Enriching Bovine Milk Fat with Alpha-Linolenic Acid, an Omega-3 Fatty Acid, Through Feeding of a Rumen Protected Flax-Based Supplement

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Haley Kathryn Peterson

Major Professor: Mark McGuire, Ph.D.

Committee Members: Amin Ahmadzadeh, Ph.D.; Gwinyai Chibisa, Ph.D.

Department Administrator: Amin Ahmadzadeh, Ph.D.

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Authorization to Submit Thesis

This thesis of Haley Kathryn Peterson, submitted for the degree of Master of Science with a major in Animal Science and titled "Enriching Bovine Milk Fat with Alpha-Linolenic Acid, an Omega-3 Fatty Acid, Through Feeding of a Rumen Protected Flax-Based Supplement," has been reviewed in final form. Permission, as indicated by the signatures and dates given below, is now granted to submit final copies to the College of Graduate Studies for approval.

Major Professor: _____Date_____
Mark McGuire, Ph.D.

Committee Members: _____Date_____
Amin Ahmadzadeh, Ph.D.

_____Date_____
Gwinyai Chibisa, Ph.D.

Department Administrator: _____Date_____
Amin Ahmadzadeh, Ph.D.

Abstract

The objective of this study was to increase the alpha-linolenic acid (ALA) concentration in milk fat by feeding a rumen protected flax-based (RPF) supplement. Eight lactating cows were randomly assigned to four treatment sequences in a replicated 4 x 4 Latin Square design with 16 d periods. The four treatments were 0, 1, 2, and 3 kg of RPF added to a base ration daily. Milk samples were collected on d 15 and 16 of each period. RPF did not affect dry matter intake, milk yield, or most milk components. Many fatty acids in milk were altered by treatment with generally decreased short- and medium-chain saturated fatty acids and increased long-chain fatty acids. RPF increased ALA in milk linearly with a 5-fold greater concentration in cows fed 3 kg/d of RPF compared to the unsupplemented control. This novel rumen protected ALA source enriched milk ALA greater than any previous method.

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CHAPTER 1

Chapter 1 – Literature Review

1.1 Introduction

Evolving consumer perceptions and government guidelines on what constitutes a well-balanced diet are constantly altering the definition of a healthy lifestyle. To keep up with these shifting demands, agricultural research is redirecting some of its focus to increasing the quality of food components and their nutrient profiles. The goal is to continue to improve production while enhancing components that are beneficial to human health. The Dietary Guidelines for Americans (2010) suggests a reduction in consumption of saturated fatty acids and replacement with polyunsaturated fatty acids (PUFA) by the human consumer. For years, efforts have been underway to reduce the consumption of milk fat through reduced fat products such as 1% and skim milk to address the recommendations. More recent efforts are specifically aimed at changing the fatty acid profile of milk fat. In general, milk fat can be manipulated through dietary means to produce a product more desirable by consumers today; however, implementing direct effects of diet on milk fatty acids is difficult. Supplemental fat is the main driver in altering milk fatty acids with oilseeds being a popular choice as they are an excellent source of PUFA. Many studies (Kennelly, 1996; Caroprese et al., 2010; Gonthier et al., 2005) have been conducted looking at the effects of feeding different lipid sources (oilseeds in particular) on milk yield and composition. Ruminal biohydrogenation greatly affects the transfer of dietary unsaturated fatty acids to milk fat and so is an obstacle that must be overcome in order to successfully alter milk composition. Rumen protected lipid sources are a promising solution to this problem as they are designed to resist biohydrogenation and enhance the unsaturated fatty acid portion of milk fat. Future research aims to continue testing and improving technologies that alter the milk fatty acid profile of milk fat to address recommendations for human health.

1.2 Milk and human health

Milk has long been considered an important dietary source of essential nutrients such as high-quality protein and important vitamins and minerals that cannot be synthesized by the body. Guidelines for milk and dairy product consumption, implemented by the 2010 Dietary Guidelines American Advisory Committee (DGAC), recommend 3 cups of low-fat milk and milk products every day as part of a well-balanced diet (Weaver, 2014). However, recommendations and perceptions about milk fat, specifically saturated fat, may lead the consumer to avoid purchasing milk and other dairy products for fear of potential negative effects on health. For the average American, only about 15% of total dietary fat comes from dairy products and of that, only about 25% is saturated fat (O'Donnell, 1993). Saturated fat has been linked to an increase in the concentration of LDL cholesterol which leads to an increased risk of cardiovascular disease (CVD) (Siri-Tarino et al., 2010). Most studies have shown that replacing saturated fat with PUFA in the diet can reduce this risk but that is not the case in every instance. Some studies have observed no effect on the occurrence of CVD events when saturated fats are swapped with PUFAs (Siri-Tarino et al., 2010). Recent confounding issues with the role of saturated fat in CVD risk need to be addressed to perhaps alleviate some of the negativity associated with this type of fat (Astrup et al., 2011). First, the potential problems with specific macronutrient energy sources being compared to saturated fatty acids must be identified. Second, replacing saturated fatty acids with *trans* fatty acids or highly processed carbohydrates could have very little positive effect or perhaps even a negative effect on lowering the incidence of CVD. Third, specific saturated fatty acids may play a more significant role in CVD risk than as a group. Fourth, other components in major food sources of saturated fatty acids may have an effect on CVD risk as saturated fat content may only be a marker for the risk. Lastly, changes in population obesity rates may influence the effect of replacing saturated fatty acids with carbohydrates (Astrup et al., 2011). As most of the evidence points towards improved cardiovascular health by limiting

the amount of saturated fat in the diet, increasing the proportion of unsaturated fat is still of significant interest.

Recent focus has been centered on omega-3 fatty acids such as alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexanoic acid (DHA). EPA and DHA have been clinically proven to have beneficial effects in people with diseases such as rheumatoid arthritis, cardiovascular issues, ulcerative colitis, and psoriasis (Simopoulos, 1991). Recently, omega-3 fatty acids have been shown to potentially reduce the risk of Alzheimer's disease (Swanson et al., 2012). Both EPA and DHA may have the ability to affect cancer risk by delaying the appearance of tumors and reducing the growth rate as well as the number of tumors (Simopoulos, 1991). In addition to reducing the risk of certain chronic diseases, omega-3 fatty acids are essential during pregnancy for proper fetal growth and development as well as full-term gestation (Swanson et al., 2012). Supplementation of EPA and DHA during pregnancy aids in correct development of the fetal brain and retina which leads to normal brain function and eyesight. An increased concentration of EPA and DHA in fetal tissues through supplementation has also been associated with decreased risk of preterm delivery (Swanson et al., 2012). There is also evidence suggesting supplementation during pregnancy and breastfeeding can reduce the incidence of a child developing allergies (Swanson et al., 2012). EPA and DHA also have anti-inflammatory effects including inhibition of the 5-lipoxygenase pathway in neutrophils and monocytes, the pathway responsible for producing proinflammatory lipid signaling molecules that regulate the immune system, and decreasing the proinflammatory cytokine family, interleukin-1 (Simopoulos, 1991). Due to these effects, EPA and DHA supplementation may lower inflammation in patients suffering from inflammatory and autoimmune disorders.

EPA and DHA are usually most abundant in fish and fish oils so increasing the concentrations of these omega-3 fatty acids in the diet can be achieved by increasing dietary fish intake and taking supplements derived from fish oils (Simopoulos, 1991). Synthesis of EPA and DHA from ALA has also been demonstrated in humans although rates are greatest

in infancy and nearly non-existent in adults (Brenna et al., 2009). Dietary ALA generally undergoes two metabolic processes, β-oxidation and carbon recycling, which use the majority of ALA with very little remaining for conversion to EPA and DHA (Barcelo-Coblijn and Murphy, 2009). The available ALA is first desaturated to C18:4n-3 (stearidonic acid) followed by elongation to C20:4n-3 and then desaturated again to EPA (C20:5n-3). EPA is elongated to C22:5n-3 and then to C24:5n-3 followed by desaturation into C24:6n-3 which is finally formed into DHA (C22:6n-3) through β-oxidation (Barcelo-Coblijn and Murphy, 2009). Linoleic acid (C18:2n-6), an omega-6 fatty acid, is thought to inhibit this conversion pathway since it competes for the same enzymes to convert to arachidonic acid (20:4n-6) and docosapentaenoic acid (C22:5n-6). Decreasing the omega-6-to-omega-3 fatty acid ratio would reduce this inhibition (Brenna et al., 2009). Accumulation of EPA and DHA converted from ALA is generally tissue-specific with EPA mostly found in plasma, liver, and heart tissues and DHA usually found in the brain (Barcelo-Coblijn and Murphy, 2009). Despite the existence of this conversion pathway, most research in humans show that dietary supplementation of ALA induces only slight increases in concentrations of EPA and has almost no impact on concentrations of DHA (Brenna et al., 2009; Rodriguez-Leyva et al., 2010). The conversion pathway is also present in other mammalian species, including cattle, but biohydrogenation of ALA in ruminant animals will limit the bioavailability of ALA resulting in concentrations of EPA and DHA being virtually nonexistent compared to the already low concentrations in monogastrics (Barcelo-Coblijn and Murphy, 2009).

ALA, one of the two essential fatty acids for humans, may have positive effects on health in addition to being a precursor for EPA and DHA. Limited studies have shown that ALA has antiarrhythmic, anti-inflammatory, and neuroprotective properties (Barcelo-Coblijn and Murphy, 2009). Clinical trials (Siri-Tarino et al., 2010; Barcelo-Coblijn and Murphy, 2009) and systematic reviews (Astrup et al., 2011; Rodriguez-Leyva et al., 2010) conclude that a diet rich in ALA may lower heart rate and reduce the risk of myocardial infarction and fatal ischemic heart disease. An increase in phospholipid and serum ALA was related to a

reduction in the risk of stroke (Rodriguez-Leyva et al., 2010). ALA can also suppress expression of inflammatory cytokines through inhibiting the DNA binding activity of nuclear factor kappa-beta (Barcelo-Coblijn and Murphy, 2009). Through these actions, dietary supplementation with ALA may lower chronic inflammation associated with obesity. ALA also has the potential to limit central nervous system injury by positively altering the function of ionic channels (Barcelo-Coblijn and Murphy, 2009). Consuming moderate amounts of ALA-rich dietary sources have been shown to provide similar effects on health as those provided by sources rich in EPA and DHA (Rodriguez-Leyva et al., 2010). Flaxseed is one of the richest plant sources of ALA with high bioavailability depending on how it is processed, making it a popular alternative for consumers with an aversion to fish (Rodriguez-Leyva et al., 2010). Foods enriched with omega-3 fatty acids, either naturally or strategically, provide the option of obtaining desired biochemical effects without resorting to ingesting supplements or drastically changing dietary habits. Opportunities exist to increase the content of these specific fatty acids in milk and dairy products through improved understanding of the complex relationships between rumen fermentation, lipid metabolism, and milk fat synthesis (Lock and Bauman, 2004). Even though they are present in limited concentrations, omega-3 fatty acids also benefit dairy cattle with similar anti-inflammatory effects and have positive impacts on reproduction including increased fertility, postpartum enhanced follicular development, and improved oocyte and embryo quality (Santos et al., 2008). Effectively supplementing the diet with omega-3 fatty acids would benefit the animal as well as the human consumer. By manipulating the diets of dairy cows, researchers may be able to modify the milk fatty acid profile to better comply with health recommendations.

1.3 Milk fatty acid profile

Before discussing different methods of altering the fatty acid profile of milk, it is important to understand what fatty acids are actually present and what their functional properties are. Short- and medium-chain fatty acids (<16 carbons) are synthesized in the mammary gland

and long-chain fatty acids (>16 carbons) are supplied by the diet. Palmitic acid (C16:0) can be synthesized in the mammary gland or supplied by the diet. Saturated fatty acids constitute the majority (65%) of the fatty acids present in milk fat with the rest being unsaturated (35%) (Table 1.1). The long-chain fatty acids that make up 40-60% of the total milk fatty acids are mostly provided by the diet. These fatty acids are predominantly the C18 fatty acids with some saturated and others unsaturated (Palmquist, 2006). Oleic (C18:1), linoleic (C18:2) and linolenic (C18:3) acids, composed of various *cis*-isomers, make up the majority of the C18 unsaturated fatty acids. The reduced proportions of these dietary unsaturated fatty acids in milk fat are because of ruminal biohydrogenation, which will be discussed in more detail below.

