen microbes, along with microbial phospholipids (which are hydrolyzed in the small intestine), they are absorbed, re-esterified, and transported via the lymphatic system and plasma to adipose tissue using lipoproteins (chylomicrons and VLDL) as carriers (Bauchart, 1993).

Figure 1.2: Lipolysis and biohydrogenation of dietary fatty acids by rumen bacteria. MGDG: monoglycerides. DGDG: diglycerides. PL: phospholipids. TG: triglycerides (Geay et al., 2001).



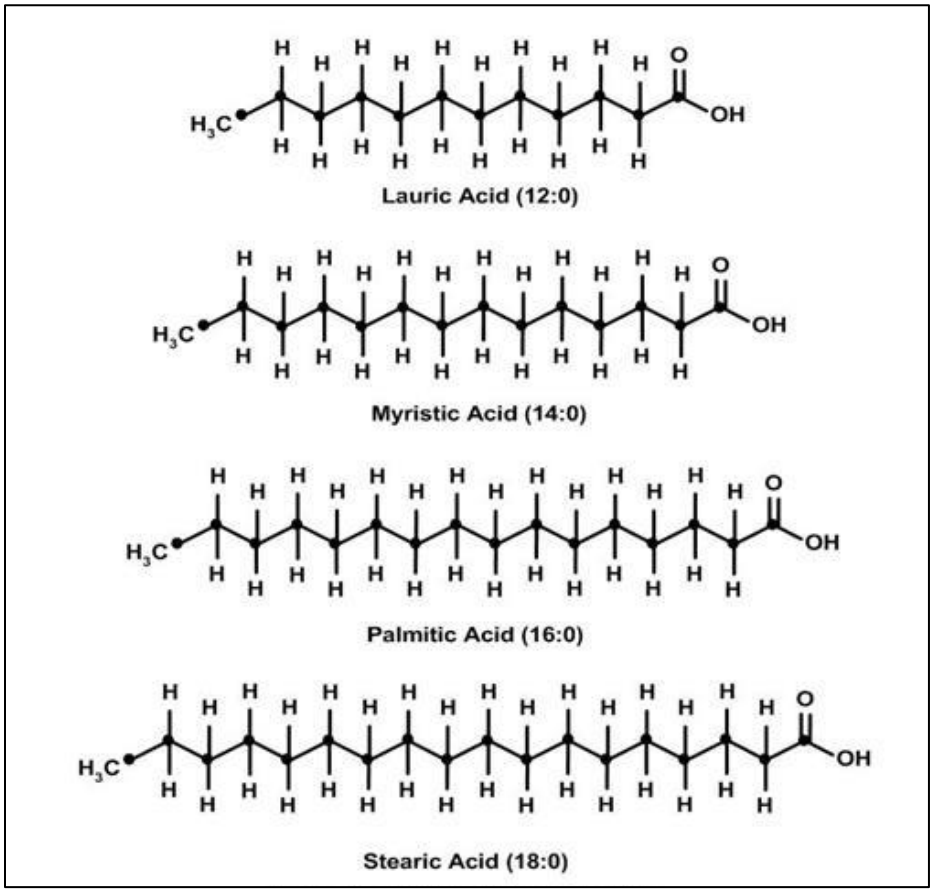
Importance of fat and fatty acids to human health

The fat content of meat is related to its organoleptic characteristics, and the absence of intramuscular fat (marbling) could result in poor acceptability by consumers. Though some amount of fat is deemed desirable, the World Health Organization (2003) has recommended limits on daily energy intake from fats, suggesting 15 to 30% of daily energy intake from fat and less than 10% from saturated fatty acids (SFA). Recommendations of the American Heart Association (2014) allow for somewhat higher total fat intake (25-35% of daily energy intake), but are more restrictive in SFA intake (7% of daily energy intake). The reasoning for this is the direct relationship that has been observed between high intake of fat (and SFA) and increased risks of cardiovascular diseases, obesity, and diabetes.

Saturated fats

The main saturated fats in beef are lauric, myristic, palmitic, and stearic acids (Figure 1.3). Talbot (2011) found a positive correlation between the total intake of SFA across European countries in 1998 and deaths for coronary heart disease during the same year. There is evidence, however, that the SFA have varying effects depending on the number of carbons. For example, palmitic acid (C16:0) has been correlated with an increase in total:HDL cholesterol, effectively decreasing the HDL (or "good") cholesterol as a proportion of total cholesterol. On the other hand, lauric acid (C12:0) has been shown to have relatively little effect, or possible even decreasing this ratio (Mensink et al., 2003).

Figure 1.3: Structures of more common saturated fatty acid (Blake, 2010).



Saturated fatty acids have been related to insulin sensitivity and type 2 diabetes. Several studies cited by Pedersen (2011) provide evidence that decreasing SFA intake improves insulin sensitivity and is likely to reduce risk of type 2 diabetes; however, some SFA (i.e., lauric and myristic) shown a positive correlation with insulin sensitivity.

Unsaturated fats

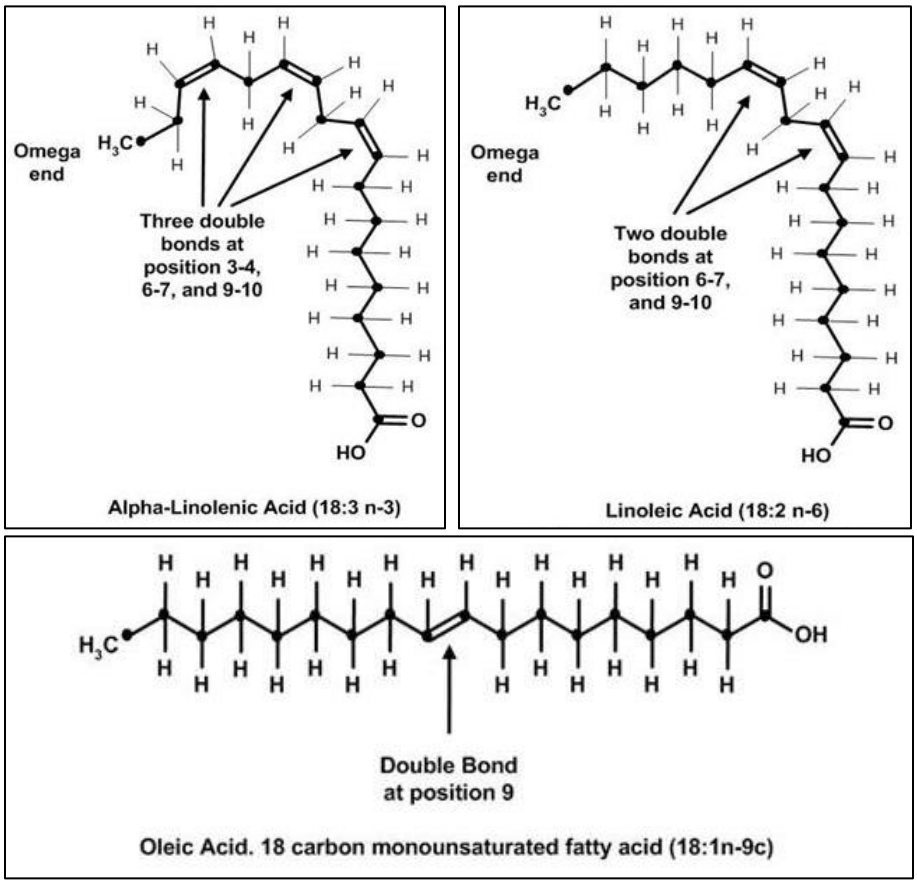
Some examples of UFA are presented in Figures 1.4 and 1.5, including those that have one (MUFA, Figure 1.4) or more (PUFA, Figures 1.4 and 1.5) double bonds in their structure. Unsaturated fatty acids, especially PUFA, in general have health effects opposite those of saturated fatty acids. Studies have shown that risk of cardiovascular disease decreases, insulin sensitivity is improved, and risk of type 2 diabetes is decreased when intake of PUFA are increased (Mensink et al., 2003; Pedersen, 2011; Talbot, 2011). Exceptions to this observation are *trans* fatty acids. The more common in beef is elaidic acid (*trans*-C18:1), which is found in artificially hydrogenated oils. Figure 1.5 shows *trans* fatty acids having a structure more similar to SFA, and their effects on human health are more similar to SFA compared to other PUFA. For example, Mensink et al. (2003) reported that *trans* fatty acids have the largest adverse effect (increasing) on total:HDL cholesterol ratio, even more than SFA.

Conjugated linoleic acid (CLA)

A special group of PUFA commonly found in milk and beef are CLA. They are intermediate products derived through ruminal biohydrogenation of C18:2 and C18:3 fatty acids. Fatty acids are conjugated when two double bonds are separated by 1 single bond (see Figure 1.6) with no methyl groups between the double bonds. Conjugated bonds are uncommon in

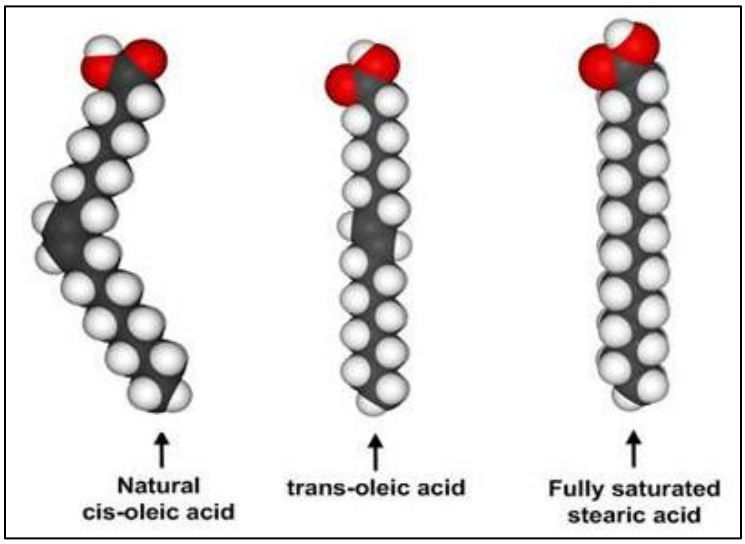
nature; far more common are the non-conjugated bonds, where 2 double bonds are separated by 2 or more single bonds with 1 or more methyl groups between.

Figure 1.4: The structure of oleic acid, linoleic acid, α-linolenic acid (Blake, 2010).



There are about 28 isomers of CLA, depending on position and configuration of the double bond. More abundant is the *cis*-9 *trans*-11 CLA (rumenic acid), which has been noted for its functional properties against cancer and atherosclerosis (Bauman et al., 2000). Another important isomer is *trans*-10, *cis*-12 CLA, which purportedly modifies fat accretion and thus has been promoted for its anti-obesity effect (Bauman et al., 2008).

Figure 1.5: The structure of *cis* and *trans* oleic acid, and the fully saturated stearic acid (Blake, 2010).

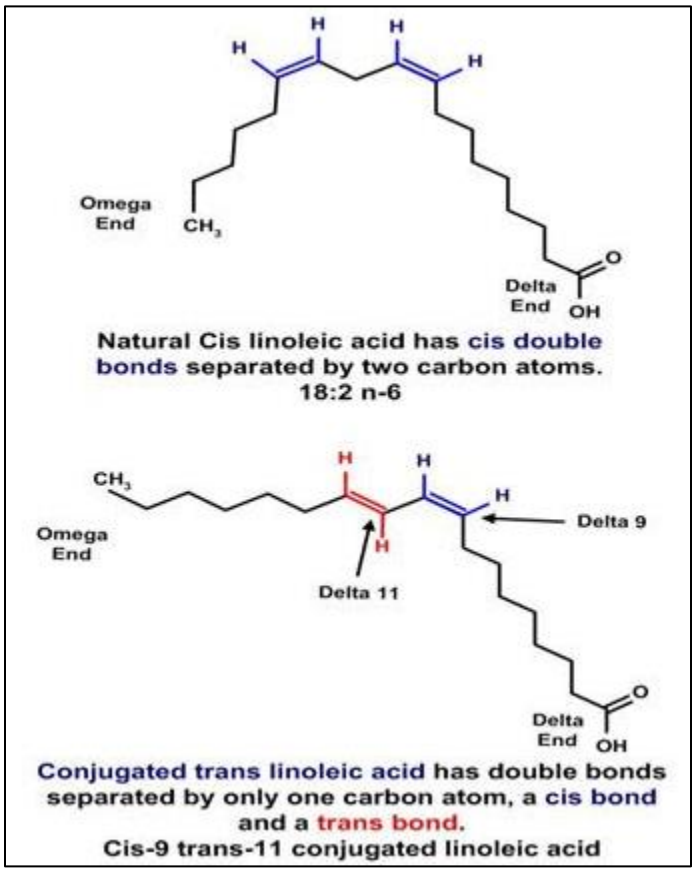


Omega-3 and omega-6 fatty acids

The omega-3 and omega-6 fatty acids are another subgroup of UFA, and α-linolenic acid (C18:3 *n-3*, ALA) and linoleic acid (C18:2 *n-6*, LA) presented in Figure 1.4 are fairly common in human diets. Both are essential fatty acids, meaning they must be incorporated in the human diet, as humans and other mammals lack enzymes needed to introduce double bonds further than carbon 9 and 10. For example, to synthesize ALA *de novo* from LA the enzyme Δ^{15} desaturase is needed, and only plants possess this enzyme. The terminology "omega" is used to describe the position of the first double bond related to the methyl end (also called omega end) of the acyl chain. For example, the omega-3 fatty acids (abbreviated *ω-3* or *n-3* FA) have the first double bond between carbon 3 and 4 relative to the methyl end (Figure 1.4). Other *n-3* and *n-6* fatty acids, listed in Table 1.1, are not essential because mammals have the enzymes needed to elongate ALA and LA to synthesize the longer chain products. Alpha linolenic acid and LA are important as precursors for synthesis of longer chain *n-3* PUFA, including C20:5 *n-3* (EPA) and C22:6 *n-3* (DHA). These fatty acids often are limited in human diets, especially if ALA is not

included in the diet, consumption of natural sources of EPA and DHA (fish and other marine products) is inadequate, or LA is consumed in excess (Palmquist, 2009).

Figure 1.6: Structure of a conjugated fatty acid (Blake, 2010).



The incorporation of EPA and DHA to cell membranes has a large modifying impact on membrane structure, altering fluidity and activity of receptors, transporters, ion channels, and signaling enzymes (Calder, 2013). The elongation of LA produces arachidonic acid (C20:4 *n-6*, AA) which is the main precursor for the synthesis of proinflammatory eicosanoids (prostaglandins, thromboxanes, and leukotrienes) and these are chemical messengers associated with inflammation and immunity (Calder, 2001). The enzyme Δ6 desaturase is common to the *n-3* pathway (to produce EPA and DHA) and *n-6* pathway (to produce AA), and it is considered a

rate-limiting step. Furthermore, there is evidence that affinity of this enzyme is higher for *n-3* than for *n-6* fatty acids, implying that an adequate (high) intake of ALA could decrease production of proinflammatory eicosanoids (Calder, 2001; Palmquist, 2009). Additionally, marine sources of EPA and DHA in the diet also decrease the production of proinflammatory eicosanoids because their precursor (AA) is partially replaced in the cell membrane and there is an inhibitor effect of EPA and DHA against AA metabolism (Calder, 2013).

Consumption of EPA and DHA has been associated with reduced risk of cardiovascular disease (Wilson, 2004) and there is evidence illustrating that intake of ALA has similar effects, albeit to a lesser magnitude compared to marine sources due to limited conversion of ALA to DHA (Calder, 2013). Medeiros et al. (2007) used a rat model to demonstrate that meat rich in ALA, obtained from cattle whose diets were supplemented with flaxseed during the finishing period, increased the concentration of DHA in the liver and heart when consumed by the rats.

Fatty acid composition of beef

The biohydrogenation of PUFA has a large impact on the fatty acid profile of meat. In general, bovine and ovine meats have greater proportions of SFA and MUFA fatty acids compared to meats from pigs or poultry. The SFA can account for as much as 50% of total fat in ruminant adipose tissue, and MUFA (mostly C18:1) can account for 40% or more of total fat (Geay et al., 2001; Wood et al., 2008; Table 1.1).

This composition could be affected by the nature of diet. For example, Rule et al. (2002) compared the fatty acid composition of muscle from bison and beef cattle under a range diet or feedlot finishing diet. The results illustrate a greater proportion of PUFA and *n-3* FA in range-fed animals, but the total fat content was lower than in meat from feedlot animals.

Table 1.1: Fatty acid composition of muscle from beef, lamb, and pork expressed as percentage of total fatty acid (Enser at al., 1996).

Fatty acid	Beef	Lamb	Pork
C12:0 lauric	0.08	0.31	0.12
C14:0 myristic	2.66	3.3	1.33
C16:0 palmitic	25.0	22.2	23.2
C16:1 *cis*	4.54	2.20	2.71
C18:0 stearic	13.4	18.1	12.2
C18:1 *trans*	2.75	4.67	–
C18:1 *n-9* oleic	36.1	32.5	32.8
C18:1 *n-7* vaccenic	2.33	1.45	3.99
C18:2 *n-6* linoleic	2.42	2.70	14.2
C18:3 *n-6* γ-linolenic	–	–	0.06
C18:3 *n-3* α-linolenic	0.7	1.37	0.95
C20:2 *n-6*	–	–	0.42
C20:3 *n-6*	0.21	0.05	0.34
C20:3 *n-3*	0.007	–	0.12
C20:4 *n-6* arachidonic	0.63	0.64	2.21
C20:4 *n-3*	0.08	–	0.009
C20:5 *n-3* EPA	0.28	0.45	0.31
C22:4 *n-6*	0.04	–	0.23
C22:5 *n-3*	0.45	0.52	0.62
C22:6 *n-3* DHA	0.05	0.15	0.39

Supplementation with lipid sources has been used as an approach to alter fatty acid composition of muscle. Common sources are plant oils, oil seeds, algae, fish, and other marine sources of oil, supplemented directly to diets of finishing cattle. Cooper et al. (2004) used linseed oil (high in 18:3 *n-3*), fish oil (high in 20:5 *n-3* and 22:6 *n-3*) and a mixture of fish oil and marine algae (high in 20:5 *n-3* and 22:6 *n-3*) as sources of PUFA in diets of finishing lambs, and all were effective for increasing *n-3* FA in meat. Meat from steers that were supplemented with 3% fish oil in diets for 70 days prior to harvest had increased *n-3* FA and decreased *n-3*:*n-6* ratio (Wistuba et al., 2007).

Flaxseed and flax oil as PUFA supplements

A frequently used source of PUFA in beef cattle diets are flaxseed or flaxseed oil. Flax (*Linum usitatissimum* L.) is an oilseed member of the genus Linum and family Linaceae that contains about 41% oil, 20% CP, and 20% NDF, and it is an important source of ALA (~50% total oil content; Maddock et al., 2006). Several studies have reported that use of flaxseed or flax oil as supplement in beef cattle increase the concentration of *n-3* fatty acid in meat without adversely affecting feedlot performance or meat quality (Mach et al., 2006; Farran et al., 2008; He et al., 2012). Mach et al. (2006) used two lipid sources (whole canola and flaxseed) at three levels of inclusion (5, 8, and 11% of diet DM) and reported a linear increase in *n-3* fatty acid concentrations within loins of Holstein bulls fed with flaxseed, whereas those that fed canola remained unchanged. Performance and carcass quality were unaffected in Mach's study; however, positive effects have been described with the addition of flaxseed (Drouillard et al., 2004; Maddock et al., 2006). Maddock et al. (2006), feeding whole flaxseed, rolled flaxseed, and ground flaxseed in the finishing diet of beef heifers reported greater ($P < 0.01$) ADG (1.53 vs. 1.35 kg), gain to feed ratio (134.7 vs. 117.5 g/kg), hot carcass weight (330.4 vs. 319.6 kg), marbling score (468 vs. 432), and *n-3* FA in meat (0.57 vs. 0.26 nmol/100 nmol of total fatty acid) compared to the control where no flaxseed was included in the diet.

Quinn et al. (2008) fed steers steam-flaked corn diets with no supplemental fat, flax oil supplement (4% of DM), and other derivatives of flax oil (lipase-treated flax oil and linseed soapstock). In this study the DMI was decreased with no effect on feedlot performance and the concentration of *n-3* FA in meat was increased ($P < 0.05$).

Kronberg et al. (2011) evaluated the effects of flaxseed in steers finished in a grazing system. They grazed growing forage and were supplemented with ground flaxseed (0.2% of

BW), ground corn plus soybean meal (0.28% of BW), or no supplement. A 62% greater content of ALA in meat was observed when flaxseed was included with no differences in carcass or organoleptic characteristics of meat (slight off-flavor detected in steaks). Furthermore, the rate of growth was 25% greater than the control. The author concluded that flaxseed can be a good energy source to increase the growth rate and the *n-3* FA content.

Protection of dietary PUFA against biohydrogenation

The use of unprotected flaxseed or other sources of PUFA as a feed additive to increase *n-3* in meat is inefficient due to the rumen biohydrogenation previously discussed. Scollan et al. (2001) used fistulated steers (rumen, proximal duodenum and terminal ileum) to evaluate the biohydrogenation of PUFA by rumen bacteria of different fat sources (palm oil, whole flaxseed, and fish oil). The biohydrogenation of C18:2 *n-6* and ALA were 89.8 and 92.1% respectively across treatments, and the seed coat of flaxseed was unable to protect it. In another study flaxseed oil was directly infused into the proximal duodenum of steers, the plasma concentration of ALA was 5.7 times greater than the control (no oil supplemented) and 2.2 times greater than the treatment that was supplemented with extruded flaxseed (Scislowski et al., 2005).

Several methods have been proposed to protect PUFA against biohydrogenation. In an *in vitro* study Sinclair et al. (2005) demonstrated that flaxseed pre-treated with sodium hydroxide, formic acid, or ammonium tetraformate and then with formaldehyde was effective in protecting PUFA against biohydrogenation compared to unprotected flax oil and to other protective methods (i.e., directly formaldehyde or xylose treated). A blend of flaxseed, casein, and soybean (70.8, 10, and 19.2%, respectively) treated with formaldehyde has been considered the most effective method, and was estimated to provide 80% protection to the fatty acid against biohydrogenation. Formaldehyde forms a complex with proteins that is resistant to bacterial

degradation, thus protecting the oil against hydrogenation. Later, in the abomasum, the low pH hydrolyzes this complex and PUFA are available for digestion and absorption. There is concern about use of formaldehyde in food systems, as it is a known carcinogenic agent (Scott and Ashes, 1993; Scollan et al., 2003; Dewhurst and Moloney, 2013).

The micronization of flaxseed was evaluated in an *in situ* study and compared to ground flaxseed (Mustafa et al., 2002). They utilized ruminal and duodenally cannulated steers where samples were incubated in plastic bags in order to determine ruminal, postruminal, and total tract digestibility. Conclusions of the authors were as follows: micronization is an effective method to protect flaxseed against ruminal degradation of DM and CP, but unfortunately PUFA were not evaluated. Gonthier et al. (2005) utilized a similar processing method for flaxseed, which was compared to ground flaxseed and extruded. The analysis of plasma and milk concentrations of PUFA in dairy cattle showed no positive effects (protection) when flaxseed was micronized.

