AN ABSTRACT OF THE THESIS OF

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Title: Improving the Nutritional and Textural Properties of Dairy Products by Feeding Holstein Cows Processed Flaxseed

Abstract approved:

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There is growing public concern about the high proportion of saturated fatty acids in milk fat; however, feed intake, energy partitioning toward milk synthesis, and milk fat concentrations can decrease when cows are fed high concentrations of unsaturated lipids. The objective of this study was to identify the optimal rate for feeding OmegaBoost™ (a flaxseed supplement that was processed using a proprietary technique by Double Pass LLC, Tualatin, OR) to dairy cows. The central hypothesis was that supplementation with OmegaBoost will decrease the proportion of saturated fatty acids in milk fat in a dose dependent manner. Using a latin-square design, 10 Holstein cows in mid to late lactation were fed for two-week periods 0, 2, 4, or 6 lbs/d of OmegaBoost or 4lbs/d ground flax as top dressing to their total mixed ration. Feed intake, body weight, activity and resting time, milk production and milk composition were measured daily. At the end of each two-week period, milk and serum samples were taken and analyzed for fatty acid composition using gas chromatography. In addition, fresh Mozzarella cheese and butter was manufactured and tested to determine the fatty acid composition and the effects of flaxseed supplementation on texture. Feeding OmegaBoost at 2, 4, and 6 lbs/d linearly decreased the proportion of saturated fatty acids in milk by 6, 15, and 18%,

respectively, and linearly increased the proportion of mono-unsaturated fatty acids (14, 32, and 35%), poly-unsaturated fatty acids (16, 49, and 82%), and α-linolenic acid (26, 52, and 70%). Similar changes in fatty acid composition were observed in butter and cheese samples, resulting in butter that was less hard and adhesive at refrigeration temperature in response to feeding cows increasing concentrations of OmegaBoost. Feed intake, body weight, serum metabolite concentrations, milk production and composition, and butter and cheese yield were not significantly affected by feeding processed flaxseed. Therefore, feeding 4 or 6 lbs/d of OmegaBoost to dairy cows is effective in improving the nutritional and textural profile of dairy products without negatively affecting feed intake, milk production, or weight gain.

Improving the Nutritional and Textural Properties of Dairy Products by Feeding Holstein Cows Processed Flaxseed

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

Scott P. Oeffner, Author

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CONTRIBUTION OF AUTHORS

Chapters 3& 4:

Nathalie Quezada – Provided technical assistance during sample analysis.

Erica Ramsing – Provided technical assistance during data collection and sample analysis.

Dr. Bobe – obtained funding, oversaw project design sample collection and analysis, aided in data interpretation, and performed statistical analysis and manuscript writing assistance.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

IMPROVING THE NUTRITIONAL AND TEXTURAL PROPERTIES OF DAIRY PRODUCTS BY FEEDING HOLSTEIN COWS PROCESSED FLAXSEED

CHAPTER 1. LITERATURE REVIEW

A growing public concern regarding dairy products is the high ratio of saturated to unsaturated fatty acids because dietary intake of saturated fatty acids is associated with increased risk of several chronic diseases. Composition of animal feed has been shown to be effective in altering milk fatty acid composition in order to make it healthier for people who enjoy consuming milk and milk products (Collomb et al., 2004; Kudrna and Marounek, 2008; Soita et al., 2003). Increasing concentrations of poly-unsaturated fatty acids, particularly linoleic and α-linolenic acids, in the dairy cow's feed decreases the synthesis of saturated fatty acids in the mammary gland (lauric, myristic, and palmitic acid). This results in decreased blood concentrations of total and low density lipoprotein cholesterol in humans consuming the modified dairy products (Palmquist et al., 1993; Soita et al., 2003; Ward et al., 2002). The objective of the literature review is to summarize current knowledge about ruminant lipid metabolism, milk fat and human health, and flaxseed feeding on animal performance and fatty acid composition of dairy products.

RUMINANT LIPID METABOLISM

Rumen Lipid Metabolism

Dietary lipid intake and composition is only a rough starting point when beginning to account for diversity of fatty acids found in milk fat. Once ingested, triacylglycerols become the substrate for microbial conversion. The degree of change that any single fatty acid achieves is dependent upon a number of variables that can alter ruminal environment, microbial population, and the accessibility of the fatty acid to microbes. The degree of saturation will also have a significant effect on how the lipids will change as they flow through the rumen.

The agents of change in the rumen are primarily fiber digesting bacteria (B. fibrisolvens), but it is important not to forget that there is a large degree of interdependence (cross feeding) that sustains the ruminal ecosystem. As feed enters the rumen, large buoyant particles form a fiber mat that floats on top of the ruminal fluid while small dense particles sink in the ruminal fluid. The interface between the solid and liquid phase is where the bulk of fiber digestion occurs as fungi and fiber digesting bacteria work together with ruminal contractions and regurgitation to physically and chemically increase surface area and decrease particle size. As digestion is taking place, the decreasing particle size allows more surface area for microbial attachment that further increases solubilization of dietary nutrients.

The hydrolysis of triacylglycerols (TAG) in the rumen by microbial lipoprotein lipase releases non-esterified fatty acids (NEFA). Fatty acids that are completely saturated have very little impact on rumen function, but as the number of double bonds in a fatty acid increases, the more disruptive it becomes to microbial membranes. Because of their toxic properties, the double bonds of unsaturated fatty acids are targeted by fiber digesting bacteria to be hydrogenated. Hydrogenation primarily occurs in a stepwise fashion because very few bacterial species are capable of performing the necessary steps to produce complete hydrogenation.

For example, the hydrogenation of a poly-unsaturated fatty acid such as linolenic acid can produce a number of intermediate products, including trans fatty acids and conjugated linolenic acids (CLA), because of variations that are possible as each of its three double bonds are reduced to varying degrees. Although some variations do exist, especially as ruminal pH decreases and dietary poly-unsaturated fatty acids increase, the

primary fatty acids that are produced by the hydrogenation of linolenic acid are fairly consistent (Figure 1).

Very little linolenic acid (or linoleic acid) passes through the rumen intact once it has been cleaved from TAG. If the cleavage is prevented, hydrolysis and thus hydrogenation cannot take place. The prevention of fatty acid cleavage from TAG is a common strategy used in research to manipulate fatty acid availability in the ruminant diet. In addition to altering the flow of fatty acids from the rumen, protection allows us to incorporate fat at a higher concentration without negative effects on rumen microbial populations. The ability to feed more fat enables the production of feed with higher energy density and lower heat of fermentation that can be used during the transition period when energy demands are high or during periods of heat stress when feed intake is low.

Intestinal Lipid Absorption

In mono-gastric animals little digestion of dietary lipids occurs prior to their arrival in the duodenum and only after mixing with bile and pancreatic secretions does digestion begin. In ruminants, rumen microbes begin the digestion of many dietary lipids by a) releasing NEFA from TAG, b) bio-hydrogenation of poly-unsaturated FA, and c) microbial biosynthesis of lipids (Garton, 1965).

After conversion in the rumen, NEFA (70-80% of the lipids) and TAG flow through the reticulum and omasum into the abomasum, where the acidic environment kills all microbes and stops any lingering hydrogenation. The acidic environment of the abomasum and proximal jejunum also allows for the dissociation of many protective compounds (encapsulation and calcium salts) so that TAG can be hydrolyzed by gastric

and pancreatic lipases prior to absorption. However, absorption of lipids in the ruminant small intestine occurs in stages. Lipid absorption primarily occurs in the proximal jejunum as NEFA (hydrolyzed by rumen microbes). Because pancreatic secretions are much less in ruminants, the pH remains higher in the proximal jejunum than in non-ruminants, increasing the rate of absorption of NEFA but adversely effecting pancreatic lipase activity. Pancreatic lipase is secreted but is largely inactive in the pH ranges of the proximal jejunum. However, as the digesta move to toward the distal jejunum and pH rises and lipase activity increases releasing NEFA from TAG that escaped absorption in the proximal jejunum. Absorption is enhanced as NEFA and monoacylglycerol form micelles with bile salts and diffuse through the unstirred water layer overlying the intestinal epithelial microvilli (Caple and Heath, 1975; Garton, 1965; Leat and Harrison, 1975).

When the micelle arrives at the surface of the enterocyte, fatty acids can enter the cell via passive or carrier-mediated uptake. The fatty acids dissolve in the brush border membrane as they contact the enterocyte and due to the concentration gradient diffuse into the cell. The NEFA concentration gradient is maintained by the rapid re-esterification of NEFA to TAG by enzymes on the cytosolic surface of the endoplasmic reticulum; i.e. monoacylglycerol acyltransferase and diacylglycerol acyltransferase associated with a complex called TAG synthetase; thus favoring continued uptake or diffusion of NEFA into the enterocyte (Lehner and Kuksis, 1995). Fatty acid transport can also be mediated by FATP4 but this facilitate transport appears to be significant only during periods of starvation (Stahl et al. 1999).

Water-soluble components of lipid digestion and more soluble short chain fatty acids can exit the enterocyte and enter the portal blood system via diffusion. However

less soluble lipids must first be packaged into chylomicrons and secreted into the lymphatic system prior to entry into the blood stream. Pre-chylomicron formation involves the incorporation of apolipoproteins (primarily A and B) and lipids to form a soluble complex. The prechylomicron then moves from the endoplasmic reticulum (N-glycosylation occurs), through the Golgi apparatus (terminal glycosylation occurs) and finally out of the enterocyte, into the intracellular space via excocytosis becoming a chylomicron (Sabesin and Frase, 1977). Chylomicrons enter the blood stream via the lymph system and can be utilized by other body tissues.

Hepatic Lipid Metabolism

Other than NEFA released by adipose tissue, the ruminant liver can utilize fatty acids from hepatic de-novo synthesis, cytoplasmic TAG reserves, and TAG transported in LDL (Nguyen et al., 2008). The primary source is NEFA during lactation (Cuvelier et al., 2005b; Emery et al., 1992; Hocquette and Bauchart, 1999) (Figure 1.2). Unlike adipose and mammary tissue, the ruminant liver cannot efficiently hydrolyze fatty acids for transport into the hepatocyte (due to low hepatic lipoprotein lipase activity) (Bauchart et al., 1996). Although the esterification capacity of the ruminant liver is similar to that of other species (Emery et al., 1992), lower precursor availability causes ruminant hepatic fatty acid synthesis to be relatively low (Bauchart et al., 1996).

Once NEFA enter the liver they flow through one of three pathways: secretion, storage, or oxidation (Emery et al., 1992; Hocquette and Bauchart, 1999). Hepatic free fatty acids can be secreted as NEFA into bile (minor pathway) (Emery et al., 1992) or be oxidized to produce energy, carbon dioxide, acetate, and ketone bodies. Fatty acids that

are re-esterified to TAG can either be stored in the liver or repackaged with lipoproteins and secreted back into the blood for utilization by other tissues (Nguyen and al., 2008).

Like elsewhere in the body, fatty acids up to 18 carbons in length are oxidized in the mitochondria. Fatty acids 14 carbons and longer must first be activated by acyl-CoA synthetase before they can be transported into the mitochondria by carnitine acyltransferase. Fatty acids less than 14 carbons can enter directly into the mitochondria and are activated in situ (Hocquette and Bauchart, 1999; Drackley, 2000). In the mitochondria, fatty acids can then be either completely oxidized to produce ATP or partially oxidized and used in the formation of ketone bodies (Hocquette and Bauchart, 1999; Drackley, 2000). Fatty acids that are more than 18 carbons long are shunted to peroxisomes for partial oxidation and removal of acyl units to shorten the fatty acid. The shortened fatty acid that leaves the peroxisome (still in the cytosol) can be incorporated into the membrane, used for fatty acid synthesis, packaged for secretion, or activated for transport into the mitochondria for oxidation (Guzman and Geelen, 1993).

Acetyl-CoA resulting from beta-oxidation can enter the Krebs cycle to be completely oxidized and provides energy in the form of ATP or be used in the synthesis of ketone bodies (Hocquette and Bauchart, 1999; Drackley, 2000). The liver can process fatty acids through ketogenesis approximately five times faster than through the Krebs cycle to yield approximately the same amount of energy (Cuvelier et al. 2005b). The production of ketone bodies from fatty acids allows the ruminant liver to cope with the massive influx of NEFA in situations of negative energy balance (Drackley, 2000). As an alternative to beta-oxidation during periods of positive energy balance, the esterification of acyl-CoA to TAG, and to a lesser extent, phospholipids and cholesterol

esters are favored by the increased glycerol-3-phosphate and malonyl-CoA (Gruffat et al., 1996; Drackley, 2000).

The content of TAG in hepatocytes depends on the balance between the rate of absorption, de-novo synthesis, esterification, and the rate of oxidation and secretion of very low density lipoprotein (VLDL) TAG (Nguyen et al., 2008). The liver of ruminants is characterized by low VLDL export capacity in relation to other species of mammals. As indicated by Emery et al. (1992), TAG secretion is 60% higher in pregnant or lactating ewes than in open ewes, but only represents 2% of fatty acid uptake, while oxidation accounts for approximately 12%. The low capacity of VLDL secretion favors pathological accumulation of TAG in hepatocytes ("fatty liver"). This condition is most common during the final phase of the gestation and early lactation when the synthesis of TAG from NEFA exceeds the liver's export and oxidation capacity forcing storage (Grummer, 1993).

Adipose Lipid Metabolism

Lipogenesis in ruminants occurs primarily in adipose tissues (Roh et al. 2006). Preformed fatty acids enter adipocytes after hydrolysis from TAG rich lipoproteins or serum albumin by lipoprotein lipase at the endothelial surface of adipose tissue, while acetate and BHBA diffuse across the membrane and enter a cycle of activation and elongation via acetyl-CoA carboxylase (ACC) and fatty acid synthetase (FAS) to produce medium chain (12-16) NEFA in de novo synthesis. Triacylglycerol is formed by the esterification of de-novo and preformed fatty acids to glycerol-3 phosphate (synthesized de novo from propionate or preformed) to produce a dense form of storable energy: triacylglycerol (Figure 3). Triacylglycerol is stored as fat as long as the animal is in

positive energy balance. Then, as the animal transitions to negative energy balance TAG is hydrolyzed and adipocytes release NEFA and glycerol to provide the animal with reserve energy until the energy demands can again be met by dietary intake.

Mammary Lipid Metabolism:

Palmquist and Conrad (1971) concluded that 88% of long chain fatty acids (18 carbon chain length and longer) in milk were derived directly from TAG of intestinal lipoproteins while 12% were synthesized endogenously. Like in other tissues circulating triacylglycerols are hydrolyzed to NEFA and absorbed into the targeting tissue. In the mammary gland up to 40% of NEFA from hydrolyzed TAGs are released into plasma, escaping absorption by mammary cells (Mendelson and Scow, 1972). Absorption into mammary epithelial cells is achieved by diffusion (Thompson et al., 1983; Hajri and Abumrad, 2002) into and through the membrane following lipoprotein lipase hydrolysis or is mediated by carrier proteins: FAT CD 36 (Barber et al., FABP (Veercamp et al., 1991; Storch and Thumser, 2000), 1997), and acyl-CoA biding proteins (ACBP; Kundsen et al., 2000); the latter two of which are implicated primarily in long chain (16-20) fatty acid absorption with ACBP having a 10 fold greater binding affinity (Rasmussen et al., 1990). However expression of ACBP is relatively low in mammary tissue in cows.

Fatty acid synthesis in the mammary gland begins with acetate (BHBA, propionate, and other microbialy derived volatile fatty acids; Figure 4). Acetate is activated to acyl-CoA by acetyl-CoA carboxylase (ACC) and used as a building block by fatty acid synthetase (FAS) to add additional two carbon units to the original fatty acid unit. The large assortment of fatty acids found in milk fat comes in part from this ability to start from and elongate several different short chain fatty acids derived from microbial

metabolism. Additional variation in the fatty acid composition of milk is contributed by the low specificity of fatty acid synthase. Ruminant fatty acid synthase does not hold the elongating FA chain as securely as that of non-ruminants, allowing the release fatty acids of various chain lengths between 6 and 16 carbon chain with 12, 14, and 16 carbon chain being synthesized in the greatest proportion from acetate.