Several factors can influence the milk fatty acid profile including genetics, stage of lactation, and dietary components. Genetic selection for milk fat concentration can alter fatty acid composition by influencing proportions of individual fatty acids. As milk fat percentage increases, proportions of C14-C16 fatty acids usually increase and proportions of C18 unsaturated fatty acids decrease. Since short- and medium-chain fatty acids are synthesized in the mammary gland, it is expected that they are influenced by genetic selection more than the long-chain fatty acids (Stoop et al., 2008). As lactation proceeds, proportions of the *de novo* synthesized fatty acids tend to increase and proportions of the C18 fatty acids tend to decrease. This is due to increased positive energy balance and reduced adipose tissue mobilization, a source of stored C18 fatty acids. Dietary intake of grain, fat, protein, and energy all influence the fatty acid composition of milk fat (Palmquist et al., 1993). Increasing the amounts of starch and decreasing the amounts of forage in the diet will usually cause a decline in milk fat percentage, due to disrupted ruminal fermentation and a lower rumen pH, possibly leading to milk fat depression. In this case, depending on the grain source and its fat content, the concentrations of short-chain fatty acids decrease as the concentrations of the C18 fatty acids increase. Supplemental dietary fat can lower proportions of the *de novo* synthesized fatty acids (Palmquist et al., 1993). Changes to the proportions of C16:0,

C18:0, and C18:1 in milk fat depend on the content of those fatty acids in the supplement itself (Palmquist et al., 1993). The amount of supplemental fat added to the diet will also affect the absorption of fatty acids. When fat is added to the diet at 3-5%, it is about 80% digestible but when that amount is increased to 10% (adding about 5-6% fatty acids in excess), digestibility will be reduced to about 56% (Palmquist and Jenkins, 1980). Higher intakes of dietary protein have been shown to increase the proportions of long-chain fatty acids in milk fat, especially during early lactation, by increasing the mobilization of adipose tissue (Oldham, 1984). Energy intake also influences the proportion of *de novo* synthesized fatty acids depending upon whether the animal is in positive or negative energy balance (Palmquist et al., 1993).

Transfer of fatty acids from dietary fat to milk fat is affected by three main elements: ruminal biohydrogenation, absorption, and deposition in adipose tissue (energy balance). Ruminal biohydrogenation has the greatest influence on unsaturated fatty acid digestion and absorption with PUFA being particularly affected. Several studies have bypassed the rumen using abomasal and duodenal infusions to observe direct effects of unsaturated fatty acids on the milk fatty acid profile. Drackley et al. (1992) infused fatty acids of varying degrees of unsaturation directly into the abomasum of lactating dairy cows to examine the impact on milk yield, feed intake, nutrient digestibility, milk components, and blood metabolites. Treatments consisted of a control, the control and mostly saturated fatty acids, the control and a mix of saturated and unsaturated fatty acids, and the control and mostly unsaturated fatty acids. Milk yield, dry matter (DM) intake, and digestible energy intake decreased as the degree of unsaturation increased. Infusing long-chain fatty acids, regardless of degree of unsaturation, increased non-esterified fatty acid (NEFA) and cholesterol concentrations in blood plasma (Drackley et al., 1992). Overall milk components were not affected by the treatments but the fatty acid profile of milk fat was altered. Infusion of long-chain fatty acids caused proportions of C8:0, C10:0, C12:0, C14:0, C14:1, C15:0, and C16:0 to decrease and proportions of C18:1, C18:2, and C18:3 (all found in the infusate) to increase.

This study showed that it is possible to alter the milk fatty acid profile depending on the degree of unsaturation (Drackley et al., 1992).

Another experiment conducted by Khas-Erdene et al. (2010) investigated the effect of infusing ALA into the duodenum of lactating cows on milk yield and fatty acid composition. An ALA-rich infusate (82.4% ALA, 14.7% linoleic acid, 2.8% oleic acid, and 0.1% other fatty acids) infused at various rates (0, 40, 80, 120, and 160 g/d) was compared with the infusate control (xanthan gum). The results showed a slight decrease in milk yield as infusion rates increased, milk fat concentration tended to increase, and milk protein concentration remained unchanged. Increasing the amount of ALA infusate increased the concentration of ALA (25.4% at 160 g/d) and linoleic acid (4.2% at 160 g/d) in milk fat and decreased the concentration of C4:0, C14:0, C16:0, C18:1 *cis*-9, and C18:2 *cis*-9 *trans*-11 conjugated linoleic acid (CLA). Completely avoiding ruminal biohyrogenation can have a profound impact on the amount of ALA in milk fat. Bernal-Santos et al. (2010) infused stearidonic acid (C18:4n-3 SDA) enhanced soybean oil into both the rumen and abomasum of lactating dairy cows to observe the effects on milk fatty acid profile. The SDA-enhanced soybean oil consisted of 27.1% SDA, 10.4% ALA, and 7.2% gamma-linolenic acid (C18:3n-6). Treatments did not affect DM intake, milk yield, milk protein, or lactose. Milk fat percentage was lower when the SDA-enhanced soybean oil was infused into the rumen compared to the control. The SDA-enhanced soybean oil infused at 57 g/d in the abomasum increased the amount of ALA in milk fat (1.55%) compared to the control (0.44%) while no difference was detected for the SDA-enhanced soybean oil infused in the rumen (0.43%) compared with the control. Concentration of EPA was increased in milk fat (0.18%) when the SDA-enhanced soybean oil was infused into the abomasum compared to the control (0.06%) but no difference was detected between the control and when the SDA-enhanced soybean oil was infused into the rumen (0.05%). There was no difference in the concentration of DHA for SDA-enhanced soybean oil infused into either the abomasum or the rumen compared to the control. Further research is needed but enhancing supplemental fat with SDA has the potential to improve

the concentrations of omega-3 fatty acids in milk fat. Collectively, these studies show that unsaturated fatty acids can be transferred to milk fat as long as ruminal biohydrogenation is avoided.

1.4 Biohydrogenation pathways

Ruminant diets generally consist of forages and grains that have relatively low concentrations (<3%) of lipid with linoleic acid and linolenic acid being the predominant fatty acids present. These lipids are usually in the form of triaclyglycerols, galactosylacylglycerols, and phospholipids (Kennelly, 1996). Supplemental fat, fed to increase the energy density of the diet, is quite varied in source and fatty acid profile. Therefore, choosing what to feed should be based on production needs, cost, and availability. Another aspect to consider is that dietary lipids may have disruptive effects on rumen fermentation (Jenkins, 1993). Interrupting rumen fermentation can cause decreased digestibility of other dietary energy sources such as carbohydrates and proteins. This leads to a reduction in availability of these nutrients to the animal which can cause problems with production, reproduction, and other important body functions. Several theories, such as the lipid coating theory and the direct antimicrobial effects theory, have been proposed to explain this inhibition of rumen fermentation but none have been definitively proven (Jenkins, 1993).

Once dietary lipids enter the rumen, they are metabolized through lipolysis and biohydrogenation processes by rumen bacteria (Jenkins, 1993). Lipolysis refers to the action by which free fatty acids (FFA) are released from the glycerol backbone of esterified plant lipids. Biohydrogenation then converts the unsaturated FFA to their saturated form. This action, which is not completely described, is thought to be a protection mechanism for rumen bacteria against the toxic effects of unsaturated fatty acids (Jenkins, 1993). The rates of lipolysis and biohydrogenation can potentially be influenced by several factors including the amount of added fat in the diet and the diet itself. Beam et al. (2000) conducted experiments to see what impact some of these factors had on *in vitro* lipolysis and biohydrogenation of lipids

in ruminal contents. Soybean oil, linoleic acid, canola oil and tallow were added to a ground grass hay substrate that was then mixed with inoculum obtained from a nonlactating, ruminally fistulated Holstein cow and incubated for 48 h with samples collected throughout that time period to determine effects on lipolysis and biohydrogenation. Inoculum samples were then taken across several days from the same donor cow and incubated to see if the time of inoculum sampling had any effect on rates of lipolysis and biohydrogenation. Finally, four ruminally fistulated cows were fed four different diets with inoculum samples taken at different time points and incubated to determine if the diet of the donor cow affected rates of lipolysis and biohydrogenation. In most cases, rates for both lipolysis and biohydrogenation were greater for C18:2 compared to C18:1 with lipolysis occurring at a higher rate than biohydrogenation. Different PUFAs are converted into distinct biohydrogenation intermediates before yielding the final end product, stearic acid (C18:0) (Jouany et al., 2007). The general pathway for linoleic acid (C18:2 *cis*-9 *cis*-12) starts with a shift in the double bond position through isomerization to produce conjugated linoleic acid (CLA) (C18:2 *cis*-9 *trans*-11) which is then reduced to vaccenic acid (C18:1 *trans*-11) and finally to stearic acid (C18:0). Similar to linoleic acid (C18:2 *cis*-9 *cis*-12), the general biohydrogenation pathway of alpha-linolenic acid (C18:3 *cis*-9 *cis*-12 *cis*-15) first yields a conjugated triene (C18:3 *cis*-9 *trans*-11 *cis*-15) that is hydrogenated into C18:2 *trans*-11 *cis*-15 before reduction to vaccenic acid (C18:1 *trans*-11) and finally to stearic acid (C18:0) (Figure 1.1). Biohydrogenation can produce many other intermediates depending on the amount of dietary fatty acids and whether they are in the free or esterified form (Jouany et a., 2007). These intermediates are usually *cis*- and *trans*-isomers of C18:1 and C18:2 fatty acids that contribute to new pathways of PUFA biohydrogenation. Changes to the rumen environment, particularly a drop in rumen pH, can cause the biohydrogenation of linoleic acid to occur through another pathway, yielding a different CLA isomer (C18:2 *trans*-10 *cis*-12), a cause of milk fat depression (Baumgard et al., 2001; Palmquist, 2006). All of this processing leads to a large pool of C18 saturated fatty acids and limited PUFA available for absorption by the small

intestine. The estimated extent of biohydrogenation for linoleic and linolenic acid were 82% and 86%, respectively (Jenkins and Bridges Jr., 2007). Once absorbed in the small intestine, fatty acids are packaged into triacylglycerols (TAG) and transported to the mammary gland via TAG-rich lipoproteins in the bloodstream. A highly active enzyme in the mammary gland, stearoyl-CoA desaturase (SCD), converts C18:0 into the monounsaturated fatty acid (MUFA) oleic acid (C18:1 *cis*-9) as well as converts other fatty acids into C14:1, C16:1, and CLA which are then re-packaged into TAGs and secreted in the milk (Mosley and McGuire, 2007; Palmquist, 2006). As previously stated, saturated fatty acids can be related to changes in blood lipids associated with increased CVD so reducing biohydrogenation is essential to produce a product recommended for today's consumers.

1.5 Rumen protected lipid sources

One approach to reduce ruminal biohydrogenation is to protect lipids so they can reach the small intestine for absorption with their original fatty acid profile mostly intact. Feeding oilseeds can potentially reduce ruminal biohydrogenation because their hard outer coat naturally offers limited protection from microbial enzymes. Oilseeds can also be processed through a variety of methods, including extrusion, grinding, or pelleting to prevent biohydrogenation (Jenkins and McGuire, 2006). Processing allows for improved ease of handling, increased intake, and enhanced digestibility. Kliem et al. (2016) found that feeding oilseed supplements to lactating dairy cows can have positive effects on the fatty acid profile of milk fat by decreasing the saturated fatty acid content and increasing both the MUFA and PUFA content without disrupting cow performance. There are several factors that affect whether feeding of an oilseed alters the fatty acid profile of milk fat. These factors include the extent of ruminal biohydrogenation, the makeup of the non-lipid portion of the diet, how the lipid source can influence microbial synthesis of fatty acids and *de novo* fatty acid synthesis in the mammary gland, the stage of lactation, and the desaturase activity in the intestine and mammary gland (Kennelly, 1996). All of these factors must be considered when feeding

oilseeds as a fat source.