Sterk et al. (2010) evaluated the effects of 9 processing methods for flaxseed in an *in vitro* fermentation study: flaxseed oil, ground flaxseed, formaldehyde treated ground flaxseed, sodium hydroxide/formaldehyde treated ground flaxseed, extruded whole flaxseed, extruded ground flaxseed, micronized ground flaxseed, lipid encapsulated flaxseed oil, and DHA addition to linseed oil. The more effective method to protect ALA against hydrogenation was the use of ground flaxseed treated with formaldehyde, similar to what was previously reported in other studies (Scott and Ashes, 1993; Dewhurst and Moloney, 2013). There was no additional protection of ALA when flaxseed was pretreated with sodium hydroxide and then with formaldehyde. Other methods used in this study were not able to decrease extent of *in vitro* biohydrogenation.

Kronberg et al. (2007) studied effects of tannin treated flaxseed with or without casein as an approach to reduce biohydrogenation of ALA *in vitro* and *in vivo*. The *in vitro* study showed a positive effect of tannins on protection of ALA, however, when these products were evaluated *in vivo* with steers fed high-forage or a high-concentrate diets, no effect on plasma concentrations of ALA were detected. The authors concluded the reason for this was low ruminal pH observed in feedlot cattle, which could decrease the capacity of tannins as a protective method.

A gel-based whey protein was developed at the University of California, Davis, and it has been used as a protective barrier of PUFA against biohydrogenation (Carrol et al., 2006; Heguy et al., 2006). A mix of soybean oil and flaxseed oil (1:1) was protected with two alternatives of this gel (whey protein concentrate gel and whey protein isolate gel) and they were compared to unprotected and calcium salts of the same oil mix (Heguy et al., 2006). Dairy cows where fed with a total mixed ratio and supplemented with equal amounts of oil. Results revealed no protective effect of calcium salts of the oils or by treatment with whey protein concentrate gel, but there was a protective effect ($P < 0.01$) when the whey protein isolate gel was used, which was reflected in higher plasma and milk concentration of ALA.

LITERATURE CITED

American Heart Association. 2014. Fats and Oils: AHA Recommendation. http://www.heart.org/HEARTORG/GettingHealthy/FatsAndOils/Fats101/Fats-and-Oils-AHA-Recommendation_UCM_316375_Article.jsp. (Accessed 20 March 2014.)

Annison, E. F. 1993. Fat deposition in ruminants. In: D. J. Farrel, editor. Recent Advances in Animal Nutrition in Australia. The University of New England Publishing Unit, Armidale. p. 51–60.

Bauchart, D. 1993. Lipid absorption and transport in ruminants. J. Dairy Sci. 76(12):3864–3881.

Bauman, D. E., K. J. Harvatine, and A. L. Lock. 2011. Nutrigenomics, rumen-derived bioactive fatty acids, and the regulation of milk fat synthesis. Annu. Rev. Nutr. 31(Aug 21):299–319.

Bauman, D. E., J. W. Perfield II, K. J. Harvatine, and L. H. Baumgard. 2008. Regulation of fat synthesis by conjugated linoleic acid: lactation and the ruminant model. J. Nutr. 138(2):403–409.

Bauman, D. E., L. H. Baumgard, B. A. Corl, and J. M. Griinari. 2000. Biosynthesis of conjugated linoleic acid in ruminants. J. Anim. Sci. 77(E-Suppl):1–15.

Bergen, W.G., and H. J. Mersmann. 2005. Comparative aspects of lipid metabolism: impact on contemporary research and use of animal models. J. Nutr. 135(11):2499–2502.

Blake, S. 2010. Understanding Dietary Fats and Oils: A Scientific Guide to their Health Effects. http://www.naturalhealthwizards.com/Understanding.htm. (Accessed 20 March 2014.)

Brun, R.P., and B. M. Spiegelman. 1997. PPARγ and the molecular control of adipogenesis. J. Endocrinol. 155(2):217–218.

Carroll, S. M., E. J. DePeters, and M. Rosenberg. 2006. Efficacy of a novel whey protein gel complex to increase the unsaturated fatty acid composition of bovine milk fat. J. Dairy Sci. 89(2):640–650.

Calder, P. C. 2013. Nutritional benefits of omega-3 fatty acids. In: C. Jacobsen, N. S. Nielsen, A. F. Horn, and A.-D. M. Sørensen, editors. Food Enrichment with Omega-3 Fatty Acids. Woodhead. Cambridge, UK. p. 3–26.

Calder, P. C. 2001. Polyunsaturated fatty acids, inflammation, and immunity. Lipids 36(9):1007–1024.

Cooper, S. L., L. A. Sinclair, R. G. Wilkinson, K. G. Hallett, M. Enser, and J. D. Wood. 2004. Manipulation of the *n-3* polyunsaturated fatty acid content of muscle and adipose tissue in lambs. J. Anim. Sci. 82(5):1461–1470.

Dewhurst, R. J., and A. P. Moloney. 2013. Modification of animal diets for the enrichment of dairy and meat products with omega-3 fatty acids. In: C. Jacobsen, N. S. Nielsen, A. F. Horn, and A.-D. M. Sørensen, editors. Food Enrichment with Omega-3 Fatty Acids. Woodhead. Cambridge, UK. p. 257–287.

Drouillard, J. S., M. A. Seyfert, E. J. Good, E. R. Loe, B. Depenbusch, and R. Daubert. 2004. Flaxseed for finishing beef cattle: Effects on animal performance, carcass quality, and meat composition. In: Proc. 60th Flax Institute. Fargo, N.D. p. 108–117.

Du, M., and M. V. Dodson. 2011. Chapter 5. Advanced techniques to enhance marbling in meat. In: S.T. Joo, editor. Control of Meat Quality. Research Signpost, India. p. 105–115.

Enser, M., K. Hallett, B. Hewitt, G. A. J. Fursey, and J. D. Wood. 1996. Fatty acid content and composition of English beef, lamb, and pork at retail. Meat Sci. 42(4):443–456.

Farran, T. B., C. D. Reinhardt, D. A. Blasi, J. E. Minton, T. H. Elsasser, J. J. Higgins, and J. S. Drouillard. 2008. Source of dietary lipid may modify the immune response in stressed feeder cattle. J. Anim. Sci. 86(6):1382–1394.

Geay, Y., D. Bauchart, H. F. Hocquette, and J. Culioli. 2001. Effect of nutritional factors on biochemical, structural and metabolic characteristics of muscles in ruminants, consequences on dietetic value and sensorial qualities of meat. Reprod. Nutr. Dev. 41(1):1–26.

Gonthier, C., A. F. Mustafa, D. R. Ouellet, P. Y. Chouinard, R. Berthiaume, and H. V. Petit. 2005. Feeding micronized and extruded flaxseed to dairy cows: effects on blood parameters and milk fatty acid composition. J. Dairy Sci. 88(2):748–756.

Harfoot, C. G., and G. P. Hazlewood. 1997. Lipid metabolism in the rumen. In: P. N. Hobson and C. S. Stewart, editors. The Rumen Microbial Ecosystem. Chapman and Hall, London, UK. p. 382–426.

Hausman, G. J., M. V. Dodson, K. Ajuwon, M. Azain, K. M. Barnes, L. L. Guan, Z. Jiang, S. P. Poulos, R. D. Sainz, S. Smith, M. Spurlock, J. Novakofski, M. E. Fernyhough, and W. G. Bergen. 2009. Board-invited review: the biology and regulation of preadipocytes and adipocytes in meat animals. J. Anim. Sci. 87(4):1218–1246.

He, M. L., T. A. McAllister, J. P. Kastelic, P. S. Mir, J. L. Aalhus, M. E. R. Dugan, N. Aldai, and J. J. McKinnon. 2012. Feeding flaxseed in grass hay and barley silage diets to beef cows increases alpha-linolenic acid and its biohydrogenation intermediates in subcutaneous fat. J. Anim. Sci. 90(2):592–604.

Heguy, J. M., S. O. Juchem, E. J. DePeters, M. Rosenberg, J. E. P. Santos, and S. J. Taylor. 2006. Whey protein gel composites of soybean and linseed oils as a dietary method to

modify the unsaturated fatty acid composition of milk lipids. Anim. Feed Sci. Technol. 131(3–4):370–388.

Hocquette, J. F., F. Gondret, E. Baeza, F. Medale, C. Jurie, and D. W. Pethick. 2010. Intramuscular fat content in meat-producing animals: development, genetic and nutritional control, and identification of putative markers. Animal 4(2):303–319.

Jenkins, T. C. 1993. Lipid metabolism in the rumen. J. Dairy. Sci. 76(12):3851–3863.

Jenkins, T. C., R. J. Wallace, P. J. Moate, and E. E. Mosley. 2008. Board-invited review: Recent advances in biohydrogenation of unsaturated fatty acids within the rumen microbial ecosystem. J. Anim. Sci. 86(2):397–412.

Kronberg, S. L., E. J. Scholljegerdes, G. Barceló-Coblijn, and E. J. Murphy. 2007. Flaxseed treatments to reduce biohydrogenation of α-linolenic acid by rumen microbes in cattle. Lipids 42(12):1105–1111.

Kronberg, S. L., E. J. Scholljegerdes, A. N. Lepper, and E. P. Berg. 2011. The effect of flaxseed supplementation on growth, carcass characteristics, fatty acid profile, retail shelf life, and sensory characteristics of beef steers finished on grasslands of the Northern Great Plains. J. Anim. Sci. 89(9):2892–2903.

Lawrence, T. L. J. and V. R. Fowler. 2002. Tissues: Basic Structure and Growth. In: Growth of Farm Animals (2nd Ed.). CABI, Wallingford, UK. p. 21–85.

Mach, N., M. Devant, I. Díaz, M. Font-Furnols, M. A. Oliver, J. A. García, and A. Bach. 2006. Increasing the amount of *n-3* fatty acid in meat from young Holstein bulls through nutrition. J. Anim. Sci. 84(11):3039–3048.

Maczulak, A. E., B. A. Dehority, and D. L. Palmquist. 1981. Effects of Long-Chain Fatty Acids on Growth of Rumen Bacteria. Appl. Environ. Microbiol. 42(5):856–862.

Maddock, T. D., M. L. Bauer, K. B. Koch, V. L. Anderson, R. J. Maddock, G. Barceló-Coblijn, E. J. Murphy, and G. P. Lardy. 2006. Effect of processing flax in beef feedlot diets on performance, carcass characteristics, and trained sensory panel ratings. J. Anim. Sci. 84(6):1544–1551.

Maia, M. R. G., L. C. Chaudhary, L. Figueres, and R. J. Wallace. 2007. Metabolism of polyunsaturated fatty acids and their toxicity to the microflora of the rumen. Anton. Leeuw. Int. J. G. 91(4):303–314.

Mensink, R. P., P. L. Zock, A. D. Kester, and M. B. Katan. 2003. Effects of dietary fatty acids and carbohydrates on the ratio of total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. Am. J. Clin. Nutr. 77(5):1146–1155.

Mustafa, A. F., J. J. McKinnon, D. A. Christensen, and T. He. 2002. Effects of micronization of flaxseed on nutrient disappearance in the gastrointestinal tract of steers. Anim. Feed Sci. Technol. 95(3–4):123–132.

Palmquist, D. L. 2009. Omega-3 fatty acids in metabolism, health, and nutrition and for modified animal product foods. Prof. Anim. Sci. 25(3):207–249.

Pedersen, J. I. 2011. Health aspects of saturated fatty acids. In: G. Talbot, editor. Reducing Saturated Fats in Foods. Woodhead. Padstow, Cornwall, UK. p. 77–97.

Quinn, M. J., E. R. Loe, B. E. Depenbusch, J. J. Higgins, and J. S. Drouillard. 2008. The effects of flaxseed oil and derivatives on in vitro gas production, performance, carcass characteristics, and meat quality of finishing steers. Prof. Anim. Sci. 24(2):161–168.

Rule, D. C., K. S. Broughton, S. M. Shellito, and G. Maiorano. 2002. Comparison of muscle fatty acid profiles and cholesterol concentrations of bison, beef cattle, elk, and chicken. J. Anim. Sci. 80(5): 1202-1211.

Scislowski, V., D. Bauchart, D. Gruffat, P. M. Laplaud, and D. Durand. 2005. Effects of dietary *n-6* or *n-3* polyunsaturated fatty acids protected or not protected against ruminal hydrogenation on plasma lipids and their susceptibility to peroxidation in fattening steers. J. Anim. Sci. 83(9):2162–2174.

Scollan, N. D., M. S. Dhanoa, N. J. Choi, W. J. Maeng, M. Enser, and J. D. Wood. 2001. Biohydrogenation and digestion of long chain fatty acids in steers fed on different sources of lipid. J. Agr. Sci. 136(3):345–355.

Scollan, N. D., M. Enser, S. K. Gulati, I. Richardson, and J. D. 2003. Wood Effects of including a ruminally protected lipid supplement in the diet on the fatty acid composition of beef muscle. Br. J. Nutr. 90(3):709–716.

Scott, T. W., and J. R. Ashes. 1993. Dietary lipids for ruminants: protection, utilization and effects on remodelling of skeletal muscle phospholipids. Aust. J. Agric. Res. 44(3):495–508.

Sinclair, L. A., S. L. Cooper, J. A. Huntington, R. G. Wilkinson, K. G. Hallett, M. Enser, and J. D. Wood. 2005. In vitro biohydrogenation of *n-3* polyunsaturated fatty acids protected against ruminal microbial metabolism. Anim. Feed Sci. Technol. 123–124(2):579–596.

Sterk, A., R. Hovenier, B. Vlaeminck, A. M. van Vuuren, W. H. Hendriks, and J. Dijkstra. 2010. Effects of chemically or technologically treated linseed products and docosahexaenoic acid addition to linseed oil on biohydrogenation of C18:3*n-3* in vitro. J. Dairy Sci. 93(11):5286–5299.

Talbot, G. 2011. Saturated fats in foods and strategies for their replacement: an introduction. In: G. Talbot, editor. Reducing Saturated Fats in Foods. Woodhead. Padstow, Cornwall, UK. p. 3–28.

Wilson, J. 2004. Balancing the risks and benefits of fish consumption. Ann. Intern. Med. 141(12):977–980.

Wistuba, T. J., E. B. Kegley, J. K. Apple, and D. C. Rule. 2007. Feeding feedlot steers fish oil alters the fatty acid composition of adipose and muscle tissue. Meat Sci. 77(2):196–203.

Wood, J. D., M. Enser, A. V. Fisher, G. R. Nute, P. R. Sheard, R. I. Richardson, S. I. Hughes, and F. M. Whittington. 2008. Fat deposition, fatty acid composition and meat quality: A review. Meat Sci. 78(4):343–358.

World Health Organization. 2003. Diet, nutrition, and the prevention of chronic diseases. Technical Report No. 916, Geneva.

Chapter 2 - Effects of Linpro and dietary copper on performance, carcass characteristics, and meat quality of feedlot cattle[1]

C. A. Alvarado,* C. C. Aperce,* K. A. Miller,* C. L. Van Bibber-Krueger,* S. Uwituze,* J. S. Drouillard,*[2] and J. J. Higgins†

*Department of Animal Sciences and Industry, Kansas State University, Manhattan, KS 66506; and †Department of Statistics, Kansas State University, Manhattan, Kansas 66506.

[1] Contribution number 14-336-J from the Kansas Agricultural Experiment Station, Manhattan, KS.

[2] Corresponding author: jdrouill@ksu.edu.

ABSTRACT

Our objective was to evaluate whether feeding elevated Cu concentrations in conjunction with Linpro, a co-extruded blend of field peas and flaxseed, affected performance and plasma lipid profiles of fattening beef heifers. The study was conducted as a randomized complete block experiment with a 2 × 2 factorial treatment arrangement. Supplementation consisted of dietary Cu (10 or 100 mg/kg added Cu) and Linpro (0 or 10% of diet, dry basis). Linpro contains 12% linolenic acid, added vitamins, and minerals (22% CP; 23% fat). Crossbred yearling heifers (n = 261; 351 ± 23 kg initial BW) were blocked by weight into heavy and light groups and assigned randomly to experimental pens containing 10 or 11 heifers each. Pens (n = 24) were assigned randomly to each of the 4 treatments. Cattle were fed once daily and had *ad libitum* access to feed and water. The 0% Linpro diets included (DM basis) 35% wet corn gluten feed, 35% cracked corn, 15.8% pelleted soybean hulls, 10% corn silage, vitamins, and minerals. For Linpro diets, the extrudate was added at 10% of DM, replacing soybean hulls. At d 64, blood samples were drawn from the jugular vein for analysis of Cu and long-chain fatty acid (LCFA) concentrations. Heavy and light blocks were harvested on d 117 and 136, respectively. There were no significant interactions between levels of Linpro and supplemental Cu. Added Cu did not affect performance ($P > 0.15$). Final body weights were similar for cattle fed 0 and 10% Linpro (581 vs. 588 kg; SEM = 5.18; $P > 0.20$), but cattle fed diets with Linpro consumed less feed (14.08 vs. 13.59 kg/d; SEM = 0.21; $P < 0.05$) and were therefore more efficient (0.131 vs. 0.141, for 0 and 10% Linpro, respectively; SEM = 0.002; $P < 0.01$). Carcass traits were not affected by treatment. Feeding elevated levels of Cu did not appreciably alter PUFA proportions in plasma. Plasma concentrations of omega-3 fatty acids, including C18:3, C20:5, and C22:5, were greater for heifers fed Linpro ($P < 0.05$). Linpro can be used effectively as an energy source

and to modify the fatty acid profile of meat. Contrary to our hypothesis, increasing dietary concentrations of Cu was not effective as a strategy for decreasing ruminal biohydrogenation and subsequent tissue deposition of PUFA.

Key words: flaxseed, biohydrogenation, protection.

INTRODUCTION

Omega-3 fatty acids (*n-3* FA), which are essential for human nutrition, have been shown to have positive effects on human health because of their anti-inflammatory and immunity-improving qualities (Calder, 2001). Human diets often are low in these fatty acids and relatively high in SFA, which are considered to have negative consequences for human health. Saturated fatty acids have been associated with elevated serum cholesterol (Hegsted et al., 1993). Feeding cattle with flax-based feeds can increase concentrations of *n-3* FA in beef (Drouillard et al., 2004) despite the fact that *n-3* fats from flaxseed are extensively hydrogenated into saturated fats by ruminal microbes (Montgomery et al., 2008). Several studies have reported a relationship between dietary Cu and fat metabolism; for example, adding Cu to the finishing diets of steers and lambs reduced total serum cholesterol and backfat (Ward and Spears, 1997; Engle and Spears, 2000a,b; Cheng et al., 2008). An increase in PUFA content of meat from steers supplemented with Cu also has been noted, which the authors attributed to Cu's ability to inhibit biohydrogenation or increase desaturase activity (Engle and Spears, 2000a; Engle, 2011). The objective of the present study was to evaluate the potential for feeding elevated Cu concentrations in conjunction with Linpro, a co-extruded blend of field peas and flaxseed, on biohydrogenation, performance, and lipid profiles of plasma and meat of fattening beef heifers.

MATERIALS AND METHODS

Procedures followed in this study were approved by the Kansas State University Institutional Animal Care and Use Committee protocol no. 2315.

Study 1

Experimental Design

The study was conducted as a randomized complete block experiment with a 2 × 2 factorial treatment arrangement. Supplementation consisted of dietary Cu (10 or 100 mg/kg added Cu in the form of Cu sulfate) and Linpro (0 or 10% of diet, DM basis). Linpro (O&T Farms; Regina, Saskatchewan, Canada) is an extruded blend of flaxseed and field peas containing 12% linolenic acid, added vitamins, and minerals (22% CP; 23% fat). Crossbred yearling heifers (n = 261; 351 ± 23 kg initial BW) were blocked by weight into heavy and light groups and assigned randomly to experimental pens containing 10 or 11 heifers each. Pens (n = 24) were assigned randomly to each of the 4 treatments.

Animal Processing and Feeding

Animals were processed 1 d after arrival in the feedlot. Heifers were implanted (Revalor-200; Intervet, Inc., Millsboro, DE), dewormed (Safeguard; Intervet, Inc.), and vaccinated against common viral and clostridial diseases (Vista 3 and Vision 7; Intervet/Schering-Plough Animal Health, De Soto, KS). Cattle were fed once daily and had *ad libitum* access to feed (Table 2.1) and water. For Linpro diets, the extrudate was added at 10% of DM, replacing soybean hulls. Cattle were gradually transitioned from their initial diets of 60% corn silage (DM basis) to their final high-concentrate diets using a series of four step-up diets that each were fed for 5 d. Starting 23 d before harvest, Zilmax (Intervet/Schering-Plough Animal Health, De Soto, KS)

was added to the diet for 20 days. Heavy and light blocks were harvested on d 117 and 136, respectively.

Samples and Laboratory Analysis

Individual feedstuffs were sampled weekly. Monthly composited samples were analyzed for DM, NDF, CP, Cu, and ether extract. Portions of ground samples of feedstuffs were dried in a forced-air oven at 105°C overnight to determine DM (Undersander et al., 1993). Determination of NDF was conducted using an Ankom fiber analyzer (Ankom Technology, Macedon, NY) according to van Soest et al. (1991). Heat-stable α-amylase (Ankom Technology) was added to remove residual starch from feedstuff samples. Determination of CP was accomplished by measuring N content with a LECO FP2000 N analyzer (LECO Corp., St Joseph, MI; minimum detection limit = 48 μg/g, intra-assay CV = 3.2%; inter-assay CV = 3.7%, Kansas State University Analytical Laboratory) following AOAC (1995) official method 990.03. Ether extract analysis was performed according to AOAC (1995) official method 920.39. Net energy for maintenance (NE_m) and gain (NE_g) were calculated using prediction equations from NRC (1984).

At d 64, two animals were randomly selected from each pen to analyze levels of Cu and long-chain fatty acids (LCFA). Blood samples were drawn from the jugular vein using heparinized vacuum tubes (BD, Franklin Lakes, NJ). Tubes were gently mixed and immediately placed on ice until centrifugation. After approximately 15 min, samples were centrifuged at 3,200 × g for 10 min. Plasma was recovered, transferred to plastic vials, and frozen at -20°C. For analysis of LCFA, an aliquot of 500 μL of plasma was lyophilized and combined with 1 mL benzene containing 1,000 μg/mL methyl-C13:0 as internal standard and 4 mL BF3-methanol reagent (Supelco B1252). Tubes were incubated at 60°C for 60 min, then cooled to room

temperature. One milliliter of hexane and 4 mL H_2O were added to each tube. The tubes were then vortexed and centrifuged at 1000 × g for 5 min. The organic (upper) solvent layer (1 to 2 mL) was then analyzed via gas chromatography (Schimadzu model 17A, Palo Alto, CA) using a Supelco SP-2560 capillary column (100 m × 0.25 mm × 0.20 μm film) and He as the carrier gas at a flow rate of 1.1 mL/min. Initial temperature was 140°C for 4 min and increased at a rate of 4°C/min to a final temperature of 240°C.