Regulation of Lipid Metabolism:

The regulation of whole body nutrient partitioning is ultimately regulated by the nutrients (glucose and NEFA) that either are or are not available to the body tissues. In the ruminant animal this process is slightly more complex because ruminal microbes extensively alter these dietary nutrients. Dietary starch is utilized (converted to propionate) by ruminal microorganisms and is generally in short supply to the body tissues. Because glucose is primarily produced via gluconeogenesis, at a fairly consistent rate, it plays only a minor role in regulation of nutrient utilization SREBP.

Peroxisome proliferator-activated receptors (PPARs) are expressed in nearly every tissue of the body, but of primary importance is the expression of PPARα in liver and heart tissues and cells of the arterial wall and of PPARγ in adipose tissue. The activation of PPARα is mediated by fibrates, eicosanoids and to a lesser degree poly-unsaturated fatty acids, namely α-linolenic, eicosapentaenoic, docosahexaenoic acids (Chinetti et al., 2001). Peroxisome proliferator-activated receptors control lipid transport and oxidation by regulating gene expression: PPARα regulates the expression of genes involved in the peroxisomal and mitochondrial β-oxidation pathways such as Acyl-CoA oxidase, Enoyl-CoA hydratase/dehydrogenase multifunctional enzyme, Keto-Acyl-CoA

thiolase, Malic enzyme, medium chain Acyl-CoA dehydrogenase, and mitochondrial hydroxy methylglutaryl-CoA synthase.

Peroxisome proliferator-activated receptor-α also regulates fatty acid transport protein (FATP), fatty acid translocas FAT/CD36, and liver cytosolic fatty acid-binding protein (FABP), which regulate fatty acid transport pathways. By altering transcription of these genes, activated PPARα leads to increased hydrolysis of TAG, fatty acid oxidation, increased cellular fatty acid uptake, and reduced TAG and fatty acid synthesis. Peroxisome proliferator-activated receptor-γ is activated by fatty acids or their oxidized derivatives and promotes insulin sensitivity and adipocyte differentiation. Peroxisome proliferator-activated receptor-γ activates transcription in concert with coactivators including steroid receptor coactivator-1 (SRC1), and has also been implicated in a variety of neoplastic processes, including colorectal cancer (Boitier, 2003).

HUMAN HEALTH IMPLICATION

Cancer

Early recommendations, in the 1980's, based on animal studies and international comparisons recommended the reduction of dietary fat based on the strong correlations between national *per capita* fat consumption and the incidence of cancer (Willett, 2001a; Kushi and Giovannucci, 2002). Later studies by Seidell (1998), and Willett (2002), have not supported an important role for total fat intake in the development of cancer separate from excess energy intake. Other studies have shown that physical inactivity or excess energy intake relative to requirements strongly increased the risk of malignancy in cases of colon cancer (Giovannucci and Goldin, 1997), breast cancer (Ip et al., 1990) and prostate cancer (Kolonel, 2001). However neither total, saturated nor mono- or poly-

unsaturated fat was associated with risk of colorectal cancer (Howe et al., 1997) or breast cancer (Hunter et al., 1996). A weak inverse correlation between animal fat/saturated fat and prostate cancer is likely related to the products formed by the charring of red meat rather than the type of fat per se (Kolonel, 2001).

Conjugated linolenic acid has been proposed to decrease the risk of cancer by reducing body fat accumulation (inhibition of fatty acid synthesis by cis-10, trans-12 CLA) and inhibition of inflammation (inhibition cyclooxgenase activity and prostaglandin synthesis by cis-10, trans-12 and cis-9, trans-11 CLA). Similar anticarcinogenic effects have been proposed for α-linolenic acid.

Atherosclerosis

Studies by Kritchevsky (1999 and 2003) established that dietary CLA inhibits the development of cholesterol-induced atherosclerosis. The authors observed a dcrease in incidence and severity of atherosclerotic lesions. However, the studies used a CLA mixture, so that one could not distinguish cs-10, trans-12 and cis-9, trans-11 CLA, which differ in their effects.

In most cases improvements were attributed to CLA's anti-inflammatory effects (i.e. reduced: expression of cyclooxygenase -2, production of nitric oxide, and production of tumor necrosis factor α, interleukin 1 and 6) rather than any effect on the levels of plasma cholesterol and fatty acid synthesis (Yu et al., 2002). Desvergne and Wahli (1999) and Kersten et al. (2000) proposed that these anti-inflammatory effects of CLA are the result of peroxisome proliferator-activated receptor (PPAR) γ modulation, decreasing the abundance of transcription factors with key roles in the production of cytokines. The PPAR pathway proposal is supported by Belury et al. (2002) who found

that cis-9, trans-11 CLA is a potent activator of both PPAR-α and PPAR-γ and by Toomey et al. (2003) who found that regression of pre-established atherosclerosis in apo $E^{-/-}$ mice fed RA is associated with an increased expression of PPAR-γ.

Coronary Heart Disease

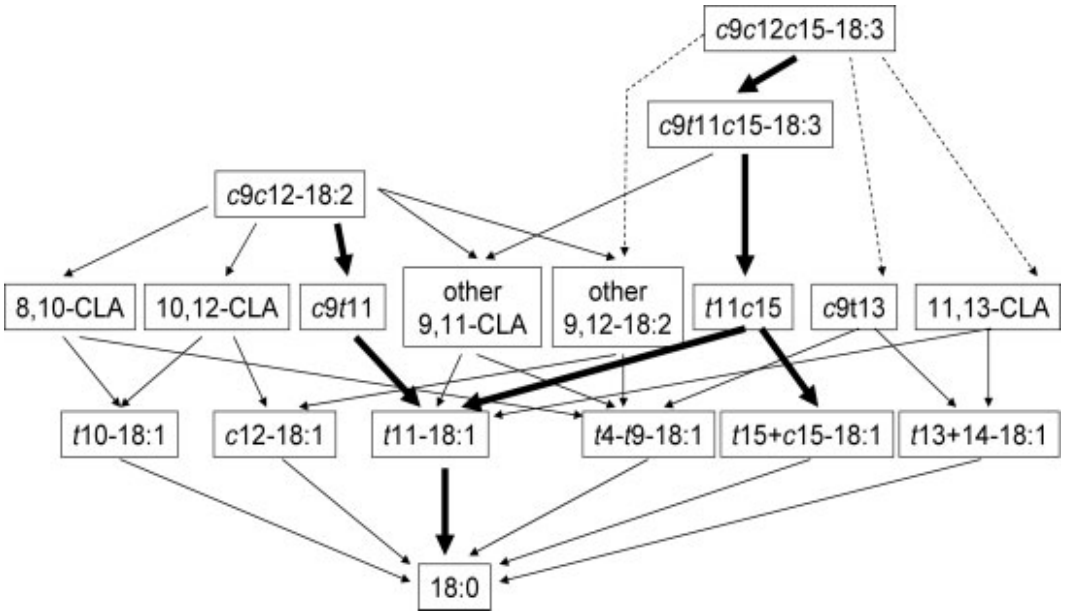
Coronary heart disease (CHD) was linked to beef and dairy products in the early fifties when studies reported that diets containing saturated fats resulted in higher plasma cholesterol concentrations than diets containing unsaturated fat from safflower or corn oil (Ahrens, 1957). Since then we have learned that not all saturated or unsaturated fatty acids have the same effect on plasma cholesterol levels. Lauric , myristic and palmitic acid increase plasma cholesterol concentrations with myristic exhibiting the greatest potency, while butyric, caproic, caprylic, capric, and stearic acids have no discernable effect on plasma cholesterol levels (Mensink et al., 2003). Also, CLA and linolenic acids help to alleviate the chronic inflammation associated with CHD by modulating PPARs to reduce the expression of cyclooxygenase-2, production of nitric oxide, and production of tumor necrosis factor α, interleukin 1 and 6 while many unsaturated omega-6 fatty acids in vegetable oils (depending on the source) increase the expression of pro-inflammatory products (Yu et al., 2002). Even still in studies where fat type and rate of consumption are compared between individuals who have CHD and those without CHD and studies where saturated fatty acids were grouped there was no clear association linking saturated fatty acids or groups of saturated fatty acids to CHD (Hu et al., 1999; Ravnskov, 1998; McNamara, 2000; Schaefer, 2002; Pietinen et al., 1997).

While lauric, myristic and palmitic acid raise total plasma cholesterol by increasing low density lipoprotein cholesterol, early studies investigating the effects of

trans fats found that total plasma cholesterol was relatively unchanged. They found that plasma low density lipoprotein : high density lipoprotein ratios doubled when partially hydrogenated lipids were consumed (Ascherio et al., 1999; Mauger et al., 2003). More recent studies have found that trans fats from ruminant products (CLA, trans-11 oleic acid from rumen microbial metabolism) and those derived from hydrogenated vegetable oils (from heating vegetable oils) exhibit differential effects on plasma cholesterol and on the risk of CHD (Ascherio et al., 1994; Bolton-Smith et al., 1996; Willett et al., 1993). The authors found that the detrimental effects of trans fatty acids could be entirely explained by the intake of trans fatty acids from hydrogenated vegetable oils. This difference has been explained by the predominance of CLA and trans-11 oleic acid in ruminant products and their near or complete absence in hydrogenated vegetable products. In addition to the presence of the variety of other C18:1 trans isomers in hydrogenated vegetable products do not affect or increase plasma cholesterol concentrations (Parodi, 2004).

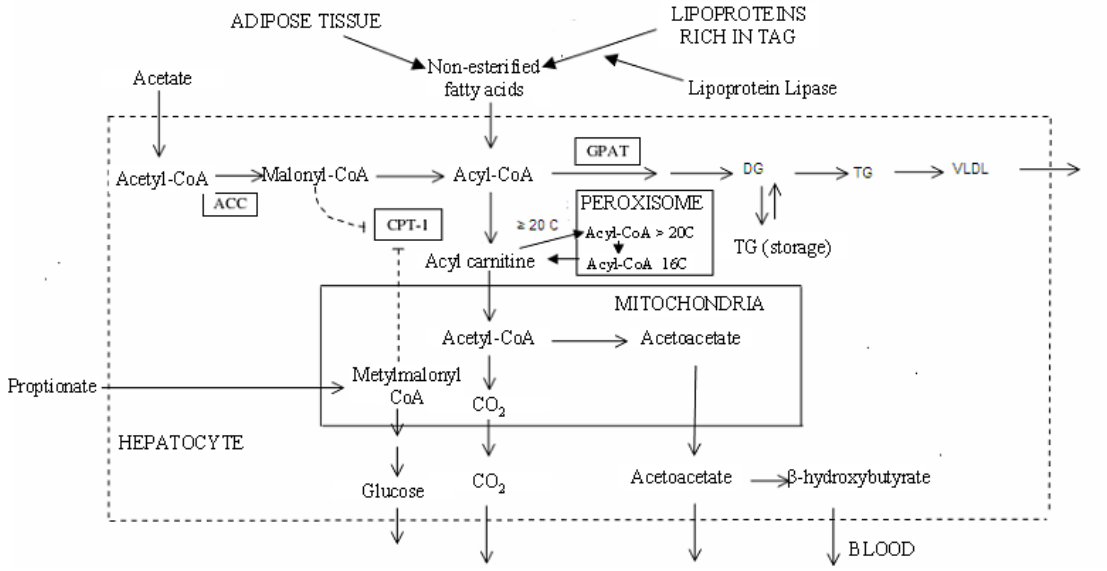
Milk has been cited for producing many general health benefits. Parodi (2004) summarizes the benefits of inclusion of milk fat in the diet: anti bacterial and anti viral properties, protection against stress, bacterial and chemically induced gastric mucosal damage, promotion of bone formation, reduced plaque formation on tooth surfaces and lower incidence of asthma and other allergic disorders. Milk is the most basic dietary staple with various beneficial effects to human health, yet it can be improved. Increasing the concentrations of fatty acids in milk that are essential to the human body will allow milk to supply a larger portion of the dietary requirements for humans. Furthermore, increased amounts of trans-10, cis-12 CLA can decrease fatty acid synthesis and prevent obesity.

Figure 1.1 Biohydrogenation pathway in the rumen



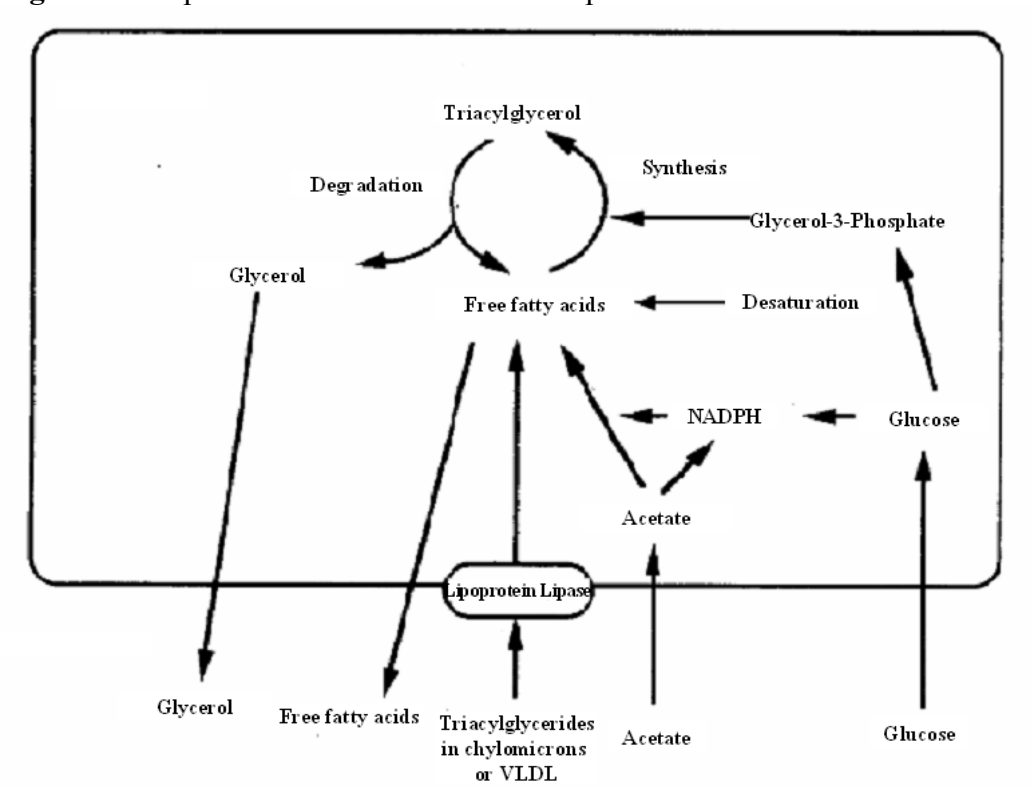
LA – c9c12c15-18:3; VA – t11-18:1; CLA = Conjugated linoleic acid; LA = linoleic acid; LNA = Linolenic acid; VA = Vaccinic acid. Adapted from Chilliard et al. (2007).