There are a variety of oilseeds, whole or processed, as well as oils derived from oilseeds to choose from including peanut, canola, linseed, micronized (infra-red heat-treated) soybeans, sunflower seed, cottonseed, rapeseed, and flaxseed. In 2002, Petit conducted a study looking at the effects of feeding whole flaxseed to lactating dairy cows. Total mixed rations (TMR) with added supplemental fat sources of whole flaxseed, Megalac (calcium salts of palm oil), or micronized soybeans were compared. Rates of feeding (on a DM basis) were 2.0 kg/d whole flaxseed, 0.7 kg/d Megalac, and 3.3 kg/d micronized soybeans for each of the respective treatments. Concentrations of C18:3 fatty acids varied among treatment diets: flaxseed diet (43.9%), Megalac diet (8.3%) and micronized soybean diet (13.0%). Milk yield and milk protein percentage were higher in the animals fed the whole flaxseed compared to in the animals fed either Megalac or micronized soybeans. Milk fat percentage was higher in the cows fed Megalac (4.14%) compared to those on the flaxseed (3.81%) or micronized soybean diets (3.70%). Milk from cows fed the micronized soybean diet exhibited the greatest increase in ALA concentration of milk fat (1.04%) compared to cows fed the flaxseed diet (0.90%) and the Megalac diet (0.44%). The researchers also noted feeding flaxseed resulted in a lower omega-6-to-omega-3 fatty acid ratio in the milk compared to the other two supplements. Later Petit et al. (2004) tested feeding whole, unprocessed sunflower seeds and flaxseeds. The treatments were diets based on sunflower seeds (6.6% ALA), flaxseed (38.4% ALA), Megalac (7.2% ALA), and an unsupplemented control (14.8% ALA). Feeding rates (on a DM basis) for each of the respective treatments were 2.0 kg/d sunflower seed, 2.0 kg/d flaxseed, and 1.0 kg/d Megalac. The concentration of ALA was highest in the milk fat from cows fed the flaxseed diet (1.1%) compared to those fed either the sunflower seed diet (0.5%), the Megalac diet (0.6%), or the control diet (0.6%). Again they found the omega-6-to-omega-3 fatty acid ratio to be lower in milk fat of cows fed the flaxseed. Moallem (2009) specifically looked at the effects of feeding lactating cows an extruded flaxseed supplement (50.4% ALA) on milk yield and fatty acid composition. The extruded flaxseed supplement

was fed at 40 g/kg DM. Milk yield was slightly higher in the group of cows fed the extruded flaxseed diet (45.4 kg/d) compared to the group of cows fed the control diet (44.2 kg/d). Cows fed the diet containing the extruded flaxseed supplement had increased ALA in milk fat (0.93%) compared to cows fed the control diet (0.30%). Caroprese et al. (2010) fed whole flaxseed (7.01% ALA on DM basis) at a rate of 1.2 kg/d and fish oil (3.87% ALA on DM basis) at a rate of 0.2 kg/d. The fish oil diet resulted in the greatest increase in ALA concentration (0.84%) followed by the whole flaxseed diet (0.81%) compared to the unsupplemented control (0.75%). The fish oil diet also increased the concentration of EPA and DHA in milk fat (0.06% and 0.12%, respectively) compared to the flaxseed diet (0.02% and 0.001%, respectively) and the unsupplemented control (0.003% and 0.001%, respectively). Supplementing rations with fish oil or other marine sources can directly impact EPA and DHA concentrations in milk fat.

Other studies have compared processed oilseeds with whole oilseeds and oils to determine their effectiveness on improving the fatty acid profile of milk. Chilliard et al. (2009) compared the effects of different physical forms of linseed on milk fatty acid profile. Four treatments consisting of a control diet (16.2% ALA), the control diet and whole crude linseed (40.3% ALA), the control diet and extruded linseed (46.4% ALA), and the control diet and linseed oil (49.2% ALA) were fed to lactating dairy cows. Diets were isolipidic with feeding rates (on a DM basis) of 1.5 kg/d crude linseed, 1.9 kg/d extruded linseed, and 0.4 kg/d linseed oil. Milk fat from cows fed the extruded linseed diet had the highest concentration of ALA (1.20%) followed by cows fed the whole crude linseed diet (0.65%) and cows fed the linseed oil diet (0.54%) compared to cows fed the control diet (0.67%). Dang Van et al. (2011) evaluated how well extruded rapeseed paired with an alfalfa protein concentrate (APC) could improve the fatty acid profile of milk fat. Both linseed and rapeseed are high in ALA but rapeseed has not been found to have an impact on ALA concentration in milk fat (Bayourthe et al., 2000; Collomb et al., 2004). Dang Van et al. (2011) paired the extruded rapeseed with the APC in the hopes of increasing ALA in the milk fat. One treatment con-

sisted of a TMR with extruded rapeseed as the supplemental fat source (3.6% ALA on DM basis) and the APC as a protein source (3.1% ALA on DM basis) and the other treatment was a TMR with extruded linseed as the supplemental fat source (12.2% ALA on DM basis) and soybean meal as a protein source (0.1% ALA on DM basis). The treatment diets were isolipidic with feeding rates (on DM basis) of 2.2 kg/d extruded linseed and 3.7 kg/d extruded rapeseed-APC combination. The milk fat from cows fed the extruded rapeseed-APC diet had a greater concentration of ALA (1.31%) compared to milk fat from cows fed the extruded linseed diet (1.18%). They found the apparent transfer efficiency of ALA from the diet of the extruded rapeseed-APC combination to milk (7.4% of intake) to be almost double that of the extruded linseed diet (3.8% of intake). As the researchers did not test extruded linseed paired with the APC nor the APC by itself, the need for the extruded rapeseed-APC combination is not certain.

Altering lipids to provide protection is most often achieved by physical or chemical treatments. These treatments allow the lipid source to be fed in ruminant diets with reduced biohydrogenation occurring in the rumen. Examples of protection methods include encapsulation, treatment with formaldehyde, and formation of amides and calcium salts of fatty acids (Jenkins and Bridges Jr., 2007). Protection methods for ALA are commonly calcium salts of oilseeds but a recent treatment using a urea formaldehyde condensation polymer (UFCP) may offer another option (Hawkins et al., 2013). Cortes et al. (2010) compared the effects of feeding whole flaxseed and calcium salts of flaxseed oil to lactating dairy cows on milk yield, composition and fatty acid profile. Four different treatments consisted of a control diet (no flaxseed products; 7.8% ALA), a diet with whole flaxseed (21.8% ALA), a diet with calcium salts of flaxseed oil (23.5% ALA), and a diet with a mix of both whole flaxseed and calcium salts of flaxseed oil (22.6% ALA). Dietary inclusions were 1.0 kg/d whole flaxseed, 0.4 kg/d calcium salts of flaxseed oil, and 0.7 kg/d mix (0.2 kg/d calcium salts of flaxseed oil and 0.5 kg/d whole flaxseed) on a DM basis. Milk yield and composition were not affected by the treatments. The concentration of ALA in milk fat was greatest in

the cows fed the calcium salts of flaxseed oil (1.03%) followed by the mixed diet (0.95%) and then the whole flaxseed diet (0.84%) compared with the control (0.59%). Calcium salts of flaxseed oil appear to offer better protection against biohydrogenation than whole flaxseed. Hawkins et al. (2013) tested the effects of feeding UFCP-treated flaxseed on lactation in dairy cows. Cows were fed a diet containing either UFCP-treated flaxseed or heated ground flaxseed as a control (both were 53.7% ALA) at a rate of 3 kg/d. The proportion of ALA in milk fat from cows fed the UFCP-treated flaxseed diet was higher (1.77%) than for cows fed the heated ground flaxseed diet (1.49%); however, there was no treatment difference in milk ALA yield (g/d). Overall they concluded that the transfer of ALA to milk was not improved by feeding the UFCP-treated flaxseed compared to heated ground flaxseed.

A meta-analysis conducted by Glasser et al. (2008) looked at differences in milk fatty acid profiles of cows fed either linseed, rapeseed, soybean, and sunflower seed lipid sources supplied in different forms (i.e., whole seeds, oils, and protected). The oils category included oils, amides, and calcium salts and the protected category included encapsulated or formaldehyde-treated. The mean ALA concentrations of the oilseed supplements (all forms included) were as follows: linseed supplements 54.4%, rapeseed supplements 9.2%, soybean supplements 7.0%, and sunflower seed supplements 0.2%. Protected linseed forms increased the ALA amount in milk fat (1.56%) followed by whole seeds (1.06%) and then oils (0.91%) compared to an unsupplemented control (0.59%). Whole seeds of rapeseed increased ALA in milk fat (0.63%) compared to oils (0.56%) which were not different from the control (no data for protected rapeseed). Whole soybeans increased ALA in the milk fat (0.72%) whereas oils decreased the amount of ALA (0.44%) compared to the control (no data for protected soybeans). Sunflower seeds in either whole (0.53%) or oil (0.54%) forms did not alter the ALA amount in milk fat compared to the control (no data for protected sunflower seed). Linseed improved the amount of ALA in milk fat (1.18%) with the protected form (1.56%) having the most significant effect. It is important to note that some protection methods have not been highly successful in decreasing ruminal loss of unsaturated fatty acids. Ac-

cording to Palmquist (2007), escape of unsaturated fatty acids from biohydrogenation is generally low regardless of the physical or chemical properties of the lipid source fed in the diet. Some methods do not provide complete protection so continued research is necessary. A new method, discussed in chapter 2, may offer better protection for unsaturated fatty acids through the use of a protein matrix.

1.6 Conclusion

Consumers today are more aware of the food they eat and how it affects their health and well-being (Lock and Bauman, 2004). Even though milk is an important dietary source of essential nutrients, its saturated fat content may reduce consumption of it and other dairy products. Dietary saturated fat has been associated with cardiovascular diseases. Hence, decreasing the proportions of saturated fatty acids in milk fat may be beneficial. Recent analyses have determined that the risk for coronary heart disease can be lowered by replacing saturated fatty acids with PUFAs whereas replacing saturated fatty acids with a carbohydrate had little to no effect (Astrup, 2011). It is possible to alter the milk fatty acid profile by feeding supplemental fat high in PUFA to dairy cows, as demonstrated by abomasal and duodenal infusions, to produce a product that is perceived to be healthier. Polyunsaturated lipid sources for lactating dairy cow diets are generally plant-based, with oilseeds (whole or processed) or oils as a popular choice (Kennelly, 1996). These sources are generally high in PUFA, particularly omega-3s, and offer varying degrees of protection against ruminal biohydrogenation. The efficacy and economic feasibility of including oilseeds in a dairy cow ration to alter milk fatty acids are dependent upon processing. Recent research provides strong evidence that feeding rumen protected lipid sources can increase the concentration of ALA in milk fat thereby improving the overall nutrient profile of milk.

Table 1.1: Fatty acid composition of bovine milk

Fatty Acid[1]	**(wt% of FAME**[2]**)**
C4:0	3.30
C6:0	1.40
C8:0	0.65
C10:0	1.40
C12:0	1.50
C14:0	6.90
C14:1	0.69
C15:0	0.74
C16:0	25.0
C16:1 *cis*-9	1.50
C17:0	0.64
C18:0	12.7
C18:1 *trans*-9	0.48
C18:1 *trans*-10	0.63
C18:1 *trans*-11	1.10
C18:1 *cis*-9	30.7
C18:2 *cis*-9 *cis*-12	3.30
C18:2 *cis*-9 *trans*-11	0.48
C18:3 *cis*-9 *cis*-12 *cis*-15	0.43

[1]Adapted from Mosley et al. (2006), [2]fatty acid methyl ester

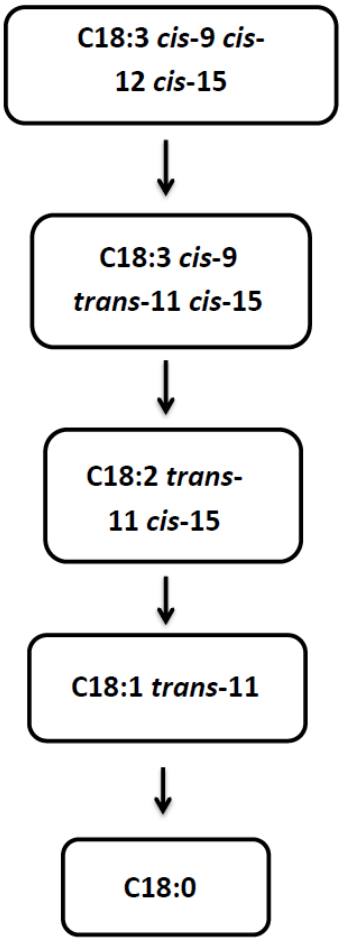


Figure 1.1: Biohydrogenation pathway for alpha-linolenic acid

The primary pathway for biohydrogenation of alpha-linolenic acid (C18:3 *cis*-9 *cis*-12 *cis*-15) (adapted from Jouany et al,. 2007)

CHAPTER 2

Chapter 2 Effects of Feeding a Rumen Protected Flax-Based (RPF) Supplement on Milk Fatty Acid Composition

2.1 Introduction

The concentration of ALA in bovine milk fat is naturally very low (<0.6%) and with recent evidence indicating ALA can have positive impacts on health, recommendations (Rodriguez-Leyva et al., 2010) have been made to enhance the content of this fatty acid in milk. Khas-Erdene et al. (2010) demonstrated that infusing an ALA-rich fatty acid mixture into the duodenum of lactating cows improved the concentration of ALA in milk fat to a quarter of total fatty acids. Infusing fatty acids into the abomasum or duodenum to bypass the rumen is an effective way to increase the amount of unsaturated fat in milk but it is not practical in a dairy production setting. Methods of protecting PUFA from rumen biohydrogenation such as calcium salts, formaldehyde, and heat have various advantages and disadvantages. Feeding processed oilseeds may increase ALA concentration in milk fat but concentrations of biohydrogenation intermediates increase as well, signaling incomplete protection (Chilliard et al., 2009; Dang Van et al., 2011). Calcium salts of ALA offer poor protection from biohydrogenation (Palmquist, 2006). An alternative protection method combining ground flaxseed and soy flour with heat may improve transfer of ALA into milk fat. Heat denatures the protein component, soy flour, to form a matrix around the lipid portion of the flaxseed. This matrix may prevent rumen bacteria from accessing the lipid and altering the fatty acids through biohydrogenation. The objective of the study was to evaluate the enrichment of milk fat with ALA when feeding cows a rumen protected flax-based (RPF) supplement. We hypothesize that feeding this supplement will offer better protection against biohydrogenation than previously tested methods and increase the amount of ALA in milk fat.