Animals (pens) were weighed and harvested on d 117 and 136 for heavy and light blocks, respectively. Orts and daily feed deliveries were weighed, and DMI and G:F were calculated for each pen of cattle. Cattle were transported 450 km to a commercial abattoir (Tyson Fresh Meats; Holcomb, KS) where HCW and liver abscess scores were obtained the day of harvest, and LM area, KPH, 12^{th}-rib fat thickness, marbling score, USDA yield grade, and USDA quality grades were collected after 48 h.

Three animals from each pen were randomly selected. Their livers were collected, placed in a container with dry ice, transported in a refrigerated truck to the Kansas State University Meats Laboratory, and then frozen until they were analyzed for Cu concentration. In addition, a 2.5-cm sample of the *Longissimus dorsi* (loin samples from one side of each carcass) muscle was taken 24 h after harvest from these selected animals, placed in dry ice, and transported to the same laboratory where the samples were frozen until analyzed for LCFA profile according the procedures of Sukhija and Palmquist (1988). Approximately 100 mg of each sample were mixed with 2 mL internal standard (methyl tridecanoic acid, C13:0, in benzene) and 3 mL methanolic-HCl before being flushed with N, capped, vortexed, heated for 120 min at 70°C, and vortexed every 30 min during heating. Tubes were cooled to room temperature and mixed with 5 mL of 6% K_2CO_3 and 2 mL benzene, vortexed, and centrifuged at 500 × g for 5 min. The organic

(upper) solvent layer was then analyzed using a Schimadzu gas chromatograph (model 17A; Schimadzu Corp., Palo Alto, CA) equipped with a Supelco SP-2560 capillary column (100 m × 0.25 mm × 0.20 μm film thickness; Supelco Inc., Bellefonte, PA) using He as the carrier gas at a flow rate of 1.1 mL/min. Initial temperature was 140°C for 4 min and increased at a rate of 4°C/min to a final temperature of 240°C.

Copper analyses of liver and plasma were conducted as described by van der Merwe et al. (2011). Livers were subsampled (2 to 3 g of wet tissue) from each lobe (left, right, caudate, and quadrate lobes) and analyzed for Cu concentration separately. One gram of liver sample (or 1 mL of plasma) was digested in nitric acid at 105°C, diluted with 18 mL of water, and analyzed by inductively coupled plasma mass spectroscopy (ICP-MS; Agilent ICP-MS 7500cx, Agilent Technologies, Wilmington, DE). Reference samples containing 1 mg/L of Cu, prepared from commercially available standards (Environmental Express, Charleston, SC), were used for data quality assurance. Acceptable quality was defined as a measured concentration between 0.8 and 1.2 times the actual concentration. Each batch of samples included digestion or processing blanks. Element concentrations in blanks were used to correct results for background contaminants.

Statistical Analyses

Continuous data were statistically analyzed using the MIXED procedures of SAS version 9.1 (SAS Inst. Inc., Cary, NC) and categorical data (USDA quality grade and liver abscesses) using the GLIMMIX procedure SAS. In both models, fixed effects included dietary concentrations of Linpro and Cu, the interaction between Linpro and Cu, and weight block; pen nested within Linpro, Cu, and block as random effects, and pen was the experimental unit.

Differences were determined using the PDIFF option of the LS Means statement. Means were determined to be different at $\alpha = 0.05$.

Study 2

Experimental design

An *in vitro* gas production trial was conducted as a randomized complete experiment with a 2 × 2 factorial treatment arrangement. Factors consisted of dietary Cu (10 or 100 mg/kg added Cu) and Linpro (0 or 10% of substrate, DM basis).

Four ruminally fistulated Holstein steers were placed into individual pens in an indoor barn and fed a high-concentrate, corn-based finishing diet. Ruminal fluid from these animals was collected and placed into a pre-warmed insulated container, transported to the Kansas State University Preharvest Food Safety Laboratory, and placed into a 37°C room. Ruminal fluid was strained through 8 layers of cheese cloth, placed into a large separatory funnel, and gassed with CO_2 continuously for 30 to 40 minutes to allow for stratification of the mat, fluid, and protozoal fractions. The protozoa-rich fraction was voided from the funnel, and the clarified liquid layer was mixed 1:2 with McDougall's buffer. Substrates were the same four experimental diets used in Study 1 (Table 2.1). Substrates (2.5 g of sample diets) were placed into 250-mL fermentation flasks equipped with Ankom (Gas Production System, Ankom Technology) pressure monitors that recorded vessel pressure (i.e., fermentative gas production) at 5-min intervals. Each experimental diet was incubated with 150 mL of the buffered ruminal fluid from each steer (steer served as the replicate). This process was repeated for 2 d.

Samples of fermentative gasses were removed from the vial after 0 and 24 h of incubation to determine concentrations of CO_2, H_2S, and CH_4 using a gas chromatograph (SRI

Instruments, Torrance, CA) equipped with a thermal-conductivity detector, a flame ionization detector, and a gas sampling valve with a 0.5-mL sample loop. Separation was achieved using a 0.3 cm × 90 cm Haye Sep D packed Teflon column (SRI Instruments, Torrance, CA) with He as the carrier gas; pressure was maintained at 69 KPa, and oven temperature was maintained at 40°C. Gas samples (10 mL) were transferred from serum bottles and manually injected into the sample loop with a gas-tight syringe (10 MDF-LL-GT; SGE, Austin, TX).

After 24 h of fermentation, contents of the flasks were chilled in an ice bath to cease microbial activity. Contents were centrifuged at 30,000 × g for 20 min, and a 4-mL sample of supernatant was combined with 1 mL of metaphosphoric acid and used to characterize concentrations of VFA and lactate. Volatile fatty acids and lactate were analyzed by gas chromatography (Hewlett-Packard 5890A, Palo Alto, CA; 2 m × 2 mm column; Supelco Carbopack B-DA 80/120 4% CW 20 m column packing, Bellefonte, PA) with He as the carrier gas, a flow rate of 24 mL/min, and a column temperature of 175°C. Total VFA production was computed by adding individual VFA.

Statistical Analyses

Data were statistically analyzed using the MIXED procedure of SAS version 9.1. In the analysis of gas production Linpro, Cu, time, and all 2- and 3-way interactions were included as fixed effects; day, animal, day × animal interaction, and animal × day × Linpro × Cu interaction were used as random effects. For gas composition, VFA, LCFA of Study 2, Linpro, Cu, and their interactions were used as fixed effects, and day, animal, and animal × day as random effects. Differences were determined using the PDIFF option of the LS Means statement. Means were determined to be different at $\alpha = 0.05$.

Study 3

Experimental Design

A third trial was conducted to determine *in vitro* dry matter disappearance (IVDMD) of the aforementioned diets (Study 1). Samples of the four experimental diets (0.5 g in duplicate, DM basis) were put into 50-mL centrifuge tubes with 20 mL of prewarmed McDougall's artificial saliva and 10 mL warm ruminal fluid. The origin and processing of the ruminal fluid was identical to study 2. Fermentation tubes were sparged with CO_2, then placed into a 39°C room. Tubes were gently swirled every 3 to 4 h during the 24-h incubation. After incubation, tubes were placed in ice water bath and pH was recorded prior to being frozen for later analysis. Frozen samples were thawed at room temperature, then centrifuged at 30,000 × g for 20 min. Centrifugate pellet were then frozen, lyophilized, and weighed. The residual dried weight was used to calculate IVDMD. This process was repeated for 2 d.

Statistical Analyses

Data were statistically analyzed using the MIXED procedure of SAS version 9.1, where Linpro, Cu, and their interactions were used as fixed effects, and day, animal, and animal × day as random effects. Differences were determined using the PDIFF option of the LS Means statement. Means were determined to be different at $\alpha = 0.05$.

RESULTS AND DISCUSSION

Study 1

Feedlot performance is summarized in Table 2.2. No interaction between Linpro and Cu level were found for measures of feedlot performance ($P > 0.10$). No detrimental effects were

observed when 100 mg/kg DM of added Cu was included, which is consistent with NRC (2000) recommendations for safe upper limits. No Cu effects on performance were found ($P > 0.10$), which agrees with the result of Engle and Spears (2000a), who reported no effect of high-Cu diets on performance of finishing cattle. We found no differences in initial or final BW ($P > 0.05$); however, decreased DMI was observed ($P = 0.03$) in animals fed Linpro, with no differences in ADG ($P = 0.20$), which resulted in improved efficiency of gain (0.129 and 0.137 for 0 and 10% Linpro groups, respectively; $P < 0.01$) and higher NE_m (2.0 and 2.12 Mcal/kg for 0 and 10% Linpro groups, respectively; $P < 0.01$) and NE_g (1.35 and 1.45 Mcal/kg for 0 and 10% Linpro groups, respectively; $P < 0.01$). Similar effects on DMI and efficiency have been reported when flaxseed is added to finishing cattle diets (Kim et al., 2009). Conversely, Maddock et al. (2006) reported increased G:F and ADG with no differences in DMI when comparing performance of cattle fed diets with 8% flaxseed to those fed diets without flaxseed. Lower DMI could be the result of increased energy density of the diets containing Linpro, or could be the result of decreases in NDF digestibility when fat is added to the diet, as noted by Jenkins (1993).

Carcass traits are presented in Table 2.3. No interaction effects between Linpro and Cu were observed for carcass traits. Based on the observations of Engle et al. (2000a,b) and Engle and Spears (2000a,b), we expected addition of 100 mg/kg Cu to the diets of finishing cattle to result in less 12^{th}-rib carcass fat, but no such effects were noted in our study. Responses to Cu supplementation have been inconsistent in published experiments. Lee at al. (2002) and Johnson and Engle (2003) found a tendency ($P < 0.11$ and $P < 0.18$, respectively) for decreased backfat thickness in cattle supplemented with Cu at 10 or 20 mg/kg DM. Engle and Spears (2001) reported no differences in backfat thickness when Simmental steers were supplemented with 10 or 40 mg Cu/kg DM and speculated that the lack of response may have been attributable to breed

effects, as Simmental cattle were previously noted to have greater requirements for Cu than other breeds.

Copper and LCFA of plasma after 64 d of feeding are shown in Table 2.4. No interaction between levels of Linpro and Cu were observed ($P > 0.2$). Copper and Linpro had no effects on blood Cu concentration ($P > 0.2$), most likely due to Cu storage in the liver (NRC, 2000). Copper is released from the liver to keep a constant blood Cu level. Sixty-four days on the high-Cu diet appear to be insufficient to alter the blood level in the current trial; however, difference were detected during longer periods of finishing (130 days, Engle et al., 2000b). High Cu reduced plasma C20:0 ($P < 0.04$) and C20:3 *n-3* ($P < 0.05$), but most effects on plasma LCFA were made by Linpro treatment, which increased the concentration of C18:3 *n-3*, C20:5 *n-3*, C22:5 *n-3*, and total *n-3* and reduced concentration of C18:3 *n-6* and C20:3 *n-6* ($P < 0.05$). An interaction between Cu and Linpro levels on the *n-6*:*n-3* ratio was observed ($P < 0.05$). The addition of Cu without Linpro decreased the *n-6*:*n-3* ratio, whereas the addition of Cu with Linpro treatments increased *n-6*:*n-3* ratio.

The effects of each treatment on liver Cu and LCFA profile of LM samples are presented in Table 2.5. Liver storage of Cu is reflected in our results, which were higher ($P < 0.01$) in high-Cu treatments. As we expected from examining previous research (Drouillard et al., 2004; LaBrune et al., 2008; Kim et al., 2009), an important effect of Linpro on FA (principally UFA) was observed. Fatty acids from LM samples in the form of C18:1 *n-11*, C18:2 *trans-6*, and C18:3 *n-3*, were increased by adding flaxseed to the diets, but other FA such as C20:0, C20:3 *n-6*, and C20:5 *n-3* were reduced ($P < 0.01$). The overall effect in meat from flaxseed-supplemented heifers was an 88.7% increase in *n-3* FA, 17.0% increase of PUFA, and a reduction in the *n-6*:*n-*

3 ratio ($P < 0.01$), which are considered to have a positive impact on human nutrition (Hegsted et al., 1993).

The main goal in this study was to evaluate the effect of supplemental Cu in finishing diets as a strategy to decrease ruminal biohydrogenation of PUFA, especially *n-3* FA. The high-Cu treatment had a minor effect on FA profile of LM. A tendency ($P = 0.11$) for increased concentrations of eicosapentaenoic acid (C22:5 *n-3*) and a lower *n-6*:*n-3* ratio ($P = 0.03$) was observed with higher levels of dietary Cu. Cummins et al. (2008) and Engle and Spears (2001) reported almost no effect of high-Cu diets on FA composition of LM in goat kids and steers, respectively. The only exception was C15:0 in the first cited work (Cummins et al., 2008), which increased linearly ($P = 0.03$) when 100 and 200 mg/d of Cu where added. Tissue concentrations of C15:0 fatty acids were not affected in our experiment ($P = 0.66$).

Another possible explanation for the discrepancies between experiments could be related to mineral composition of the basal diets. Other minerals that are antagonistic to Cu, such as S, could have affected ruminal availability of Cu. Wet corn gluten feed normally contains substantial amounts of S, and its presence may have influenced bioavailability of Cu within the rumen via formation of insoluble cupric sulfide, thereby decreasing the impact of Cu on biohydrogenation of PUFA by ruminal bacteria (Spears, 2003; Dias et al., 2013). Concentrations of Cu within the liver (Table 2.5) were, however, greater for cattle supplemented with 100 mg/kg Cu than for their counterparts fed 10 mg/kg Cu ($P < 0.01$), suggesting that supplemented Cu was at least partially available.

The potential effect of sex on fat and Cu metabolism has not been evaluated in previous studies. Most, if not all, of the previous research has been conducted supplementing Cu (10 to 40 mg/kg) to steers fed a finishing diet (Essig et al. 1972; Ward and Spears, 1997; Engle and Spears,

2000a,b; Engle and Spears, 2001; Lee at al., 2002; Dorton et al., 2003; Johnson and Engle, 2003). In contrast, we used yearling heifers. Differences in fat metabolisms and Cu requirements between steers and heifers have been reported. For instance, differences in fat deposition between bulls, steers, and heifers (ordered from leanest to fattest) have been recognized. Gooneratne and Christensen (1989) sampled steers and heifers from a commercial slaughterhouse and noted that 20% of the steers and 55% of heifers were Cu-deficient, which could indicate a higher Cu requirement in heifers.

An adaptive mechanism against Cu toxicity has been proposed in previous research (Engle and Spears, 2000; Arthington, 2005). Arthington (2005) observed that the supplementation of heifers with 15 mg/kg DM daily increased the Cu content of liver compared with the control (no Cu added), but higher doses (i.e., 60 and 120 mg/kg) were not different from the control group, which was attributed to the adaptive capacity of cattle to increase Cu excretion (increasing bile production) or by decreasing its absorption.

Studies 2 and 3 (In vitro)

In vitro DM disappearance, final pH, VFA, and gas production of studies 2 and 3 are presented in Table 2.6 and 2.7. No interactions between levels of Cu and Linpro were observed. Copper concentration alone had no impact on IVDMD ($P > 0.2$) but IVDMD increased by 1.2% when Linpro was included as part of the substrate ($P < 0.05$). A similar situation was observed for final pH after incubation: no effect of Cu was observed ($P > 0.05$), but pH increased when Linpro was added ($P > 0.05$). Total VFA were higher in high-Cu treatments ($P = 0.038$); however, the molar proportions were not affected ($P > 0.34$). In contrast, Linpro had no effect on total VFA ($P = 0.45$), but molar proportions of propionate and isobutyrate increased whereas acetate and the acetate:propionate ratio significantly decreased ($P < 0.01$). There was no

interaction between the level of Linpro and Cu for *in vitro* gas production ($P > 0.2$). Linpro increased the production of H_2S (30% higher, $P = 0.05$), and Cu inclusion slightly increased CO_2 proportion (64.06 vs. 67.58%, for Linpro and Cu treatments, respectively). Earlier work reflected both the Cu and flaxseed effect on fermentation (Engle and Spears, 2000; Scholljegerdes and Kronberg, 2008), and the extent of this effect should be related to the amount of Cu and flaxseed supplemented. For instance, no differences on IVDMD were reported by Engle and Spears (2000) when Cu was added; however, Essig et al. (1972) noted a reduction in fermentation, which manifested as decreased VFA production. Engle and Spears (2000) suggested these differences were due to the higher Cu doses in the earlier work (Essig et al., 1972), which also was reflected in our study.

Scholljegerdes and Kronberg evaluated the effects of flaxseed (2008). They found no differences in total VFA production, although, they noted a linear relationship between flaxseed supplementation and molar proportions of VFA. Similar to our results, they reported that the use of flaxseed increased the molar proportions of propionate, isobutyrate, and isovalerate; decreased acetate and the acetate:propionate ratio; and left butyrate unaffected. Data presented in Table 2.1 reflect an important difference in lipid content of diets with and without Linpro (4.76 vs. 2.70% EE, respectively), which was sufficient to change LM LCFA profile without negative effects on ruminal fermentation.

IMPLICATIONS

Linpro supplementation at a level of 10% of DM (5% flaxseed, DM basis) in beef cattle finishing diets can be used effectively as an energy source, improving efficiency and favorably affecting the LCFA profile of beef. No effects of Cu or interaction between Cu and Linpro were noted for FA profiles of blood or meat. Increased levels of Cu in finishing diets thus does not

appear to be an effective strategy for decreasing ruminal biohydrogenation and promoting subsequent assimilation of PUFA into tissues. Further research is needed to evaluate if the discrepancies with previous work are sex-related (i.e., heifers vs. steers) or attributable to interactions with other dietary minerals.

LITERATURE CITED

AOAC. 1995. Official Methods of Analysis. 16th ed. Assoc. Off. Anal. Chem., Arlington, VA.

Arthington, J. D. 2005. Effects of copper oxide bolus administration or high-level copper supplementation on forage utilization and copper status in beef cattle. J. Anim. Sci. 83(12):2894–2900.

Calder, P. C. 2001. Polyunsaturated fatty acids, inflammation, and immunity. Lipids 36(9):1007–1024.

Cheng, J., C. Fan, W. Zhang, X. Zhu, X. Yan, R. Wang, and Z. Jia. 2008. Effects of dietary copper and level on performance, carcass characteristics and lipid metabolism in lambs. Asian-Aust. J. Anim. Sci. 21(5):685–691.

Cummins, K. A., S. G. Solaiman, and W. G. Bergen. 2008. The effect of dietary copper supplementation on fatty acid profile and oxidative stability of adipose depots in Boer × Spanish goats. J. Anim. Sci. 86(2):390–396.

Dias, R. S., S. López, Y. R. Montanholi, B. Smith, L. S. Haas, S. P. Miller, and J. France. 2013. A meta-analysis of the effects of dietary copper, molybdenum, and sulfur on plasma and liver copper, weight gain, and feed conversion in growing-finishing cattle. J. Anim. Sci. 91(12):5714–5723.

Drouillard, J. S., M. A. Seyfert, E. J. Good, E. R. Loe, B. Depenbusch, and R. Daubert. 2004. Flaxseed for finishing beef cattle: Effects on animal performance, carcass quality, and meat composition. Proc. 60th Flax Institute, March 17-19. Fargo, N.D. p. 108–117.

Engle, T. E. 2011. Copper and lipid metabolism in beef cattle: A review. J. Anim. Sci. 89(2):591–596.

Engle, T. E., and J. W. Spears. 2000a. Dietary copper effects on lipid metabolism, performance, and ruminal fermentation in finishing steers. J. Anim. Sci. 78(9):2452–2458.

Engle, T. E., and J. W. Spears. 2000b. Effects of dietary copper concentration and source on performance and copper status of growing and finishing steers. J. Anim. Sci. 78(9):2446–2451.

Engle, T. E., and J. W. Spears. 2001. Performance, carcass characteristics, and lipid metabolism in growing and finishing Simmental steers fed varying concentrations of copper. J. Anim. Sci. 79(11):2920–2925.

Engle, T. E., J. W. Spears, V. Fellner, and J. Odle. 2000a. Effects of soybean oil and dietary copper on ruminal and tissue lipid metabolism in finishing steers. J. Anim. Sci. 78(10):2713–2721.

Engle, T. E., J. W. Spears, L. Xi, and F. W. Edens. 2000b. Dietary copper effects on lipid metabolism and circulating catecholamine concentrations in finishing steers. J. Anim. Sci. 78(10):2737–2744.

Essig, H. W., J. D. Davis, and L. J. Smithson. 1972. Copper sulfate in steer rations. J. Anim. Sci. 35(2):436−439.

Gooneratne, S. R. and Christensen, D. A. 1989. A survey of maternal copper status and fetal tissue copper concentrations in Saskatchewan bovine. Can. J. Anim. Sci.69(1):141–150.

Hegsted, D.M., L. M. Ausman, J. A. Johnson, and G. E Dallal. 1993. Dietary fat and serum lipids: an evaluation of the experimental data. Am. J. Clin. Nutr. 57(6):875–883.

Jenkins, T. C. 1993. Lipid metabolism in the rumen. J. Dairy Sci. 76(12):3851–3863.

Johnson, L. R., and T. E. Engle. 2003. The effects of copper source and concentration on lipid metabolism in growing and finishing Angus steers. Asian-Aust. J. Anim. Sci. 16(8):1131–1136.

Kim, C. M., J. H. Kim, Y. K. Oh, E. K. Park, G. C. Ahn, G. Y. Lee, J. I. Lee, and K. K. Park. 2009. Effects of flaxseed diets on performance, carcass characteristics and fatty acid composition of Hanwoo steers. Asian-Aust. J. Anim. Sci. 22(8):1151–1159.

LaBrune, H. J., C. D. Reinhardt, M. E. Dikeman, and J. S. Drouillard. 2008. Effects of grain processing and dietary lipid source on performance, carcass characteristics, plasma fatty acids, and sensory properties of steaks from finishing cattle. J. Anim Sci. 86(1):167–172.

Lee, S. H., T. E. Engle, and K. L. Hossner. 2002. Effects of dietary copper on the expression of lipogenic genes and metabolic hormones in steers. J. Anim. Sci. 80(7):1999–2005.

Maddock, T. D., M. L. Bauer, K. B. Koch, V. L. Anderson, R. J. Maddock, G. Barcelo-Coblijn, E. J. Murphy, and G. P. Lardy. 2006. Effect of processing flax in beef feedlot diets on performance, carcass characteristics, and trained sensory panel ratings. J. Anim. Sci. 84(6):1544–1551.

Montgomery, S. P., J. S. Drouillard, T. G. Nagaraja, E. C. Titgemeyer, and J. J. Sindt. 2008. Effects of supplemental fat source on nutrient digestion and ruminal fermentation in steers. J. Anim. Sci. 86:640–650.