Figure 1.2. Hepatic lipid metabolism



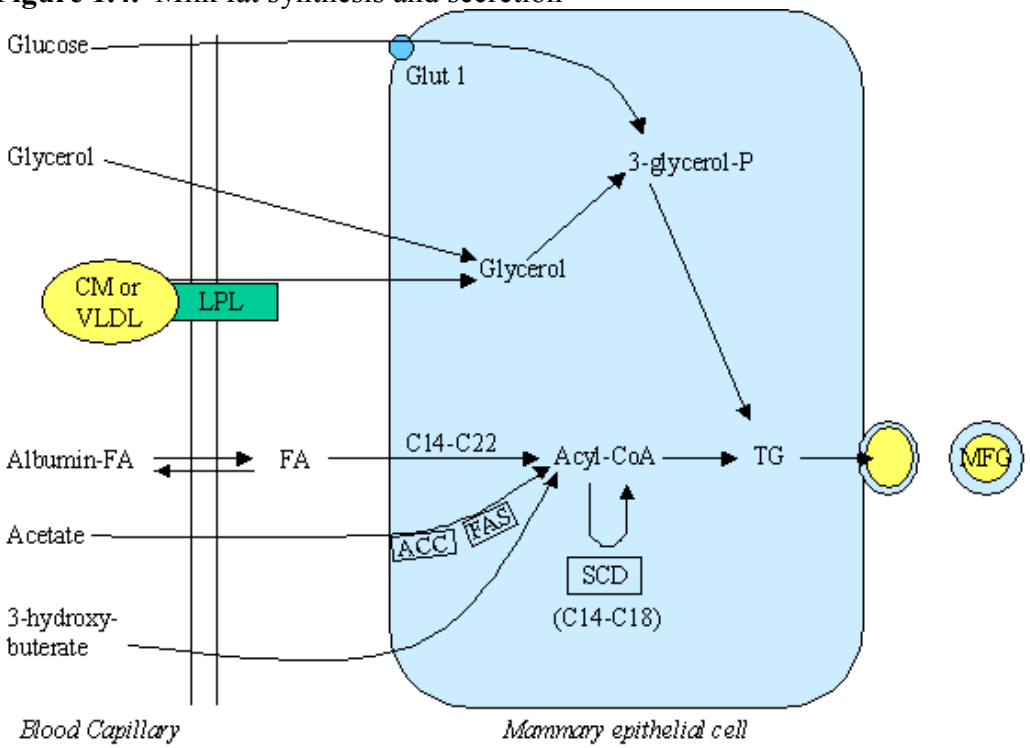
Adapted from Vernon (2005) and Cuvelier et al. (2005b). Abbreviations: ACC = Acetyl-CoA carboxylase; CPT-1 = Carnitine palmitoyltransferase; DG = Diglycerides; GPAT = glycerol-3-phosphate dehydrogenase; TG = Triglycerides; VLDL = Very low density lipoproteins. The dashed lines indicate allosteric inhibition.

Figure 1.3. Lipid metabolism in ruminant adipose tissue



Adapted from Chilliard (1993)

Figure 1.4. Milk fat synthesis and secretion



Adapted from Chilliard et al (2000)
ACC = Acetyl-CoA carboxylase; CM = Chylomicron; SCD = Stearoyl-CoA desaturase; FA = Fatty acid; FAS = Fatty acid synthase; Glut 1 = Glucose transporter 1; LPL = Lipoprotein lipase; MFG = Milk fat globule; TG = Triglyceride; VLDL = Very low density-lipoprotein.

FLAXSEED FEEDING

A review of the effects of source and supplementation rate of flaxseed on production indicators in dairy cows reveals several trends (**Table 1.1**). 1) Feeding flaxseed decreased dry matter intake in 12 of 15 studies (on average: 5% decrease). Larger decreases are observed at higher supplementation rates (>5% of dry matter intake) with raw flaxseed or with flaxseed oil. Similar results, although not significant, have been reported in a meta-analysis by Glasser et al. (2010), which are summarized in **Table 1.2**. High concentrations of lipids prevent bacteria attachment to fiber in the rumen. Furthermore, high concentrations of poly-unsaturated fatty acids are toxic to fiber digesting bacteria and protozoa in the rumen fluid and decrease fiber digestibility and rumen passage rate (Chilliard et al., 2009). As a result, dry matter intake and milk production (despite higher dietary energy content) is decreased at higher concentrations of non-protected flaxseed.

2) Feeding protected flaxseed (formaldehyde-treated or encapsulated flaxseed) increased milk fat concentration and yield (**Tables 1.1, 1.2**), as larger amounts of fatty acids escape rumen degradation and are absorbed in the jejunum. In contrast, feeding extrusion products of flaxseed and flaxseed oil result in low milk fat concentrations (i.e., milk fat depression) and yield. Higher concentrations of readily available poly-unsaturated fatty acid increase the concentration of *trans*-11 oleic acid formed from poly-unsaturated fatty acids in the rumen and decrease the cellulose digesting bacteria population and the acetate to propionate ratio of volatile fatty acids. As a result, less precursors are available for fatty acid de-novo synthesis in the mammary gland. Furthermore, higher concentrations of propionate (i.e., the precursor of glucose) and insulin divert more fatty acids from the mammary gland to adipose tissue. As a result,

cows accumulate more body fat and produce less milk. This effect is more prevalent in mid- and late lactation (Zachut et al., 2010), as milk synthesis is less of the driving force for energy partitioning.

3) Flaxseed supplementation exhibits little effect on milk protein yield or concentration (**Table 1.1**) indicating that flaxseed supplementation either has little effect on protein digestibility or that its favorable (e.g., less protein degradation in the rumen because of toxic effect on protozoa) and its unfavorable effects (e.g., slower passage rate which increases protein degradation in the rumen) balance each other out.

4) Feeding flaxseed decreased the proportion of saturated fatty acids, in particular C12:0, C14:0, and C16:0, by approximately 25 % (**Tables 1.1, 1.2**). Rumen degradation products of α-linolenic acid, in particular trans-10, cis-12 linoleic acid and trans-9, cis-11 linoleic acid, are potent inhibitors of activity and abundance of enzymes involved in *de-novo* fatty acid synthesis in the mammary gland. Since flaxseed extrusion and processing into flaxseed oil increases the ruminal availability of fatty acids, it is not surprising, that the decrease in de-novo synthesized fatty acids is greatest in cows receiving flaxseed oil or extruded flaxseed (**Table 1.2**).

5) Flaxseed supplementation increased the concentration of stearic, oleic, and linoleic acid in milk fat (**Tables 1.1, 1.2**). The reason is that a significant amount of α-linolenic acid from flaxseed is biohydrogenated in the rumen to stearic, oleic, and linoleic acid. The degree of ruminal biohydrogenation depends on the degree of ruminal availability of fatty acids from flaxseed. Ruminal biohydrogenation is stronger at greater supplementation rates with extruded flaxseed products and flaxseed oil. Higher concentrate : fiber ratio decrease ruminal biohydrogenation (Loor et al., 2004) by increasing rumen passage rate.

6) The strongest effect of feeding flaxseed is an increase in α-linolenic acid in milk fat (**Tables 1.1, 1.2**). The most abundent fatty acid in flaxseed is α-linolenic acid (approximately 50 to 60%). A proportion of α-linolenic acid from flaxseed gets hydrogenated in the rumen. The degree of ruminal biohydrogenation depends on the flaxseed processing method and the amount fed and is reflected in the fatty acid concentrations in blood. The increase in α-linolenic acid is dosage dependent and greater when protected flaxseed is used. A proportion of the absorbed of α-linolenic acid is transported to the mammary gland and is used for milk TAG synthesis. Little is known about the regulation how much of α-linolenic acid is incorporated into milk TAG.

Table 1.1 Summary of the effects of flaxseed supplementation on dry matter intake (DMI), milk production and milk fatty acid composition in the literature.

Study	Study, #	Cows, #	Flaxseed Treatment	Trt Period, wks	Trt, % DM
Flaxseeds					
Ward et al 2002	1	4	Ground	3	3.25
Petit et al 2004	2	4	Whole	35 d	9.0
Petit et al 2005	3	10	Whole (16% protein)	10	12.0
Petit et al 2005	4	10	Whole (18% protein)	10	12.0
Gonthier et al 2005	5	4	Whole (55:45)[1]	4	12.6
Chilliard et al 2009	6	8	Whole	4	5.0
Caroprese et al 2010	7	8	Whole	12	1200 g/d
Processed Flaxseed					
Petit et al 2003	10	10	Whole, Formaldehyde	10	11.4
Gonthier et al 2005	11	4	Micronized (55:45)	4	12.6
Gonthier et al 2005	12	4	Extruded (55:45)	4	12.6
Chilliard et al 2009	13	8	Extruded	4	5.0
Hurtaud et al 2010	14	12	Extruded (70:30)	7	2.0
Hurtaud et al 2010	15	12	Extruded (70:30)	7	4.3
Zachut et al 2010	16	22	Extruded	100 d	9.0
Flaxseed Oils					
Chouinard et al 1998	8	6	Ca salts of oil	4	4.0
Dhiman et al 2000	17	6	Oil	5	1.00
Chouinard et al 2001	9	6	Ca salts of oil	4	4.0
Loor et al 2005	18	4	Oil (65:35)	4	3.0
Loor et al 2005	19	4	Oil (35:65)	4	3.0
Bell et al 2006	20	10	Oil + vit E	8	6.0
Bu et al 2007	21	10	Oil	9	7.61
Flowers et al 2008	22	12	Oil	3	170 g/d
Flowers et al 2008	23	12	Oil	3	340 g/d
Flowers et al 2008	24	12	Oil	3	510 g/d
Chilliard et al 2009	25	8	Oil	4	5.0
Rego et al 2009	26	16	Oil + grazed	4	500 g/d

[1] forage : concentrate ratio in parenthesis

Table 1.1 Summary of the effects of flaxseed supplementation on dry matter intake (DMI), milk production and milk fatty acid composition in the literature (continued)

Study	DMI, kg			Milk Yield, kg		
	Control	Treatment	Δ, %	Control	Treatment	Δ, %
Flaxseeds						
Ward et al 2002	20.0	19.1	- 4.5	24.7	23.0	- 6.9
Petit et al 2004	23.8	21.1	-11.3	24.8	32.1	+29.4
Petit et al 2005	19.3	17.5	- 9.3	24.0	20.3	- 15.4
Petit et al 2005	20.8	19.5	- 6.3	24.4	24.9	+ 2.0
Gonthier et al 2005	15.9	15.8	- 0.6	21.1	20.2	- 4.3
Chilliard et al 2009	19.8	19.5	- 1.5	23.0	21.5	- 6.5
Caroprese et al 2010	NM	NM		22.5	24.0	+ 6.9
Processed Flaxseed						
Petit et al 2003	19.8	20.7	+ 4.5	22.4	24.9	+11.2
Gonthier et al 2005	15.9	15.2	- 4.4	21.1	19.6	- 7.1
Gonthier et al 2005	15.9	15.5	- 2.5	21.1	18.0	- 14.7
Chilliard et al 2009	19.8	16.7	-15.7	23.0	20.8	- 9.6
Hurtaud et al 2010	NM	NM		30.8	32.5	+ 5.5
Hurtaud et al 2010	NM	NM		30.8	33.6	+ 9.1
Zachut et al 2010	26.1	27.1	+3.8	49.5	52.9	+ 6.9
Flaxseed Oils						
Chouinard et al 1998	23.5	22.4	- 4.7	35.9	38.8	+ 8.1
Dhiman et al 2000	20.6	21.7	+5.3	27.4	28.4	+ 3.6
Loor et al 2005	20.4	19.6	- 3.9	24.2	27.7	+14.5
Loor et al 2005	20.4	20.4	0.0	28.8	26.2	- 9.0
Bell et al 2006	19.1	17.8	- 6.8	32.0	29.4	- 8.3
Bu et al 2007	16.2	15.9	- 1.9	21.7	25.0	+15.2
Flowers et al 2008	NM	NM		18.9	18.5	- 2.3
Flowers et al 2008	NM	NM		18.9	19.6	+ 3.5
Flowers et al 2008	NM	NM		18.9	19.1	+ 0.9
Chilliard et al 2009	19.8	14.7	-25.8	23.0	18.9	- 17.8
Rego et al 2009	NM	NM		22.2	22.2	0.0

NM: not measured

Table 1.1 Summary of the effects of flaxseed supplementation on dry matter intake (DMI), milk production and milk fatty acid composition in the literature (continued)

Study	Fat, kg			Fat, %		
	Control	Treatment	Δ, %	Control	Treatment	Δ, %
Flaxseeds						
Ward et al 2002	0.96	0.99	+ 3.1	3.93	3.96	+ 0.8
Petit et al 2004	0.85	1.14	+34.1	3.49	3.63	+ 4.0
Petit et al 2005	1.12	1.01	- 9.8	4.68	4.99	+ 6.6
Petit et al 2005	1.08	1.08	0.0	4.44	4.45	+ 0.2
Gonthier et al 2005	0.80	0.81	+ 1.3	3.82	4.02	+ 5.2
Chilliard et al 2009	0.95	0.97	+ 1.6	NM	NM	
Caroprese et al 2010	0.79	0.96	+21.5	3.62	3.99	+ 10.2
Processed Flaxseed						
Petit et al 2003	0.90	1.06	+17.8	4.23	4.33	+ 2.4
Gonthier et al 2005	0.80	0.79	- 1.3	3.82	3.96	+ 3.7
Gonthier et al 2005	0.80	0.65	- 18.8	3.82	3.56	- 6.8
Chilliard et al 2009	0.95	0.71	- 25.4	NM	NM	
Hurtaud et al 2010	1.32	1.24	- 6.1	4.33	3.82	- 11.8
Hurtaud et al 2010	1.32	1.03	- 21.6	4.33	3.16	- 27.0
Zachut et al 2010	1.87	1.77	- 5.3	3.63	3.23	- 11.0
Flaxseed Oils						
Chouinard et al 1998	1.43	1.32	- 7.7	4.05	3.56	- 12.1
Dhiman et al 2000	0.94	1.05	+11.7	3.44	3.72	+ 8.1
Loor et al 2005	0.79	0.95	+20.3	3.30	3.45	+ 4.5
Loor et al 2005	0.70	0.55	- 21.4	2.40	2.22	- 7.5
Bell et al 2006	1.15	0.96	- 16.5	3.66	3.30	- 9.8
Bu et al 2007	0.77	0.81	+ 5.2	3.49	3.26	- 6.6
Flowers et al 2008	0.60	0.64	+ 6.7	3.23	3.44	+ 6.5
Flowers et al 2008	0.60	0.66	+10.0	3.23	3.35	+ 3.7
Flowers et al 2008	0.60	0.63	+ 5.0	3.23	3.27	+ 1.2
Chilliard et al 2009	0.95	0.62	- 34.5	NM	NM	
Rego et al 2009	0.82	0.78	- 4.9	3.75	3.59	- 4.3

NM: not measured

Table 1.1 Summary of the effects of flaxseed supplementation on dry matter intake (DMI), milk production and milk fatty acid composition in the literature (continued)

Study	Protein, kg			Protein, %		
	Control	Treatmer	Δ, %	Control	Treatment	Δ, %
Flaxseeds						
Ward et al 2002	0.84	0.75	- 10.7	3.41	3.31	- 2.9
Petit et al 2004	0.97	1.22	+25.8	3.92	3.87	- 1.3
Petit et al 2005	0.83	0.64	- 22.9	3.45	3.12	- 9.6
Petit et al 2005	0.83	0.78	- 6.0	3.46	3.16	- 8.7
Gonthier et al 2005	0.72	0.65	- 9.7	3.41	3.23	- 5.3
Chilliard et al 2009	0.78	0.73	- 5.5	NM	NM	
Caroprese et al 2010	0.68	0.78	+14.7	3.07	3.25	+ 5.9
Processed Flaxseed						
Petit et al 2003	0.74	0.82	+10.8	3.41	3.34	- 2.1
Gonthier et al 2005	0.72	0.67	- 6.9	3.41	3.40	- 0.3
Gonthier et al 2005	0.72	0.60	- 16.7	3.41	3.33	- 2.3
Chilliard et al 2009	0.78	0.68	- 12.4	NM	NM	
Hurtaud et al 2010	1.04	1.06	+ 1.5	3.40	3.26	- 4.1
Hurtaud et al 2010	1.04	1.07	+ 2.9	3.40	3.19	- 6.2
Zachut et al 2010	1.51	1.60	+ 6.0	2.94	2.97	+ 1.0
Flaxseed Oils						
Chouinard et al 1998	1.13	1.13	0.0	3.21	3.02	- 5.9
Dhiman et al 2000	0.96	0.97	+ 1.0	3.53	3.45	- 2.3
Loor et al 2005	0.75	0.81	+ 8.0	3.10	2.95	- 4.8
Loor et al 2005	0.88	0.84	- 4.5	3.03	3.21	+ 5.9
Bell et al 2006	0.95	0.89	- 6.3	3.04	3.12	+ 2.6
Bu et al 2007	0.72	0.78	+ 8.3	3.15	3.17	+ 0.6
Flowers et al 2008	0.59	0.59	0.0	3.03	3.19	+ 5.3
Flowers et al 2008	0.59	0.58	- 1.7	3.03	3.12	+ 3.0
Flowers et al 2008	0.59	0.56	- 5.1	3.03	3.08	+ 1.7
Chilliard et al 2009	0.78	0.64	- 17.0	NM	NM	
Rego et al 2009	0.78	0.76	- 2.6	3.51	3.43	- 2.3