2.2 Materials and methods

All procedures involving animals were approved by the University of Idaho Animal Care and Use Committee (protocol 2015-20).

Eight Holstein cows in mid- to late-lactation (194 ± 16.3 DIM; 730 kg ± 1.8 kg BW) from the herd at the University of Idaho Dairy Center were randomly assigned to one of four treatment sequences in a replicated 4 x 4 Latin square design. The four treatments consisted of 0, 1, 2, or 3 kg of the RPF supplement added to a base ration containing 3% lipid (DM basis). The base ration (Table 2.1 and 2.2) was formulated to meet the nutrient requirements of cows producing 33.5 kg/d. Periods were 16 d with no washout period occurring between periods. Cows were housed in individual tie stalls in the research barn at the dairy center. The stalls were bedded with rubber mats with a feed box at the head of each stall. Cows were fed approximately 40% of the base ration in the mornings and 60% of the base ration in the evenings with extra feed offered midday if necessary to ensure cows were fed ad libitum (>5% of daily offered feed remaining).The RPF supplement (26.6% protein, 31.9% lipid, and 49.9% ALA; Table 2.3) was added to the base ration and hand mixed in the mornings once the cows were secured in the appropriate stalls. Feed intakes were recorded daily and the cows had free access to water throughout the trial. Samples of the base ration were taken once a week and samples of the supplement were obtained from each bag ($n = 40$) when opened. Samples were placed in labeled bags and frozen at $-20\,^{\circ}\mathrm{C}$ until lipid extractions could be performed.

Cows were milked at 0645 and 2000 h daily. On d 15 and 16 of each period, milk production was recorded and samples were obtained at each milking. Composite samples were collected from the milk system samplers for each cow in clean, pre-labeled 250 mL bottles. Samples from cows (6 total samples) with mastitis were taken out of a separate collection bucket using the same type of bottles. All bottles were immediately transported back to the laboratory and placed in a walk-in cooler ($4\,^{\circ}\mathrm{C}$). Milk for compositional analysis

and lipid extractions was aliquoted within 24 h of sample collection. Milk was sent to Southern Counties DHIA (Shafter, CA) for analysis of fat, protein, lactose, solids non-fat (SNF), and milk urea nitrogen (MUN) via near-infrared analysis. Two 15 mL aliquots were stored at −20 °C until analysis of fatty acids.

All milk and feed samples were extracted by a modified Folch lipid extraction procedure (Clark et al., 1982). Supplement samples were first ground using a mortar and pestle. Base ration samples were dried at 60 °C for 48 h before being ground through a 1 mm screen. Milk lipids were methylated using a base-catalyzed transesterification process (Christie, 1982). Lipids from the feed samples were methylated using a two-step methylation procedure that used methanolic-HCl and sodium methoxide (Kramer et al., 1997). Fatty acid methyl esters were analyzed on a gas chromatograph (Hewlett-Packard 6890 Series with auto injector) fitted with a flame ionization detector and a 100 mm x 0.25 mm, with 0.2 µm film, silica capillary column coated with CP-Sil 88 (Varian, Lake Forest, CA). The initial oven temperature started at 70 °C then increased 4 °C/min to 175 °C and held for 3 min. The temperature was then increased to 185 °C at a rate of 1 °C/min and held for 20 min. Temperature then increased at 3 °C/min to 220 °C then held for 3 min. A final increase at 10 °C/min occurred to reach a final temperature of 230 °C for 10 min. The identities of fatty acid peaks were established by comparing retention times to the Supelco 37 standard (Sigma-Aldrich Co., St. Louis, MO). Fatty acid composition was expressed as weight percent of total FAME (wt% of total FAME).

Statistical analysis was performed using SAS (v. 9.4). Fourteen fatty acids were chosen to be analyzed based on their abundance in the milk fat and their biological response to ruminal biohydrogenation. Fatty acid data were transformed into proportions and analyzed using the generalized linear mixed model (GLIMMIX) procedure with a beta distribution. Dry matter intake, milk yield, and milk components were also analyzed using the GLIMMIX procedure but with a normal distribution. The model included the random effect of cow nested within period and fixed variables of treatment, period, and the treatment by period interaction.

The effect of treatment on fatty acid proportions was further examined by testing for linear and quadratic contrasts. Significance was declared at $P < 0.05$.

2.3 Results

Level of RPF had no effect ($P > 0.05$) on DM intake or milk yield throughout the trial (Table 2.4). Milk fat, protein, and lactose yield also did not differ across the various RPF levels. An effect of treatment ($P = 0.02$) was detected for milk fat concentration with the greatest concentration from cows fed RPF at 3 kg/d. Treatment tended ($P = 0.07$) to affect MUN with decreased concentrations in milk as the amount of RPF supplement added to the diet increased. Fatty acid profile of milk did change with proportions of some fatty acids decreasing and proportions of other fatty acids increasing as the amount of the RPF added to the diet increased (Table 2.5). The proportions of C14:0, C14:1, C15:0, C16:0, and C17:0 decreased as the amount of supplement increased in the diet ($P < 0.0001$). The proportion of C16:1 *cis*-9 also decreased ($P = 0.0358$) as the amount of RPF supplement added to the diet increased. There was a linear decrease ($P < 0.0001$) of C14:0, C14:1, C15:0, C16:0, and C17:0 in milk fat when the amount of RPF supplement added to the diet increased; C16:1 *cis*-9 also decreased linearly ($P = 0.0109$). Conversely, the proportion of C18:0 increased with increasing RPF supplement ($P = 0.0168$) as did the proportion of C18:1 *cis*-9 ($P = 0.0014$). Increasing the amount of RPF supplement to the diet also increased the proportions of C18:1 *trans*-9 ($P < 0.0001$), *trans*-10 ($P = 0.0003$), and *trans*-11 ($P < 0.0001$). There was no effect ($P > 0.05$) of RPF treatment on the proportion of C18:2 *cis*-9 *cis*-12. The proportion of C18:2 *cis*-9 *trans*-11 CLA and the proportion of ALA both increased as the amount of RPF supplement added to the diet increased ($P < 0.0001$). There was a linear increase of C18:0 ($P = 0.0035$) and C18:1 *cis*-9 ($P = 0.0002$) as well as C18:1 *trans*-9, *trans*-10, and *trans*-11 ($P < 0.0001$). Both C18:2 *cis*-9 *trans*-11 CLA and ALA increased linearly ($P < 0.0001$) as the amount of RPF supplement increased. There was also a quadratic effect of treatment ($P < 0.0001$) on the proportion of ALA in milk fat.

An effect of period was detected for C17:0 (P = 0.0004), C18:1 *trans*-9 (P = 0.0002), and C18:2 *cis*-9 *cis*-12 (P = 0.0066), and treatment by period interactions were detected for C14:0 (P = 0.0131), C16:0 (P < 0.0001), C18:1 *cis*-9 (P = 0.0002), and C18:2 *cis*-9 *cis*-12 (P = 0.0195). These fatty acids varied across periods and treatments without clear indication of a detectable pattern (see Appendix B). Only 32 milk samples had detectable peaks of EPA and just one milk sample had a detectable peak of DHA out of 128 total milk samples. Thus, no evaluation of treatment effects could occur due to a lack of positive samples with detectable peaks for statistical evaluation.

2.4 Discussion

The proportion of ALA in milk fat increased as the amount of the RPF supplement fed increased. Compared to an unsupplemented control (0 kg with 0.53% ALA), feeding 1 kg of the RPF supplement increased the concentration of ALA in milk fat approximately 3-fold, feeding 2 kg of the RPF supplement increased the concentration of ALA in milk fat approximately 4-fold, and feeding 3 kg of the RPF supplement increased the concentration of ALA in milk fat approximately 5-fold (Figure 2.1). Results from Caroprese et al. (2010) showed that fish oil fed at a rate of 0.2 kg/d had the greatest increase in ALA concentration (0.84%) followed by whole flaxseed fed at a rate of 1.2 kg/d (0.81%) compared to the unsupplemented control (0.75%). The ALA concentrations from the RPF supplement were almost double the ALA concentrations of whole flaxseed at a similar feeding rate of 1 kg/d and fish oil supplements at one-fifth the feeding rate in the study by Caroprese et al. (2010). Compared with whole flaxseed fed at a rate of 2 kg/d with resulting ALA concentrations in milk fat of 1.0% in the first experiment (Petit, 2002) and 1.1% in the second experiment (Petit et al., 2004), the ALA concentration in milk fat in the current study was again double for the RPF supplement at an equivalent feeding rate. Results from Cortes et al. (2010) show an increased concentration of ALA in milk fat (1.03%) from cows fed calcium salts of flaxseed oil at a rate of 0.4 kg/d compared to whole flaxseed (0.84%) at 1.0 kg/d and a mixture of

whole flaxseed and calcium salts of flaxseed oil (0.95%) at 0.7 kg/d. Fed at the same rate of 1 kg/d, the RPF supplement had a greater concentration of ALA (1.43%) compared with the whole flaxseed and calcium salts of flaxseed oil in the Cortes et al. (2010) study. The urea formaldehyde condensation polymer (UFCP) treated flaxseed tested by Hawkins et al. (2013) increased the concentration of ALA in milk fat (1.77%) compared to heated ground flaxseed (1.49%) fed at 3 kg/d. At the same feeding rate, the RPF supplement increased the concentration of ALA to 2.77%, nearly twice the improvement over the UFCP-treated flaxseed. The Holstein cows used in the Hawkins et al. (2013) study were later in lactation and had a greater milk fat concentration (4.36%) than the cows used in the current study (3.91%) but lipid concentration (32.9%) and ALA concentration (53.7%) of their flaxseed product was very similar. Overall, feeding the RPF supplement lead to the greatest increase of ALA concentration in milk fat compared to these other supplemental fat sources. However there is still substantial room for improvement, as demonstrated by Khas-Erdene et al. (2010) with duodenal infusions of an ALA-rich infusate that increased the concentration of ALA in milk fat to 25.4% at 160 g/d.

Feeding the RPF supplement composed mostly of extruded flaxseed did not affect the DM intake of the cows used in the current study. This observation is consistent with other studies that have included flaxseed in their experimental diets. Gonthier et al. (2004) studied the effects of feeding micronized and extruded flaxseed on ruminal fermentation in dairy cows and found that supplementation with flaxseed had no effect on dry matter, organic matter, neutral detergent fiber, crude protein, fatty acids, and gross energy digestibilities in the rumen. No effect on feed intake was found when lactating dairy cow diets were supplemented with whole ground solin (a new cultivar of flax containing 28% linoleic acid), flax, or canola oilseeds (Ward et al., 2002). Milk yield and most milk components were not affected by the RPF supplement fed. Milk fat concentration increased and MUN decreased as the amount of RPF supplement added to the diet increased. Several studies failed to detect any changes in milk yield but have also found differences in milk composition. Caroprese et

al. (2010) found no difference in milk yield but observed greater milk fat yield in cows fed whole flaxseed than those fed either the control diet or the fish oil-supplemented diet. In a diet supplemented with calcium salts of flaxseed oil, milk yield was not affected but milk fat percentage decreased compared to the control diet (Cortes et al., 2010). Other studies did observe changes in milk yield and composition when flaxseed supplements were added to the diet of lactating dairy cows. Gonthier et al. (2005) observed reduced milk yield and milk protein yield in cows fed flaxseed compared to the control. Results from Petit et al. (2004) showed flaxseed increased milk yield compared to control while milk protein concentration did not change. The variation of milk yield and composition in response to flaxseed supplementation could be due to several factors including extent of rumen degradability, fiber content of the diet, stage of lactation, and the type of cow (breed, age, etc.). The RPF supplement fed in the current study did not affect yield of milk fat or protein.