NRC. 1984. Nutrient Requirement of Beef Cattle. 6th rev. ed. Natl. Acad. Press, Washington, DC.

NRC. 2000. Nutrient requirements of beef cattle 7th Rev: update 2000. Ed. National Academy Press, Washington, D.C.

Scholljegerdes, E. J., and S. L. Kronberg. 2008. Influence of level of supplemental whole flaxseed on forage intake and site and extent of digestion in beef heifers consuming native grass hay. J. Anim. Sci. 86(9):2310–2320.

Spears, J. W. 2003. Trace mineral bioavailability in ruminants. J. Nutr. 133(5):1506S–1509S.

Sukhija, P. S., and D. L. Palmquist. 1988. Rapid method for determination of total fatty acid content and composition of feedstuffs and feces. J. Agric. Food Chem. 36(6):1202–1206.

Undersander, D., R. D. Mertens, and N. Thiex. 1993. Forage Analysis Procedures. Natl. Forage Testing Assoc., Omaha, NE.

van der Merwe, D., J. W. Carpenter, J. C. Nietfeld, and J. F. Miesner. 2011. Adverse health effects in Canada Geese (*Branta canadensis*) associated with waste from zinc and lead mines in the tri-state mining district (Kansas, Oklahoma, and Missouri, USA). J. Wildl. Dis. 47(3):650–660.

van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74(10):3583–3597.

Ward, J. D., and J. W. Spears. 1997. Long-term effects of consumption of low-copper diets with or without supplemental molybdenum on copper status, performance, and carcass characteristics of cattle. J. Anim. Sci. 75(11):3057–3065.

Table 2.1: Composition of dry-rolled-corn-based finishing diets with or without Linpro and two Cu levels (10 or 100 mg/kg).

	0% Linpro		10% Linpro	
Item	10 Cu	100 Cu	10 Cu	100 Cu
Ingredient, % of DM				
Dry-rolled corn	35.0	35.0	35.0	35.0
Wet corn gluten feed	35.0	35.0	35.0	35.0
Corn silage	10.0	10.0	10.0	10.0
Soybean hulls	15.8	15.8	5.71	5.68
Linpro	-	-	10.0	10.0
Supplement[1]	4.16	4.19	4.29	4.32
Analyzed composition				
DM, %	67.16	67.16	67.61	67.62
CP, %	14.71	14.71	15.65	15.65
Ether extract, %	2.70	2.70	4.76	4.76
NDF, %	28.02	28.00	23.42	23.40
Ca, %	0.63	0.66	0.65	0.63
P, %	0.34	0.34	0.34	0.34
K, %	0.88	0.89	0.77	0.78
Cu, mg/kg	16.2	81.2	16.8	98.1

[1]Formulated to provide 10 or 100 mg/kg added Cu; 300 mg/d monensin; 90 mg/d tylosin; 2,200 IU/kg vitamin A; 22 IU/kg vitamin E; 0.1% added Na; 0.15% added Cl; 0.10 mg/kg Co; 0.6 mg/kg I; 60 mg/kg Mn; 0.25 mg/kg Se; and 60 mg/kg Zn.

Table 2.2: Feedlot performance of yearling heifers fed finishing diets with 0 or 10% Linpro and 10 or 100 mg/kg added Cu (DM basis).

	0% Linpro		10% Linpro			*P*-value[a]		
Item	10 Cu	100 Cu	10 Cu	100 Cu	SEM	L	Cu	L × Cu
n	6	6	6	6				
Initial BW, kg	352	351	353	350	1.81	0.70	0.34	0.50
Final BW[b], kg	580	575	588	578	4.90	0.27	0.15	0.52
Carc-adj BW[c], kg	584	579	594	583	5.18	0.20	0.13	0.59
DMI, kg	14.1	14.1	13.8	13.4	0.209	0.03	0.29	0.28
ADG[b], kg	1.83	1.80	1.90	1.83	0.035	0.20	0.21	0.64
Carc-adj ADG[c], kg	1.87	1.83	1.94	1.88	0.036	0.12	0.16	0.69
G:F[b]	0.130	0.128	0.137	0.137	0.002	<0.01	0.64	0.59
Carc-adj G:F[c]	0.133	0.130	0.141	0.140	0.002	<0.01	0.41	0.47
NE_m[d], Mcal/kg	2.02	1.98	2.12	2.11	0.022	<0.01	0.30	0.58
NE_g[d], Mcal/kg	1.36	1.33	1.45	1.44	0.019	<0.01	0.31	0.58

[a]L: effect of Linpro; Cu: effect of Cu level; L × Cu: Interaction between Linpro and Cu level.

[b]BW calculated as gross live BW × 0.96 (i.e., shrunk BW).

[c]BW calculated as HCW divided by a common dressed yield of 0.635.

[d]NE_m and NE_g: Net energy for maintenance and gain, respectively, and calculated using prediction equations from NRC (1984).

Table 2.3: Carcass traits of yearling heifers fed dry-rolled-corn-based finishing diets with 0 or 10% Linpro and 10 or 100 mg/kg added Cu (DM basis).

	0% Linpro		10% Linpro			*P*-value[a]		
	10 Cu	100 Cu	10 Cu	100 Cu	SEM	L	Cu	L × Cu
n	64	66	65	66				
HCW, kg	371	367	377	370	3.3	0.20	0.13	0.57
Dressed yield, %	64.0	63.8	64.0	64.0	0.31	0.79	0.79	0.79
USDA quality grade								
Prime, %	4.7	9.1	9.2	6.1	3.55	0.85	0.86	0.31
Premium Choice[b], %	35.9	42.4	44.6	45.5	6.73	0.39	0.57	0.67
Choice, %	53.1	33.3	32.3	30.3	6.44	0.08	0.10	0.18
Select, %	3.1	6.1	10.8	13.6	0.34	0.04	0.41	0.99
Low grade[b], %	3.1	6.1	3.1	4.5	2.78	0.78	0.44	0.79
Other grade	0.0	0.0	3.0	0.0	1.10	0.18	0.18	0.18
USDA yield grade[c]	2.66	2.48	2.66	2.64	0.10	0.43	0.32	0.47
Yield grade 1[c], %	6.3	12.1	6.2	7.6	3.38	0.48	0.30	0.53
Yield grade 2[c], %	37.5	34.8	30.8	30.3	7.03	0.44	0.84	0.88
Yield grade 3[c], %	42.2	47.0	53.8	53.0	7.67	0.26	0.80	0.72
Yield grade 4[c], %	12.5	4.5	9.2	9.1	3.55	0.86	0.26	0.28
Yield grade 5[c], %	1.6	1.5	0.0	0.0	1.09	0.18	1.00	1.00
Marbling score[d]	508	520	523	512	18.0	0.89	0.97	0.54
KPH, %	2.6	2.7	2.6	2.6	0.08	0.64	0.74	0.55
12th-rib fat, cm	2.2	1.7	1.7	1.9	0.07	0.37	0.41	0.13
LM area, cm^2	90.7	93.5	93.2	91.4	1.33	0.89	0.69	0.10
Liver abscess[e], %	10.9	19.7	12.3	7.6	4.31	0.23	0.66	0.13

[a]L: effect of Linpro; Cu: effect of Cu level; L × Cu: Interaction between Linpro and Cu level.

[b]Premium Choice: Marbling score was greater than 500 and less than 700, thus qualifying carcass for upper two-thirds of the USDA Choice quality grade. Low grade: animals with advanced bone maturity, thus carcasses were classified as USDA Commercial.

[c]Yield grade as determined by a USDA grader.

[d]Marbling score 500 to 599 = Modest.

[e]Liver Abscesses: A$^-$ = 1 or 2 small abscesses. A = 2 to 4 well-organized abscesses. A$^+$ = 1 or more large abscesses along with inflammation (Liver Abscess Technical Information AI 6288, Elanco Animal Health, Greenfield, IN).

Table 2.4: Copper and long-chain fatty acids (LCFA) concentration in plasma of yearling heifers (d 64) fed finishing diets with 0 or 10% Linpro and 10 or 100 mg/kg Cu (DM basis).

	0% Linpro		10% Linpro			*P*-value[a]		
Item	10 Cu	100 Cu	10 Cu	100 Cu	SEM	L	Cu	L × Cu
Cu, µg/mL	1.14	1.47	1.18	1.28	0.16	0.64	0.20	0.48
LCFA[b], µg/mL								
C16:0	197.5	236.2	205.1	208.0	12.11	0.41	0.11	0.16
C16:1	22.23	26.13	21.19	21.93	1.40	0.08	0.12	0.28
C17:0	20.84	25.26	20.63	21.30	1.39	0.16	0.09	0.20
C17:1	0.00	0.00	0.00	0.88	0.44	0.33	0.33	0.33
C18:0	355.9	400.9	413.9	410.6	20.21	0.11	0.32	0.25
C18:1 *trans-9*	2.92	3.68	3.37	3.40	0.37	0.83	0.31	0.34
C18:1 *n-10*	18.03	19.23	16.66	16.54	2.28	0.39	0.82	0.78
C18:1 *n-11*	9.08	8.35	6.92	7.52	1.20	0.23	0.96	0.59
C18:1 *cis-9*	90.9	112.6	110.4	111.3	7.37	0.23	0.15	0.18
C18:1 cis-11	11.5	14.3	14.4	14.5	1.03	0.16	0.17	0.21
C18:2 *cis-9, cis-12*	1,228	1,417	1,267	1,325	81.8	0.76	0.15	0.43
C18:2 *cis-9, trans-11*	1.17	1.23	0.73	0.56	0.34	0.12	0.88	0.73
C18:3 *n-6*	6.74	9.44	4.67	5.57	1.42	0.05	0.22	0.54
C18:3 *n-3*	39.22	61.32	209.8	206.6	9.73	<0.01	0.35	0.21
C20:0	1.18	0.84	0.98	0.15	0.25	0.09	0.04	0.35
C20:1	0.00	0.00	0.55	0.72	0.32	0.07	0.79	0.79
C20:2	2.63	3.41	2.89	3.02	0.28	0.83	0.12	0.26
C20:3 *n-6*	26.13	43.11	22.92	23.71	4.17	0.02	0.05	0.07
C20:3 *n-3*	0.00	0.00	0.24	0.10	0.10	0.11	0.50	0.50
C20:4 *n-6*	34.17	44.04	27.15	30.04	3.45	0.01	0.08	0.33
C20:5 *n-3*	4.28	5.55	8.92	10.86	0.93	<0.01	0.10	0.72
C22:0	0.06	0.12	0.00	0.00	0.07	0.20	0.65	0.65
C22:5 *n-3*	14.20	18.45	19.17	20.86	1.617	0.04	0.09	0.44
C22:6 *n-3*	3.85	4.72	3.67	3.97	0.38	0.24	0.14	0.46
C23:0	0.28	0.38	0.11	0.00	0.14	0.07	0.99	0.45
C24:1	0.00	0.00	1.97	0.00	0.98	0.33	0.33	0.33
Total *n-3* fatty acids[c]	61.54	90.05	241.83	242.42	11.05	<0.01	0.21	0.22
Total *n-6* fatty acids[c]	1,294	1,513	1,322	1,384	86.0	0.56	0.12	0.38
n-6:*n-3* ratio	20.9	16.9	5.5	5.8	0.32	<0.01	<0.01	<0.01
SFA[c]	598.3	689.2	665.1	663.9	33.69	0.55	0.20	0.19
MUFA[c]	154.8	184.3	175.4	179.3	10.75	0.48	0.14	0.25
PUFA[c]	1,360	1,608	1,568	1,630	94.6	0.24	0.12	0.34
Total fatty acids[c]	2,113	2,482	2,408	2,473	134.5	0.30	0.13	028

[a]L: effect of Linpro; Cu: effect of Cu level; L × Cu: Interaction between Linpro and Cu level.

[b]LCFA: long-chain fatty acids.

[c]Sum of all identified fatty acids.

Table 2.5: Copper concentration of liver and long-chain fatty acids of meat samples (% of wet sample) yearling heifers fed dry-rolled-corn-based finishing diets with 0 or 10% Linpro and 10 or 100 mg/kg added Cu (DM basis).

	0% Linpro		10% Linpro			*P*-value[a]		
Item	10 Cu	100 Cu	10 Cu	100 Cu	SEM	L	Cu	L × Cu
Cu, mg/kg	122.0	247.1	155.3	241.4	21.1	0.52	<0.01	0.37
LCFA[b], %								
C10:0	0.006	0.005	0.007	0.006	0.009	0.13	0.39	0.95
C11:0	0.004	0.001	0.001	0.002	0.001	0.44	0.39	0.32
C12:0	0.01	0.01	0.01	0.01	0.001	0.35	0.79	0.34
C14:0	0.19	0.22	0.23	0.21	0.021	0.54	0.76	0.24
C14:1	0.05	0.05	0.06	0.06	0.006	0.45	0.59	0.46
C15:0	0.03	0.03	0.03	0.03	0.004	0.97	0.66	0.21
C15:1	0.00	0.00	0.00	0.00	0.001	0.47	0.55	0.60
C16:0	1.74	1.94	1.85	1.72	0.184	0.77	0.84	0.38
C16:1	0.23	0.27	0.25	0.23	0.024	0.74	0.57	0.29
C17:0	0.13	0.15	0.14	0.12	0.017	0.54	0.92	0.32
C17:1	0.07	0.08	0.06	0.06	0.011	0.25	0.86	0.52
C18:0	1.03	1.06	1.13	0.98	0.107	0.93	0.59	0.44
C18:1 *trans-9*	0.17	0.19	0.21	0.18	0.020	0.59	0.88	0.31
C18:1 *n-11*	0.02	0.02	0.08	0.07	0.005	<0.01	0.73	0.28
C18:1 *cis-9*	3.01	3.26	3.10	2.81	0.334	0.60	0.96	0.43
C18:1 *n-7*	0.14	0.16	0.15	0.13	0.014	0.41	0.65	0.19
C18:2 *trans-6*	0.01	0.02	0.05	0.04	0.003	<0.01	0.98	0.08
C18:2 *cis-9, cis-12*	0.26	0.27	0.27	0.25	0.012	0.70	0.76	0.31
C18:2 *cis-9, trans-11*	0.002	0.003	0.003	0.003	0.001	0.26	0.42	0.06
C18:2 *trans-10, cis-12*	0.001	0.002	0.003	0.003	0.001	<0.01	0.44	0.02
C18:2 *cis-9, cis-11*	0.001	0.001	0.003	0.002	0.001	<0.01	0.81	0.07
C18:2 *trans-9, trans-11*	0.01	0.01	0.01	0.01	0.001	0.40	0.41	0.14
C18:3 *n-6*	0.01	0.01	0.01	0.01	0.001	0.37	0.79	0.31
C18:3 *n-3*	0.02	0.03	0.06	0.06	0.003	<0.01	0.93	0.12
C20:0	0.01	0.01	0.01	0.01	0.001	<0.01	0.87	0.69
C20:1	0.02	0.03	0.02	0.02	0.003	0.49	0.58	0.23
C20:2	0.01	0.01	0.01	0.01	0.001	0.79	0.78	0.23
C20:3 *n-6*	0.02	0.02	0.01	0.01	0.001	<0.01	0.91	0.31
C20:4 *n-6*	0.002	0.002	0.003	0.003	0.000	<0.01	0.92	0.92
C20:5 *n-3*	0.01	0.01	0.01	0.01	0.001	<0.01	0.67	0.58
C21:0	0.02	0.02	0.02	0.02	0.002	0.34	0.53	0.22
C22:0	0.003	0.003	0.003	0.003	0.001	0.44	0.77	0.15
C22:5 *n-3*	0.01	0.01	0.01	0.01	0.001	<0.01	0.11	0.62
C22:6 *n-3*	0.004	0.003	0.003	0.004	0.001	0.46	0.27	0.03
C24:0	0.003	0.003	0.003	0.003	0.001	0.09	0.79	0.90
C24:1	0.003	0.003	0.003	0.003	0.001	0.86	0.78	0.74

Total *n-3* fatty acids[c]	0.04	0.05	0.09	0.08	0.004	<0.01	0.89	0.15
Total *n-6* fatty acids[c]	0.30	0.32	0.34	0.32	0.015	0.19	0.83	0.20
n-6:*n-3* ratio	7.27	6.78	4.02	3.93	0.125	<0.01	0.03	0.13
SFA[c]	3.17	3.45	3.42	3.11	0.329	0.90	0.97	0.37
MUFA[c]	3.71	4.08	3.93	3.57	0.399	0.72	0.99	0.38
PUFA[c]	0.36	0.39	0.45	0.42	0.020	<0.01	0.94	0.17
Total fatty acids[c]	7.24	7.92	7.80	7.10	0.743	0.87	0.99	0.37

[a]L: effect of Linpro; Cu: effect of Cu level; L × Cu: Interaction between Linpro and Cu level.

[b]LCFA: long-chain fatty acids.

[c]Sum of all identified fatty acids.

Table 2.6: *In vitro* DM disappearance (IVDMD), final pH, and VFA production of cultures fed substrates with 0 or 10% Linpro and 10 or 100 mg/kg added Cu after 24 h of *in vitro* incubation.

	0% Linpro		10% Linpro			*P*-value[1]		
	10 Cu	100 Cu	10 Cu	100 Cu	SEM	L	Cu	L × Cu
IVDMD, %	51.47	51.26	52.51	52.70	1.94	0.02	0.98	0.69
Final pH	6.53	6.52	6.56	6.58	0.10	<0.01	0.70	0.44
Total VFA, mM	86.69	96.59	89.21	90.29	5.32	0.46	0.04	0.09
VFA, mol/100 mol								
Acetate	49.66	49.33	47.90	47.80	1.65	<0.01	0.46	0.70
Propionate	35.02	35.40	36.42	36.64	2.10	<0.01	0.37	0.80
Butyrate	11.13	11.14	11.37	11.30	2.23	0.35	0.89	0.84
Valerate	2.34	2.26	2.30	2.26	0.47	0.79	0.34	0.75
Isobutyrate	0.57	0.57	0.64	0.64	0.05	<0.01	0.89	0.94
Isovalerate 2-methyl	0.82	0.81	0.83	0.82	0.31	0.85	0.69	0.93
Isovalerate 3-methyl	0.47	0.49	0.53	0.54	0.06	<0.01	0.47	0.96
Acetate:propionate	1.46	1.43	1.36	1.35	0.12	<0.01	0.35	0.82

[1]L: effect of Linpro; Cu: effect of Cu level; L × C: Interaction between Linpro and Cu level.

Table 2.7: Amount and composition of fermentative gasses produced by *in vitro* cultures fed substrates with 0 or 10% Linpro and 10 or 100 mg/kg added Cu (DM basis).

	0% Linpro		10% Linpro			*P*-value[1]		
Item	10 Cu	100 Cu	10 Cu	100 Cu	SEM	L	Cu	L × Cu
Total gas, mL	394.42	383.98	391.01	394.45	24.99	0.65	0.66	0.38
H_2S, µg/mL	84.48	132.95	159.75	145.28	42.17	0.07	0.47	0.18
H_2S, mL	0.042	0.068	0.078	0.077	0.03	0.05	0.25	0.24
CH_4, %	10.38	10.90	10.37	10.37	0.77	0.36	0.37	0.38
CH_4, mL	42.97	44.90	43.93	43.57	5.42	0.92	0.69	0.56
CO_2, %	62.18	67.55	65.94	67.61	2.08	0.25	0.04	0.27
CO_2, mL	264.4	282.8	282.4	288.5	23.84	0.18	0.16	0.48

[1]L: effect of Linpro; Cu: effect of Cu level; L × Cu: Interaction between Linpro and Cu level.

Chapter 3 - Protection of polyunsaturated fatty acids against ruminal biohydrogenation: pilot experiments for three approaches[3]

C. A. Alvarado,* C. C. Aperce,* K. A. Miller,* C. L. Van Bibber-Krueger,* D. Klamfoth,† and J. S. Drouillard*[4]

*Department of Animal Sciences and Industry, Kansas State University, Manhattan, KS 66506; and †Lhoist North America, Fort Worth, TX 76107.

[3] Contribution number 14-337-J from the Kansas Agricultural Experiment Station, Manhattan, KS.

[4] Corresponding author: jdrouill@ksu.edu.

ABSTRACT

Three studies were conducted to evaluate different methods for protection of PUFA against biohydrogenation by ruminal microorganisms. In study 1, 60 crossbred yearling steers (BW = 475 ± 55 kg) were used in a randomized complete block design experiment. Steers were fed for 12 d with a diet consisting of 48.73% steam-flaked corn, 35% wet corn gluten feed, 12% corn silage, and 4.27% vitamins and minerals (Control). For the other 4 treatments, a portion of wet corn gluten feed was replaced with 5% of an unprocessed mixture of ground flaxseed and calcium oxide, an extruded blend of flaxseed and calcium oxide, an unprocessed mixture of flaxseed and soybean meal, or an extruded blend of soybean meal and flaxseed pre-treated with live yeast and water. Animals were weighed and blood samples taken from the jugular vein for analysis of long-chain fatty acid (LCFA) concentrations on d 0 and 12 of the study. Co-extrusion of flaxseed with soybean meal or lime failed to improve resistance of PUFA against biohydrogenation compared with unprocessed controls ($P > 0.1$). In study 2, in situ DM disappearance (ISDMD) and in situ fatty acid disappearance were evaluated for ground flaxseed (Flaxseed) or ground flaxseed embedded within a protective matrix of dolomitic lime hydrate (L-Flaxseed) using 6 ruminally fistulated Holstein steers, 3 of which were fed a 50:50 forage:concentrate diet, and 3 of which were fed a 90:10 concentrate:forage diet. The proportion of α-linolenic acid that was resistant to ruminal biohydrogenation was approximately 2-fold greater for L-Flaxseed than for Flaxseed ($P < 0.05$). In study 3, 45 steers (269 ± 19.5 kg initial BW) were used in a randomized complete block design. Steers were fed diets containing 0% Flaxseed (No Flaxseed), and in treatments 2 and 3, a portion of flaked corn was replaced with Flaxseed or L-Flaxseed. Animals were weighed and blood samples were taken on days 0, 7, and 14 of the study and analyzed similarly to study 1. The third approach, the use of L-Flaxseed in

studies 2 and 3, was effective and increased the proportion of polyunsaturated omega-3 fatty acids that reached the small intestine. These results were confirmed in our third study, where plasma concentrations of omega-3 fatty acids in cattle fed embedded flaxseed were more than 4 times the level observed in cattle fed unprotected flaxseed, suggesting the dolomitic lime hydrate was effective as a protective matrix.

Key words: flaxseed, processing methods, omega-3 fatty acids.