NM: not measured

Table 1.1 Summary of the effects of flaxseed supplementation on dry matter intake (DMI), milk production and milk fatty acid composition in the literature (continued)

Study	C12:0 (lauric acid)			C14:0 (myristic acid)		
	Control	Treatment	Δ, %	Control	Treatment	Δ, %
Flaxseeds						
Ward et al 2002	3.01	2.18	- 27.6	9.53	7.29	- 23.5
Petit et al 2004	5.80	4.70	- 19.0	15.40	14.00	- 9.1
Petit et al 2005	3.70	2.58	- 30.3	11.34	9.07	- 20.0
Petit et al 2005	3.60	2.40	- 33.3	11.06	8.90	- 19.5
Gonthier et al 2005	4.20	2.20	- 47.6	11.80	7.80	- 33.9
Chilliard et al 2009	4.22	3.22	- 23.7	12.59	10.80	- 14.2
Caroprese et al 2010	3.09	2.87	- 7.1	10.96	10.14	- 7.5
Processed Flaxseed						
Petit et al 2003	3.10	3.10	0.0	11.40	11.50	+ 0.9
Gonthier et al 2005	4.20	2.60	- 38.1	11.80	8.30	- 29.7
Gonthier et al 2005	4.20	2.10	- 50.0	11.80	8.00	- 32.2
Chilliard et al 2009	4.22	2.36	- 44.1	12.59	8.83	- 29.9
Hurtaud et al 2010	4.65	3.81	- 18.1	12.40	11.50	- 7.3
Hurtaud et al 2010	4.65	3.33	- 28.4	12.40	10.60	- 14.5
Zachut et al 2010	3.16	3.12	- 1.3	10.31	10.22	- 0.9
Flaxseed Oils						
Chouinard et al 1998	4.50	2.71	- 39.8	12.55	9.20	- 26.7
Chouinard et al 2001	3.51	2.07	- 41.0	9.99	7.37	- 26.2
Loor et al 2005	4.13	2.15	- 48.0	12.12	8.32	- 31.4
Loor et al 2005	4.37	2.81	- 35.8	11.64	8.79	- 24.4
Bell et al 2006	2.87	1.64	- 42.9	11.64	8.48	- 27.1
Bu et al 2007	4.78	3.75	- 21.5	15.13	12.82	- 15.3
Flowers et al 2008	1.87	1.75	- 6.4	7.91	7.48	- 5.4
Flowers et al 2008	1.87	1.69	- 9.6	7.91	7.38	- 6.7
Flowers et al 2008	1.87	1.51	- 19.3	7.91	6.71	- 15.2
Chilliard et al 2009	4.22	1.52	- 64.0	12.59	5.88	- 53.3
Rego et al 2009	2.83	1.75	- 38.2	10.20	7.09	- 30.5

NM: not measured

Table 1.1 Summary of the effects of flaxseed supplementation on dry matter intake (DMI), milk production and milk fatty acid composition in the literature (continued)

Study	C16:0 (palmitic acid)		
	Control	Treatment	Δ, %
Flaxseeds			
Ward et al 2002	24.87	21.66	- 12.9
Petit et al 2004	38.10	31.20	- 18.1
Petit et al 2005	35.76	23.31	- 34.8
Petit et al 2005	31.96	22.34	- 30.1
Gonthier et al 2005	31.70	20.40	- 35.6
Chilliard et al 2009	29.06	25.00	- 14.0
Caroprese et al 2010	25.81	23.74	- 8.0
Processed Flaxseed			
Petit et al 2003	26.10	27.70	+ 6.1
Gonthier et al 2005	31.70	21.70	- 31.5
Gonthier et al 2005	31.70	21.00	- 33.8
Chilliard et al 2009	29.06	19.62	- 32.5
Hurtaud et al 2010	32.70	29.40	- 10.1
Hurtaud et al 2010	32.70	26.80	- 18.0
Zachut et al 2010	32.63	22.14	- 32.1
Flaxseed Oils			
Chouinard et al 1998	26.21	19.09	- 27.2
Dhiman et al 2000	40.80	37.50	- 8.1
Chouinard et al 2001	25.90	16.23	- 37.3
Loor et al 2005	27.16	14.71	- 45.8
Loor et al 2005	23.63	18.75	- 20.7
Bell et al 2006	30.60	17.87	- 41.6
Bu et al 2007	34.85	30.19	- 13.4
Flowers et al 2008	23.27	22.67	- 2.6
Flowers et al 2008	23.27	21.20	- 8.9
Flowers et al 2008	23.27	19.76	- 15.1
Chilliard et al 2009	29.06	15.94	- 45.1
Rego et al 2009	24.10	17.00	- 29.5

NM: not measured

Table 1.1 Summary of the effects of flaxseed supplementation on dry matter intake (DMI), milk production and milk fatty acid composition in the literature (continued)

Study	C18:1 t11 (vaccenic acid)			C18:2 cis-9 trans-11		
	Control	Treatment	Δ, %	Control	Treatment	Δ, %
Flaxseeds						
Ward et al 2002	2.53	2.16	- 14.6	1.40	1.16	- 17.1
Petit et al 2005	2.01	2.00	- 0.5	0.56	0.77	+ 37.5
Petit et al 2005	0.93	2.19	+135.5	0.63	0.77	+ 22.2
Gonthier et al 2005	2.30	4.30	+ 87.0	NM	NM	
Chilliard et al 2009	1.49	0.98	- 34.2	0.77	0.44	- 42.9
Caroprese et al 2010	1.82	2.05	+ 12.6	0.37	0.45	+ 21.6
Processed Flaxseed						
Gonthier et al 2005	2.30	4.20	+ 82.6	NM	NM	
Gonthier et al 2005	2.30	5.90	+156.5	NM	NM	
Chilliard et al 2009	1.49	2.75	+ 84.6	0.77	1.27	+ 64.9
Hurtaud et al 2010	1.23	1.40	+ 13.8	0.52	0.62	+ 19.2
Hurtaud et al 2010	1.23	1.32	+ 7.3	0.52	0.60	+ 15.4
Flaxseed Oils						
Chouinard et al 1998	1.84	10.18	+453.3	NM	NM	
Loor et al 2005	1.12	3.23	+188.4	0.62	1.34	+ 116.1
Loor et al 2005	1.32	4.53	+243.2	0.81	2.54	+ 213.6
Bell et al 2006	1.41	6.67	+373.0	NM	NM	
Bu et al 2007	1.48	3.04	+105.4	0.64	1.60	+ 150.0
Flowers et al 2008	3.39	3.62	+ 6.8	1.12	1.18	+ 5.4
Flowers et al 2008	3.39	4.25	+ 25.4	1.12	1.39	+ 24.1
Flowers et al 2008	3.39	4.89	+ 44.2	1.12	1.65	+ 47.3
Chilliard et al 2009	1.49	1.08	- 27.5	0.77	0.65	- 15.6
Rego et al 2009	2.70	3.70	+ 36.8	1.19	1.54	+ 30.2

NM: not measured

Table 1.1 Summary of the effects of flaxseed supplementation on dry matter intake (DMI), milk production and milk fatty acid composition in the literature (continued)

Study	C18:2 (linoleic acid)			C18:3 n-3 (α-linolenic acid)		
	Control	Treatment	Δ, %	Control	Treatment	Δ, %
Flaxseeds						
Ward et al 2002	2.97	2.25	- 24.2	0.46	1.21	+163.0
Petit et al 2004	NM	NM		0.60	1.10	+ 83.3
Petit et al 2005	NM	NM		0.54	1.00	+ 85.2
Petit et al 2005	NM	NM		0.67	1.01	+ 50.7
Gonthier et al 2005	2.00	2.70	+ 35.0	0.40	1.30	+225.0
Chilliard et al 2009	NM	NM		0.67	0.65	- 3.0
Caroprese et al 2010	NM	NM		0.75	0.81	+ 8.0
Processed Flaxseed						
Petit et al 2003	NM	NM		1.08	0.93	- 13.9
Gonthier et al 2005	2.00	2.90	+ 45.0	0.40	1.30	+225.0
Gonthier et al 2005	2.00	3.10	+ 55.0	0.40	0.70	+ 75.0
Chilliard et al 2009	NM	NM		0.67	1.20	+ 79.1
Hurtaud et al 2010	1.73	7.87	+354.9	0.23	0.44	+ 91.3
Hurtaud et al 2010	1.73	1.91	+ 10.4	0.23	0.67	+191.3
Zachut et al 2010	3.28	3.43	+ 4.6	0.29	1.47	+406.9
Flaxseed Oils						
Chouinard et al 1998	2.43	3.30	+ 35.8	0.24	0.31	+ 29.2
Dhiman et al 2000	2.80	2.60	- 7.1	0.63	0.82	+ 30.2
Chouinard et al 2001	1.75	2.38	+ 36.0	0.20	0.28	+ 40.0
Loor et al 2005	NM	NM		0.96	1.30	+ 35.4
Loor et al 2005	NM	NM		0.91	1.86	+104.4
Bell et al 2006	1.75	2.01	+ 14.9	0.41	0.73	+ 78.0
Bu et al 2007	2.35	2.13	- 9.4	NM	NM	
Flowers et al 2008	NM	NM		0.59	0.78	+ 32.2
Flowers et al 2008	NM	NM		0.59	1.01	+ 71.2
Flowers et al 2008	NM	NM		0.59	1.03	+ 74.6
Chilliard et al 2009	NM	NM		0.67	0.54	- 19.4
Rego et al 2009	1.12	0.99	- 11.6	0.60	0.53	- 11.5

NM: not measured

Table 1.1 Summary of the effects of flaxseed supplementation on dry matter intake (DMI), milk production and milk fatty acid composition in the literature (continued)

Study	Saturated fatty acids, %			Mono-unsaturated fatty acids, %			Poly-unsaturated fatty acids, %		
	Cont	Trt	Δ, %	Cont	Trt	Δ, %	Cont	Trt	Δ, %
Flaxseeds									
Petit et al 2004	71.30	68.10	- 4.5	24.20	27.70	+ 14.46	4.50	4.20	- 6.7
Petit et al 2005	70.20	60.36	-14.0	26.55	36.03	+ 35.71	3.25	3.61	+ 11.1
Petit et al 2005	67.80	57.94	-14.5	28.48	38.06	+ 33.64	3.72	4.00	+ 7.5
Gonthier et al 2005	70.60	58.80	-16.7	26.00	35.80	+ 37.69	3.40	5.40	+ 58.8
Chilliard et al 2009	68.95	66.27	- 3.9	26.63	30.28	+ 13.71	4.42	3.45	- 21.9
Caroprese et al 2010	65.58	63.47	- 3.2	30.12	31.81	+ 5.61	4.30	4.72	+ 9.8
Processed Flaxseed									
Gonthier et al 2005	70.60	59.80	-15.3	26.00	34.60	+ 33.08	3.40	5.60	+ 64.7
Gonthier et al 2005	70.60	55.10	-22.0	26.00	39.20	+ 50.77	3.40	5.70	+ 67.6
Chilliard et al 2009	68.95	53.74	-22.1	26.63	39.32	+ 47.65	4.42	6.94	+ 57.0
Hurtaud et al 2010	74.10	69.30	- 6.5	22.68	26.79	+ 18.12	3.22	3.91	+ 21.4
Hurtaud et al 2010	74.10	64.70	-12.7	22.68	30.58	+ 34.83	3.22	4.72	+ 46.6
Zachut et al 2010	69.43	62.62	- 9.8	26.10	31.43	+ 20.42	4.47	5.95	+ 33.1
Flaxseed Oils									
Bu et al 2007	72.06	63.61	-11.7	24.19	31.31	+ 29.43	3.75	5.08	+ 35.5
Chilliard et al 2009	68.95	42.38	-38.5	26.63	49.14	+ 84.53	4.42	8.48	+ 91.9

NM: not measured

Table 2.2. Effect of flaxseed supplement form (Unprotected: variously processed; Protected: encapsulated or formaldehyde-treated; Oils: oils, amides, and Ca salts) on mean percentages ± standard deviations of main milk fatty acids of Holstein cows (adapted from meta-analysis of Glasser et al., 2010)

		Flaxseed		
Variable	Control	Unprotected	Protected	Oils
Supplement DM		1.87± 0.88 (30)[1]	2.25 ± 0.98 (9)	0.63 ± 0.32 (9)
Supplement fat, g/d		649 ± 204 (18)	692 ± 307 (9)	613 ± 299 (10)
Supplement lipids, % of DMI		3.6 ± 1.2 (18)	3.7 ± 1.9 (9)	3.2 ± 1.7 (10)
Lipid intake, % of DMI	3.13 ± 0.83 (73)	6.5 ± 1.7 (24)	5.7 ± 1.3 (4)	7.2 ± 2.0 (6)
DMI, kg/d	20 ± 4.2 (122)	18.9 ± 2.5 (30)	19.7 ± 2.1 (9)	19.5 ± 3.1 (10)
Milk yield, kg/ d	27.1 ± 7.9 (134)	26.8 ± 7.8 (31)	29.2 ± 4.3 (9)	27.2 ± 6.4 (10)
Milk fat, %	3.73± 0.56 (134)	3.79 ± 0.54 (30)	3.95 ± 0.65 (9)	3.30 ± 0.71 (10)
Milk fat, kg/d	0.99±0.27^{ab}(133)	1.00± 0.24^{ab}(30)	1.13 ± 0.16^{b} (9)	0.88 ± 0.23^{a} (10)
Fatty acid, wt %				
Saturated FA				
C4:0	3.3 ± 1.3^{a} (101)	2.58± 0.87^{b} (21)	2.2 ± 1.4^{ab} (3)	2.9 ± 1.1^{ab} (7)
C6:0	2.28± 0.73^{a}(114)	1.99± 0.65^{ab}(24)	1.73 ± 0.24^{ab}(3)	1.59 ± 0.68^{b} (8)
C8:0	1.42± 0.44^{a}(118)	1.16± 0.37^{b} (24)	1.06 ± 0.22^{ab}(3)	1.06 ± 0.6^{b} (9)
C10:0	3.37± 0.88^{a}(124)	2.53± 0.92^{b} (29)	2.91 ± 0.3^{ab} (4)	2.07 ± 0.98^{b} (9)
C12:0	4.1 ± 1.0^{a} (125)	3.1 ± 1.0^{b} (29)	3.35 ± 0.32^{b} (4)	2.36 ± 0.89^{c} (9)
C14:0	12.3 ± 1.9^{a} (130)	9.9 ± 2.2^{b} (30)	9.5 ± 1.2^{b} (8)	9.4 ± 2.5^{b} (9)
C16:0	31.3 ± 4.7^{a} (135)	24.1 ± 3.9^{b} (32)	21.8 ± 3.2^{b} (9)	21.9 ± 7.4^{b} (9)
C18:0	9.7 ± 2.3^{b} (136)	14.4 ± 3.1^{a} (32)	14.3 ± 4.0^{a} (5)	11.9^{a} ± 2.6 (9)
Mono-unsaturated FA				
C14:1c	1.45 ± 0.59^{a} (93)	1.18± 0.69^{b} (24)	1.22± 0.36^{abc}(4)	0.80 ± 0.22^{c} (8)
C16:1	2.12± 0.97^{a}(114)	1.42± 0.75^{b} (29)	2.13 ± 0.44^{a} (4)	1.48 ± 0.65^{ab} (9)
C18:1	22.0 ± 4.2^{c} (140)	29.2 ± 5.4^{a} (33)	25.3 ± 3.6^{b} (9)	33.2 ± 6.2^{a} (9)
C18:1c	19.8 ± 3.8^{c} (80)	26.8 ± 5^{a} (28)	22.4 ± 2.9^{b} (7)	23.6 ± 5.6^{ab} (6)
C18:1t	2.1 ± 1.2^{c} (81)	3.1 ± 1.9^{b} (26)	2.56 ± 0.72^{b} (8)	10.3 ± 4.2^{a} (7)
C18:1 t10,11	1.78 ± 0.87^{c} (40)	2.54 ± 0.63^{b} (5)	2.3 ± 1.3^{bc} (18)	5.9 ± 2.9^{a} (7)
Poly-unsaturated FA				
C18:2	2.9 ± 1.3^{a} (134)	3.3 ± 1.1^{b} (32)	3.7 ± 1.8^{abc} (9)	8.1^{a} ± 5.7 (9)
CLA	0.67 ± 0.33^{c} (52)	1.1 ± 0.37^{b} (7)	1.0 ± 0.4^{b} (21)	1.94 ± 0.85^{a} (8)
C18:2 c9t11	0.58 ± 0.29^{c} (32)		0.79± 0.25^{b} (14)	1.75 ± 0.84^{a} (7)
C18:3	0.67± 0.49^{c}(121)	1.11 ± 0.35^{b}(33)	2.4 ± 1.7^{a} (9)	0.85 ± 0.51^{bc} (9)
C18:3 n-3	0.59 ± 0.26^{c} (40)	1.06 ± 0.4^{b} (19)	1.56 ± 0.41^{a} (7)	0.91 0.45^{bc} (7)

[1] number of studies in parenthesis; superscripts indicate group differences at $P \leq 0.05$.