Supplementing lactating dairy cow diets with oilseeds as the lipid source can alter the fatty acid profile of milk fat in order to make milk more appealing to today's consumers. The ultimate goal would be to transfer a high percentage of polyunsaturated fatty acids in the diet into milk. A meta-analysis performed by Glasser et al. (2008) compared different types (linseed, rapeseed, soybeans, and sunflower seed) and forms (whole, oil, and protected) of oilseeds as lipid supplements to determine their effect on milk fatty acid composition. They concluded that percentages of C14:0, C14:1, C15:0, C16:0, C16:1, and C17:0 were decreased by all supplements and the percentage of total C18 fatty acids was increased. Feeding the RPF supplement in the current study resulted in similar observations regarding those specific fatty acids. This pattern of milk fatty acids is not greatly affected by the type and form of oilseed fed in the diet. Increases in the percentages of C18:2 and C18:3 fatty acids did depend on the type of oilseed fed and what form that oilseed was delivered to the animal (Glasser et al., 2008). Feeding cows the RPF supplement followed this general trend with the exception of the C18:2 fatty acids. The RPF supplement did not affect C18:2 *cis*-9 *cis*-12 but did cause an increase in the proportion of the C18:2 *cis*-9 *trans*-11 CLA isomer. This CLA isomer in

particular has been shown to have some beneficial effects on health as well, most notably as an anticarcinogen (Bauman et al., 2000). C18:2 *cis*-9 *trans*-11 CLA is an intermediate of biohydrogenation along with C18:1 *trans*-11 so the concentration increases are possibly indicative of incomplete ruminal biohydrogenation. Increased concentrations of CLA in milk fat could be beneficial so complete biohydrogenation or complete protection from biohydrogenation may not be desired. The amount of C18:0 also slightly increased as the amount of RPF supplement added increased, further suggesting biohydrogenation occurred (Jouany et al., 2007). However, since the amount of ALA increased 5-fold as the amount of supplement added increased, modest protection against biohydrogenation was achieved.

The apparent transfer efficiencies of ALA from the RPF supplement to milk fat were 6.3% for 1 kg, 5.5% for 2 kg, and 4.6% for 3 kg. These results are similar to other apparent transfer efficiencies of ALA such as those calculated for the extruded linseed (3.8% of intake) and extruded rapeseed-APC diets (7.4% of intake) by Dang Van et al. (2011). Moallem (2009) estimated the transfer efficiency of ALA from a diet supplemented with extruded flaxseed to milk fat to be approximately 6.2% which is also similar to the transfer efficiency of the RPF supplement.

The RPF supplement reduced the concentration of saturated fatty acids in milk fat and increased the concentration of unsaturated fatty acids (Figure 2.2). This reduction is similar to that achieved by Chilliard et al. (2009) with extruded linseed (53.7% saturated fatty acids) compared to the control (69.0% saturated fatty acids) but more pronounced than that achieved by Moallem (2009) with extruded flaxseed (62.2% saturated fatty acids) compared to the control (65.8% saturated fatty acids). Reducing the amount of saturated fat consumed in the diet is thought to lower the risk of cardiovascular disease (Siri-Tarino et al., 2010). Therefore, decreasing the concentration of saturated fatty acids in milk fat may be desired. The RPF supplement also lowered the omega-6-to-omega-3 fatty acid ratio from 6.6 at 0 kg/d to 2.4 at 1 kg/d, 1.7 at 2 kg/d, and 1.3 at 3 kg/d. Brenna et al. (2009) reported that high concentrations of omega-6 fatty acids can replace and reduce concentrations of

omega-3 fatty acids by competing for incorporation into complex lipids that are present in mammalian tissues. According to Simopoulos (1991), lowering this ratio is imperative for maintaining the balance between omega-6 and omega-3 fatty acids for it impacts homeostasis and normal development throughout life.

2.5 Conclusions

The objective of this study was met as the RPF supplement did increase the concentration of ALA in milk fat. The RPF supplement offered better protection against ruminal biohydrogenation compared to other methods such as calcium salts of flaxseed oil (Cortes et al., 2010) and a urea formaldehyde condensation polymer treatment of flaxseed (Hawkins et al., 2013). In fact, enrichment of ALA in milk fat was the most extensive of any previous feeding study. Cow performance and milk production were not adversely affected by the RPF supplement, making it an optimal supplemental fat source for lactating cow rations. The RPF supplement altered the milk fatty acid profile by increasing concentrations of unsaturated fatty acids and decreasing concentrations of saturated fatty acids, thus lowering the potential risk for cardiovascular disease thought to be associated with high intakes of saturated fat (Astrup et al., 2011). With emerging information on the health benefits of ALA, milk enriched with this essential omega-3 fatty acid will be a product that appeals to today's consumers and meets recommendations for human health.

Table 2.1: Ingredients and composition of total mixed ration

Ingredient	**% DM**
Alfalfa silage	20.9
Alfalfa hay	5.77
Grass hay	9.18
Barley silage	26.8
Cracked corn	3.72
Barley grain (rolled)	8.63
Corn dry distiller grain + solubles	14.2
Canola meal	5.35
Performix[1]	4.61
Sodium bicarbonate	0.49
Salt	0.32
Chemical composition	
CP	18.0
ADF	23.4
NDF	36.5
Lipid	3.0
NEL , Mcal/kg DM	1.64
Ca	1.0
P	0.4

[1]Performix contained rr molasses, water, limestone, atta flow, com steep, salt, dolomite, ammonium polyphosphate, magnesium oxide, fat, zinc sulfate, manganese sulfate 32%, zin pro 180, vitamin E premix 60%, ferrous sulfate, copper sulfate 25%, vitamin A 1000, selenium 4%, vit D3 500, cobalt sulfate 32%, and EDDI 79.5%

Table 2.2: Fatty acid composition of total mixed ration

Fatty acid	**wt% of FAME**	**SEM**
C14:0	4.4	0.87
C16:0	16.6	0.12
C18:0	2.4	0.04
C18:1 *cis*-9	21.6	0.69
C18:2 *cis*-9 *cis*-12	41.2	0.56
C18:3 *cis*-9 *cis*-12 *cis*-15	7.9	0.52

Table 2.3: Fatty acid composition of rumen protected flax-based (RPF) supplement

Fatty acid	**wt% of FAME**	**SEM**
C16:0	6.7	0.03
C18:0	4	0.02
C18:1 *cis*-9	22.6	0.12
C18:2 *cis*-9 *cis*-12	14.2	0.06
C18:3 *cis*-9 *cis*-12 *cis*-15	49.9	0.15

Table 2.4: Effect of rumen protected flax-based (RPF) supplement on dry matter intake, milk production and composition

Variable[1]	**0 kg**	**1 kg**	**2 kg**	**3 kg**	**SEM**	**P > F**
Dry matter intake, kg/d	22.8	22	22.1	21.2	0.82	0.5534
Milk yield, kg/d	27.6	29.4	28.9	25.1	2.67	0.5935
Milk fat, %	3.75	3.65	3.72	3.91	0.001	0.02
Milk fat, kg/d	1.03	1.07	1.08	0.98	0.11	0.4608
Milk protein, %	3.1	3.15	3.09	3.07	0.001	0.7285
Milk protein, kg/d	0.85	0.93	0.89	0.77	0.09	0.2461
Lactose, %	4.64	4.65	4.62	4.59	0.001	0.9783
Lactose, kg/d	1.28	1.37	1.33	1.15	0.14	0.3068
Milk urea nitrogen, mg/dL	14.2	13.5	13.1	11.9	0.58	0.0761

[1]Milk samples were collected at each milking on d 15 and 16 of each period

Table 2.5: Effect of rumen protected flax-based (RPF) supplement on milk fatty acids (wt% of fatty acid methyl ester (FAME))

Fatty Acid	**0 kg**	**1 kg**	**2 kg**	**3 kg**	**SEM**[1]	**P > F**	**Linear**[2]	**Quadratic**[2]
C14:0	10.8	9.97	9.08	8.18	2.63	0.0001	<.0001	–
C14:1	0.46	0.41	0.38	0.34	0.0094	<.0001	<.0001	–
C15:0	1.05	0.94	0.85	0.74	0.0239	<.0001	<.0001	–
C16:0	25.7	22.8	20.6	18.7	0.3581	<.0001	<.0001	–
C16:1 *cis*-9	1.00	0.84	0.72	0.72	0.0612	0.0358	0.0109	–
C17:0	0.62	0.55	0.52	0.49	0.0101	<.0001	<.0001	–
C18:0	14.1	15.6	16.2	16.3	0.4863	0.0168	0.0035	–
C18:1 *cis*-9	23.2	24.8	25.7	26.3	0.4052	0.0014	0.0002	–
C18:1 *trans*-9	0.35	0.41	0.46	0.56	0.0203	<.0001	<.0001	–
C18:1 *trans*-10	0.43	0.49	0.60	0.72	0.0380	0.0003	<.0001	–
C18:1 *trans*-11	1.84	2.11	2.10	4.09	0.2219	<.0001	<.0001	–
C18:2 *cis*-9 *cis*-12	3.51	3.48	3.56	3.53	0.0901	0.8464	–	–
C18:2 *cis*-9 *trans*-11	0.83	0.92	1.14	1.46	0.0549	<.0001	<.0001	–
C18:3 *cis*-9 *cis*-12 *cis*-15	0.53	1.43	2.14	2.77	0.0598	<.0001	<.0001	<.0001

[1] Pooled SEM, average of SEM from each treatment,[2]orthogonal contrasts

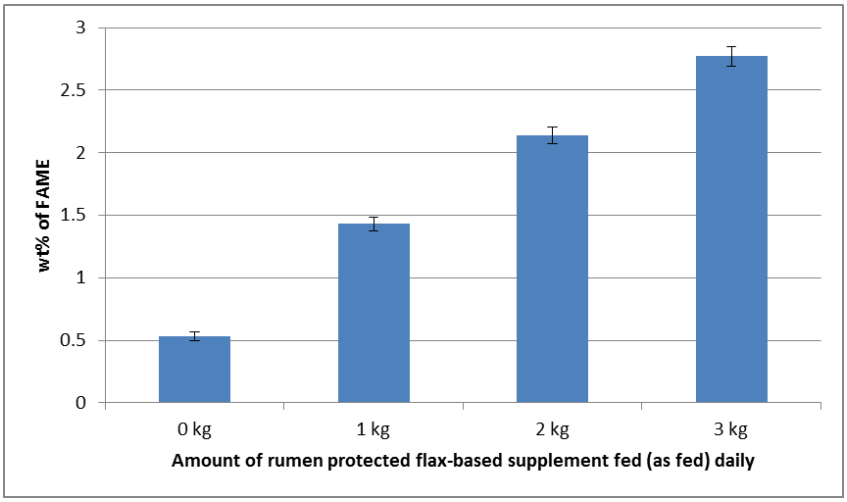


Figure 2.1: Concentration of alpha-linolenic acid in milk fat

Response of alpha-linolenic acid (C18:3 *cis*-9 *cis*-12 *cis*-15) in milk fat of cows fed varying levels of a rumen protected flax-based (RFP) supplement. Least square means ± SEM are presented for each level of RFP feeding

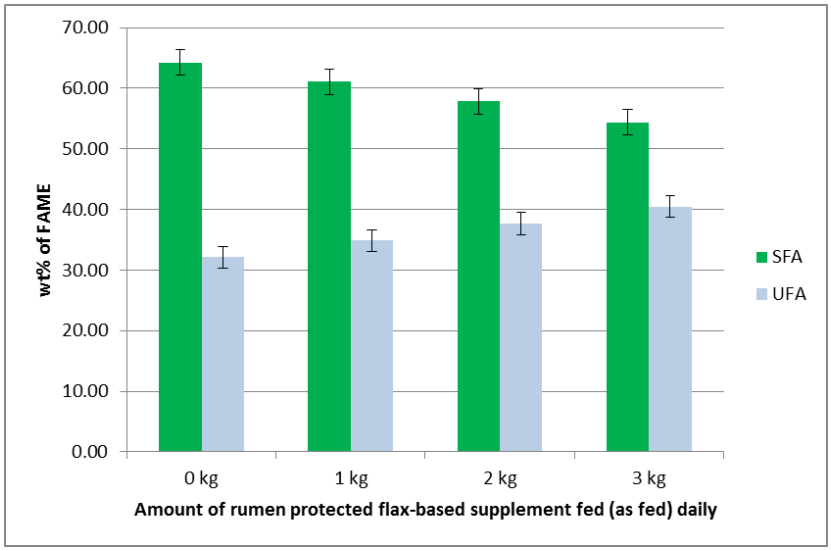


Figure 2.2: Concentrations of saturated fatty acids (SFA) and unsaturated fatty acids (UFA) in milk fat

The concentration of total SFA (sum of C4:0, C6:0, C8:0, C10:0, C12:0, C14:0, C15:0, C16:0, C17:0, and C18:0) was reduced ($P < 0.0001$) and the concentration of total UFA (sum of C14:1, C16:1 *cis*-9, C18:1 *cis*-9, C18:1 *trans*-9, C18:1 *trans*-10, C18:1 *trans*-11, C18:2 *cis*-9 *cis*-12, C18:2 *cis*-9 *trans*-11, and C18:3 *cis*-9 *cis*-12 *cis*-15) was increased ($P < 0.0001$) as the amount of RPF supplement added to the diet increased.

