THE IMPACT OF FEEDING PALMITIC AND STEARIC ACID TO LACTATING DAIRY COWS

By

Jorge Eduardo Rico Navarrete

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ABSTRACT

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Milk components as well as milk yield are key factors driving producer milk prices. Fat supplements can increase the efficiency of milk production; therefore, their addition to diets has the potential to be economically advantageous. The set of studies in this thesis evaluated the effects of supplementing palmitic (C16:0) and stearic (C18:0) acid, as well as their interaction with other dietary and animal factors. In the first experiment C16:0 and C18:0 where supplemented at 2% of ration dry matter; compared to C18:0, supplementation with C16:0 increased milk fat concentration and yield, as well as 3.5% fat corrected milk, and the efficiency of milk production. These effects were independent of cow production level, and the increase in milk fat yield was completely accounted for by an increase in the secretion of C16:0 into milk. In the second experiment, the effect of increasing doses of supplemental C16:0 (fed at 0, 0.75, 1.50, or 2.25% of ration DM) was characterized in diets that had either a low or a high fat content (2.7% or 4.2% ether extract, respectively). C16:0 increased milk fat concentration, fat yield, 3.5% FCM yield, and the efficiency of milk production, and tended to increase the yield of milk and milk protein. These responses were maximal when C16:0 was included at 1.5% of ration DM. There was no effect of basal dietary fat content on the yield of milk or milk components, however, significant interactions between C16:0 and the basal fat content of the diet were apparent. In addition, an increased secretion of 16-carbon FA as a result of C16:0 supplementation was observed which explained the majority of the increased secretion of milk fat. Collectively, these results demonstrate the potential of C16:0 to improve cow performance and provides a framework for optimizing its use to maximize productions responses and milk income.

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KEY TO ABBREVIATIONS

ACC	Acetyl-CoA carboxylase
BCS	Body condition score
BHBA	Beta-hydroxybutyric acid
BH	Biohydrogenation
BS	Bile salts
BW	Body weight
CCK	Cholecystokinin
CLA	Conjugated linoleic acid
CoASH	Coenzyme A
CP	Crude protein
CV	Coefficient of variation
DGAT	Diacylglycerol transferase
DIM	Days in milk
DM	Dry matter
DMI	Dry matter intake
ECM	Energy corrected milk
EDTA	Ethylenediaminetetraacetic acid
EE	Ether extract
FA	Fatty acids
FABP	Fatty acid binding protein
FA-CoA	Fatty acid CoA
FAME	Fatty acid methyl ester
FAS	Fatty acid synthase
FAT	Fatty acid translocator

FCM	Fat corrected milk
FCM	Fat corrected milk
GL	Glycolipids
GLC	Gas liquid chromatography
GLP	Glucagon like peptide
GLUT	Glucose transporter
GPAT	AcylCoA:glycerol-sn-3-phosphate acyl transferase
G3P	Glycerol-3-phosphate
LA	Linoleic acid
LN	Alpha linolenic acid
LPL	Lipoprotein lipase
ME	Metabolizable energy
MFD	Milk fat depression
MUFA	Monounsaturated fatty acids
MUN	Milk urea nitrogen
NADPH	Nicotinamide adenine dinucleotide phosphate
NDF	Neutral detergent fiber
NEFA	Non esterified fatty acids
OL	Oleic acid
PA	Palmitic acid treatment
PL	Phospholipid
PUFA	Polyunsaturated fatty acids
SA	Stearic acid treatment
SCD	Stearoyl-CoA desaturase
SEM	Standard error of the mean
SD	Standard deviation

SFA	Saturated fatty acids
SREBP	Sterol regulatory element binding protein
TG	Triglycerides
TMR	Total mixed ration
UFA	Unsaturated fatty acids
VLDL	Very low density lipoprotein
VFA	Volatile fatty acids

Chapter 1

INTRODUCTION

Elevated genetic merit for milk production and milk components has led to a concomitant increase in energy requirements of cows and has imposed an unavoidable negative energy balance status for extended periods of time in early lactation (Coppock and Wilks, 1991). Nutritional strategies to alleviate negative energy balance and to support elevated milk production include increasing the energy density of the diet through the use of concentrates and supplemental fat, the latter being of particular interest because it is the nutrient with the highest energy concentration (wt/wt). In addition, fats can have practical importance in diets fed to heat stressed cows as they can supply additional energy while having little effect on caloric increment of ruminal fermentation (Wang *et al*. 2010).

Although the specific requirements for fat have not been determined (NRC, 2001), some authors have suggested that because of their important nutritional role, fats should be included in diets to supply 15 to 25% of total metabolizable energy intake (ME; Kronfeld, 1976) and particularly in diets for high producing dairy cows (Palmquist and Jenkins, 1980) to increase energy content of the diet. Fat sources most commonly included in diets for dairy cows are oilseed, such as whole cottonseed and full-fat soybeans, animal fat (*e.g*. tallow), palm oils, and various modifications of these designed to reduce availability of fatty acids (FA) in the rumen (Rabiee *et al*., 2012). In addition, commercial fat supplements enriched in specific saturated fatty acids (SFA) acids have recently become available, however, fat supplements highly-enriched in individual SFA have not been extensively studied as a supplement for dairy cows. Therefore, the main objective of this dissertation was to characterize the effects of supplementing palmitic (C16:0) and stearic

(C18:0) acid to the diet of lactating dairy cows on milk production parameters, as well as blood metabolites and milk fat composition.