INTRODUCTION

Interest in increasing PUFA in human diets is growing because of the linkage between diets high in saturated fats and cardiovascular disorders, obesity, and diabetes. Some unsaturated fatty acids (i.e., conjugated linoleic acid and omega-3 fatty acid) have been reported to have positive effects on health. For example, conjugated linoleic acids (CLA) have shown anti-carcinogen effects, reduction of body fat accretion, and decrease in the development of atherosclerosis (Bauman et al., 2000). Omega-3 fatty acids (*n-3* FA), on the other hand, have been shown to have anti-inflammatory properties, to improve immunity, and to decrease the risk factor of diseases such as diabetes and cancer (Calder, 2001; Palmquist, 2009). Unfortunately, the fatty acids found in beef fat are mostly saturated due to animal diets and extensive biohydrogenation of PUFA in the rumen (Montgomery et al., 2008). Using oilseeds rich in PUFA is a feasible alternative to increasing PUFA content in beef, but it is an inefficient process because more than 90% of *n-3* FA could be biohydrogenated (Scollan et al., 2001; Drouillard et al., 2004). Several methods have been proposed as a protective barrier against biohydrogenation. Heguy et al. (2006) used a protein gel to isolate flaxseed oil, and Ashes et al. (1992) used formaldehyde as a protective method. In both studies, a positive effect was observed, but

applicability of the methods and end users' acceptance could be questioned (Kronberg et al., 2007).

The objective of this work was to evaluate three novel methods of forming a protective barrier for PUFA against rumen biohydrogenation, thus increasing PUFA concentrations in blood.

MATERIAL AND METHODS

Procedures followed in these studies were approved by the Kansas State University Institutional Animal Care and Use Committee protocol no. 2315.

Study 1

Crossbred yearling steers (n = 60; initial BW = 475 ± 55 kg) were stratified by weight, randomly assigned to individual pens in 3 different barns, and pens were assigned to dietary treatments (12 replicates). Diets are presented in Table 3.1. Steers from treatment 1 were fed for 12 d with diets containing steam-flaked corn, wet corn gluten feed, corn silage, vitamins, and minerals. In treatments 2 and 3, a portion of wet corn gluten feed was replaced by a combination of ground flaxseed, calcium oxide, and molasses (93, 2, and 5%, respectively) and fed in the unprocessed or extruded form before feeding. Similarly, for treatments 4 and 5, a portion of wet corn gluten feed was replaced by a blend of ground flaxseed, soybean meal, molasses, and baker's yeast (46.1, 46.1, 6.7, and 1.1%, respectively), which were offered in an unprocessed or processed form. The product in the processed form was moistened and prewarmed (1 h at 55°C), allowing enzymes from yeast to produce reducing sugars, and the preconditioned mixture subsequently was processed through an extruder to promote a non-enzymatic browning reaction with the aim of thereby improving resistance of proteins to ruminal degradation.

Animals were weighed, and blood samples were taken from the jugular vein for analysis of long-chain fatty acid (LCFA) concentrations on d 0 and at the end of the 12-d study. Blood samples were collected in heparinized vacuum tubes (green top, BD, Franklin Lakes, NJ) and immediately placed on ice, centrifuged (3,200 × g for 20 min), and frozen for later analysis. Five hundred microliters of plasma were lyophilized and combined with 1 mL benzene containing 1 mg/mL methyl-C:13 as internal standard and 4 mL BF3-Methanol reagent (Supelco B1252). Tubes were incubated at 60°C for 60 min, then cooled to room temperature. One milliliter of hexane and 4 mL H_2O were added to each tube, vortexed, then centrifuged at 1,000 × g for 5 min. The organic (upper) solvent layer (1 to 2 mL) was then analyzed by gas chromatography (Schimadzu model 17A, Palo Alto, CA) using a Supelco SP-2560 capillary column (100 m × 0.25 mm × 0.20 μm film) and He as the carrier gas at a flow rate of 1.1 mL/min. Initial temperature was 140°C for 4 min, and temperature was increased at a rate of 4°C/min to a final temperature of 240°C.

Study 2

In study 2, *in situ* dry matter disappearance (ISDMD) was performed on ground flaxseed (Flaxseed) or ground flaxseed embedded within a protective matrix of dolomitic lime hydrate (L-Flaxseed) as a protective barrier against biohydrogenation. Six ruminally fistuled steers were used, 3 of which were fed a 50:50 forage:concentrate diet and 3 of which were fed a 90:10 concentrate:forage diet. Briefly, 1 g (in duplicate) of Flaxseed or L-Flaxseed was placed into Dacron bags (10 cm × 20 cm; 50 ± 15 μm pore size; Ankom Technology, Macedon, NY), sealed, and incubated in the rumens of fistulated cattle for 16 h (two empty bags were included as blanks). Bags were then removed from the rumen, rinsed, and dried to determine ISDMD, which was calculated by the difference in weight of Dacron bags before and after rumen incubation and

corrected by blank. Long-chain fatty acid analysis was performed on the feed ingredient before and after incubation, and protection of ALA and other fatty acids was determined by difference.

Study 3

Crossbred yearling steers (n = 45; 269 ± 19.5 kg initial BW) were blocked by weight, randomly assigned to individual pens, and pens were assigned to dietary treatments (15 replicates). Steers were fed diets (Table 3.2) with no flaxseed, 2.79% ground flaxseed (Flaxseed), or 8.13% ground flaxseed embedded within a protective matrix of dolomitic lime hydrate (L-Flaxseed). In diets containing flaxseed, a portion of the steam-flaked corn was replaced with flaxseed or L-Flaxseed, and corn oil was included to make diets isolipidic. These inclusion levels were determined on the basis of preliminary analyses of L-Flaxseed and flaxseed, which were determined to have ether extract contents of 11.66 and 40.80%, respectively (DM basis). Subsequent analyses revealed, however, that ether extraction failed to recover a substantial portion of lipid within L-Flaxseed, and recoveries were improved (17.2%) using chloroform/methanol extraction (Nelson, 1991, Table 3.3).

Weights of unconsumed feed (orts) were determined daily. Animals were weighed, and blood samples were taken from the jugular vein for analysis of LCFA concentrations on d 0, 7, and 14 of the study using heparinized vacuum tubes (green top, BD, Franklin Lakes, NJ). Samples were immediately placed on ice for approximately 15 min and centrifuged (3200 × g for 20 min). Then plasma was removed, placed into plastic vials, and frozen at -20°C for later analysis. The LCFA analyses were conducted by gas chromatography as described for study 1.

Statistical analyses

The three studies were statistically analyzed using the MIXED procedure of SAS (Version 9.1, SAS Inst. Inc., Cary, NC). In study 1, treatments and day were the fixed effects, barn nested within strata and barn were used as random effects, and animal was the experimental unit. The two days of sampling were included in the model as repeated measures. Three contrasts were used to compare No Flaxseed vs. other treatments (C_1), unprocessed flaxseed/lime vs. processed flaxseed/lime (C_2), and unprocessed flaxseed/soybean meal vs. processed flaxseed/soybean meal (C_3). In study 2, treatment was the fixed effect, animal the random effect, and bag the experimental unit. In study 3, treatment and day were the fixed effects, feeding barn nested within strata and barn served as random effects, and animal was the experimental unit. Differences were determined using the PDiff option of the LS Means statement of SAS, and a *P*-value < 0.05 was considered to be significant.

RESULTS

Study 1

The plasma LCFA concentrations on d 12 are presented in Tables 3.4 and 3.5, expressed as μg/ml plasma and as % of total fatty acids, respectively. Contrasts showed no effects in most of the LCFA ($P > 0.05$) expressed as μg/ml of plasma, with the exception of *n-3* and omega-6 (*n-6*) fatty acids. When No Flaxseed was compared with other treatments, α-linolenic acid (C18:3 *n-3*), eicosapentaenoic acid (C20:5 *n-3*), and the total *n-3* increased ($P < 0.01$), and γ-linolenic acid (C18:3 *n-6*) decreased ($P < 0.01$). However, no differences were observed between untreated vs. extruded Flaxseed/lime (C_2, $P > 0.2$) or untreated vs. extruded Flaxseed/soybean meal (C_3, $P > 0.2$).

Long-chain fatty acids expressed as a percentage of total fat had similar results: a significant increase in the proportion of *n-3* FA ($P < 0.01$), and a decrease in C18:3 *n-6* ($P < 0.01$). In addition, other fatty acids were altered when flaxseed was included in the diet: the proportion of 3 saturated fatty acids (C14, C16, and C17), the *n-6* fatty acid C18:3 *n-6*, and total *n-6* decreased ($P < 0.05$), and C22:5 *n-3* increased ($P < 0.05$). No differences were observed between untreated and extruded flaxseed.

Study 2

The ISDMD from Dacron bags are presented in Figure 3.1. After 24 h of incubation, results showed that encapsulating ground flaxseed within a dolomitic hydrate lime matrix improved resistance to ruminal degradation. There was a significant interaction ($P < 0.01$) between diet of the animals (low or high concentrate) and the type of ground flaxseed (non-protected or protected). The ISDMD of L-Flaxseed (protected flaxseed) was unaffected by the basal diet of the fistulated steers (85.6 and 87.7% retention for low and high concentrate diets, respectively; $P > 0.1$), but ISDMD was greater for Flaxseed when the low concentrate diet was fed ($P < 0.01$). Percentage retention of the encapsulated products was greater ($P < 0.01$) compared with their unprotected counterparts (52.1 and 38.6% retention for samples incubated in cattle fed high and low concentrate diets, respectively).

Total fatty acids retained in the residual after *in situ* incubation is presented in Figure 3.2. There was no interaction or basal diet effect ($P > 0.05$); however, the proportion of fatty acids retained in bags throughout the incubation was greater ($P < 0.01$) in the L-Flaxseed samples (average of 74.3%) than in unprotected flaxseed (average of 33.6%). This same protective effect was extended to the main *n-3* fatty acid, C18:3 *n-3* (Figure 3.3), which was higher ($P < 0.01$)

when flaxseed was presented within the protective lime matrix (65.4% retained) compared with unprotected flaxseed (30.3% retained).

Study 3

Feedlot performances presented in Table 3.6 revealed no effect of treatments ($P > 0.15$). Average daily gain was 0.956, 0.937, and 0.661 kg, daily DMI was 6.48, 6.27, and 6.08 kg, and G:F was 0.148, 0.144, and 0.106 for No Flaxseed, Flaxseed, and L-Flaxseed treatments, respectively. Table 3.7 summarizes the concentrations of major LCFA in blood plasma on d 0, 7, and 14 after feeding the experimental diets. Day 0 values represent the baseline and thus are similar for all treatment groups, with relatively low content of ALA (an average of 14.32 µg/mL of plasma). After 7 d of feeding the experimental diets, concentrations of ALA remained relatively low in the No Flaxseed group (15.09 µg/mL of plasma) but increased markedly in blood plasma of cattle fed flaxseed (30.66 µg/mL of plasma). The increase in plasma concentrations of ALA was even more dramatic in cattle fed the dolomitic hydrate-flaxseed mixture (66.94 µg/mL of plasma). Plasma concentrations of *n-3* FA in cattle fed the embedded flaxseed were more than 4 times the level observed in cattle fed no flaxseed after 7 d, suggesting the dolomitic lime hydrate was effective as a protective matrix. The differences among treatments remained after 14 d. Plasma ALA was 1.46-fold greater ($P < 0.01$) for L-flaxseed than for Flaxseed after 14 d (16, 35, and 85 µg/mL for No Flaxseed, Flaxseed, and L-Flaxseed, respectively).

DISCUSSION

Our main goal in these three pilot studies was to find a simple and feasible method to protect *n-3* FA against the action of rumen microorganisms. Ground flaxseed was used as our

positive control and plasma concentration ALA as our main biological indicator. The results of our studies confirmed the previously reported capacity of ground flaxseed to increase the plasma concentration of ALA (Drouillard et al., 2004), demonstrating that it is feasible to increase tissue concentrations of *n-3* FA by incorporating high-*n-3* ingredients into an animal's diet.

Using PUFA sources unprotected against rumen microorganism biohydrogenation is an inefficient process (Scollan et al., 2001), and neither extrusion of flaxseed blended with lime nor extrusion of ground flaxseed with bypass protein used in our first experiment were able to protect *n-3* FA; plasma concentration of ALA and other PUFA did not differ from the positive control (unprotected). Our hypothesis was the extrusion of flaxseed blended with lime could possibly form calcium salts and protect PUFA against biohydrogenation, but it was not effective. Calcium salt formed between a PUFA and a calcium ion alter the properties of those fatty acids, making them more similar to saturated fatty acids and less sensitive to rumen biohydrogenation. Wu et al. (1991) showed that hydrogenation of C18:1 could be decreased by 30% when Ca salts were used as a protective method. The rumen pH, however, is negatively related to the stability of Ca salts in the rumen, which means if the pH is too low, the calcium salt loses its protective capacity (the average pKa of calcium salts is about 4.5, and it is higher for unsaturated fatty acids); rumen pH of feedlot cattle could be low enough to dissociate an important proportion of the Ca salts (Palmquist et al., 1986).

Extrusion of ground flaxseed with bypass protein provided by soybean meal was also ineffective. Our hypothesis was that heat applied during the extrusion process of flaxseed, molasses, and soybean meal could be high enough for the formation of Maillard reaction products. During Maillard reactions, amino acids, peptides, or proteins react with reducing sugars in a non-enzymatic reaction, forming products resistant to rumen degradation (Can et al.,

2011). Augustin et al. (2006) demonstrated that microencapsulation of fish oil with a carbohydrate-protein mixture that was heated to produce Maillard reaction products confer additional protection to PUFA against oxidation. Microencapsulation is not a common or practical process used in the feedlot industry, but extrusion could have been an alternative. No positive effects of extrusion were observed in our study, however, and additional research is necessary to evaluate the effectiveness of our extrusion process to produce Maillard products and increase fat bypass.

The third approach, embedding ground flaxseed into a matrix of dolomitic lime hydrate, which was used in studies 2 and 3, was effective and increased the proportion of polyunsaturated *n-3* FA that were not biohydrogenated. In our *in vitro* study, the proportion of total fatty acid and ALA that escaped ruminal degradation was about 2-fold greater for protected than for unprotected flaxseed. These results were confirmed in our third study, in which plasma concentrations of *n-3* FA in cattle fed lime-encapsulated flaxseed were 3.3- to 3.7-fold greater than those of cattle fed unprotected flaxseed, suggesting the dolomitic lime hydrate was effective as a protective matrix. This increase is attributable, in part, to differences in lipid content of the diets, but even taking differences in lipid content of diets into consideration there is a greater increase in plasma concentrations of *n-3* FA when flax is embedded within the lime matrix. Scislowski et al. (2005) infused flax oil directly into the abomasum (bypassing the rumen activity of microorganisms) and reported a 5.7-fold improvement in ALA concentration within plasma.

Despite the efficiency of the dolomitic lime hydrate matrix to protect *n-3* against biohydrogenation, its effect on growth performance remains to be fully evaluated. The short-term experiments reported herein were not designed for this purpose, nor were they adequate for

evaluating effects on feed intake and growth characteristics. Further validation of the impact of the lime-encapsulation process on PUFA tissue concentrations and cattle performance is therefore recommended.

IMPLICATIONS

The processing strategies in our first study, extrusion of flaxseed blended with lime and extrusion of ground flaxseed with bypass protein, did not improve efficiency of *n-3* fatty acid utilization. Studies 2 and 3 revealed that a matrix consisting of dolomitic lime hydrate is an effective barrier to ruminal biohydrogenation of unsaturated fats. This technology may also have application for protecting a broader range of nutrients that are otherwise susceptible to premature degradation by ruminal microbes.

LITERATURE CITED

Ashes, J. R., B. D. Siebert, S. K. Gulati, A. Z. Cuthbertson, and T. W. Scott. 1992. Incorporation of *n-3* fatty acids of fish oil into tissue and serum lipids of ruminants. Lipids 27:629–631.

Augustin, M. A., L. Sanguansri, and O. Bode. 2006. Maillard reaction products as encapsulants for fish oil powders. J. Food Sci. 71(2):E25–E32.

Bauman, D. E., L. H. Baumgard, B. A. Corl, and J. M. Griinari. 2000. Biosynthesis of conjugated linoleic acid in ruminants. J. Anim. Sci. 77(E-Suppl):1–15.

Calder, P.C. 2001. Polyunsaturated fatty acids, inflammation, and immunity. Lipids 36(9):1007–1024.

Can, A., J. Hummel, N. Denek, and K.-H. Südekum. 2011. Effects of non-enzymatic browning reaction intensity on in vitro ruminal protein degradation and intestinal protein digestion of soybean and cottonseed meals. Anim. Feed Sci. Technol. 163(2–4):255–259.

Drouillard, J. S., M. A. Seyfert, E. J. Good, E. R. Loe, B. Depenbusch, and R. Daubert. 2004. Flaxseed for finishing beef cattle: Effects on animal performance, carcass quality, and meat composition. In: Proc. 60th Flax Institute. Fargo, N.D. p. 108–117.

Heguy, J. M., S. O. Juchem, E. J. DePeters, M. Rosenberg, J. E. P. Santos, and S. J. Taylor. 2006. Whey protein gel composites of soybean and linseed oils as a dietary method to modify the unsaturated fatty acid composition of milk lipids. Anim. Feed. Sci. Tech. 131:370–388.

Kronberg, S. L., E. J. Scholljegerdes, G. Barceló-Coblijn, and E. J. Murphy. 2007. Flaxseed treatments to reduce biohydrogenation of α-linolenic acid by rumen microbes in cattle. Lipids 42(12):1105–1111.

Montgomery, S. P., J. S. Drouillard, T. G. Nagaraja, E. C. Titgemeyer, and J. J. Sindt. 2008. Effects of supplemental fat source on nutrient digestion and ruminal fermentation in steers. J. Anim. Sci. 86:640–650.

Nelson, G. J. 1991. Isolation and purification of lipids from biological matrices. Pages 20–59 in Analyses of Fats, Oils and Lipoproteins. E. G. Perkins, ed. Am. Oil Chem. Soc., Champaign, IL.

Palmquist, D. L. 2009. Omega-3 fatty acids in metabolism, health, and nutrition and for modified animal product foods. Prof. Anim. Sci. 25(3):207–249.

Palmquist, D. L., T. C. Jenkins, A. E. Joyner Jr. 1986. Effect of dietary fat and calcium source on insoluble soap formation in the rumen. J. Dairy Sci. 69(4):1020–1025.

Scislowski V., D. Bauchart, D. Gruffat, P. M. Laplaud, and D. Durand. 2005. Effects of dietary *n-6* or *n-3* polyunsaturated fatty acids protected or not protected against ruminal hydrogenation on plasma lipids and their susceptibility to peroxidation in fattening steers. J. Anim. Sci. 83:2162–2174.

Scollan, N. D., M. S. Dhanoa, N. J. Choi, W. J. Maeng, M. Enser, and J. D. Wood. 2001. Biohydrogenation and digestion of long chain fatty acids in steers fed on different sources of lipid. J. Agr. Sci. 136:345–355.

Wu, Z., O. A. Ohajuruka, and D. L. Palmquist. 1991. Rumen synthesis, biohydrogenation and digestibility of fatty acids by dairy cows. J. Dairy Sci. 74:3025–3034.

Table 3.1: Composition of experimental diets with no flaxseed or blends of ground flaxseed with calcium hydroxide or soybean meal in the untreated and extruded forms.

Item, % of dry matter	Control	Flaxseed/lime[1]		Flaxseed/soybean meal[2]	
		Untreated	Extruded	Untreated	Extruded
Ingredients					
Steam-flaked corn	48.7	48.7	48.7	48.7	48.7
Wet corn gluten feed	35.0	29.9	29.9	24.4	24.4
Corn silage	12.0	12.0	12.0	12.0	12.0
Flaxseed	-	5.0	5.0	5.0	5.0
Lime	-	0.1	0.1	-	-
Soybean meal	-	-	-	5.0	5.0
Molasses	-	0.3	0.3	0.7	0.7
Supplement[3]	4.3	4.1	4.1	4.2	4.2
Composition					
DM	64.94	66.25	66.43	67.05	67.72
CP	14.17	14.00	14.00	14.76	14.76
Ether extract	3.60	5.25	5.25	4.93	4.93
NDF	20.39	19.26	19.26	17.67	17.67
Ca	0.72	0.70	0.70	0.70	0.70
P	0.52	0.50	0.50	0.47	0.47
K	0.71	0.70	0.70	0.75	0.75

[1]For the flaxseed/lime combination, ground flaxseed was blended with calcium oxide and molasses and fed in the untreated form or extruded before feeding.

[2]For the flaxseed/soybean meal combination, ground flaxseed was blended with molasses and baker's yeast (*Saccaromyces cerevisea*) and fed in the untreated or extruded form. The extruded product was previously moistened and prewarmed, allowing enzymes from yeast to produce reducing sugars. Product was subsequently processed through an extruder to promote non-enzymatic browning, thereby improving resistance of proteins to ruminal degradation.

[3]Formulated to provide 300 mg/d monensin (Rumencin, Elanco Animal Health, Greenfield, IN), 2,200 IU/kg vitamin A, 22 IU/kg vitamin E, 0.3% salt, 0.7% Ca, 0.7% K, 0.1 mg/kg added Co, 10 mg/kg added Cu, 0.6 mg/kg added I, 60 mg/kg added Mn, 0.25 mg/kg added Se, and 60 mg/kg added Zn in the total diet on a 100% DM basis.

Table 3.2: Composition of experimental diets without flaxseed, with ground flaxseed, or ground flaxseed embedded within a protective matrix of dolomitic lime hydrate (L-Flaxseed) used in study 3

Item, % of dry matter	No Flaxseed	Flaxseed	L-Flaxseed
Ingredients			
Wet corn gluten feed	30.00	30.00	30.00
Wheat straw	25.00	25.00	25.00
Prairie hay	25.00	25.00	25.00
Steam-flaked corn	12.78	12.86	8.50
Linseed meal	3.01	1.22	1.51
Corn oil	1.19	0.10	--
Flaxseed	--	2.79	--
Lime/flaxseed	--	--	8.13
Supplement[1]	3.02	3.03	1.87
Composition			
DM	77.04	77.13	77.56
CP	12.00	12.00	11.99
Ether extract	3.81	3.81	3.81
NDF	49.81	49.73	49.05
Ca	0.70	0.70	1.48
P	0.41	0.41	0.38
K	1.06	1.05	1.01

[1]Formulated to provide 300 mg/d monensin (Elanco Animal Health, Greenfield, IN); 2,200 IU/kg vitamin A, 22 IU/kg vitamin E, 0.3% salt, 0.7% K, 0.1 mg/kg added Co, 10 mg/kg added Cu, 0.6 mg/kg added I, 60 mg/kg added Mn, 0.25 mg/kg [1]added Se, and 60 mg/kg added Zn on a DM basis.

Table 3.3: Total lipid content (chloroform/methanol extraction) and fatty acid composition of flaxseed and L-Flaxseed used in experiments 2 and 3.