2.6 References

Astrup, A., J. Dyerberg, P. Elwood, K. Hermansen, F. B. Hu, M. U. Jakobsen, F. J. Kok, R. M. Krauss, J. M. Lecerf, P. LeGrand, P. Nestel, T. Sanders, A. Sinclair, S. Stender, T. Tholstrup, and W. C. Willet. 2011. The role of reducing intakes of saturated fat in the prevention of cardiovascular disease: where does the evidence stand in 2010?. Am. J. Clin. Nutr. 93:684-688

Barcelo-Coblijn, G., and E. J. Murphy. 2009. Alpha-linolenic acid and its conversion to longer chain n-3 fatty acids: benefits for human health and a role in maintaining tissue n-3 fatty acid levels. Recent Prog. Lipid Res. 48:355-374

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

Baumgard, L. H., J. K. Sangster, and D. E. Bauman. 2001. Milk fat synthesis in dairy cows is progressively reduced by increasing supplemental amounts of trans-10, cis-12 conjugated linoleic acid (CLA). J. Nutr. 131:1764-1769

Bayourthe, C., F. Enjalbert, and R. Moncoulon. 2000. Effects of different forms of canola oil fatty acids plus canola meal on milk composition and physical properties of butter. J. Dairy Sci. 83:690-696

Beam, T. M., T. C. Jenkins, P. J. Moate, R. A. Kohn, and D. L. Palmquist. 2000. Effects of amount and source of fat on the rates of lipolysis and biohydrogenation of fatty acids in ruminal contents. J. Dairy Sci. 83:2564-2573

Bernal-Santos, C., A. M. O'Donnell, J. L. Vicini, G. F. Hartnell, and D. E. Bauman. 2010. Enhancing omega-3 fatty acids in milk fat of dairy cows by using stearidonic acid-enriched soybean oil from genetically modified soybeans. J. Dairy Sci. 93:32-37

Brenna, J. T., N. Salem Jr., A. J. Sinclair, and S. C. Cunnane. 2009. Alpha-linoleic acid supplementation and conversion to n-3 long-chain polyunsaturated fatty acids in humans. Prostaglandins, Leukotrienes, and Essential Fatty Acids. 80:85-91

Caroprese, M., A. Marzano, R. Marino, G. Gliatta, A. Muscio, and A. Sevi. 2010. Flaxseed supplementation improves fatty acid profile of cow milk. J. Dairy Sci. 93:2580-2588

Chilliard, Y., C. Martin, J. Rouel, and M. Doreau. 2009. Milk fatty acids in dairy cows fed whole crude linseed, extruded linseed, or linseed oil, and their relationship with methane output. J. Dairy Sci. 92:5199-5211

Christie, W. W. 1982. A simple procedure for rapid transmethylation of glycerolipids and cholesteryl esters. J. Lipids Res. 23:1072-1075

Clark, R. M., A. M. Ferris, M. Fey, P. B. Brown, K. E. Hundriscer, and R. G. Jensen. 1982. Changes in lipids of human milk from 2-16 weeks postpartum. J. Pediatr. Gastroenterol. Nutr. 1:311-315

Collomb, M., H. Sollberger, U. Butikofer, R. Sieber, W. Stoll, and W. Schaeren. 2004. Impact of a basal diet of hay and fodder beet supplemented with rapseed, linseed and sunflowerseed on the fatty acid composition of milk fat. Int. Dairy J. 14:549-559

Cortes, C., D. C. Silva-Kazama, R. Kazama, N. Gagnon, C. Benchaar, G. T. D. Santos, L.

M. Zeoula, and H. V. Petit. 2010. Milk composition, milk fatty acid profile, digestion, and ruminal fermentation in dairy cows fed whole flaxseed and calcium salts of flaxseed oil. J. Dairy Sci. 93:3146-3157

Dang Van, Q. C., L. Bejarano, E. Mignolet, D. Coulmier, E. Froidmont, Y. Larondelle, and M. Focant. 2011. Effectiveness of extruded rapeseed associated with an alfalfa protein concentrate in enhancing the bovine milk fatty acid composition. J. Dairy Sci. 94:4005-4015

Drackley, J.K., T. H. Klusmeyer, A. M. Trusk, and J. H. Clark. 1992. Infusion of long-chain fatty acids varying in saturation and chain length into the abomasum of lactating dairy cows. J. Dairy Sci. 75:1517-1526

Glasser, F., A. Ferlay, and Y. Chilliard. 2008. Oilseed lipid supplements and fatty acid composition of cow milk: a meta-analysis. J. Dairy Sci. 91:4687-4703

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. 88:748-756

Gonthier, C., A. F. Mustafa, R. Berthiaume, H. V. Petit, R. Martineau, and D. R. Ouellet. 2004. Effects of feeding micronized and extruded flaxseed on ruminal fermentation and nutrient utilization by dairy cows. J. Dairy Sci. 87:1854-1863

Hawkins, A., K. Yuan, C. K. Armendariz, G. Highland, N. M. Bello, T. Winowiski, J. S. Drouillard, E. C. Titgemeyer, and B. J. Bradford. 2013. Effects of urea formaldehyde condensation polymer treatment of flaxseed on ruminal digestion and lactation in dairy cows. J. Dairy Sci. 96:3907-3915

Jenkins, T. C., and M. A. McGuire. 2006. Major advances in nutrition: impact on milk composition. J. Dairy Sci. 89:1302-1310

Jenkins, T. C. 1993. Lipid metabolism in the rumen. J. Dairy Sci. 76:3851-3863

Jenkins, T. C., and W. C. Bridges Jr. 2007. Protection of fatty acids against ruminal biohydrogenation in cattle. Eur. J. Lipid Sci. 109:778-789

Jouany, J., B. Lassalas, M. Doreau, and F. Glasser. 2007. Dynamic features of the rumen metabolism of linoleic acid, linolenic acid, and linseed oil measured in vitro. Lipids. 42:351-360

Kennelly, J. J. 1996. The fatty acid composition of milk fat as influenced by feeding oilseeds. Anim. Feed Sci. Technol. 60:137-152

Khas-Erdene, J. Q. Wang, D. P. Bu, L. Wang, J. K. Drackley, Q. S. Liu, G. Yang, H. Y. Wei, and L. Y. Zhou. 2010. Responses to increasing amounts of free alpha-linolenic acid infused into the duodenum of lactating dairy cows. J. Dairy Sci. 93:1677-1684

Kliem, K. E., D. J. Humphries, C. K. Reynolds, R. Morgan, and D. I. Givens. 2016. Effect of oilseed type on milk fatty acid composition of individual cows, and also bulk tank milk fatty acid composition from commercial farms. Animal. 1-11

Kramer, J. K., V. Fellner, M. E. R. Dugan, F. D. Sauer, M. M. Mossoba, M. P. Yurawecz. 1997. Evaluating acid and base catalysts in the methylation of milk and rumen fatty acids with special emphasis on conjugated dienes and total trans fatty acids. Lipids. 32:1219-1228

Lock, A. L. and D. E. Bauman. 2004. Modifying milk fat composition of dairy cows to enhance fatty acids beneficial to human health. Lipids. 39:1197-1206

Moallem, U. 2009. The effects of extruded flaxseed supplementation to high-yielding dairy cows on milk production and milk fatty acid composition. Anim. Feed Sci. Technol. 152:232-242

Mosley, E. E., B. Shafii, P. J. Moate, and M. A. McGuire. 2006. cis-9, trans-11 conjugated linoleic acid is synthesized directly from vaccenic acid in lactating dairy cattle. J. Nutr. 136:570-575

Mosley, E. E. and M. A. McGuire. 2007. Methodology for the in vivo measurement of the delta-9 desaturation of myristic, palmitic, and stearic acids in lactating dairy cattle. Lipids. 42:939-945

O'Donnell, J. 1993. Future of milk fat modification by production or processing: integration of nutrition, food science, and animal science. J. Dairy Sci. 76:1797-1801

Oldham, J. D. 1984. Protein-energy interrelationships in dairy cows. J. Dairy Sci. 67:1090

Palmquist, D. L. 2006. Milk fat: origin of fatty acids and influence of nutritional factors thereon. Lipids. 2:43-92

Palmquist, D. L. and T. C. Jenkins. 1980. Fat in lactation rations: review. J. Dairy Sci. 63:1-14

Palmquist, D. L., A. D. Beaulieu, and D. M. Barbano. 1993. Feed and animal factors influ-

encing milk fat composition. J. Dairy Sci. 76:1753-1771

Palmquist, D. L. 2007. Biohydrogenation then and now. Eur. J. Lipid Sci. 109:737-739

Petit, H. V., C. Germiquet, and D. Lebel. 2004. Effect of feeding whole, unprocessed sunflower seeds and flaxseed on milk production, milk composition, and prostaglandin secretion in dairy cows. J. Dairy Sci. 87:3889-3898

Petit, H. V. 2002. Digestion, milk production, milk composition, and blood composition of dairy cows fed whole flaxseed. J. Dairy Sci. 85:1482-1490

Rodriguez-Leyva, D., C. M. C. Bassett, R. McCullogh, and G. N. Pierce. 2010. The cardiovascular effects of flaxseed and its omega-3 fatty acid, alpha-linolenic acid. Can. J. Cardiol. 26:489-496

Santos, J. E. P., T. R. Bilby, W. W. Thatcher, C. R. Staples, and F. T. Silvestre. 2008. Long chain fatty acids of diet as factors influencing reproduction in cattle. Reprod. Dom. Anim. 43:23-30

Simopoulos, A. P. 1991. Omega-3 fatty acids in health and disease and in growth and development. Am. J. Clin. Nutr. 54:438-463

Siri-Tarino, P. W., Q. Sun, F. B. Hu, and R. M. Krauss. 2010. Saturated fat, carbohydrate, and cardiovascular disease. Am. J. Clin. Nutr. 91:502-509

Stoop, W. M., J. A. M. van Arendonk, J. M. L. Heck, H. J. F. van Valenberg, and H. Bovenhuis. 2008. Genetic parameters for major milk fatty acids and milk production traits

of dutch Holstein-Friesians. J. Dairy Sci. 91:385-394

Swanson, D., R. Block, and S. A. Mousa. 2012. Omega-3 fatty acids EPA and DHA: health benefits throughout life. Adv. Nutr. 3:1-7

U.S. Department of Agriculture and U.S. Department of Health and Human Services. 2010. Dietary Guidelines for Americans. 7th ed. U.S. Government Printing Office, Washington DC.