CHAPTER 2. INTRODUCTION

Dairy products have a high ratio of saturated to unsaturated fatty acids, which is associated with increased risk of cardiovascular diseases. Elevated blood pressure, diabetes, hyperlipidemia, and hypercholesterolemia, particularly of low-density lipoprotein cholesterol, can be attenuated by a lower ratio of dietary saturated to unsaturated fatty acids (Noakes et al., 1996). Composition of animal feed has been shown to be effective in altering milk fatty acid composition in order to make it healthier for people who enjoy consuming milk and milk products (Collomb et al., 2004; Kudrna and Marounek, 2008; Soita et al., 2003).

Increasing concentrations of poly-unsaturated fatty acids, particularly linoleic and α-linolenic acids, in the dairy cow's feed decreases the synthesis of saturated fatty acids in the mammary gland (lauric, myristic, and palmitic acid). This results in decreased blood concentrations of total and low density lipoprotein cholesterol in humans consuming the modified dairy products (Palmquist et al., 1993; Soita et al., 2003; Ward et al., 2002). Added benefits of increasing α-linolenic acid concentrations in dairy cow's feed are decreasing the n-6 : n-3 ratio in dairy products, which is assumed to decrease the risk of cardiovascular diseases and other chronic inflammation associated diseases. The objective of the current study is to test a newly developed flaxseed supplement, OmegaBoost (Double Pass LLC, Tualatin, OR), and its effects on animal performance and fatty acid composition of dairy products. The hypothesis is that feeding increasing amounts of OmegaBoost will improve the nutritional fatty acid profile and the textural properties of butter and cheese at refrigeration temperature in a dose dependent manner without negatively affecting feed intake and milk production.

CHAPTER 3.MATERIALS AND METHODS

Experimental Design and Diets

Ten confirmed pregnant, lactating Holstein dairy cows (DIM at beginning of study: 168 to 285, 5 cows first lactation, 3 cows second lactation and 2 cows third lactation) that were housed at Oregon State University Dairy Center were used for the feeding study. Cows were fed individually via Calan gates (American Calan, Northwood, NH) twice daily (7:00, 19:00) a total mixed ration that was formulated to meet NRC requirements (2001) for lactating Holstein cows (**Table 3.1**). All procedures involving animals were conducted in accordance with Oregon State University Institutional Animal Care and Use (ACUP No 4013). The experiment was performed in accordance with guidelines established in the Federation of Animal Sciences Societies Guide for the Care and Use of Agricultural Animals in Animal Research and Teaching.

The study design was a 5 x 5 latin square with one replication at the same time. Cows were blocked by parity (primiparous, multiparous) and randomly assigned to 5 treatment groups. Cows received each of the 5 supplements, 0, 2, 4, 6 lbs/d of OmegaBoost™ (flaxseed that was processed by a proprietary technique by Double Pass LLC, Tualatin, OR that likely utilizes the incorporation protein with ground flaxseed followed by a heat treatment and extrusion to increase passage rate through the rumen and protect against ruminal biohydrogenation), or 4 lbs/d of ground flaxseed (from the same batch of flaxseed that was used to make OmegaBoost) for 14 d without wash-out period in between (**Table 3.2**). The total study length was 10 wk. During morning feeding OmegaBoost or ground flaxseed at the assigned amounts was added as top dressing to the total mixed ration. OmegBoost was processed into cylindrical pellets (3 cm x 0.4 cm thick), while ground flaxseed was a powder with a lighter brown color than

OmegaBoost. Feed intake was monitored daily by refusal weigh backs. Feed intake was adjusted to have at least 10% feed refusal. One cow injured her leg in the fourth feeding period and dropped from the study. The results of the first three feeding periods were included in the statistical analysis.

Table 3.1. Composition of total mixed ration

Item	TMR
Ingredient, % DM	
Alfalfa	29.9
Grass Silage	29.6
Concentrate	40.5
Barley	29.5
Corn	29.46
Wheat	17
Dried Distillers Grain	11.75
Canola Meal	4.1
Soybean Meal (high protein)	3.1
Calcium Carbonate	1.75
Sodium Chloride	1.205
Bicarbonate	0.795
Magnesium Oxide, 58%	0.475
Urea	0.4
LDH Fortifier®[1]	0.3
RU-Max Plus®[1]	0.139
Sel-Plex 2700®[1]	0.024
Chemical Composition, g/kg unless otherwise noted	
DM, %	55.1
NE_L , Mcal/kg of DM	1.68
CP	185
RUP; % of CP	30.9
NDF	174
ADF	96
NFC	395
Fat	34.8

[1] Agri-King Inc, Fulton, IL

Abbreviations: DM = Dry matter; NE_L = Net energy of lactation; CP = Crude protein; RUP = Rumen un-degradable protein; NDF = Neutral detergent fiber; ADF = Acid detergent fiber; NCF = Neutral detergent fiber

Table 3.2. Fatty acid composition of flaxseed supplements

	Ground Flaxseed	OmegaBoost
Fatty Acid Composition, wt%		
C16:0	6.64	5.77
C16:1	0.51	0.19
C18:0	4.78	4.15
C18:1c9	15.77	18.2
C18:2	13.21	15.4
C18:3 n-3	59.08	56.29
Lipid Content, g/kg DM		
Total Lipid	365	306
C18:1c9	58	66
C18:2	48	47
C18:3, g/kg of DM	215	172

Sample Collection

Activity and resting times between milkings, body weight, and milk production and composition from each cow were measured after each milking (6:00, 18:00) using the Affimetrix system (Kibbutz Afikim, Israel) and averaged for each cow and 14-d feeding period for statistical analysis. To determine the milk fatty acid composition, a composite milk sample was taken from each cow on the last day of each 14-d feeding period and stored at -20 °C until analysis. To determine serum metabolite and fatty acid concentrations, a blood sample from each cow was collected from the coccygeal (tail) vein or artery on the last morning (6:00) of each 14-d feeding period. Blood samples were collected in serum vacutainer tubes (BD Vacutainer Plus Plastic Serum Tubes, BD Diagnostics, Franklin Lakes, NJ) and placed on ice immediately after collection. Within 30 min of collection, serum was transferred after centrifugation at 1600 *x g* for 20 min, and stored at -20 °C until analysis.

To determine the fatty acid composition of butter and mozzarella cheese, milk was collected from each cow on the last morning (6:00) of each 14-d feeding period for manufacture into butter and cheese. Butter and cheese was stored at -80 °C until

analysis. To determine the lipid content and fatty acid composition of the supplement, OmegaBoost, which was pelleted, was ground in a Proctor-Silex coffee grinder (model E160B, Hamilton Beach/Proctor-Silex Inc. Southern Pines, NC). Lipid content of the supplements was determined using an ether extract (AOAC Official Methods of Analysis, 1990). The feed lipid was stored at -20 °C until further analysis.

Butter and Mozzarella Cheese Manufacture

To manufacture butter and cheese, milk was collected in stainless steel milk cans on the last morning of each 14-d feeding period from each cow. Milk from each cow (8 L/cow) was pasteurized in double boiler pots at 63 °C for 30 min and then cooled to 15 °C. The pasteurized milk from the two cows receiving the same treatment were combined and then used at equal amounts (8 L) for butter and Mozzarella cheese manufacture.

For butter manufacture, cream was separated using a De Laval Model 100 (The De Laval Separator Co., Poughkeepsie, NY) electric cream separator and stored overnight at 4 °C. The following day, an electric mixer (KitchenAid Model KHM7GTN2, St. Joseph, MI) that was operated at the speed setting 3 or 4 at 5 °C room temperature was used to churn the cream into butter (time range for churning: 5 to 48 min). One cream sample did not churn into butter (4 lbs/d Omegaboost, 3rd feeding period). The next morning, butter was vacuum-packed using a FoodSaver® V2450 bag sealer (Jarden Consumer Solutions, Boca Raton, FL), weighed, and stored at -80°C until further analysis.

For cheese manufacture, 8 L of milk was acidified with 237 mL of distilled white vinegar (5% acidity). The acidified milk was then gently stirred while the temperature

was raised to 33 °C in a double boiler pot. While stirring, 1.23 mL of single strength liquid rennet (The Dairy Connection Inc., Madison, WI) was diluted 1:10 (vol:vol) with distilled water and slowly poured into the milk. Stirring was continued for 60 sec to ensure uniform incorporation into the milk. Stirring was discontinued to allow curd formation. Once the curd formed a clean break (~5 min), it was cut into ½ inch cubes. The temperature was raised over a 30 min period to 40.5 °C, while the curd was gently lifted from the bottom of the boiler to prevent matting. After 30 minutes, the whey was drained through a food safe plastic colander until the curd completely matted.

The matted curd was then cut into 4 approximately equal pieces, which were immersed each separately in 77 °C water until the curd became soft and malleable. Once malleable, the curd was worked together (smashing together between fingers and palms) until the structure was semi-firm and the surface glossy (this process usually required heating one additional time because the curd cooled and hardened while being worked). The four cheese loaves were cooled for 30 min in ice water baths, two of which were saturated with ionized salt. The four cheese loaves were stored overnight at 4 °C and the next morning vacuum-packed using a FoodSaver® V2450 bag sealer. Cheese loaves were weighed and stored at -80°C until further analysis.

Fatty Acid Analysis: Butter, Cheese, Supplement, Milk, and Serum

Lipids were extracted from butter, cheese, milk, and serum according to a modified method of Folch et al (1957) (Cherian et al., 1996). In short, 9 mL chloroform:methanol (2:1; vol:vol) was added to the sample (3 ml) and combined using an analog vortex mixer for 30 s and left overnight at 4 °C. Four ml of 0.88% NaCl solution was added to the sample tubes and inverted to mix. Sample tubes were then

centrifuged for 10 minutes at 2000 rpm. After centrifugation, the aqueous top layer was removed by suction and lipid extract was obtained by filtration through Whatman 1 (Whatman International, England) filter paper.

Internal standard solution (1% C13:0 wt/wt in butanol; Nu-Chek Prep, Inc., Elysian, MN) was added at 25 L (for serum or ether extract of feed), 50 L (for butter), or 150 L (for cheese) to 1.5 ml of lipid. The mixture was dried under nitrogen and methylated using 2 mL boron-trifluoride solution. Test tubes were incubated at 90 to 100 °C for 1 h. Fatty acid methylesters were recovered by washing the sample with 2 mL of hexane and then 2 mL of water followed by the removal of the top hexane layer for fatty acid analysis using a pipette.

Fatty acid methyl esters were separated and quantified by an automated gas chromatograph Agilent 6890(Agilent Technologies Inc., Palo Alto, CA) equipped with an auto sampler and flame ionization detector, using a 100m x 0.25 mm x 0.20 μm film thickness Supelco 2560 fused silica capillary column (Supelco, Bellefonte, PA). The initial column temperature was set at 60 °C for 4 min, increased to 165 °C at 4 °C/min, and maintained for 2 min. Next the column temperature was elevated to 200 °C at a rate of 15°C/min and maintained at the final temperature for 30 min. Helium was used as the carrier gas at a flow rate of 1.0 mL/min. The flame ionization detector was set at 250 °C.

Texture Analysis of Butter and Cheese

Butter and cheese texture analysis was done using a Stable Micro-Systems TA-XT2® texture analyzer (Texture Technologies, Scarsdale, NY.) after all samples had been manufactured. Butter and cheese samples were cut in 2.5 cm wide x 10 cm long x 2 cm deep rectangular boxes and the texture was analyzed at refrigeration temperature (4 °C)

and room temperature (23 °C). For textural analysis a 6mm cylindrical probe (P6) was advanced at 1mm/sec until it reached a depth of 12 mm for butter and 10 mm for cheese. Force measurements were recorded after the probe contacted that sample and reached the minimum trigger force: cheese 5 gr, butter 3 gr. The hardness was measured as the maximum force required for initial penetration and the force required to retract the probe after penetration was reported as measure of adherence. Measures of hardness and adhesiveness were taken in triplicate at 23 °C and the average was used for statistical analysis. At 4 °C, the hardness and adhesiveness decreased proportionally to the amount of time that the samples spent out of the refrigerator. Therefore, we used only the first measurement for statistical analysis.

Serum Analysis

Serum concentrations of glucose (Stanbio-Glucose Procedure No. 1075; Stanbio Laboratory; Boerne, TX), NEFA (ACS ACOD method, WAKO Diagnostics, Richmond, VA), β-hydroxybuterate (BHBA) (Stanbio BHBA LiquiColor® Procedure No. 2440; Stanbio Laboratory), and urea nitrogen (Stanbio Urea Nitrogen Liqui-UV® Test; Stanbio Laboratory) were measured according to manufacturer's instructions using a FLUOstar Omega microplate autoreader (BMG Labtech Inc., San Francisco, CA).

Statistical Analysis

Data were analyzed as a 5 x 5 latin-square design using PROC MIXED in SAS. The fixed effects were supplement (0, 2, 4, 6 lbs/d OmegaBoost, 4 lbs/d ground flax seed), feeding period (1, 2, 3, 4 or 5), and, except for butter and cheese measurements, parity (primiparous, multiparous), and the interaction between supplement and feeding period. A first order autoregressive variance-covariance matrix was used to adjust for

variation within cow over time. To determine the dose-dependency of the OmegaBoost supplement, linear, quadratic, and cubic contrasts were constructed using the ESTIMATE statement. The averages shown in the tables are least-squares means. The standard error of the mean shown in the table is the largest standard error of the 5 treatment groups.