Item	Flaxseed	L-Flaxseed
Total fat, % of DM	37.8	17.2
Fatty acids, % of total fatty acids		
C15:0	0.1	0.2
C16:0	5.8	8.4
C18:0	3.5	4.7
C18:1	20.1	25.8
C18:2	16.2	16.3
C18:3 *n-3*	54.2	43.3
C20:0	0.1	0.2
C22:0	0.1	0.3
C22:1	0.1	0.6
C22:2	0.1	0.1
C24:0	0.1	0.1

Table 3.4: LCFA profiles (μg/mL of plasma) of steers after feeding experimental diets without flaxseed, or with untreated and extruded blends of flaxseed with lime or soybean meal (study 1).

Fatty acids	No Flaxseed	Flaxseed/lime[1]		Flaxseed/soybean meal[2]		SEM	Contrasts[3]		
		Untreated	Extruded	Untreated	Extruded		C_1	C_2	C_3
C14:0	8.46	8.57	8.77	8.06	8.27	0.608	0.94	0.79	0.78
C14:1	3.18	2.99	3.59	4.28	2.81	0.947	0.81	0.62	0.25
C15:0	8.56	7.56	7.94	7.92	7.65	0.533	0.11	0.54	0.66
C15:1	2.17	3.23	3.36	2.37	2.60	0.837	0.45	0.92	0.84
C16:0	136.3	132.4	133.6	126.5	128.8	7.57	0.40	0.89	0.80
C16:1	9.13	7.94	8.69	8.97	8.21	0.618	0.27	0.34	0.33
C17:0	12.06	11.21	11.91	11.55	11.48	0.680	0.45	0.42	0.94
C18:0	204.7	214.9	217.3	212.2	211.2	12.9	0.44	0.87	0.95
C18:1 *trans-9*	4.05	5.36	5.20	3.32	4.84	0.718	0.44	0.88	0.14
C18:1 *trans*-8	9.93	11.08	13.18	7.51	13.00	1.39	0.42	0.29	0.01
C18:1 *trans-7*	5.41	7.46	6.83	4.93	6.87	0.602	0.10	0.46	0.03
C18:1 *cis*-9	78.14	75.47	80.53	74.74	75.04	4.50	0.71	0.37	0.96
C18:1 *cis*-7	9.45	9.34	9.25	8.49	9.73	0.504	0.65	0.90	0.08
C18:2 *cis-9,cis12*	640.9	644.9	638.7	626.8	663.3	35.87	0.95	0.90	0.46
C20:0	2.76	3.50	3.82	3.79	3.30	1.102	0.48	0.83	0.75
C18:3 *n-6*	7.39	4.51	4.76	5.00	4.80	0.828	0.00	0.83	0.86
C18:3 *n-3*	18.56	88.83	85.48	92.15	95.11	3.30	<0.01	0.46	0.52
C18:2 *cis*-9,*trans*-11	5.95	6.56	6.13	4.62	5.03	1.27	0.78	0.79	0.80
C21:0	2.66	3.35	2.77	3.75	2.95	1.06	0.63	0.69	0.58
C20:2	0.12	0.18	0.26	0.31	0.17	0.162	0.56	0.71	0.55
C22:0	3.43	4.05	4.27	4.77	3.37	0.91	0.48	0.86	0.26
C20:3 *n-6*	18.56	14.01	15.32	16.26	14.79	1.73	0.05	0.56	0.51
C20:4 *n-6*	28.60	28.72	28.67	27.50	25.93	1.99	0.61	0.98	0.48
C22:2	0.00	0.43	0.00	0.38	1.04	0.414	0.32	0.47	0.26
C24:0	1.59	2.04	2.01	2.57	1.57	0.633	0.51	0.97	0.26
C20:5 *n-3*	6.04	7.30	7.94	7.75	7.28	0.725	0.01	0.36	0.51
C22:5 *n-3*	14.99	15.47	14.89	15.18	13.73	1.07	0.86	0.62	0.22

C22:6 *n-3*	5.73	4.43	5.27	4.90	4.37	0.763	0.11	0.28	0.50
Total *n-3*	45.3	116.0	113.6	120.0	121.1	4.59	<0.01	0.68	0.84
Total *n-6*	695.5	692.2	687.5	675.5	708.8	37.97	0.91	0.93	0.52
n-6:*n-3*	15.98	5.95	6.10	5.64	5.90	0.780	<0.01	0.88	0.80
SFA	380.5	387.6	392.4	381.1	378.6	22.38	0.84	0.86	0.93
MUFA	121.5	122.9	130.6	114.6	123.7	6.730	0.83	0.39	0.31
PUFA	746.9	815.4	807.5	800.8	836.2	41.39	0.12	0.89	0.52
Total fatty acids	1,249	1,326	1,331	1,297	1,339	60.51	0.23	0.95	0.59

[1]For the flaxseed/lime combination, ground flaxseed was blended with calcium oxide and molasses and fed in the untreated form or extruded before feeding.

[2]For the flaxseed/soybean meal combination, ground flaxseed was blended with molasses and baker's yeast (*Saccaromyces cerevisea*) and fed in the untreated or extruded form. The extruded product was previously moistened and prewarmed, allowing enzymes from yeast to produce reducing sugars. Product was subsequently processed through an extruder to promote non-enzymatic browning, thereby improving resistance of proteins to ruminal degradation.

[3]Orthogonal contrasts. C_1 = no flaxseed vs. all others; C_2: untreated vs. extruded flaxseed/lime; C_3: untreated vs. extruded flaxseed/soybean meal.

Table 3.5: Long-chain fatty acid profile (% of total fatty acids) of blood plasma from steers after feeding experimental diets without , or with untreated and extruded blends of flaxseed with lime or soybean meal (study 1).

Fatty acids, %	No Flaxseed	Flaxseed/lime[1]		Flaxseed/soybean meal[2]		SEM	Contrasts[3]		
		Untreated	Extruded	Untreated	Extruded		C_1	C_2	C_3
C14:0	0.68	0.66	0.66	0.62	0.63	0.041	0.41	1.00	0.86
C14:1	0.26	0.23	0.28	0.33	0.20	0.081	0.95	0.70	0.24
C15:0	0.70	0.57	0.60	0.61	0.57	0.029	0.00	0.54	0.34
C15:1	0.16	0.24	0.25	0.18	0.18	0.061	0.40	0.85	0.96
C16:0	10.95	10.16	10.08	9.78	9.74	0.353	0.01	0.88	0.94
C16:1	0.74	0.61	0.66	0.69	0.62	0.045	0.07	0.44	0.26
C17:0	0.98	0.84	0.89	0.89	0.86	0.036	0.01	0.29	0.46
C18:0	16.35	16.34	16.30	16.41	15.85	0.530	0.83	0.95	0.46
C18:1 *trans-9*	0.33	0.42	0.40	0.25	0.35	0.065	0.69	0.83	0.28
C18:1 *trans*-8	0.81	0.83	1.01	0.58	0.95	0.115	0.79	0.29	0.03
C18:1 *trans-7*	0.43	0.57	0.53	0.39	0.51	0.056	0.23	0.53	0.08
C18:1 *cis*-9	6.27	5.82	6.11	5.77	5.69	0.317	0.23	0.52	0.85
C18:1 *cis-7*	0.77	0.71	0.70	0.66	0.73	0.037	0.11	0.80	0.15
C18:2 *cis-9,cis-12*	51.37	48.11	47.91	48.28	49.32	1.349	0.05	0.92	0.59
C20:0	0.21	0.27	0.29	0.29	0.24	0.092	0.54	0.85	0.71
C18:3 *n-6*	0.58	0.33	0.35	0.38	0.36	0.064	0.00	0.87	0.84
C18:3 *n-3*	1.47	6.70	6.47	7.11	7.11	0.162	<0.01	0.32	1.00
C18:2 *cis*-9,*trans*-11	0.49	0.51	0.47	0.36	0.37	0.108	0.56	0.81	0.91
C21:0	0.21	0.26	0.21	0.29	0.22	0.088	0.69	0.64	0.51
C20:2	0.01	0.01	0.02	0.02	0.01	0.011	0.76	0.68	0.45
C22:0	0.27	0.31	0.32	0.37	0.25	0.077	0.58	0.90	0.24
C20:3 *n-6*	1.46	1.05	1.12	1.25	1.11	0.116	0.01	0.65	0.41
C20:4 *n-6*	2.26	2.18	2.14	2.12	1.94	0.104	0.12	0.76	0.17
C22:2	0.00	0.03	0.00	0.03	0.07	0.033	0.40	0.58	0.39
C24:0	0.12	0.16	0.15	0.20	0.11	0.053	0.60	0.88	0.23
C20:5 *n-3*	0.47	0.55	0.59	0.60	0.55	0.053	0.03	0.47	0.38
C22:6 *n-3*	0.46	0.33	0.38	0.38	0.32	0.064	0.23	0.41	0.09

C22:5 *n-3*	1.20	1.17	1.10	1.17	1.03	0.060	0.03	0.45	0.36
Total *n-3*	3.60	8.76	8.55	9.26	9.05	0.210	<0.01	0.48	0.47
Total *n-6*	55.66	51.68	51.52	52.03	52.74	1.301	0.01	0.94	0.70
n-6:*n-3*	15.98	5.95	6.10	5.64	5.90	0.780	<0.01	0.88	0.80
SFA	30.48	29.58	29.51	29.46	28.47	0.907	0.23	0.95	0.44
MUFA	9.75	9.44	9.93	8.84	9.29	0.467	0.47	0.46	0.51
PUFA	59.77	60.98	60.56	61.70	62.24	1.250	0.254	0.82	0.76

For the flaxseed/lime combination, ground flaxseed was blended with calcium oxide and molasses and fed in the untreated form or extruded before feeding.

[2]For the flaxseed/soybean meal combination, ground flaxseed was blended with molasses and baker's yeast (*Saccaromyces cerevisea*) and fed in the untreated or extruded form. The extruded product was previously moistened and prewarmed, allowing enzymes from yeast to produce reducing sugars. Product was subsequently processed through an extruder to promote non-enzymatic browning, thereby improving resistance of proteins to ruminal degradation.

[3]Orthogonal contrast of interest: C_1: control vs. others; C_2: untreated vs. extruded flaxseed/lime; C_3: untreated vs. extruded flaxseed/soybean meal.

Table 3.6: Feedlot performance of cattle fed diets without flaxseed (No Flaxseed), with ground flaxseed (Flaxseed), or with ground flaxseed embedded within a protective matrix of dolomitic lime hydrate (L-Flaxseed)

	No Flaxseed	Flaxseed	L-Flaxseed	SEM	*P*-value
Initial BW	268.4	268.3	270.7	11.8	0.722
Final BW	283.7	283.3	281.3	11.9	0.756
Gain, kg/d	0.956	0.937	0.661	0.185	0.166
DMI, kg/d	6.48	6.27	6.08	0.408	0.203
G:F	0.148	0.144	0.106	0.029	0.249

Table 3.7: Concentrations (µg/mL of plasma) of major long-chain fatty acids in blood plasma of cattle fed diets without flaxseed (Control), with ground flaxseed (Flaxseed), or with ground flaxseed embedded within a protective matrix of dolomitic lime hydrate (L-Flaxseed, study 3)

Fatty acid	Day 0			Day 7			Day 14			SEM	*P*-value[1]		
	Control	Flaxseed	L-Flaxseed	Control	Flaxseed	L-Flaxseed	Control	Flaxseed	L-Flaxseed		Day	Trt	Day×Trt
C12:0	1.15	0.26	0.24	0.63	0.14	0.55	0.30	0.33	0.54	0.34	0.82	0.20	0.43
C14:0	5.54	5.97	4.84	5.03	4.73	5.13	5.08	5.65	5.53	0.42	0.09	0.83	0.08
C14:1	4.21^{a,b}	5.1^{a,b}	3.31^{b}	3.73^{b}	4.64^{a,b}	5.03^{a,b}	4.14^{a,b}	4.92^{a,b}	6.03^{a}	0.72	0.17	0.50	0.05
C15:0	5.93^{b}	7.99^{ab}	6.8^{b}	4.94^{b}	7.6^{a,b}	8.79^{a}	6.61^{b}	7.98^{ab}	9.07^{a}	0.56	0.28	0.02	0.46
C15:1	10.52^{b}	6.34^{b}	11.53^{b}	17.02^{a,b}	21.13^{a,b}	29.15^{a}	4.13^{b}	9.89^{b}	24.81^{a,b}	3.72	0.03	0.12	0.62
C16:0	73.14^{c}	76.61^{b,c}	74.66^{b,c}	79.12^{a,b}	78.88^{a,b}	84.44^{a}	76.93^{b}	74.64^{b,c}	88.92^{a}	3.22	0.02	0.01	0.08
C17:0	29.62^{c}	25.01^{c}	29.96^{c}	87.9^{a}	50.78^{b}	78.02^{a,b}	61.1^{a,b}	43.25^{b}	55.56^{b}	5.71	<0.01	0.04	0.45
C17:1	18.80^{a}	14.34^{ab}	20.12^{a}	16.07^{a}	15.94^{a}	8.61^{b}	7.96^{b}	17.98^{a}	12.09^{ab}	2.62	0.03	0.59	0.00
C18:0	120.4^{c}	111.0^{c}	119.4^{c}	163.9^{a,b}	167.8^{a,b}	173.6^{a,b}	176.5^{a,b}	183.8^{a}	185.8^{a}	5.96	<0.01	0.73	0.93
C18:1	73.19^{c}	74.04^{c}	76.38^{c}	94.96^{a,b}	86.5^{b}	81.37^{b,c}	92.21^{a,b}	99.42^{a}	91.63^{a,b}	3.15	<0.01	0.68	0.16
C18:2	271.7^{c}	249.8^{c}	263.2^{c}	310.7^{b}	276.6^{b,c}	316.9^{b}	334.5^{a}	287.3^{b,c}	363.7^{a}	14.3	<0.01	0.01	0.10
C18:3 *n-3*	15.66^{d}	13.63^{d}	13.68^{d}	15.09^{d}	30.66^{c}	66.94^{b}	16.14^{d}	34.74^{c}	85.34^{a}	2.25	<0.01	<0.01	<0.01
C20:5/C22:0	95.50^{bc}	42.68^{c}	84.19^{bc}	315.6^{a}	212.9^{ab}	293.6^{a}	295.8^{a}	282.2^{a}	308.4^{a}	27.0	<0.01	0.24	0.93

[a,b,c,d]Means without a common superscript letter in a raw are different, $P < 0.05$.

[1]*P*-values of d effect, treatment effect (Trt), and interaction day treatment (Day×Trt).

Figure 3.1: Dry matter disappearance from nylon bags after 24 h of ruminal incubation of ground flaxseed (Flaxseed) and ground flaxseed embedded within a protective matrix of dolomitic lime hydrate (L-Flaxseed, study 3). Means without a common superscript letter are different, $P < 0.05$. Effect of basal diet, $P = 0.053$; Effect of protective matrix, $P < 0.001$; interaction effect: $P < 0.01$; SEM = 2.93.

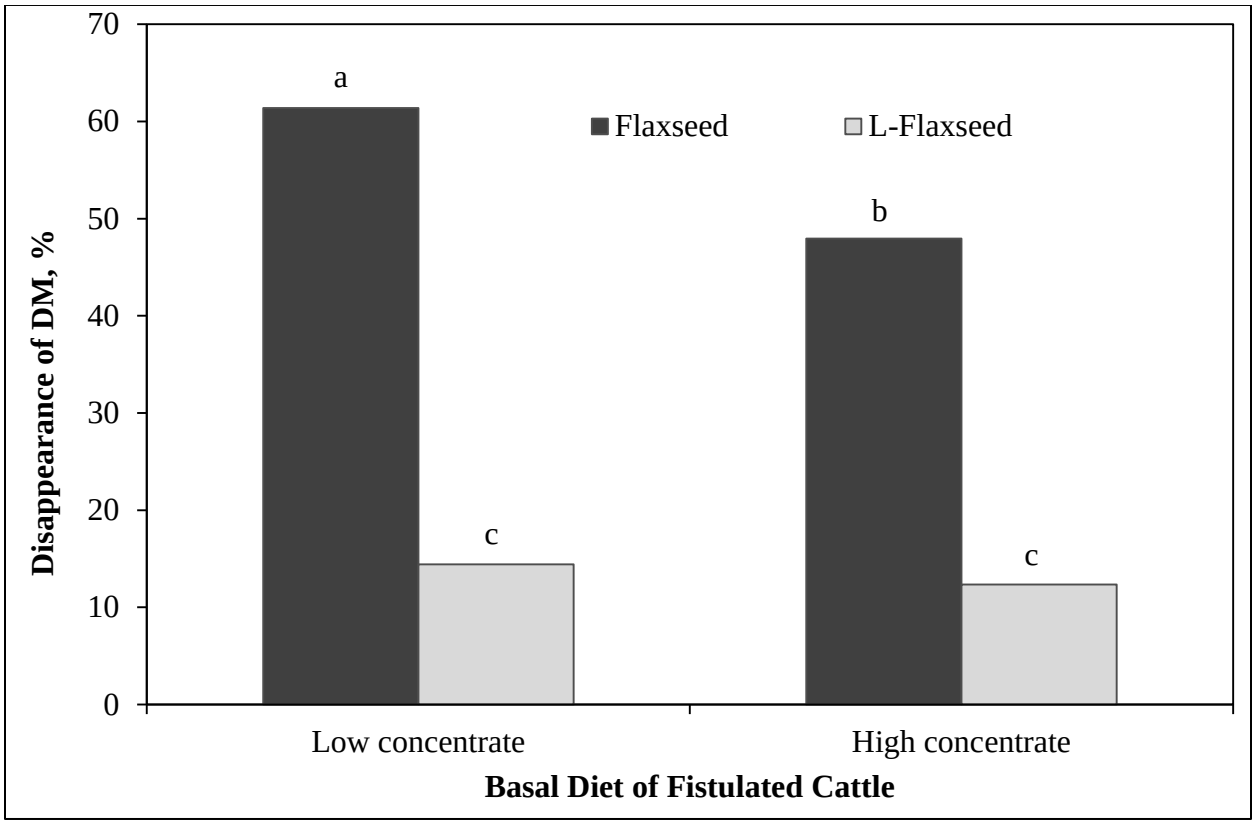


Figure 3.2: Total fatty acids protected from ruminal degradation after 24 h of *in situ* incubation of ground flaxseed (Flaxseed) and ground flaxseed embedded within a protective matrix of dolomitic lime hydrate (L-Flaxseed, study 3). Means without a common superscript letter are different, $P < 0.05$. Effect of basal diet, $P = 0.066$; effect of protective matrix, $P < 0.001$; interaction effect: $P > 0.20$; SEM = 3.27.

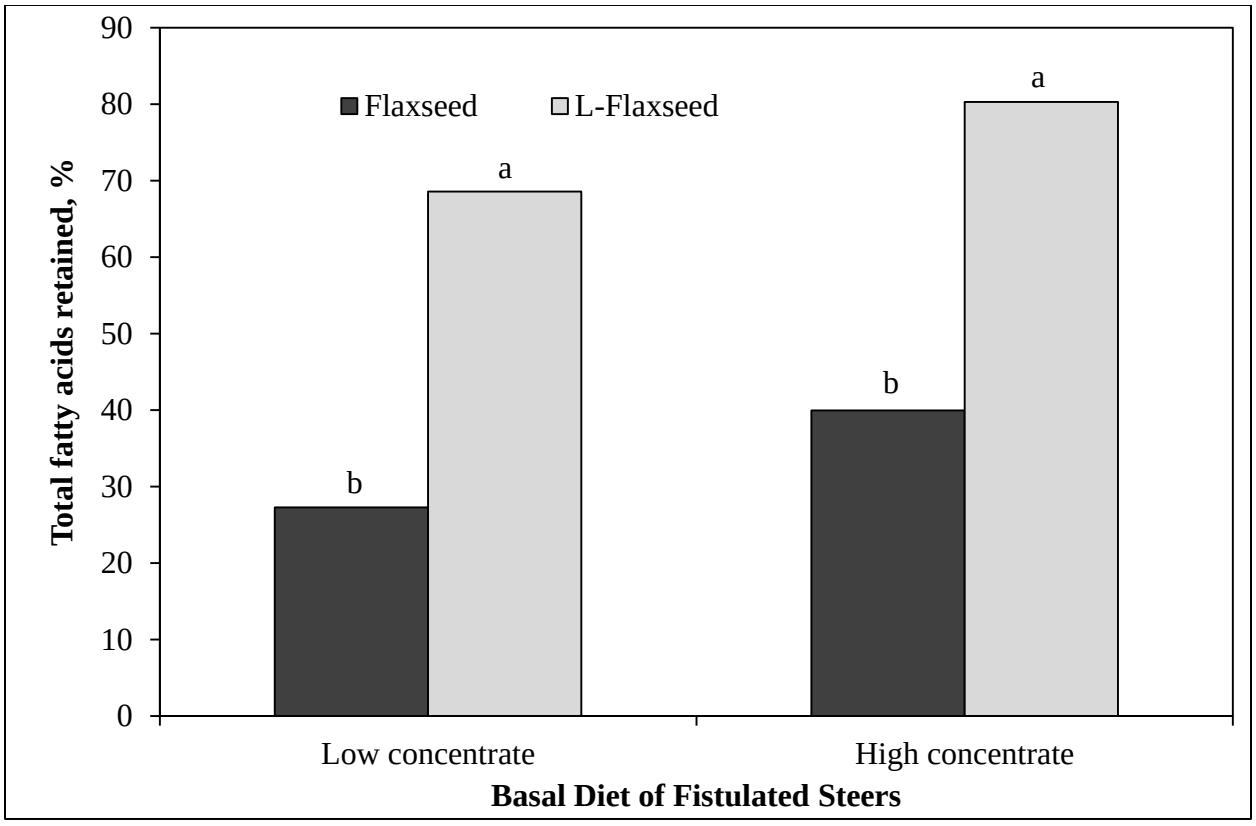
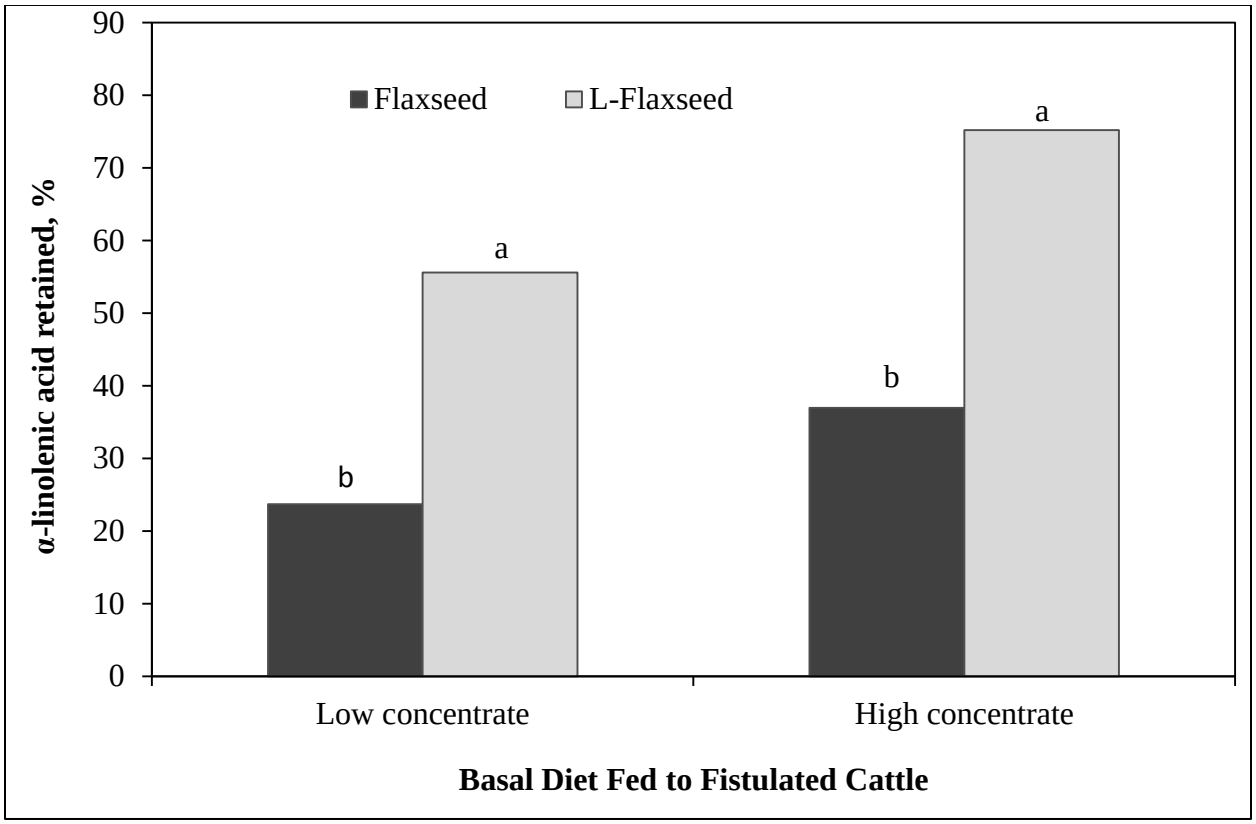


Figure 3.3: α-linoleic acid retained in residues after 24 h of *in situ* incubation of ground flaxseed (Flaxseed) and ground flaxseed embedded within a protective matrix of dolomitic lime hydrate (L-Flaxseed, study 3). Means without a common superscript letter are different, $P < 0.05$. Effect of basal diet, $P > 0.10$; effect of protective matrix, $P < 0.001$; interaction effect: $P > 0.10$; SEM = 5.09.