Ward, A. T., K. M. Wittenberg, and R. Przybylski. 2002. Bovine milk fatty acid profiles produced by feeding diets containing solin, flax, and canola. J. Dairy Sci. 85:1191-1196

Weaver, C. M. 2014. How sound is the science behind the dietary recommendations for dairy?. Am. J. Clin. Nutr. 99:1217-1222

Appendix A: Research Protocol Approval

Tuesday, October 4, 2016 at 3:08:33 PM Pacific Daylight Time

Subject: IACUC Protocol 2015-20 Approval
Date: Thursday, August 25, 2016 at 3:21:05 PM Pacific Daylight Time
From: Institutional Animal Care and Use Committee (iacuc@uidaho.edu)
To: McGuire, Mark (mmcguire@uidaho.edu)
Attachments: image001.jpg

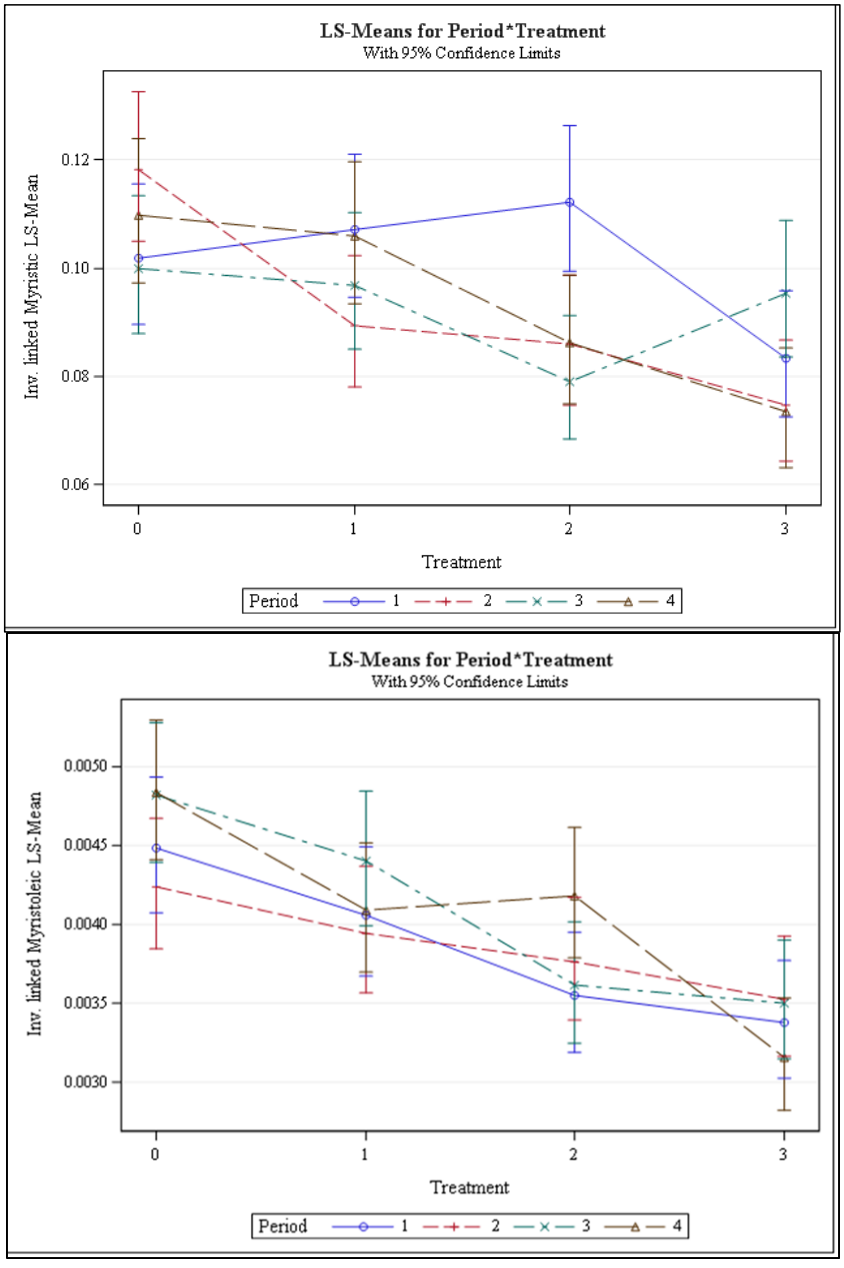
University of Idaho
Institutional Animal Care and Use Committee

Date: Thursday, August 18, 2016
To: Mark McGuire
From: University of Idaho
Institutional Animal Care and Use Committee
Re: Protocol 2015-20
Dietary lipid sources for dairy cows; effect on milk fat and fatty acid composition

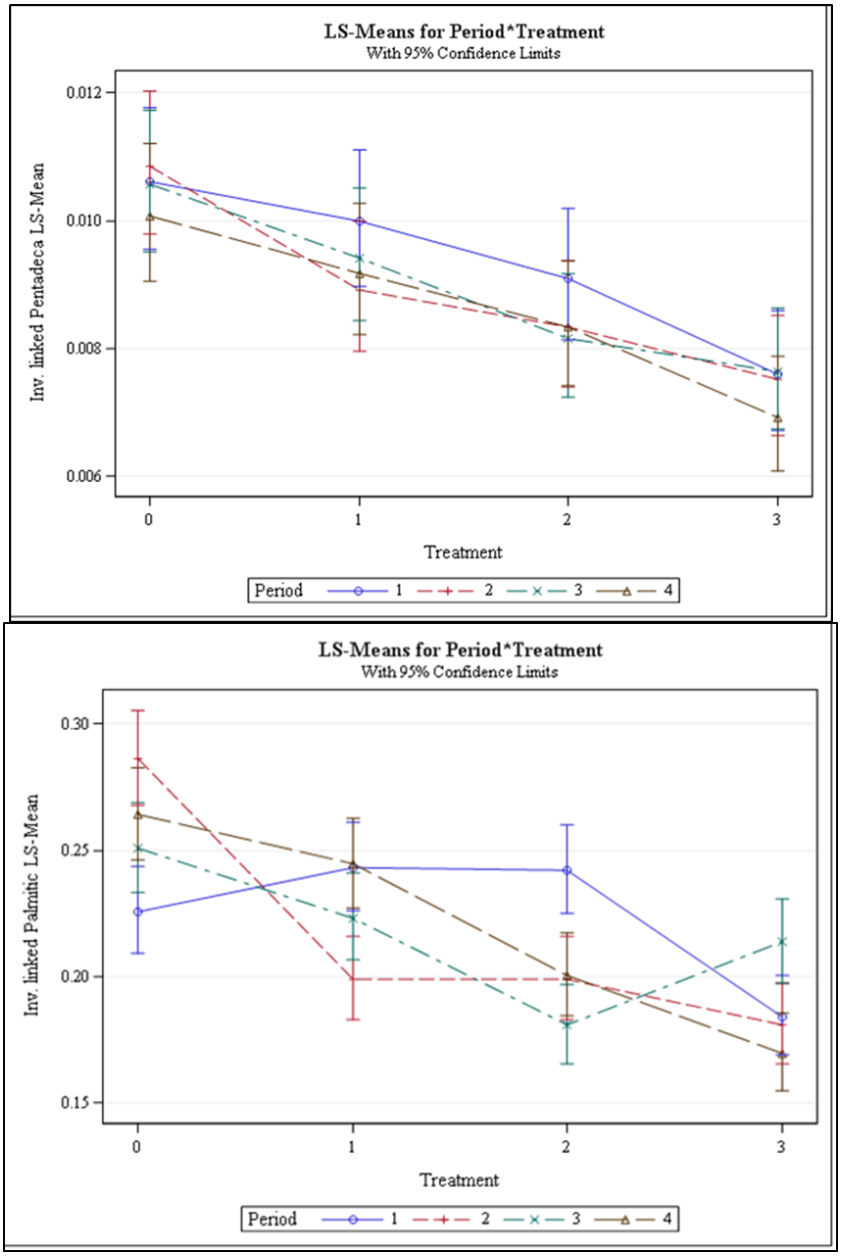
Your requested renewal of the animal care and use protocol shown above was reviewed and approved by the Institutional Animal Care and Use Committee on Thursday, August 18, 2016.
This protocol was originally submitted for review on: Wednesday, March 18, 2015
The original approval date for this protocol is: Thursday, March 26, 2015
This approval will remain in affect until: Friday, August 18, 2017
The protocol may be continued by annual updates until: Monday, March 26, 2018
Federal laws and guidelines require that institutional animal care and use committees review ongoing projects annually. For the first two years after initial approval of the protocol you will be asked to submit an annual update form describing any changes in procedures or personnel. The committee may, at its discretion, extend approval for the project in yearly increments until the third anniversary of the original approval of the project. At that time, the protocol must be replaced by an entirely new submission.

Barrie Robison, IACUC Chair

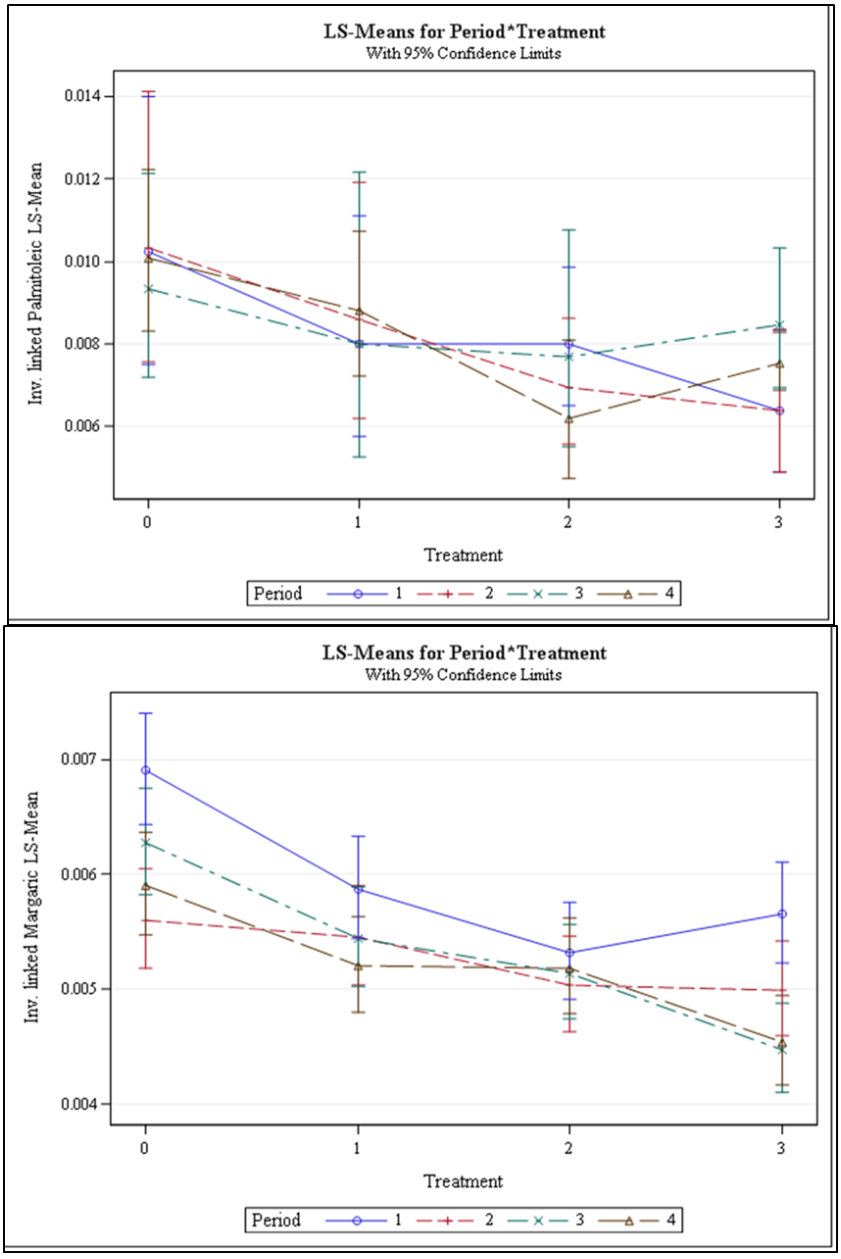
Appendix B: Interaction Plots



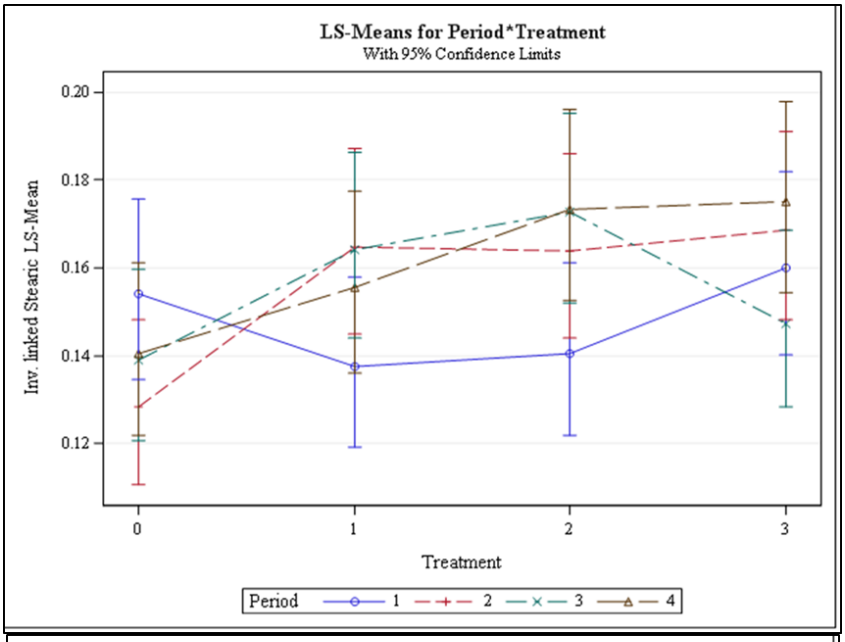
Interaction plots for myristic (C14:0) and myristoleic (C14:1) fatty acids