CHAPTER 4. RESULTS

Supplement Composition

Although manufactured from the same batch of flaxseed, OmegaBoost contained 19% less fat per kg of dry matter than ground flaxseed (30.6 vs. 36.5%; **Table 3.2**). In addition, the proportion of α-linolenic acid was lower in the lipid fraction of OmegaBoost (56.3 wt%) than in ground flaxseed (59.1 wt%), resulting in a 25% lower intake of α-linolenic acid with OmegaBoost (172 g/kg DM) when fed at the same amount of ground flaxseed (215 g/kg DM). The decrease in α-linolenic acid coincided with an increase in oleic acid in OmegaBoost (66 vs. 58 g/kg DM in ground flaxseed) and similar concentrations of linoleic acid (47 vs. 48 g/kg DM in ground flaxseed).

Production Performance

Feeding OmegaBoost tended to increase linearly dry matter intake ($P = 0.06$: **Table 4.1**). Although cows received OmegaBoost together with the total mixed ration, cows consumed OmegaBoost before the total mixed ration within 5 min. The consumption of the complete 6 lbs of OmegaBoost and of ground flaxseed was slower and took approximately 30 min. Despite the greater dry matter intake and higher energy concentrations at higher OmegaBoost supplementation amounts, no significant differences in milk production and body weight were observed for source and amount of processed flaxseed. Similarly, no differences in activity and resting time were detected.

Feeding processed flaxseed increased dietary lipid intake from 3.5% of dry matter intake (DMI) at 0 lbs/d of OmegaBoost to 6.5% of DMI at 6 lbs/d of OmegaBoost (**Table 4.1**). The increase in dietary lipid intake with increasing dietary amounts of OmegaBoost was not reflected in increasing concentrations or yields of milk fat.

Similarly, milk protein and lactose concentrations and yield were not significantly different by source and amount of processed flaxseed.

Serum concentrations of glucose, BHBA, urea N, and non-esterified fatty acids (NEFA) were not significantly affected by source and amount of processed flaxseed. The closest to significant is a decrease in serum BHBA concentrations at 6 lbs/d of OmegaBoost (0.70 mM/L) compared to 2 lbs/d of OmegaBoost (0.50 mM/L; $P = 0.06$).

Serum Fatty Acid Concentration and Composition

Feeding OmegaBoost tended to increase linearly total serum fatty acid concentrations from 291 μg/mL at 0 lbs/d OmegaBoost to 342 μg/mL at 6 lbs/d OmegaBoost ($P = 0.09$; **Table 4.2**). The linear increase in serum concentrations of α-linolenic acid from 22.5 μg/mL at 0 lbs/d OmegaBoost to 44.0 μg/mL at 6 lbs/d of OmegaBoost ($P < 0.001$) nearly doubled α-linolenic acid serum concentrations and contributed for 42% of the increase in total serum fatty acid concentration. In addition, the increase in serum concentrations of α-linolenic acid in response to feeding increasing amounts of OmegaBoost, resulted in a linear increase in the proportion of α-linolenic acid on total serum fatty acids from 7.6 wt% at 0 lbs/d OmegaBoost to 11.9 wt% at 6 lbs/d of OmegaBoost ($P < 0.001$; **Table 4.3**).

The next greatest contributor to the increase in total serum fatty acid concentrations was oleic acid (contributed for 20% of the increase in total serum fatty acid concentration; **Table 4.2**), which is the second most abundant fatty acid in flaxseed behind α-linolenic acid (**Table 3.2**) and increased linearly from 30.5 μg/mL at 0 lbs/d OmegaBoost to 40.9 μg/mL at 6 lbs/d OmegaBoost ($P = 0.07$). Another intriguing finding was that serum concentrations of trans oleic acid increased from 7.4 μg/mL at 4

lbs/d OmegaBoost to 13.0 μg/mL at 6 lbs/d OmegaBoost (P = 0.03), while serum CLA concentrations tended to decrease from 24.7 μg/mL at 2 lbs/d OmegaBoost to 14.6 μg/mL at 6 lbs/d OmegaBoost (P = 0.10).

Milk Fatty Acid Composition

Feeding increasing amounts of OmegaBoost altered milk fatty acid composition toward a more unsaturated fatty acid profile (**Table 4.4**). Feeding OmegaBoost at 2, 4, and 6 lbs/d linearly decreased the proportion of saturated fatty acids in milk by 6, 15, and 18%, respectively ($P_{Linear} < 0.001$). Feeding 4 lb/d of ground flaxseed decreased the proportion of saturated fatty acids by 12%, which is intermediate between 2 and 4 lbs/d of OmegaBoost but not significantly different at $P \leq 0.05$ from either one of them. The lower proportion of saturated fatty acids was a result of a decrease in the proportion of *de-novo* synthesized fatty acids (C6:0 to C16:0). For example, the atherogenic fatty acids lauric, myristic, and palmitic acid combined decreased 9, 18, and 23% by feeding 2, 4, and 6 lbs/d of OmegaBoost, respectively ($P_{Linear} < 0.001$).

Feeding 2, 4, and 6 lbs/d of OmegaBoost linearly increased the proportion of mono-unsaturated fatty acids in milk fat by 14, 32, and 35%, respectively ($P_{Linear} < 0.001$; **Table 4.4**). The higher proportion of mono-unsaturated fatty acids was caused by an increase in the proportion of oleic acid, which increased 16, 33, and 39%, when feeding 2, 4, and 6 lbs/d of OmegaBoost, respectively ($P_{Linear} < 0.001$). In particular, the proportion of the in the rumen formed trans oleic acid isomers increased 43, 116, and 124%, when feeding 2, 4, and 6 lbs/d of OmegaBoost, respectively ($P_{Linear} < 0.001$).

Furthermore, feeding 2, 4, and 6 lbs/d of OmegaBoost linearly increased the proportion of mono-unsaturated fatty acids in milk fat by 16, 49, and 82%, respectively

($P_{Linear} < 0.001$; **Table 4.4**). The proportion of all major poly-unsaturated fatty acids increased linearly with increasing amounts of OmegaBoost. The proportion of the primary fatty acid in flaxseed α-linolenic acid increased 26, 52, and 70%, when feeding 2, 4, and 6 lbs/d of OmegaBoost, respectively ($P_{Linear} < 0.001$).

Butter and Cheese Fatty Acid Composition

Similar relative changes in fatty acid profile as in milk were observed in butter samples in response to feeding increasing amounts of OmegaBoost (**Tables 4.4, 4.5**). In butter fat, the proportion of saturated fatty acids in butter decreased 6, 15, and 17% after feeding 2, 4, and 6 lbs/d of OmegaBooost, respectively ($P_{Linear} < 0.001$), while the proportion of mono-unsaturated fatty acids increased by 9, 26, and 28% ($P_{Linear} = 0.001$).and the proportion of poly-unsaturated fatty acids by 27, 61, and 74% ($P_{Linear} < 0.001$; **Table 4.4**). In addition, the changes in fatty acid profile were similar in significance in milk and butter fat, as indicated by the probability values (**Tables 4.4, 4.5**). Butter manufacture may have decreased the proportion of short-chain saturated fatty acids, as indicated by the lower proportion of C4:0 to C14:0 in butter fat than in milk fat, which was proportionally largest in C4:0 (approximately 20% lower; **Tables 4.4, 4.5**).

Relative changes in fatty acid profile were similar in milk as in fresh Mozzarella cheese samples after feeding increasing amounts of OmegaBoost, except for a smaller relative change in the proportion of poly-unsaturated fatty acids (**Tables 4.4, 4.6**). In cheese fat, the proportion of saturated fatty acids decreased by 8, 12, and 18% after feeding 2, 4, and 6 lbs/d of OmegaBooost, respectively ($P_{Linear} = 0.01$), while the proportion of mono-unsaturated fatty acids increased by 16, 24, and 35% ($P_{Linear} = 0.01$),

and the proportion of poly-unsaturated fatty acids by 19, 20, and 43% (P_{Linear} = 0.02; **Table 4.6**). The relative change in poly-unsaturated fatty acids in cheese fat was smaller because the proportion of poly-unsaturated fatty acid in cheese fat was higher than in milk fat (3.94 wt% in milk fat compared to 6.02 wt% in cheese fat when cows were fed 0 lbs/d of OmegaBoost; **Tables 4.4, 4.6**). The increase in the proportion of poly-unsaturated fatty acids in cheese fat compared to milk fat was counterbalanced by a decrease in the proportion of saturated fatty acids C4:0 to C14:0 in cheese fat (**Tables 4.4, 4.6**). Cheese manufacturing may have also increased the variability of the fatty acid profile because the probability values were higher for cheese than milk or butter (**Tables 4.4, 4.5, 4.6**).

Butter and Cheese Texture Analysis

Feeding OmegaBoost to cows improved the spreadability of refrigerated butter made from their milk as indicated by the decreased hardness and adhesiveness (**Table 4.7**). At refrigeration temperature, the hardness decreased 37, 48, and 58% after feeding 2, 4, and 6 lbs/d of OmegaBooost, respectively (P_{Linear} = 0.003) and the adhesiveness decreased 82, 84, and 86% (P_{Linear} = 0.03). At room temperature, the butter hardness (P_{Linear} = 0.05) and adhesiveness (P_{Linear} = 0.17) also decreased with feeding increasing amounts of OmegaBoost but the decrease was smaller and less significant. The effect of OmegaBoost feeding on spreadability was smaller and less significant in fresh Mozzarella cheese, which has a softer structure than butter.

Table 4.1 Effect of increasing concentrations of processed (OmegaBoost) or ground flaxseed on production of Holstein cows

	OmegaBoost[1]				Ground		Contrast[4]		
Variable	0	2	4	6	Flaxseed[2]	SEM[3]	O	L	Q
Dry Matter Intake, kg	23.0	23.9	24.3	25.5	24.8	1.1	0.06	0.06	0.78
Body Weight, kg	749	757	755	755	753	15	0.89	0.62	0.43
Activity, min	130	121	124	122	123	11	0.94	0.64	0.64
Rest, min	730	698	719	741	745	25	0.12	0.60	0.12
Supplement									
Intake, kg/d	0.00	0.85	1.71	2.56	1.71				
Fat intake, kg/d	0.00	0.26	0.52	0.78	0.62				
C18:3 intake, g/d	0.00	0.15	0.29	0.44	0.37				
Lipids, % of DM	0.00	1.25	2.43	3.45	2.82				
Total Lipid Intake, % of DM	3.48	4.60	5.64	6.54	6.03				
Milk Amount (kg/d):									
Milk	30.0	30.4	30.3	30.2	30.0	1.8	0.99	0.95	0.79
Fat	1.26	1.30	1.29	1.28	1.28	0.06	0.97	0.80	0.54
Protein	0.93	0.93	0.93	0.93	0.91	0.05	0.97	0.95	0.90
Lactose	1.45	1.47	1.47	1.47	1.45	0.09	1.00	0.91	0.84
Total Solids	3.63	3.71	3.68	3.68	3.65	0.20	0.99	0.87	0.75
Milk Composition (%):									
Fat	4.20	4.31	4.28	4.27	4.30	0.16	0.84	0.73	0.41
Protein	3.11	3.07	3.10	3.09	3.07	0.06	0.86	0.88	0.71
Lactose	4.84	4.83	4.84	4.86	4.84	0.03	0.85	0.63	0.44
Total Solids	12.15	12.21	12.22	12.21	12.21	0.20	0.97	0.71	0.67
Serum Metabolites:									
Glucose, mg/dL	71.5	73.0	71.6	70.8	72.3	1.7	0.83	0.64	0.40
BHBA, mM/L	0.65	0.70	0.59	0.50	0.54	0.08	0.17	0.15	0.36
Urea N, mg/dL	15.1	15.7	16.1	16.9	15.4	1.2	0.85	0.28	0.97
NEFA, mEq/L	127	104	91	135	99	27	0.66	0.93	0.22

[1] 0 = no additional fat supplementation above that supplied by mixed ration; 2 = 0.28 kg/d supplemented fat from rumen protected flaxseed with 56% C18:3; 4 = 0.56 kg/d supplemented fat from rumen protected flaxseed with 56% C18:3; 6 = 0.83 kg/d supplemented fat from rumen protected flaxseed with 56% C18:3.

[2] Ground Flax = 0.66 kg/d supplemented fat from ground flaxseed with 59% C18:3.

[3] SEM = standard error of the mean; the largest standard error of the 5 treatment groups is shown.

[4] L = contrast testing linear effect between 0, 2, 4 and 6; Q = contrast testing quadratic effect between 0, 2, 4 and 6.

Table 4.2 Effect of increasing concentrations of processed (OmegaBoost) or ground flaxseed on serum fatty acid cooncentrations (g/ml) of Holstein cows

Fatty acid,	OmegaBoost[1]				Ground		Contrast[4]		
g/ml	0	2	4	6	Flaxseed[2]	SEM[3]	O	L	Q
Total	291	293	329	342	298	25	0.37	0.09	0.78
SFA[5]	111	107	118	127	112	12	0.70	0.29	0.53
$Total_{C12, 14, 16:0}$	45.3	42.2	45.8	51.1	43.9	6.3	0.74	0.45	0.42
C14:0	7.88	6.51	7.17	7.85	8.53	1.21	0.80	0.92	0.35
C16:0	37.8	36.0	38.7	43.2	35.4	5.5	0.70	0.44	0.50
C18:0	41.8	43.2	46.3	47.5	41.8	3.7	0.78	0.21	0.98
MUFA[6]	37.4	37.9	42.2	50.0	38.8	5.4	0.23	0.08	0.39
C16:1 c9	6.96	7.48	8.43	9.11	6.12	1.14	0.41	0.24	0.95
C18:1	30.5	30.5	33.7	40.9	32.7	4.3	0.21	0.07	0.29
C18:1c	22.0	22.1	26.2	27.9	24.0	2.8	0.29	0.05	0.68
C18:1t	8.72	8.73	7.51	13.0	8.72	1.95	0.20	0.20	0.14
C18:1 t10,11	8.57	8.11	7.42	11.4	7.89	1.82	0.32	0.35	0.18
PUFA[7]	143	148	169	165	147	11	0.36	0.09	0.67
C18:2	120	120	130	121	113	9	0.62	0.75	0.56
C18:2 UC[8]	101	96	108	107	96	8	0.66	0.48	0.79
CLA[9]	19.2	24.7	22.1	14.6	16.7	4.3	0.43	0.37	0.15
C18:2c9t11	7.29	8.87	7.24	6.64	7.12	2.88	0.98	0.78	0.66
C18:3	22.5	27.5	38.9	44.1	34.6	3.2	<0.001	<0.001	0.98
C18:3 n-3	22.5	27.3	38.9	44.0	34.6	3.2	<0.001	<0.001	0.95

[1] 0 = no additional fat supplementation above that supplied by mixed ration; 2 = 0.28 kg/d supplemented fat from rumen protected flaxseed with 56% C18:3; 4 = 0.56 kg/d supplemented fat from rumen protected flaxseed with 56% C18:3; 6 = 0.83 kg/d supplemented fat from rumen protected flaxseed with 56% C18:3.

[2] Ground Flax = 0.66 kg/d supplemented fat from ground flaxseed with 59% C18:3.

[3] SEM = standard error of the mean; the largest standard error of the 5 treatment groups is shown.

[4] L = contrast testing linear effect between 0, 2, 4 and 6; Q = contrast testing quadratic effect between 0, 2, 4 and 6.

[5] SFA = Saturated fatty acids (Cn:0).

[6] MUFA = Mono-unsaturated fatty acids (Cn:1).

[7] PUFA = Poly-unsaturated fatty acids (Cn:2+).

[8] UC = Unconjugated linoleic acid.

[9] CLA = Conjugated linoleic acid.