Chapter 4 - Effects of flaxseed encapsulation on biohydrogenation of polyunsaturated fatty acids by ruminal microorganisms: feedlot performance, carcass quality, and tissue fatty acid composition[5]

C. A. Alvarado,* C. C. Aperce,* K. A. Miller,* C. L. Van Bibber-Krueger,* D. Klamfoth,† and J. S. Drouillard*[6]

*Department of Animal Sciences and Industry, Kansas State University, Manhattan, KS 66506

†Lhoist North America, Fort Worth, TX 76107.

[5] Contribution number 14-338-J from the Kansas Agricultural Experiment Station, Manhattan, KS.

[6] Corresponding author: jdrouill@ksu.edu.

ABSTRACT

The objective of this study was to evaluate the efficacy of protecting PUFA within ground flaxseed (Flaxseed) against ruminal biohydrogenation by encapsulating them in a matrix consisting of a 1:1 blend of ground flaxseed and dolomitic lime hydrate (L-Flaxseed). Crossbred heifers (n = 454, 346±19.9 kg) were blocked by weight and randomly assigned to individual pens, and pens were assigned to 6 dietary treatments (11 replicates) in a randomized complete block design. Treatment 1 consisted of a combination of 54.58% steam-flaked corn, 30.0% wet corn gluten feed, and 8.0% roughage, supplemented with vitamins, minerals, monensin, and tylosin (0% flaxseed). In treatments 2 and 3, a proportion of steam-flaked corn was replaced with 3 and 6% flaxseed, respectively, and in treatments 4, 5, and 6 with 2, 4, or 6% L-Flaxseed, respectively. Cattle were fed once daily *ad libitum* for 154 d, then harvested in a commercial abattoir where carcass data were collected. Approximately 24 h after harvest, carcasses were evaluated for 12th-rib subcutaneous fat thickness, KPH, LM area, marbling score, and USDA yield and quality grades. Samples of *longissimus lumborum* were also obtained for determination of long-chain fatty acid profiles. Data were statistically analyzed using the MIXED procedure of SAS (Version 9.2) with diet as the fixed effect, block as a random effect, and pen as the experimental unit. Cattle that were fed diets with 4 and 6% L-Flaxseed consumed less feed than other treatments ($P < 0.05$), which adversely affected ADG. Compared with cattle fed 0% flaxseed, cattle in these treatments had lower final BW (18.1 and 45.3 kg less for the 4 and 6% L-Flaxseed treatments, respectively), less ADG (0.16 and 0.48 kg/d less for the 4 and 6% L-Flaxseed treatments, respectively), and lower carcass weights, dressing percentages, LM area, backfat thickness, and marbling scores. The addition of flaxseed or 2% L-Flaxseed did not affect performance or carcass traits ($P > 0.05$). Supplementation with flaxseed increased ($P < 0.05$) the

concentration of α-linolenic acid (ALA) in meat (0.173, 0.482, 0.743 mg/g for 0, 3%, and 6% flaxseed, respectively). Furthermore, proportionate increases in the omega-3 fatty acid content of muscle tissue were 47% greater when flaxseed was encapsulated within the dolomitic lime matrix (0.288, 0.433, 0.592 mg/g for 2, 4, and 6% L-Flaxseed, respectively). Both products showed a linear response in ALA concentration (R^2 > 99%; increases for Flaxseed and L-Flaxseed of 0.095 and 0.140 mg of ALA/g of tissue for each percentage of flaxseed added). This study indicates that a matrix consisting of dolomitic lime hydrate is an effective barrier to ruminal biohydrogenation of PUFA; however, adverse effects on DMI limit the amounts that can be fed.

Key words: encapsulation, matrix, omega-3 fatty acids.

INTRODUCTION

Omega-3 fatty acids (*n-3* FA) are essential nutrients for humans, but human dietary intake of these nutrients is often inadequate due to low consumption of *n-3* FA-rich foods such as fish, walnuts, and flaxseed. The *per capita* consumption of red meats is, in contrast, relatively high, but provides only small amounts of *n-3* FA. Feeding cattle diets containing *n-3* FA has consistently increased the proportion of these desirable fats in beef (Drouillard et al., 2004). Unfortunately, the proportions of dietary *n-3* FA that are actually deposited into beef tissues is relatively low, because rumen microorganisms extensively biohydrogenate unsaturated *n-3* FA to the saturated fats characteristic of beef fat (Montgomery et al., 2008). Encapsulation of fats has been proposed as a method for improving transfer efficiency of *n-3* FA into beef tissues. Encapsulation processes consist of applying a protective barrier to the surface of fats or fat-containing feeds, which theoretically decreases their susceptibility to microbial biohydrogenation. Protective coatings must remain intact prior to and in the rumen to retain their

functionality. Physical damage to the coatings during handling will expose the core material to rumen microorganisms and result in poor efficacy. Embedding feed particles within a homogeneous protective matrix constitutes a potentially useful alternative to protective surface barriers. The matrix is created by mixing feed particles that are to be protected with a suitable matrix material that is resistant to microbial digestion and subsequently forming prills with the mixture. In contrast with encapsulation, when the matrix incurs physical damage, exposure of the core material is confined to the broken surface, and the remainder of the matrix retains its ruminal stability.

The objectives of this study were to compare feedlot performance, carcass characteristics, blood plasma fatty acid profiles, and meat lipids of cattle fed traditional finishing diets to those supplemented with ground flaxseed or ground flaxseed that has been co-prilled with dolomitic lime hydrate as a protective matrix.

MATERIAL AND METHODS

Procedures in this study were approved by the Kansas State University Institutional Animal Care and Use Committee protocol no. 2315.

Animals and Diets

Crossbred heifers (n = 454, BW = 345.9±19.9 kg) were blocked by weight and randomly assigned to pens, and pens were assigned to dietary treatments (11 replicates). This experiment included 6 treatment groups in a randomized complete block design. Treatment 1 (0% flaxseed) consisted of animals fed a finishing diet (Table 4.1) containing a combination of steam-flaked corn, wet corn gluten feed, and roughage, supplemented with vitamins A and E, macro minerals (calcium, potassium), inorganic trace minerals (Na, Co, Cu, I, Mn, Se, and Zn), monensin, and

tylosin (Elanco Animal Health; Greenfield, IN). Treatments 2 and 3 included 3 and 6% ground flaxseed, respectively (Flaxseed treatments). In treatments 4, 5, and 6, 2, 4, or 6% of a prilled mixture of ground flaxseed and dolomitic lime hydrate (L-Flaxseed treatments) served as a protective barrier against biohydrogenation and which total fat and fatty acid composition are presented in Table 4.2. Dolomitic lime was blended with flaxseed, water was added, and the mix was subjected to mixing in a high speed turbulizer to form densified prills, which were then dried to a final DM of approximately 98%. Cattle were fed once daily with *ad libitum* access to feed and water. Heifers were implanted (Component TE-200, Zoetis Inc., Florham Park, NJ), dewormed (Dectomax, Zoetis Inc.), and vaccinated against common viral and clostridial diseases (Ultra-Bac 7 and Bovi-Shield Gold, Zoetis Inc.).

Sampling Procedures and Harvest Data Collection

Animals were weighed approximately every 28 d for the duration of the experiment. At the beginning of the experiment and 29 d later, blood samples were collected from the jugular vein of each animal using heparinized vacuum tubes (BD, Franklin Lakes, NJ). Tubes were immediately placed in ice and centrifuged (3,200 × g for 20 min), and plasma was collected and frozen for subsequent analysis of long-chain fatty acids (LCFA) by gas chromatography. Starting 23 d before harvest, zilpaterol hydrochloride (Zilmax, Intervet/Schering-Plough Animal Health, De Soto, KS) was added to the diet for 20 d. The 6 heaviest pens from each treatment were harvested at a commercial abattoir on d 140, and the remaining pens were harvested at the same location on d 168. Hot carcass weight and liver abscesses were collected at slaughter. After a 24-h chill period, carcasses were evaluated for fat thickness over the 12th rib, KPH, LM area, marbling score, and USDA yield and quality grades. Entire loins from one side of each carcass were collected, transported in a refrigerated truck to the Kansas State University Meat

Laboratory, refrigerated overnight at 0 ± 2°C, subsampled the next day, and kept frozen for further analysis.

On weighing day, unconsumed feed remaining in feed bunks was measured for each pen, and DMI was estimated using the as-fed deliveries and actual feedstuff DM values minus the amount of unconsumed DM. Daily gain was calculated as kilograms of gain on a shrunk basis (4%). Feed efficiency was calculated as kilograms of gain per kilogram of DM consumed.

Analyses of Plasma Fatty Acids

One milliliter of plasma was freeze-dried and combined with 1 mL benzene containing methyl tridecanoate as the internal standard (400 μg/mL of benzene, Fluka 91558, Sigma-Aldrich, St. Louis, MO) and 4 mL of a boron triflouride-methanol solution. Tubes were incubated at 60°C for 60 min and cooled to room temperature before the addition of 1 ml hexane and 4 ml H_2O. Finally, tubes were vortexed and centrifuged at 1000 × g for 5 min before the organic solvent layer (1 to 2 mL) was collected to be analyzed via gas chromatography. An Agilent gas chromatograph (model 7890A, Santa Clara, CA) equipped with a HP-88 J&W Agilent GC capillary column (30 m × 0.25 mm × 0.20 μm film) was used for the analysis. The injection temperature was 250°C, the split ratio was 1:100. The flame-ionization detector was set at 280°C, using H (35 mL/min), air (400 mL/min), makeup He (25 mL/min), and He carrier gas at constant flow (0.91 mL/min). The oven temperature program was set as follows: the initial temperature was 80°C, held 1 min, increased 14°C/min to 240°C, and held 3 min. Supelco 37 Component FAME Mix (47885-U Supelco, Sigma-Aldrich) was used as standard.

Muscle Sample Analyses

The LCFA profiles were analyzed according to the procedure of Sukhija and Palmquist (1988). Briefly, 40- to 50-g samples were freeze-dried and ground, and about 0.2 g of dry sample was mixed with 2 mL of benzene containing methyl tridecanoate as the internal standard (2 mg/mL of benzene, Fluka 91558) and 5 mL methanolic-HCl, then flushed with N. Tubes were then capped, vortexed, heated for 2 h at 70°C, and vortexed every 30 min during heating. Tubes were cooled to room temperature, mixed with 5 mL 6% K_2CO_3 and 2 mL benzene, vortexed, and centrifuged at 500 × g for 5 min. The organic solvent layer was then analyzed by gas chromatography as previously described for plasma LCFA.

Statistical analyses

Continuous data (LCFA, growth performance, and carcass characteristics) were analyzed using the MIXED procedure of SAS (version 9.2; SAS Inst. Inc., Cary, NC). Categorical data (USDA quality grade and liver abscesses) were analyzed using the GLIMMIX procedure of SAS. In both models, pens were the experimental units, diet the fixed effect, and block the random effects. Treatment means were separated using the LSMEANS statement and the PDIFF option of SAS. Means were considered different at P-value ≤ 0.05, and a P-value ≤ 0.10 was considered as a tendency.

RESULTS

Long-Chain Fatty Acids in Plasma

The main plasma LCFA are presented in Table 4.3. At the beginning of the experiment (day 0), LCFA were not different among treatments ($P > 0.15$), but after 29 d of feeding dietary treatments, LCFA profiles were altered ($P < 0.05$). The predominant *n-3* FA, α-linolenic acid

(ALA, C18:3 *n-3*), increased from 21.4 µg/mL of plasma with the 0% flaxseed diet to 145.9 and 278.0 µg/mL of plasma when 3 and 6% flaxseed were fed, respectively. Similar responses were observed with the protected flaxseed (L-Flaxseed), where ALA concentrations were 72.3, 138.7, and 208.1 µg/mL of plasma for cattle fed 2, 4, and 6% L-Flaxseed, respectively. Other fatty acids were also affected. The C16:0, C18:0, C18:1 *n-9 cis*, and C18:2 *n-6 cis* increased ($P < 0.01$) when flaxseed was included in the diet, and C18:3 *n-6* decreased ($P < 0.01$).

Feedlot Performance and Carcass Characteristics

Feedlot performance and carcass traits results are reported in Tables 4.4 and 4.5, respectively. Flaxseed treatments and 2% L-Flaxseed did not affect the performance of heifers ($P > 0.05$), and these treatments were not different from the 0% flaxseed diet. Inclusion of 4 and 6% L-Flaxseed had a negative impact on performance. Final shrunk body weights decreased by 11 and 34 kg, respectively, compared with the control, and similar effects were observed with adjusted final BW, which was decreased by 14.5 and 45.7 kg, respectively, compared with the control. In addition, 4 and 6% L-Flaxseed had lower ADG ($P < 0.01$) compared with the control, with 0.07 and 0.21 kg/d decrease, respectively. Dry matter intake of 4 and 6% L-Flaxseed treatments also decreased ($P < 0.01$) compared with the control, by 0.49 and 1.5 kg/d, respectively. Gain-to-feed ratio was unaffected by treatments ($P = 0.72$).

The hot and cold carcass weights were not affected ($P > 0.05$) when flaxseed or 2% L-Flaxseed was added to diet, but carcasses resulting from the addition of 4 or 6% L-Flaxseed were approximately 10 and 30 kg lighter than those fed the 0% flaxseed treatment, and they also had a decreased dressing percentage ($P < 0.01$). The LM area increased ($P < 0.05$) in those animals treated with 6% flaxseed and 2% L-Flaxseed, decreased when 6% L-Flaxseed was included ($P < 0.05$), and stayed the same with other treatments ($P > 0.05$). Backfat thickness was 1.23 cm for

animals fed the 6% L-Flaxseed treatment, which was significantly lower ($P < 0.01$) than those fed other treatments, which were not different from each other (1.54 cm average, $P > 0.05$). Marbling score and yield grade were unaffected by the addition of flaxseed or 2 and 4% L-Flaxseed but were lower ($P < 0.05$) in the 6% L-Flaxseed group. Animals with marbling scores greater than 500 and less than 700, thus qualifying carcasses for the upper two-thirds of the USDA Choice quality grade (Premium Choice), were lower ($P < 0.05$) in the 6% L-Flaxseed group. Other carcass traits were unaffected ($P < 0.05$) by treatment.

Long-Chain Fatty Acids in Loins

Long-chain fatty acid compositions for loin steaks are reported in Tables 4.6 (concentration, mg/g) and 4.7 (as percentage of total fatty acids). The ALA concentration increased ($P < 0.01$) when flaxseed or L-Flaxseed were added to the diets. The addition of 3 and 6% flaxseed resulted in ALA concentrations in meat that were 2.8- and 4.3-fold higher than those in animals fed the control diet, respectively. Addition of 2, 4, and 6% L-Flaxseed increased ALA concentrations by 0.7-, 1.5-, and 2.4-fold, respectively. This positive effect was also reflected when it was expressed as percentage of the total fat ($P < 0.01$). The *Trans* C18:1 *n-9* concentration was increased with the inclusion of L-Flaxseed in the diet ($P = 0.002$). The proportions of *trans* C18:2 *n-6* and C20:2 in total fat were increased with the inclusion of flaxseed and L-Flaxseed in the diet ($P < 0.02$). Finally, the concentration and proportion of C20:0 increased with the inclusion of flaxseed and L-Flaxseed in the diet ($P < 0.01$). Other LCFA concentrations or proportions were not affected ($P > 0.1$).

DISCUSSION

Adipose tissue from feedlot cattle normally contains low concentrations of PUFA and, more specifically, low concentrations of *n-3* FA, compared with adipose tissue from grazing cattle (Rule et al., 2002; Montgomery et al., 2008). Low concentrations are due in part to extensive biohydrogenation of PUFA by ruminal bacteria. Previous experiments have shown that increasing the concentration of PUFA via the addition of flaxseed in the diet is an effective way to increase the plasma *n-3* FA concentrations (Farran et al., 2008; He et al., 2012; Zachut et al., 2012). Unfortunately, using flaxseed without a protective barrier against rumen microorganism biohydrogenation is an inefficient process (Scollan et al., 2001). Using ground flaxseed embedded within a protective matrix of dolomitic lime hydrate in this study yielded an increase of 62.6 μg of ALA/mL of plasma for every 1% of protected flaxseed added to the diet, which was a 46% improvement over results obtained with unprotected ground flaxseed (Figure 4.1). This improvement is far less than the 5.7-fold improvement shown when flaxseed oil is infused directly into the proximal duodenum (thus avoiding biohydrogenation), as was reported by Scislowski et al. (2005). The use of L-Flaxseed may, however, be more practical than using a protein gel to isolated flaxseed oil (Heguy et al., 2006) and more acceptable to consumers than formaldehyde-treated products (Ashes et al., 1992; Kronberg et al., 2007)

Meat sample analyses confirmed our earlier observation in plasma profiles after 29 days on feed. Increasing PUFA via addition of flaxseed in the diet was reflected by higher concentrations of *n-3* FA, particularly ALA, and the rate at which this FA was incorporated into meat tissues was 47% greater when ground flaxseed was protected with dolomitic lime hydrate (Figure 4.2). The ALA concentrations in meat from animals receiving 6% L-Flaxseed in their diets was 23% greater than in animals fed diets containing 3% unprotected flaxseed. It is

important to consider that these two treatments supply equal amounts of flaxseed (3%), which demonstrates the effectiveness of our method (co-prilling ground flaxseed with dolomitic hydrate lime) to protect LCFA against biohydrogenation.

Our first goal in this study was to protect LCFA against the action of microorganisms without adversely affecting animal performance. Previous studies where flaxseed was used as the source of LCFA showed no negative effects on animal performance when finishing diets contained 5% ground flaxseed (Drouillard et al., 2004). One study even reported improvement in feedlot performance when 8% flaxseed was included, reflected by no change in DMI but greater ADG and improved efficiency (Kronberg et al., 2007). In the present study, we observed no adverse effect of unprotected flaxseed (Flaxseed treatments) on feedlot performance but a negative effect when flaxseed was protected with a matrix of dolomitic hydrate lime (L-Flaxseed treatment), specifically with 4 and 6% inclusion rates, which decreased DMI by 0.49 and 1.50 kg/d, respectively. Decreases in DMI resulted in commensurate changes in ADG and final BW of the animals (Table 4.4). The exception was the treatment that included 2% L-Flaxseed, which had no adverse effects on feedlot performance.

The negative effects of 4 and 6% L-Flaxseed treatments were also observed in carcass traits: HCW, dressing percentage, and LM area decreased, presumably due to poorer intake and ADG during the feedlot phase. The addition of 2% L-Flaxseed resulted in carcass measurements comparable to the 0, 3, and 6% flaxseed treatments, which could imply that this level of L-Flaxseed could be a feasible alternative, but unfortunately the improvement in ALA concentration in meat is lower than the concentration obtained with the unprotected flaxseed.

In earlier studies, $Ca(OH)_2$ and $Mg(OH)_2$, which are the primary components of dolomitic lime hydrate, were evaluated as rumen buffers in cows fed high-concentrate/low-

roughage diets (Thomas and Emery, 1969; Thomas et al., 1984) to prevent milk fat depression syndrome in dairy cows. These studies revealed a capacity for the hydrates to buffer the rumen, but a negative effect on voluntary DMI was also observed. More recently, Oddy et al. (2003) compared the effects of $CaCO_3$ and $Ca(OH)_2$ additions to feedlot steer diets on the deposition of intramuscular fat. There were no differences among treatments with respect to fat deposition, but feeding the lime hydrate decreased DMI, ultimately depressing growth rate. These results are in agreement with our observations, although the specific cause is not presently known.

IMPLICATIONS

This study indicates that a matrix consisting of dolomitic lime hydrate is an effective barrier to ruminal biohydrogenation of unsaturated fats. This technology also may have application for protecting a broad range of nutrients that are otherwise susceptible to premature degradation by ruminal microbes. The adverse effects of lime hydrates on feed intake may limit application of the technology to use with ingredients that are fed in relatively small quantities. Further studies are needed to explain why DMI is depressed when hydroxide forms of Mg and Ca are used in cattle feeding without adverse effects.

LITERATURE CITED

Ashes J. R., B. D. Siebert, S. K. Gulati, A. Z. Cuthbertson, and T. W. Scott. 1992. Incorporation of *n-3* fatty acids of fish oil into tissue and serum lipids of ruminants. Lipids 27:629–631.

Corazzin, M., S. Bovolenta, A. Sepulcri, and E. Piasentier. 2012. Effect of whole linseed addition on meat production and quality of Italian Simmental and Holstein young bulls. Meat Sci. 90(1):99–105.