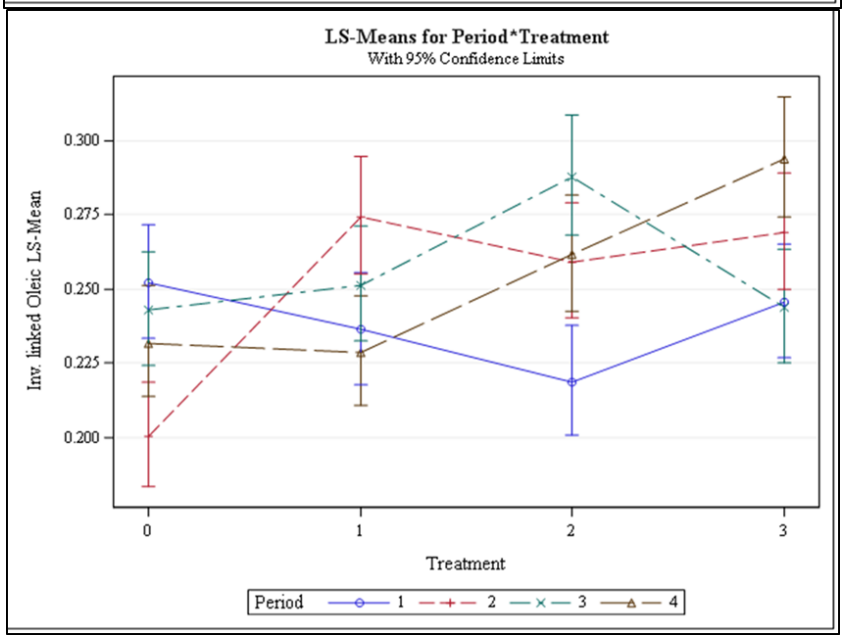


Interaction plots for pentadeca (C15:0) and palmitic (C16:0) fatty acids

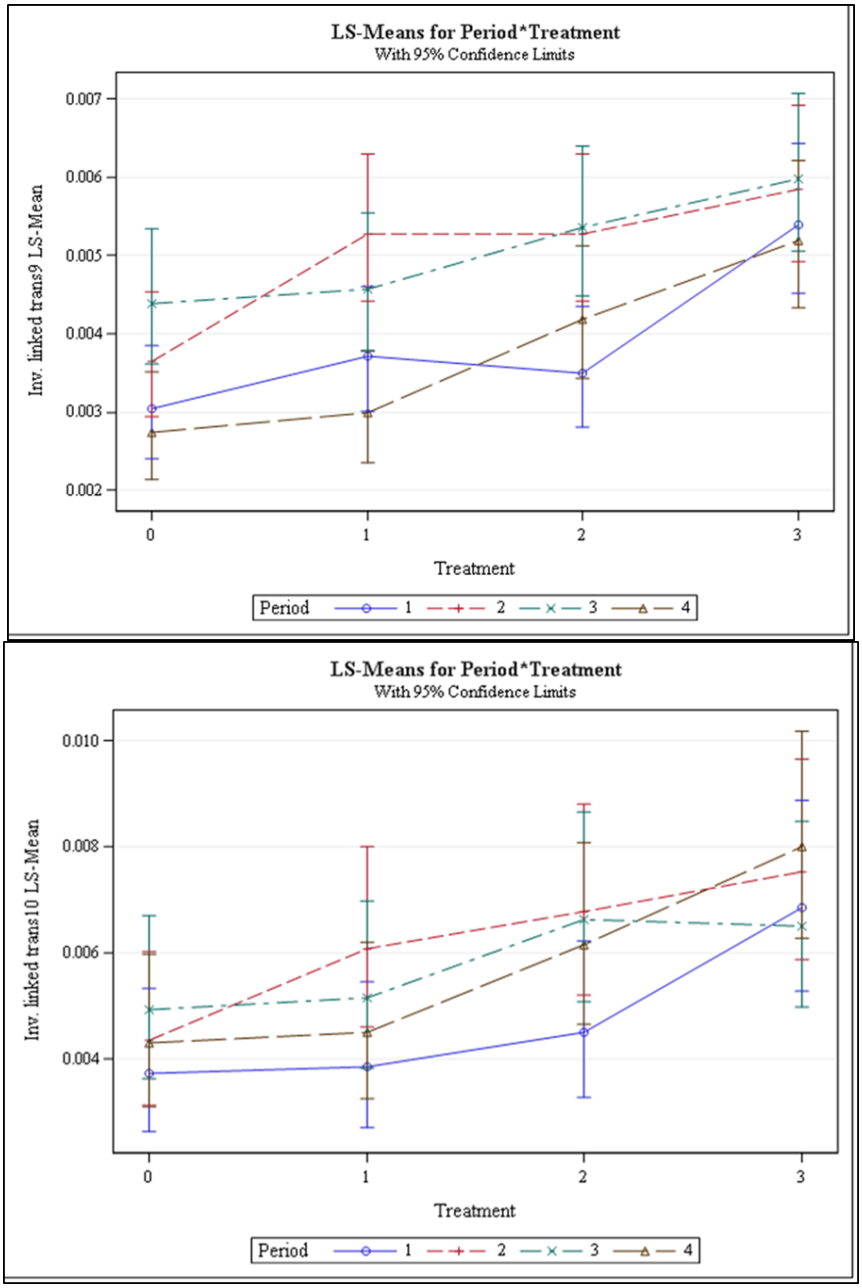


Interaction plots for palmitoleic (16:1 *cis*-9) and margaric (C17:0) fatty acids

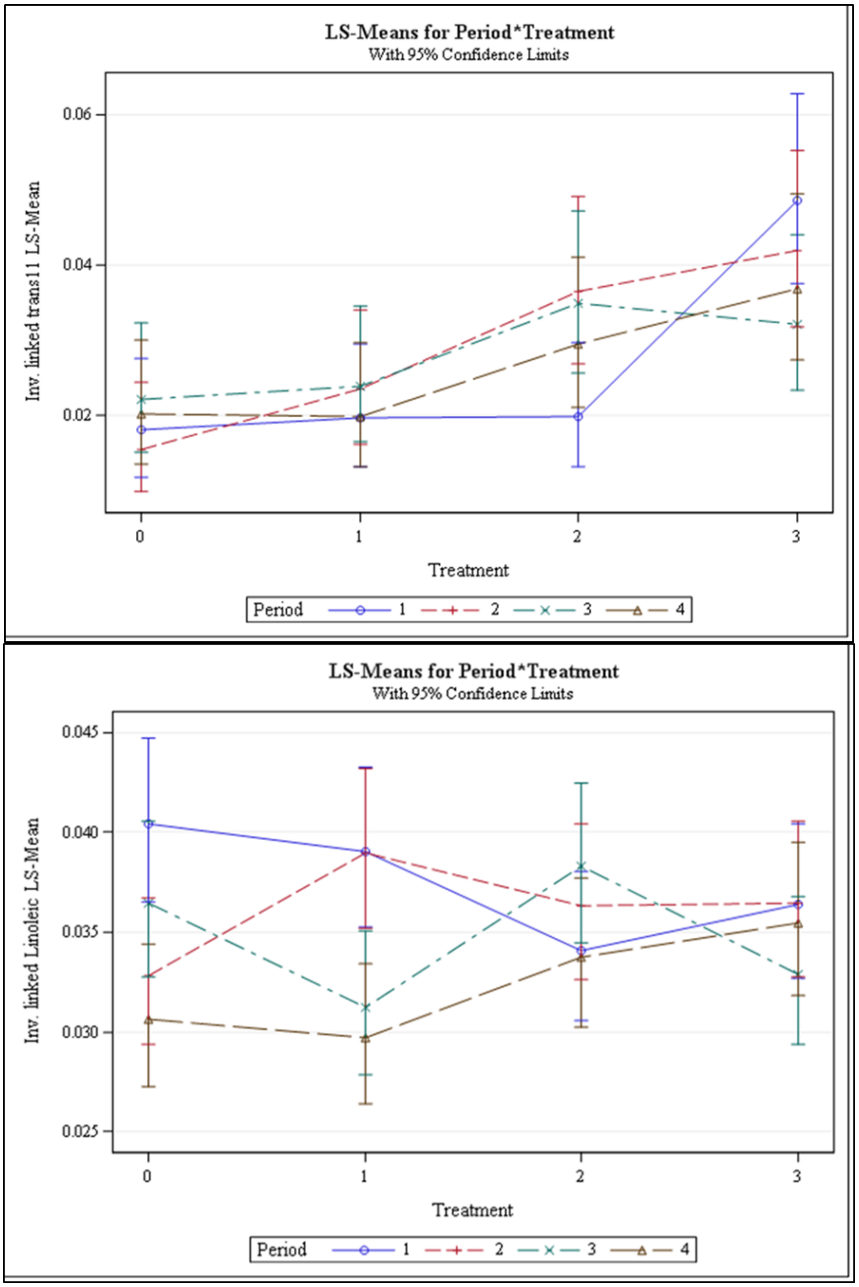




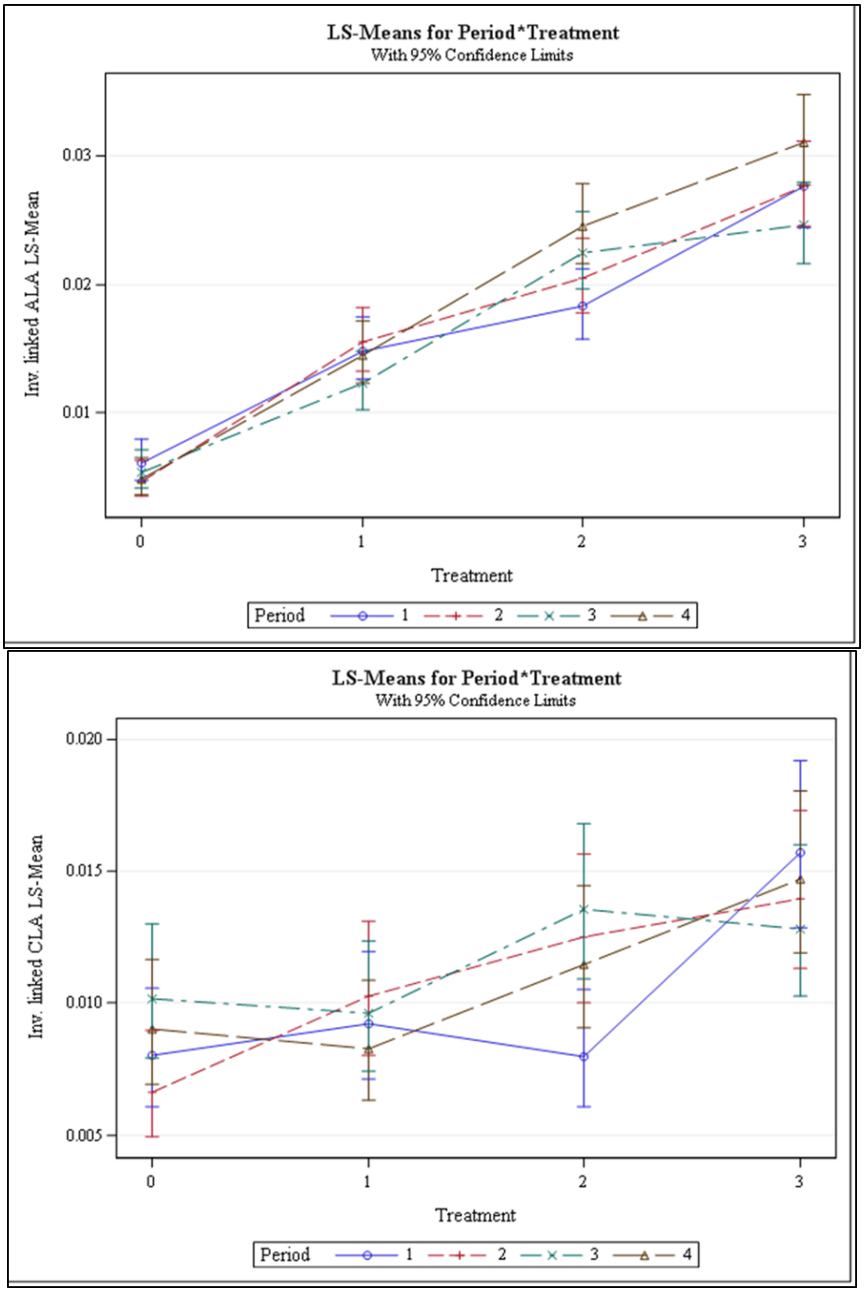
Interaction plots for stearic (C18:0) and oleic (C18:1 *cis*-9) fatty acids



Interaction plots for C18:1 *trans*-9 and *trans*-10 fatty acids



Interaction plots for C18:1 *trans*-11 and linoleic (C18:2 *cis*-9 *cis*-12) fatty acids



Interaction plots for alpha-linolenic acid (C18:3 *cis*-9 *cis*-12 *cis*-15) and conjugated linoleic acid (C18:2 *cis*-9 *trans*-11 CLA) fatty acids