Table 4.3. Effect of increasing concentrations of processed (OmegaBoost) or ground flaxseed on serum (weight %) fatty acid composition of Holstein cows

Fatty acid,	OmegaBoost[1]				Ground		Contrast[4]		
% wt	0	2	4	6	Flaxseed[2]	SEM[3]	O	L	Q
SFA[5]	38.1	37.1	35.4	36.5	38.6	2.0	0.80	0.50	0.58
$Total_{C12, 14, 16:0}$	15.5	14.4	13.6	14.7	15.1	1.2	0.80	0.53	0.29
C14:0	2.74	2.24	2.15	2.24	3.13	0.44	0.44	0.43	0.48
C16:0	12.9	12.4	11.4	12.4	12.0	1.0	0.83	0.60	0.42
C18:0	14.2	15.0	14.0	13.9	15.2	0.8	0.45	0.64	0.50
MUFA[6]	12.7	13.0	12.6	13.9	13.1	0.9	0.75	0.43	0.53
C16:1 c9	2.35	2.45	2.46	2.55	2.05	0.37	0.85	0.72	0.98
C18:1	10.3	10.5	10.1	11.4	11.1	0.74	0.56	0.40	0.40
C18:1c	7.51	7.44	7.89	7.95	8.18	0.43	0.61	0.35	0.84
C18:1t	2.81	3.13	2.21	3.42	2.90	0.58	0.56	0.73	0.43
C18:1 t10,11	2.77	2.77	2.18	3.11	2.61	0.50	0.61	0.85	0.33
PUFA[7]	49.3	50.0	52.0	49.6	48.3	2.7	0.87	0.82	0.52
C18:2	41.8	41.0	40.1	36.8	37.2	2.2	0.32	0.11	0.49
C18:2 UC[8]	34.7	32.3	33.4	32.1	31.3	2.3	0.82	0.55	0.80
CLA[9]	6.86	8.22	6.66	4.62	5.83	1.35	0.40	0.15	0.23
C18:2 c9t11	2.50	2.51	2.04	1.93	2.46	0.71	0.95	0.51	0.93
C18:3	7.63	8.98	11.9	12.8	11.1	0.84	<0.001	<0.001	0.82
C18:3 n-3	7.62	8.88	11.9	12.8	11.1	0.82	<0.001	<0.001	0.86

[1] 0 = no additional fat supplementation above that supplied by mixed ration; 2 = 0.28 kg/d supplemented fat from rumen protected flaxseed with 56% C18:3; 4 = 0.56 kg/d supplemented fat from rumen protected flaxseed with 56% C18:3; 6 = 0.83 kg/d supplemented fat from rumen protected flaxseed with 56% C18:3.

[2] Ground Flax = 0.66 kg/d supplemented fat from ground flaxseed with 59% C18:3.

[3] SEM = standard error of the mean; the largest standard error of the 5 treatment groups is shown.

[4] L = contrast testing linear effect between 0, 2, 4 and 6; Q = contrast testing quadratic effect between 0, 2, 4 and 6.

[5] SFA = Saturated fatty acids (Cn:0).

[6] MUFA = Mono-unsaturated fatty acids (Cn:1).

[7] PUFA = Poly-unsaturated fatty acids (Cn:2+).

[8] UC = Unconjugated linoleic acid.

[9] CLA = Conjugated linoleic acid.

Table 4.4. Effect of increasing concentrations of processed (OmegaBoost) or ground flaxseed on milk fatty acid composition of Holstein cows

Fatty acid,	OmegaBoost[1]				Ground		Contrast[4]		
% wt	0	2	4	6	Flaxseed[2]	SEM[3]	O	L	Q
SFA[5]	70.0	65.6	59.7	57.6	61.5	2.0	0.001	<0.001	0.49
C4:0	3.43	3.48	3.11	3.05	3.39	0.26	0.61	0.19	0.81
C6:0	2.72	2.60	2.25	2.15	2.46	0.17	0.10	0.008	0.92
C8:0	1.78	1.65	1.40	1.33	1.55	0.11	0.03	0.001	0.68
C10:0	4.22	3.73	3.16	2.96	3.46	0.26	0.007	<0.001	0.45
$Total_{C12, 14, 16:0}$	44.1	39.9	36.0	34.1	36.6	1.3	<0.001	<0.001	0.31
C12:0	4.64	4.04	3.49	3.23	3.75	0.23	0.002	<0.001	0.34
C14:0	13.5	12.6	11.2	10.6	11.4	0.4	<0.001	<0.001	0.71
C16:0	26.0	23.3	21.3	20.3	21.4	0.9	0.003	<0.001	0.29
C18:0	9.85	10.9	10.8	11.1	10.9	0.6	0.34	0.15	0.42
MUFA[6]	26.1	29.8	34.5	35.2	32.4	1.5	<0.001	<0.001	0.21
C14:1c	1.12	1.11	1.58	1.05	1.03	0.32	0.45	0.87	0.32
C16:1 c9	1.08	1.04	1.04	1.02	0.12	0.91	0.91	0.78	0.93
C16:1 c11	0.32	0.26	0.32	0.37	0.28	0.06	0.30	0.33	0.21
C18:1	23.6	27.4	31.4	32.8	30.1	1.3	<0.001	<0.001	0.25
C18:1c	20.2	22.6	24.2	25.3	24.1	0.7	<0.001	<0.001	0.23
C18:1t	3.35	4.78	7.22	7.49	6.05	0.88	0.02	0.001	0.45
C18:1t10,11	1.56	1.74	3.33	3.43	2.57	0.71	0.20	0.03	0.95
PUFA[7]	3.94	4.56	5.89	7.17	6.11	0.66	0.02	0.001	0.56
C18:2	2.93	3.24	4.36	5.46	4.56	0.61	0.04	0.003	0.43
C18:2 UC[8]	2.31	2.62	3.45	4.27	3.66	0.44	0.03	0.002	0.49
CLA[9]	0.62	0.62	0.91	1.19	0.90	0.19	0.17	0.02	0.37
C18:2 c9t11	0.62	0.62	0.91	1.19	0.90	0.19	0.17	0.02	0.37
C18:3	1.00	1.27	1.53	1.71	1.55	0.13	0.008	<0.001	0.70
C18:3 n-3	1.00	1.26	1.52	1.70	1.55	0.13	0.01	<0.001	0.70

[1] 0 = no additional fat supplementation above that supplied by mixed ration; 2 = 0.28 kg/d supplemented fat from rumen protected flaxseed with 56% C18:3; 4 = 0.56 kg/d supplemented fat from rumen protected flaxseed with 56% C18:3; 6 = 0.83 kg/d supplemented fat from rumen protected flaxseed with 56% C18:3.

[2] Ground Flax = 0.66 kg/d supplemented fat from ground flaxseed with 59% C18:3.

[3] SEM = standard error of the mean; the largest standard error of the 5 treatment groups is shown.

[4] L = contrast testing linear effect between 0, 2, 4 and 6; Q = contrast testing quadratic effect between 0, 2, 4 and 6.

[5] SFA = Saturated fatty acids (Cn:0).

[6] MUFA = Mono-unsaturated fatty acids (Cn:1).

[7] PUFA = Poly-unsaturated fatty acids (Cn:2+)

[8] UC = Unconjugated linoleic acid.

[9] CLA = Conjugated linoleic acid.

Table 4.5. Effect of increasing concentrations of processed (OmegaBoost) or ground flaxseed on butter fatty acid composition of Holstein cows

Fatty acid,	OmegaBoost[1]				Ground		Contrast[4]		
% wt	0	2	4	6	Flaxseed[2]	SEM[3]	O	L	Q
SFA[5]	66.5	62.8	56.3	55.2	57.6	2.2	0.004	<0.001	0.44
C4:0	2.40	2.53	2.18	2.21	2.23	0.14	0.16	0.09	0.60
C6:0	1.87	1.88	1.63	1.61	1.66	0.12	0.13	0.03	0.85
C8:0	1.27	1.18	0.98	0.98	1.03	0.09	0.05	0.006	0.49
C10:0	3.21	2.85	2.41	2.31	2.54	0.21	0.01	0.001	0.40
$Total_{C12,14,16:0}$	43.1	38.8	33.3	32.7	34.4	1.9	0.003	<0.001	0.25
C12:0	3.89	3.25	2.84	2.75	3.00	0.24	0.006	<0.001	0.22
C14:0	12.6	11.8	10.2	9.77	10.7	0.5	0.004	<0.001	0.63
C16:0	26.6	23.7	20.3	20.1	20.7	1.3	0.005	<0.001	0.20
C18:0	10.8	11.6	11.6	12.0	11.9	0.6	0.46	0.13	0.66
MUFA[6]	28.9	31.4	36.4	37.1	35.0	1.8	0.02	0.001	0.53
C14:1	1.12	0.85	0.84	0.86	0.89	0.10	0.17	0.06	0.15
C16:1 c9	1.25	0.99	0.85	1.02	0.88	0.08	0.003	0.02	0.006
C16:1 c11	0.32	0.25	0.22	0.33	0.24	0.03	0.16	0.95	0.02
C18:1	26.2	29.3	34.5	34.8	33.0	1.7	0.008	<0.001	0.33
C18:1c	22.8	23.5	24.1	26.2	25.7	1.2	0.19	0.04	0.50
C18:1t	3.23	5.78	10.3	8.71	7.47	1.2	0.005	<0.001	0.05
C18:1t10,11	1.04	2.27	3.47	3.87	2.83	0.69	0.04	0.003	0.44
PUFA[7]	4.51	5.74	7.27	7.83	7.39	0.68	0.006	<0.001	0.52
C18:2	3.11	3.88	5.10	5.57	4.89	0.68	0.006	<0.001	0.52
C18:2 UC[8]	2.42	2.95	3.75	4.27	3.67	0.47	0.04	0.003	0.98
CLA[9]	0.68	0.93	1.35	1.31	1.22	0.16	0.01	0.002	0.22
18:2 c9t11	0.68	0.93	1.35	1.31	1.22	0.16	0.01	0.002	0.22
C18:3	1.35	1.86	2.18	2.27	2.50	0.14	<0.001	<0.001	0.12
C18:3 n-3	1.23	1.67	1.97	2.04	2.13	0.14	0.002	<0.001	0.14
C18:3 n-6	0.17	0.19	0.22	0.23	0.36	0.03	0.007	0.10	0.80

[1] 0 = no additional fat supplementation above that supplied by mixed ration; 2 = 0.28 kg/d supplemented fat from rumen protected flaxseed with 56% C18:3; 4 = 0.56 kg/d supplemented fat from rumen protected flaxseed with 56% C18:3; 6 = 0.83 kg/d supplemented fat from rumen protected flaxseed with 56% C18:3.

[2] Ground Flax = 0.66 kg/d supplemented fat from ground flaxseed with 59% C18:3.

[3] SEM = standard error of the mean; the largest standard error of the 5 treatment groups is shown.

[4] L = contrast testing linear effect between 0, 2, 4 and 6; Q = contrast testing quadratic effect between 0, 2, 4 and 6.

[5] SFA = Saturated fatty acids (Cn:0).

[6] MUFA = Mono-unsaturated fatty acids (Cn:1).

[7] PUFA = Poly-unsaturated fatty acids (Cn:2+).

[8] UC = Unconjugated linoleic acid.

[9] CLA = Conjugated linoleic acid.

Table 4.6. Effect of increasing concentrations of processed (OmegaBoost) or ground flaxseed on fresh Mozarella cheese fatty acid composition of Holstein cows

Fatty acid,	OmegaBoost[1]				Ground		Contrast[4]		
% wt	0	2	4	6	Flaxseed[2]	SEM[3]	O	L	Q
SFA[5]	67.3	61.6	59.5	55.2	58.7	2.7	0.10	0.01	0.80
C4:0	2.68	2.66	2.48	2.71	2.82	0.18	0.77	0.91	0.49
C6:0	2.09	1.94	1.88	1.85	2.07	0.16	0.86	0.41	0.61
C8:0	1.40	1.24	1.19	1.16	1.31	0.11	0.64	0.17	0.58
C10:0	3.45	2.96	2.84	2.62	3.04	0.25	0.30	0.04	0.58
$Total_{C12, 14, 16:0}$	43.0	37.2	36.0	32.2	34.7	1.9	0.02	0.002	0.59
C12:0	4.01	3.39	3.32	2.95	3.42	0.23	0.09	0.009	0.58
C14:0	12.6	11.8	11.0	9.5	11.1	0.51	0.04	0.002	0.69
C16:0	26.4	21.9	21.6	19.3	20.2	1.3	0.01	0.003	0.37
C18:0	9.81	11.6	10.9	11.0	11.2	0.3	0.03	0.04	0.03
MUFA[6]	26.8	31.2	33.3	36.2	33.4	2.2	0.12	0.01	0.71
C14:1c	1.05	0.95	1.03	0.91	0.95	0.07	0.28	0.17	0.54
C16:1 c9	1.26	1.07	0.95	1.02	0.92	0.08	0.02	0.03	0.06
C16:1 c11	0.30	0.29	0.24	0.29	0.28	0.03	0.33	0.45	0.20
C18:1	24.2	31.3	28.9	31.0	33.9	2.2	0.09	0.009	0.68
C18:1c	19.5	22.3	24.1	24.9	24.1	1.3	0.11	0.01	0.45
C18:1t	4.69	6.55	6.94	9.00	7.26	1.1	0.17	0.02	0.93
C18:1 t10,11	1.56	2.34	1.95	3.69	2.55	0.67	0.23	0.08	0.48
PUFA[7]	6.02	7.17	7.20	8.61	7.79	0.63	0.12	0.02	0.83
C18:2	3.58	4.06	3.99	5.31	4.50	0.53	0.23	0.06	0.41
C18:2 UC[8]	2.73	3.15	3.16	4.15	3.48	0.39	0.18	0.04	0.44
CLA[9]	0.85	0.92	0.84	1.15	1.01	0.17	0.68	0.33	0.47
C18:2 c9t11	0.85	0.92	0.84	1.15	1.01	0.17	0.68	0.33	0.47
C18:3	2.43	3.12	3.20	3.30	3.29	0.14	0.005	<0.001	0.05
C18:3 n-3	1.93	2.55	2.60	2.74	2.69	0.13	0.006	0.001	0.08
C18:3 n-6	0.50	0.57	0.60	0.57	0.60	0.06	0.70	0.38	0.37

[1] 0 = no additional fat supplementation above that supplied by mixed ration; 2 = 0.28 kg/d supplemented fat from rumen protected flaxseed with 56% C18:3; 4 = 0.56 kg/d supplemented fat from rumen protected flaxseed with 56% C18:3; 6 = 0.83 kg/d supplemented fat from rumen protected flaxseed with 56% C18:3.

[2] Ground Flax = 0.66 kg/d supplemented fat from ground flaxseed with 59% C18:3.

[3] SEM = standard error of the mean; the largest standard error of the 5 treatment groups is shown.

[4] L = contrast testing linear effect between 0, 2, 4 and 6; Q = contrast testing quadratic effect between 0, 2, 4 and 6.

[5] SFA = Saturated fatty acids (Cn:0).

[6] MUFA = Mono-unsaturated fatty acids (Cn:1).

[7] PUFA = Poly-unsaturated fatty acids (Cn:2+).

[8] UC = Unconjugated linoleic acid.

[9] CLA = Conjugated linoleic acid.