Drouillard, J. S., M. A. Seyfert, E. J. Good, E. R. Loe, B. Depenbusch, and R. Daubert. 2004. Flaxseed for finishing beef cattle: Effects on animal performance, carcass quality, and meat composition. In: Proc. 60th Flax Institute. Fargo, N.D. p. 108–117.

Farran, T. B., C. D. Reinhardt, D. A. Blasi, J. E. Minton, T. H. Elsasser, J. J. Higgins, and J. S. Drouillard. 2008. Source of dietary lipid may modify the immune response in stressed feeder cattle. J. Anim. Sci. 86:1382–1394.

He, M. L., T. A. McAllister, J. P. Kastelic, P. S. Mir, J. L. Aalhus, M. E. R. Dugan, N. Aldai, and J. J. McKinnon. 2012. Feeding flaxseed in grass hay and barley silage diets to beef cows increases alpha-linolenic acid and its biohydrogenation intermediates in subcutaneous fat. J. Anim. Sci. 90:592–604.

Heguy J. M., S. O. Juchem, E. J. DePeters, M. Rosenberg, J. E. P. Santos, and S. J. Taylor. 2006. Whey protein gel composites of soybean and linseed oils as a dietary method to modify the unsaturated fatty acid composition of milk lipids. Anim. Feed. Sci. Tech. 131:370–388.

Kronberg, S. L., E. J. Scholljegerdes, G. Barceló-Coblijn, and E. J. Murphy. 2007. Flaxseed treatments to reduce biohydrogenation of α-linolenic acid by rumen microbes in cattle. Lipids 42(12):1105–1111.

Montgomery, S. P., J. S. Drouillard, T. G. Nagaraja, E. C. Titgemeyer, and J. J. Sindt. 2008. Effects of supplemental fat source on nutrient digestion and ruminal fermentation in steers. J. Anim. Sci. 86:640–650.

Oddy, V. H., S. H. Bird, and L. G. Walker. 2003. Evaluation of calcium and canola oil in the diet of feedlot steers. Aust. J. Exp. Agr. 43(5):459–465.

Rule, D. C., K. S. Broughton, S. M. Shellito, and G. Maiorano. 2002. Comparison of muscle fatty acid profiles and cholesterol concentrations of bison, beef cattle, elk, and chicken. J. Anim. Sci. 80:1202–1211.

Scislowski V., D. Bauchart, D. Gruffat, P. M. Laplaud, and D. Durand. 2005. Effects of dietary *n-6* or *n-3* polyunsaturated fatty acids protected or not protected against ruminal hydrogenation on plasma lipids and their susceptibility to peroxidation in fattening steers. J. Anim. Sci. 83:2162–2174.

Scollan, N. D., M. S. Dhanoa, N. J. Choi, W. J. Maeng, M. Enser, and J. D. Wood. 2001. Biohydrogenation and digestion of long chain fatty acids in steers fed on different sources of lipid. J. Agr. Sci. 136:345–355.

Sukhija, P.S. and D.L. Palmquist. 1988. Rapid method for determination of total fatty acid content and composition of feedstuffs and feces. J. Agric. Food Chem. 36:1202–1206.

Thomas, J. W. and R. S. Emery. 1969. Effects of sodium bicarbonate, magnesium oxide, and calcium hydroxide on milk fat secretion. J. Dairy Sci. 52(1):60–63.

Thomas, J. W., R. S. Emery, J. K. Breaux, and J. S. Liesman. 1984. Response of milking cows fed a high concentrate, low roughage diet plus sodium bicarbonate, magnesium oxide, or magnesium hydroxide. J. Dairy Sci. 67(11):2532-2545.

Zachut, M., A. Arieli, H. Lehrer, L. Livshitz, S. Yakoby, and U. Moallem. 2010. Effects of increased supplementation of *n-3* fatty acids to transition dairy cows on performance and fatty acid profile in plasma, adipose tissue, and milk fat. J. Dairy Sci. 93:5877–5889.

Table 4.1: Composition of experimental diets containing 0, 3, and 6% ground flaxseed (Flaxseed) or 2, 4, and 6% of a 1:1 blend of dolomitic lime hydrate and ground flaxseed formed into a prilled matrix (L-Flaxseed)

Item, % of DM	Flaxseed			L-Flaxseed		
	0%	3%	6%	2%	4%	6%
Ingredients						
Steam-flaked corn	54.58	52.47	50.49	53.47	52.37	51.26
Wet corn gluten feed	30.00	30.00	30.00	30.00	30.00	30.00
Corn silage	5.00	5.00	5.00	5.00	5.00	5.00
Wheat straw	3.00	3.00	3.00	3.00	3.00	3.00
Soybean meal	1.66	0.84	--	1.46	1.26	1.06
Ground flaxseed	--	3.00	6.00	--	--	--
L-Flaxseed	--	--	--	2.00	4.00	6.00
Supplement[1]	5.76	5.69	5.51	5.07	4.37	3.68
Calculated composition						
DM	68.36	68.52	68.57	68.42	68.48	68.54
CP	14.00	14.00	14.00	14.00	14.00	14.00
P	0.48	0.48	0.49	0.47	0.47	0.47
Ca	0.88	0.88	0.88	0.88	0.88	0.88
Total fat	3.59	4.65	5.72	3.87	4.15	4.42
NDF	20.47	20.61	20.75	20.36	20.24	20.13

[1]Formulated to provide 300 mg/d monensin (Elanco Animal Health, Greenfield, IN); 2,200 IU/kg vitamin A; 22 IU/kg vitamin E; 0.3% salt; 0.7% Ca; 0.7% K; 0.1 mg/kg added Co; 10 added mg/kg Cu; 0.6 mg/kg I; 60 mg/kg Mn; 0.25 mg/kg added Se; and 60 mg/kg added Zn in the total diet on a 100% DM basis.

Table 4.2: Total lipid contents (chloroform/methanol extraction) and fatty acid composition of flaxseed and L-Flaxseed

Item	Flaxseed	L-Flaxseed
Total fat, % of DM	37.8	20.1
Fatty acids, % of total fatty acids		
C12:0	0.40	0.21
C15:0	0.02	0.03
C16:0	5.13	6.39
C16:1	0.07	0.09
C17:0	0.06	0.07
C18:0	3.89	4.68
C18:1 *n-9*	20.77	22.72
C18:2 *n-6*	15.55	16.35
C20:0	0.16	0.19
C18:3 *n-3*	53.26	48.32
C20:1	0.15	0.24
C21:0	0.02	0.14
C20:2	0.10	0.07
C22:0	0.18	0.20
C24:0	0.13	0.19

Table 4.3: Fatty acid concentrations in plasma of heifers fed 0, 3, and 6% ground flaxseed (Flaxseed) or 2, 4, and 6% of a 1:1 blend of dolomitic lime hydrate and ground flaxseed formed into a prilled matrix (L-Flaxseed)

	Flaxseed			L-Flaxseed				
Fatty acid, µg/mL	0%	3%	6%	2%	4%	6%	SEM	*P*-value[1]
Day 0								
C16:0	324.9	334.9	319.4	298.5	315.3	316.4	11.92	0.18
C18:0	418.2	440.6	423.9	392.8	423.8	423.1	15.80	0.21
C18:1 *cis-9*	247.9	245.5	252.3	223.1	241.7	240.7	13.13	0.33
C18:2 *cis-9, cis-12*	1342	1398	1303	1293	1328	1326	62.43	0.65
C18:3 *n-6*	10.81	11.46	10.39	9.77	11.51	10.97	1.34	0.94
C18:3 *n-3*	41.47	40.18	43.20	36.74	41.77	39.55	2.59	0.37
Day 29								
C16:0	216.6^{b}	241.9^{a}	243.6^{a}	227.2^{ab}	221.1^{b}	238.3^{ab}	7.37	0.01
C18:0	325.2^{d}	401.9^{ab}	422.6^{a}	347.9^{cd}	363.1^{c}	375.9^{bc}	14.22	<0.01
C18:1 *cis-9*	116.4^{b}	132.9^{a}	144.3^{a}	135.7^{a}	134.8^{a}	142.9^{a}	5.20	<0.01
C18:2 *cis-9, cis-12*	1207^{b}	1423^{a}	1473^{a}	1234^{b}	1266^{b}	1227^{b}	43.61	<0.01
C18:3 *n-6*	8.57^{a}	4.48^{c}	1.36^{d}	7.83^{ab}	5.98^{bc}	4.74^{c}	0.73	<0.01
C18:3 *n-3*	21.42^{e}	145.9^{c}	278.0^{a}	72.34^{d}	138.7^{c}	208.1^{b}	5.86	<0.01

[a,b,c]Within a row, means without a common superscript differ ($P < 0.05$).

Table 4.4: Feedlot performance of heifers fed 0, 3, and 6% ground flaxseed (Flaxseed) or 2, 4, and 6% of a 1:1 blend of dolomitic lime hydrate and ground flaxseed formed into a prilled matrix (L-Flaxseed)

	Flaxseed			L-Flaxseed				
Item	0%	3%	6%	2%	4%	6%	SEM	*P*-value*
Initial BW, kg	346.9	345.7	342.3	346.2	347.2	346.8	6.23	0.26
Final BW†, kg	546.5[a]	548.0[a]	542.2[a]	543.1[a]	535.8[b]	513.0[c]	6.57	<0.01
Adjusted final BW‡, kg	552.3[a]	559.8[a]	554.9[a]	555.1[a]	537.8[b]	506.6[c]	6.42	<0.01
ADG†, kg/d	1.31[a]	1.33[a]	1.32[a]	1.30[a]	1.24[b]	1.10[c]	0.035	<0.01
Adjusted ADG‡, kg/d	1.35[a]	1.41[a]	1.40[a]	1.37[a]	1.26[b]	1.05[c]	0.034	<0.01
DMI, kg/d	8.93[a]	8.82[a]	8.79[a]	8.89[a]	8.44[b]	7.43[c]	0.160	<0.01
G:F†	0.1470	0.1511	0.1499	0.1458	0.1470	0.1473	0.0011	0.72

[a,b,c]Within a row, means without a common superscript differ ($P < 0.05$).

*P-values for treatment effect.

†BW calculated as gross live BW × 0.96 (i.e., shrunk BW).

‡BW calculated as HCW divided by a common dressed yield of 0.635.

Table 4.5: Carcass traits of heifers fed 0, 3, and 6% ground flaxseed (Flaxseed) or 2, 4, and 6% of a 1:1 blend of dolomitic lime hydrate and ground flaxseed formed into a prilled matrix (L-Flaxseed)

	Flaxseed			L-Flaxseed				
Item	0%	3%	6%	2%	4%	6%	SEM	*P*-value[*]
n	75	75	77	75	75	77		
HCW, kg	350.7[a]	354.5[a]	352.4[a]	351.9[a]	341.0[b]	321.7[c]	4.09	<0.01
CCW[†], kg	351.5[a]	355.3[a]	353.0[a]	352.8[a]	341.9[b]	322.3[c]	4.09	<0.01
Dressed yield, %	64.2[ab]	64.9[a]	65.0[a]	64.9[a]	63.7[b]	62.7[c]	0.239	<0.01
LM area, cm^2	87.26[b]	88.04[ab]	90.02[a]	89.79[a]	85.09[bc]	84.59[c]	1.04	<0.01
12^{th}-rib fat thickness, cm	1.54[a]	1.60[a]	1.54[a]	1.53[a]	1.51[a]	1.23[b]	0.053	<0.01
KPH, %	2.63	2.59	2.88	2.87	2.56	2.69	2.08	0.44
Marbling score[‡]	493.2[a]	498.8[a]	490.7[a]	490.1[a]	496.9[a]	448.6[b]	12.4	0.04
USDA yield grade	2.73[a]	2.76[a]	2.62[a]	2.57[ab]	2.76[a]	2.32[b]	0.101	0.01
Yield grade 1, %	5.33	5.33	9.09	9.33	8.00	18.18	3.40	0.10
Yield grade 2, %	30.67	28.00	29.87	36.00	30.67	35.06	5.42	0.85
Yield grade 3, %	50.67	52.00	50.65	42.67	40.00	42.86	5.81	0.51
Yield grade 4, %	12.00	14.67	10.39	12.00	20.00	3.90	3.90	0.08
Yield grade 5, %	1.33	0.00	0.00	0.00	1.33	0.00	0.77	0.51
Liver abscesses[§], %	14.67	16.00	7.79	14.67	12.00	10.39	3.92	0.63
A^-, %	8.00	10.67	6.49	8.00	8.00	6.49	3.16	0.94
A, %	6.67	5.33	1.30	6.67	4.00	3.90	2.57	0.60
A^+, %	0.00	0.00	0.00	0.00	0.00	0.00		
Prime, %	4.00	1.33	9.09	5.33	2.67	1.30	2.26	0.12
Premium Choice[¥], %	44.0[a]	49.3[a]	36.4[a]	38.7[a]	37.3[a]	16.9[b]	5.64	<0.01
Choice, %	42.7	41.3	40.3	36.0	42.7	58.4	5.79	0.10
Select, %	8.00	6.67	14.29	16.00	13.33	18.18	4.12	0.14
Low grade[¥], %	1.33	1.33	0.00	1.33	0.00	0.00	0.95	0.69
No roll, %	0.00	0.00	0.00	0.00	1.33	2.60	0.94	0.23
Other grade, %	0.00	0.00	0.00	2.67	2.67	2.60	1.35	0.38

[a,b, c]Within a row, means without a common superscript differ ($P < 0.05$).

[*] *P*-values for treatment effect.

†CCW = Cold carcass weight.

[‡]Marbling score 400 to 499 = Small.

[¥]Premium Choice: Marbling score was greater than 500 and less than 700, thus qualifying carcass for upper two-thirds of the USDA Choice quality grade. Low grade: animals with advanced bone maturity, thus carcasses were classified as USDA Commercial.

[§]Liver Abscesses: A^- = 1 or 2 small abscesses. A = 2 to 4 well organized abscesses. A^+ = 1 or more large abscesses along with inflammation (Liver Abscess Technical Information AI 6288, Elanco Animal Health, Greenfield, IN).

Table 4.6: Long-chain fatty acid composition for loin steaks derived from heifers fed 0, 3, and 6% ground flaxseed (Flaxseed) or 2, 4, and 6% of a 1:1 blend of dolomitic lime hydrate and ground flaxseed formed into a prilled matrix (L-Flaxseed) expressed as mg/g of wet tissue

Fatty acid, mg/g	Flaxseed			L-Flaxseed			SEM	*P*-value[1]
	0%	3%	6%	2%	4%	6%		
C10:0	0.041	0.035	0.040	0.037	0.041	0.039	0.01	0.81
C12:0	0.048	0.044	0.048	0.045	0.048	0.044	0.01	0.96
C14:0	2.11	1.89	2.18	2.00	2.15	1.89	0.21	0.82
C14:1	0.619	0.572	0.638	0.585	0.620	0.503	0.06	0.64
C15:0	0.292	0.258	0.287	0.262	0.260	0.222	0.03	0.55
C16:0	18.00	16.51	17.96	17.34	17.78	16.00	1.56	0.90
C16:1	1.41	1.17	1.14	1.20	1.28	1.28	0.29	0.90
C17:0	0.80	0.73	0.75	0.72	0.71	0.56	0.07	0.30
C17:1	0.71^{a}	0.62^{ab}	0.60^{ab}	0.64^{ab}	0.54^{b}	0.46^{b}	0.05	0.02
C18:0	8.11	8.17	8.70	7.87	8.44	7.81	0.75	0.95
C18:1 *trans-9*	1.60	1.57	1.65	1.96	2.11	1.84	0.20	0.29
C18:1 *cis-9*	19.60	20.57	20.21	20.50	16.66	15.33	3.51	0.75
C18:2 *trans-9,trans12*	0.031	0.034	0.039	0.029	0.034	0.035	0.00	0.45
C18:2 *cis-9,cis-12*	1.22	1.09	1.13	0.95	1.09	1.24	0.29	0.87
C20:0	0.043^{b}	0.047^{b}	0.065^{a}	0.044^{b}	0.047^{b}	0.048^{b}	0.01	0.05
C18:3 *n-3*	0.173^{e}	0.482^{c}	0.743^{a}	0.288^{d}	0.433^{c}	0.592^{b}	0.04	<0.01
C20:1	0.142	0.144	0.164	0.151	0.153	0.167	0.02	0.83
C20:2	0.077	0.090	0.084	0.070	0.069	0.079	0.01	0.34
C20:3 *n-6*	0.224	0.222	0.203	0.203	0.182	0.213	0.02	0.32
C20:3 *n-3*/C22:1 *n-9*	0.551^{a}	0.544^{a}	0.479^{b}	0.517^{ab}	0.486^{b}	0.506^{ab}	0.02	0.03
Total fatty acids	55.81	54.80	57.15	55.39	53.14	48.88	5.98	0.92

[a,b,c,d,e] Within a row, means without a common superscript differ ($P < 0.05$).

[1]*P*-values for treatment effect.

Table 4.7: Long-chain fatty acid composition for loin steaks derived from heifers fed 0, 3, and 6% ground flaxseed (Flaxseed) or 2, 4, and 6% of a 1:1 blend of dolomitic lime hydrate and ground flaxseed formed into a prilled matrix (L-Flaxseed) expressed as % of total fatty acid

Fatty acid, %	Flaxseed			L-Flaxseed			SEM	*P*-value[1]
	0	3%	6%	2%	4%	6%		
C10:0	0.08	0.07	0.08	0.07	0.08	0.09	0.01	0.11
C12:0	0.09	0.08	0.09	0.08	0.10	0.10	0.01	0.37
C14:0	4.07	3.74	4.13	3.80	4.29	4.19	0.33	0.46
C14:1	1.19	1.14	1.20	1.13	1.24	1.10	0.10	0.77
C15:0	0.54	0.51	0.54	0.49	0.53	0.51	0.04	0.85
C16:0	34.35	33.90	35.04	33.13	35.96	35.71	2.64	0.88
C16:1	2.46	2.10	1.85	2.13	2.38	2.34	0.41	0.52
C17:0	1.47	1.46	1.44	1.33	1.42	1.28	0.11	0.61
C17:1	1.33	1.32	1.24	1.21	1.21	1.10	0.11	0.48
C18:0	15.45	16.53	17.38	15.20	17.02	17.67	1.32	0.36
C18:1 *trans-9*	3.03^{b}	3.20^{b}	3.15^{b}	3.69^{ab}	4.07^{a}	4.32^{a}	0.33	0.00
C18:1 *cis-9*	31.21	30.34	28.11	33.11	26.43	24.44	4.49	0.49
C18:2 *trans-9,trans-12*	0.06^{b}	0.07^{ab}	0.08^{a}	0.05^{b}	0.07^{ab}	0.08^{a}	0.01	0.02
C18:2 *cis-9,cis-12*	2.03	2.05	1.84	1.93	2.16	2.80	0.56	0.50
C20:0	0.08^{c}	0.09^{bc}	0.13^{a}	0.09^{bc}	0.09^{bc}	0.11^{b}	0.01	<0.01
C18:3 *n-3*	0.36^{c}	1.02^{b}	1.56^{a}	0.57^{c}	0.92^{b}	1.45^{a}	0.11	<0.01
C20:1	0.29	0.30	0.34	0.29	0.32	0.42	0.04	0.06
C20:2	0.14^{b}	0.18^{a}	0.16^{ab}	0.14^{b}	0.14^{b}	0.18^{a}	0.01	0.02
C20:3 *n-6*	0.49	0.50	0.46	0.44	0.40	0.58	0.07	0.41
C20:3 *n-3*/C22:1 *n-9*	1.26	1.37	1.17	1.12	1.13	1.50	0.19	0.58

[a,b,c] Within a row, means without a common superscript differ ($P < 0.05$).

[1]*P*-values for treatment effect.

Figure 4.1: α-Linolenic acid in plasma of heifers fed 0, 3, and 6% ground flaxseed (Flaxseed) or 2, 4, and 6% of a 1:1 blend of dolomitic lime hydrate and ground flaxseed formed into a prilled matrix (L-Flaxseed). Means without a common superscript letter are different, $P < 0.05$. Treatment effect: $P < 0.001$; SEM = 5.86.

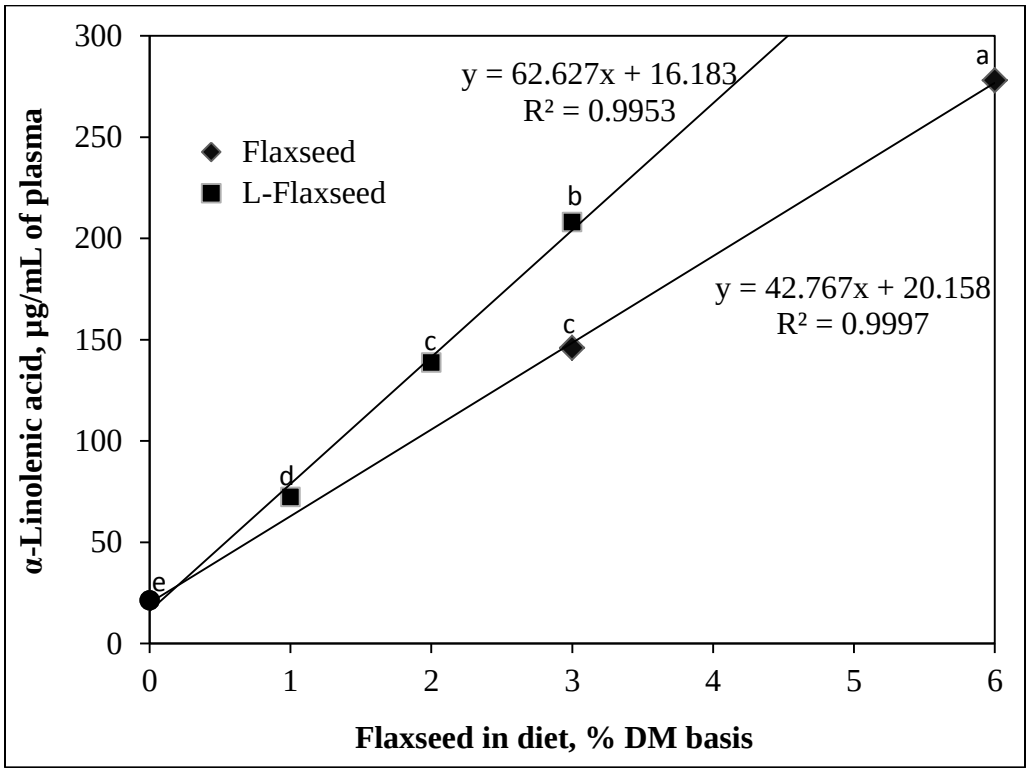


Figure 4.2: α-Linolenic acid for loin steaks derived from heifers fed 0, 3, and 6% ground flaxseed (Flaxseed) or 2, 4, and 6% of a 1:1 blend of dolomitic lime hydrate and ground flaxseed formed into a prilled matrix (L-Flaxseed). Means without a common superscript letter are different, $P < 0.05$. Treatment effect: $P < 0.001$; SEM = 0.04