Table 4.7. Effect of increasing concentrations of processed (OmegaBoost) or ground flaxseed on yield and texture of Mozarella cheese and butter made from milk produced by Holstein cows

	OmegaBoost[1]				Ground		Contrast[4]		
Variable	0	2	4	6	Flaxseed[2]	SEM[3]	O	L	Q
Butter:									
Amount	260	275	275	290	266	35	0.95	0.58	0.95
20°C									
Hardness	25.5	20.7	19.0	20.3	19.1	2.0	0.11	0.05	0.11
Adhesiveness	16.6	13.9	12.6	14.0	14.1	1.5	0.42	0.17	0.16
4°C									
Hardness	1459	925	756	618	721	184	0.02	0.003	0.22
Adhesiveness	1302	239	207	186	199	39	0.19	0.03	0.55
Mozarella Cheese:									
Amount	945	911	908	933	921	41	0.86	0.77	0.36
20°C									
Hardness	263	235	212	243	250	24	0.65	0.46	0.25
Adhesiveness	30.8	20.7	17.3	20.8	29.0	3.5	0.15	0.08	0.08
4°C									
Hardness	639	463	480	514	539	61	0.36	0.19	0.12
Adhesiveness	77	60	56	71	66	7	0.23	0.42	0.05

[1] 0 = no additional fat supplementation above that supplied by mixed ration; 2 = 0.28 kg/d supplemented fat from rumen protected flaxseed with 56% C18:3; 4 = 0.56 kg/d supplemented fat from rumen protected flaxseed with 56% C18:3; 6 = 0.83 kg/d supplemented fat from rumen protected flaxseed with 56% C18:3.

[2] Ground Flax = 0.66 kg/d supplemented fat from ground flaxseed with 59% C18:3.

[3] SEM = standard error of the mean; the largest standard error of the 5 treatment groups is shown.

[4] L = contrast testing linear effect between 0, 2, 4 and 6; Q = contrast testing quadratic effect between 0, 2, 4 and 6.

CHAPTER 5.DISCUSSION

Effect of OmegaBoost Processing on Flaxseed Composition

OmegaBoost processing decreased the lipid content of the supplement, as indicated by the 19% lower lipid content of OmegaBoost versus ground flaxseed (**Table 3.2**). The processing method of OmegaBoost is proprietary and unknown to us. Based on talks with the makers of OmegaBoost, a compound is added during manufacturing, which dilutes the lipid content of OmegaBoost. In addition, OmegaBoost processing alters the fatty acid profile of ground flaxseed towards a higher proportion of oleic acid and a lower proportion of α-linolenic acid. The shift in fatty acid profile suggest that manufacturing of OmegaBoost may result in a partial loss of α-linolenic acid due to its lower melting temperature, due to conversion to oleic and linolenic acid, or by both.

Effect of OmegaBoost Feeding on Production Parameters

Besides an increase in dry matter intake, we did not observe any changes in production parameters in response to feeding increasing amounts of OmegaBoost (**Table 4.1**). The reason for the increase in dry matter intake was because feeding increasing amounts of OmegaBoost as supplement did not affect the consumption of the total mixed ration (results not shown). Most studies evaluate the effect of increasing amounts of a dietary lipid source by substituting the lipid for concentrate, such as corn or soybean meal (Chilliard et al, 2009 and Gonthier et al 2005). Substituting like this does not only change the energy content of the diet but also change the nutrient profile of the diet, including the amounts of undesirable fatty acids. Therefore, the observed effects in a substitution study could be either due to the increasing amounts of desirable fatty acids from the lipid source, the decreased amounts of the undesirable fatty acids or other nutrients from the substituted concentrate, or a combination of both. We chose lipid

supplementation instead of substitution to minimize potential effects of decreased amounts of another feed component on production parameter and product fatty acid profile.

Many of the substitution studies report a decrease in dry matter intake, especially if flaxseed oil or extruded flaxseed is fed (**Tables 2.1, 2.2**). The decrease in dry matter intake is generally attributed to increase rumen availability of poly-unsaturated fatty acids, which are toxic to cellulolytic bacteria and protozoa and, thus, delay fiber digestion (Chilliard et al., 2009). Little is known how fatty acids in the small intestine affect satiety signals in the ruminant. Thus, longer chain fatty acids may also decrease dry matter intake by increased chylomicron formation, as suggested by Stipanuk (2006).

In our study, we did not observe a decrease in dry matter intake by feeding increasing amounts of flaxseed. There are several potential reasons: 1) The amounts of flaxseed supplement tested in this study were too low to reduce a decrease in dry matter intake. A decrease in dry matter intake with lipid supplementation is usually not seen until 3% of fatty acid are added to the dry matter (Allen, 2000), which was the highest proportion added in our study (**Table 4.1**). We intentionally set up the highest dosage to this percentage to have a detrimental effect on feed intake because the focus of our study was to examine the effect of OmegaBoost on fatty acid profile of milk and dairy products and not how much lipid supplement was needed to negatively affect feed intake. In addition, we did not observe that feeding OmegaBoost increased serum concentrations of non-esterified fatty acids, which at high circulating concentrations limit feed intake. We, however, cannot exclude the possibility that feeding 6 lbs/d OmegaBoost for more than 2 wks negatively affects dry matter intake, which must be tested in future trials.

2) OmegaBoost was top-dressed rather than included in the TMR. Since cows consumed the supplement before the total mixed ration, the supplement may have passed the rumen before the bulk of the dietary fiber entered the rumen and slowed down the passage rate and increased the rumen availability of poly-unsaturated fatty acids. The detrimental effect of poly-unsaturated fatty acids on dry matter intake is usually greater at greater dietary fiber concentrations (Loor et al., 2005).

One would expect that the increased dry matter intake of the more energy-dense diet would have increased either body weight and milk production. Neither of those was observed in this study (**Table 4.1**). Since also resting and activity time were not affected by the dietary changes (**Table 4.1**), we hypothesize that more energy was excreted in urine and feces, which was not tested in this study. We were concerned that increasing the energy density of the diet by lipid supplementation may divert more energy in the tested late-lactation from milk production to body accretion. It is not uncommon that cows gain on a high-energy diet in late lactation and during advanced pregnancy a lot of weight and become obese, which negatively impact their health status around parturition. We did not observe an increase in serum concentrations of glucose (**Table 4.1**), which may suggest that the supplementation amounts tested in this study are insufficient to promote weight gain. Zachut et al. (2010), however, observed a body weight increase in lactating cows in response to feeding high amounts of extruded flaxseed starting at 10 wks of the diet. Thus, we cannot explain the possibility that feeding high amounts of OmegaBoost for extended time may increase body weight.

Effect of OmegaBoost Feeding on Serum Fatty Acid Concentrations and Profile

Serum fatty acid concentrations, specifically long-chain unsaturated fatty acids, tended to increase linearly with feeding increasing amount of OmegaBoost (**Table 4.2**). Gonthier et al. (2005) wrote that the increases in serum fatty acid concentrations are reflective of the duodenal concentrations and thus a good measure of the degree of protection against ruminal biohydrogenation by treatment diets. Thus, our results may indicate that feeding OmegaBoost increases the intestinal absorption of fatty acids. The primary increase in serum fatty acid concentrations was for α-linolenic acid (**Table 4.2**), which was expected because it is the primary fatty acid in flaxseed (**Table 3.2**) and because similar results had been reported previously (Gonthier et al., 2005).

When the amount of OmegaBoost was increased from 4 to 6 lbs/d, CLA concentrations in serum dropped while trans oleic acid concentrations increased (**Table 4.2**). Similar results had been reported by Gonthier et al. (2005) and suggest changes in the rumen microflora, pH, or both that allow for a biohydrogenation of poly-unsaturated fatty acids beyond the initial trans-isomerization step at higher amounts of flaxseed. Further studies are required to examine how different amounts of flaxseed supplement affect the different steps of the transformation of poly-unsaturated fatty acids in the rumen.

Effect of OmegaBoost Processing on Milk Fatty Acid Composition

Feeding increasing amounts of OmegaBoost altered milk fatty acid composition toward a more unsaturated fatty acid profile in a dose-dependent manner (**Table 4.4**). Specifically, the proportion of fatty acids, which are synthesized *de novo* in the mammary gland (C6:0 to C6:0), was decreased in milk fat. This is consistent with the literature as

reviewed by Glasser et al. (2008). Potential reasons for the decrease in *de novo* synthesized fatty acids in the mammary gland are that acetyl CoA carboxylase and fatty acid synthase gene expression and activities in the mammary gland are decreased by feeding high concentrations of unsaturated lipids (Piperova et al., 2000; Ahnadi et al., 2002; Harvatine and Bauman, 2006) either directly or by conjugated linoleic acid isomers trans-10, cis-12 and trans-9, cis-11 that are formed by rumen biohydrogenation. The decrease in saturated fatty acid could be also caused by a decrease in the precursors of these fatty acids as acetate or BHBA. We did observed a 29% decrease in serum BHBA concentrations when 6 compared to 2 lbs/d of OmegaBoost were fed ($P = 0.06$), which may indicate that high concentrations of OmegaBoost may inhibit ruminal formation of acetate and butyrate.

We did not observe a change in desaturase index in milk fat, using C14:1 to C14:0 and C16:1 to C16:0 as indicators (**Table 4.4**). Our results are consistent with other studies feeding flaxseed (as reviewed by Glasser et al., 2008), which suggests that feeding flaxseed at the amounts in this study, does not alter desaturase activity in the mammary gland. Future studies are warranted to examine the effect of flaxseed on protein expression and activity of enzymes involved in the synthesis of milk fatty acids and TAG.

Feeding increasing amounts of OmegaBoost increased the proportion of mono- and in particular poly-unsaturated fatty acids (**Table 4.4**). Our results are consistent with previous studies (as reviewed by Glasser et al., 2008) and suggest a beneficial effect of feeding high amounts of OmegaBoost onto the nutritional profile of milk fat. The higher proportion of trans oleic acid isomers and conjugated linoleic acid in milk fat at the higher amounts of OmegaBoost indicate that part of α-linolenic acid is converted in the

rumen. The proportion of dietary α-linolenic acid that is converted in the rumen is similar in OmegaBoost and in ground flaxseed, which suggest that the proprietary processing method of OmegaBoost does not effectively "bypass" flaxseed lipids through the rumen. Future studies in rumen-fistulated cows are warranted to examine how the processing method of OmegaBoost affects flaxseed digestion in the rumen and how to improve the method to achieve "rumen-protected" flaxseed supplement.

Previous studies showed that the absorption efficiency of poly-unsaturated fatty acids from duodenum to milk decrease with increasing amounts of flaxseed (Gonthier et al., 2005; Hurtaud et al., 2010), suggesting that there is a rate-limiting amount of ingested poly-unsaturated fatty acids above which the proportion of poly-unsaturated fatty acids cannot be increased. This limitation could be either explained by: limited poly-unsaturated fatty acid absorption in ruminants, specificity of mammary lipoprotein lipase activity, limited incorporation in milk TAG, or a combination of those factors. In our study, changes in milk fatty acid profile were gradual even at the higher amounts of flaxseed (**Table 4.4**), which suggests that the proportion of poly-unsaturated fatty acids, including α-linolenic acid, could be increased further if more than 6 lbs/d of OmegaBoost is fed.

Effect of OmegaBoost Processing on Butter and Cheese Fatty Acid Composition and Texture

Feeding cows increasing amounts of OmegaBoost altered the fatty acid profile of butter manufactured from their milk toward a more unsaturated composition that was enriched in α-linolenic acid (**Tables 4.4**). Our results are consistent with previous reports examining the effects of feeding flaxseed on butter composition (Smet et al., 2010;

Hurtaud et al., 2010). Differences in fatty acid profile of milk fat are usually reflected in butter fat (Bobe et al., 2003) with the proportion of short-chain fatty acids being slightly lower in butter fat than in milk fat, which is consistent with our results (**Table 4.4**). The lower proportion of short-chain fatty acids in butterfat than in milk fat suggests that a proportion of short-chain fatty acids are lost during pasteurization, into butter milk during butter churning, or both. Hurtaud et al. (2010) reported significant losses of milk fat in buttermilk when cows were fed 4.3% extruded flaxseed of dry matter intake. We did not measure fat concentrations in butter milk and butter; however, we had learned from our previous studies (Bobe et al., 2003; Chen et al., 2004; Bobe et al., 2007) that highly saturated milk fat requires different churning conditions than more unsaturated milk fat to achieve similar butter yield and butter fat concentrations. Butter from milk of cows fed flaxseed, which had a more unsaturated fatty acid profile, required slower churning at refrigeration temperature to prevent foaming, loss of milk fat into buttermilk, and potentially not converting into butter. In contrast, butter from milk of cows fed no flaxseed churned without major milk fat losses at room temperature. The reason for the differences in churning behavior is that milk fatty acid melting temperature and chain length affect the incorporation of TAG into butter granules.

Feed-associated changes in butter fatty acid profile were reflected in textural properties of butter (**Table 4.6**). Consistent with previous studies (Bobe et al., 2003; Chen et al., 2004; Bobe et al., 2007), more unsaturated butterfat is more spreadable, as indicated by lower hardness and adhesiveness of butter. The beneficial textural effects of OmegaBoost are more accentuated at refrigeration temperature than at room temperature, because TAG rich in unsaturated fatty acids have their melting point between refrigeration and room temperature. While we observed beneficial textural effects of

OmegaBoost at 2 lbs/d, Hurtaud et al. (2010) only observed improved spreadability when feeding 4.3% extruded flaxseed per dry matter but not when feeding 2%.

Feeding higher concentrations of flaxseed is potentially detrimental for consumer preference because butter that is rich in poly-unsaturated fatty acid is likely to oil off and lose structure at room temperature, as the poly-unsaturated melt out of the butter granules. Furthermore, butter that is rich in poly-unsaturated fatty acids is more likely to oxidize, which negatively impacts shelf-life, flavor, and color. We did not test flavor characteristics in our study but previous studies by Hurtaud et al (2010) did not observe flavor deficiencies when feeding 4.3% extruded flaxseed per dry matter.

The effect of feeding flaxseed on cheese fatty acid composition (**Table 4.5**) and textural properties (**Table 4.6**) has been, to our knowledge, not reported in the literature. The changes in fatty acid profile and texture were similar in direction but smaller in magnitude and significance (**Table 4.4, 4.5, 4.6**). The smaller magnitude and significance in differences in cheese was expected based on previous studies (Chen et al., 2004) because the lipid content and therefore its effect on structure was smaller in Mozzarella cheese than in butter. Mozzarella cheese was softer at refrigeration temperature than butter and, therefore, textural changes will be less pronounced. Furthermore, cheese manufacture includes more steps that potentially induce increased variability than butter manufacture. Regardless of the manufacture-associated variability, butter and Mozzarella cheese had increased proportions of mono- and poly-unsaturated fatty acids, including α-linolenic acid, in their fat, which improve their nutritional value

CHAPTER 6.CONCLUSION

In conclusion, our results suggest that feeding increasing amounts of OmegaBoost improved the nutritional fatty acid profile and the textural properties of butter and cheese at refrigeration temperature without negatively affecting feed intake and milk production. The proprietary processing method of OmegaBoost did not significantly change milk fatty acid profile compared to ground flaxseed, which questions the effectiveness of the proprietary processing method to improve transfer efficiency of poly-unsaturated fatty acids from feed to milk. Further studies are warranted to improve the processing method to increase ruminal bypass before further dosage and time length studies should be conducted to determine the optimal amount of OmegaBoost for supplementation.

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List of Abbreviations

ADF	Acid detergent fiber
ALA	Alpha linolenic acid
BHBA	Beta-hydroxybutyrate
$Total_{C12,14,16}$	Sum of lauric, myristic, and palmitic acid
CLA	Conjugated linoleic acid
CoA	Coenzyme A
CP	Crude protein
DIM	Days in milk
DM	Dry matter
DMI	Dry matter intake
LA	Linoleic acid
MUFA	Mono-unsaturated fatty acid
NCF	Neutral detergent fiber
NDF	Neutral detergent fiber
NEFA	Non-esterified fatty acid
NE_L	Net energy of lactation;
NRC	National Research Council
OmegaBoost	Processed flax formulated with the intent to bypass the rumen with minimal hydrogenation of poly-unsaturated fatty acids
PPAR	Peroxisome proliferator-activated receptor
PUFA	Poly-unsaturated fatty acid
Renet	Enzyme product form the gut of young ruminants that cause coagulation of milk to form the cheese curd
RUP	Rumen un-degradable protein
SEM	Standard error of mean

SFA	Saturated fatty acid
TAG	Triacylglycerol
UC	Unconjugated linoleic acid