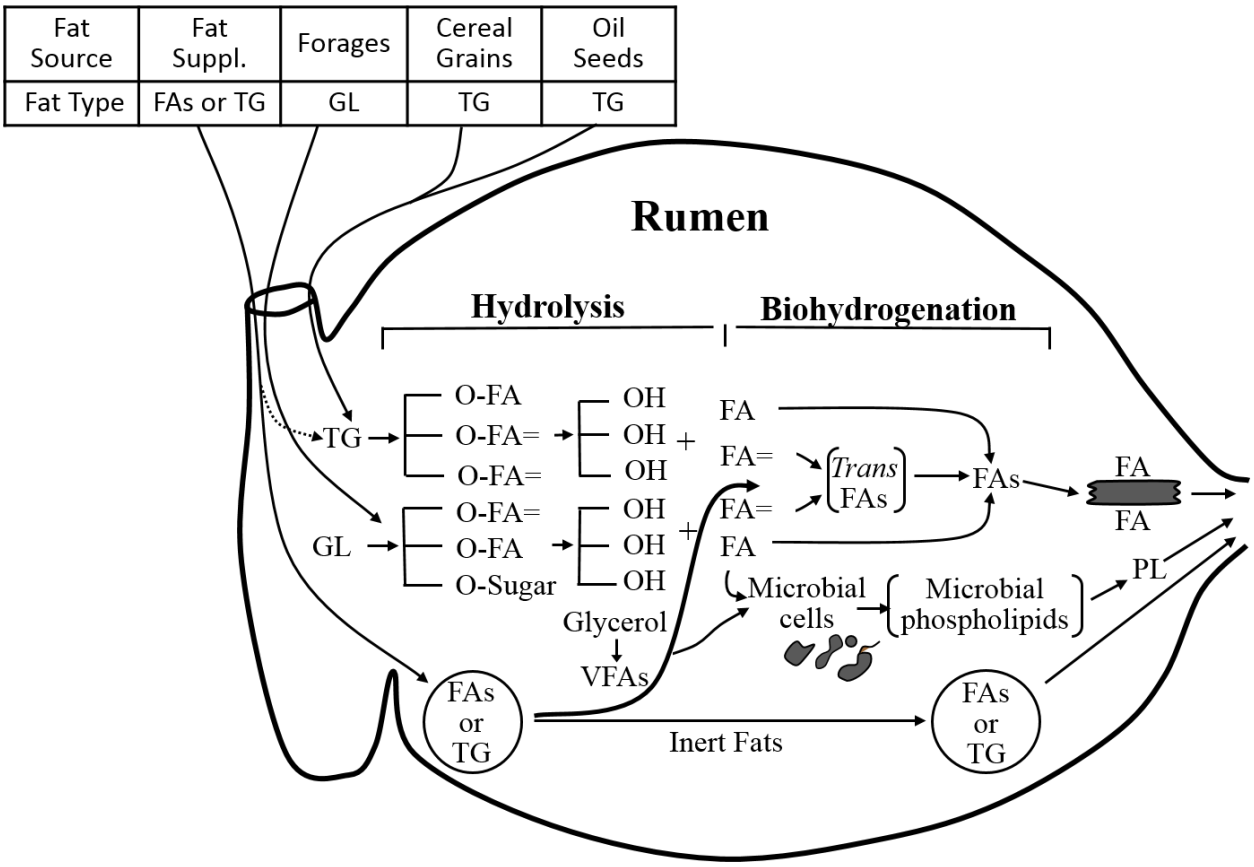
Chapter 2

LITERATURE REVIEW

Rumen Metabolism of Dietary Fats

Important modifications can occur to dietary fatty acids (FA) once they enter the rumen, and the extent of those changes will impact their composition, absorption and tissue utilization. FA in the diet of ruminants are typically supplied by forages and cereal grains, present mostly in the form of triglycerides and galactolipids, respectively. Although most of the FA entering the rumen are comprised of the unsaturated FA linoleic acid (LA, *cis*-9, *cis*-12, C18:2) and α-linolenic acid (LN, *cis*-9, *cis*-12, *cis*-15, C18:3), milk FA are mostly saturated (~70%; Jensen, 2002), which illustrates the extensive metabolism of fats in the rumen. The two major processes that dietary FA undergo in the rumen are hydrolysis of ester linkages and biohydrogenation (BH) (Figure 2-1). Before BH of FA can take place, plant lipids must become free of surrounding matrix by mastication and microbial digestive processes, followed by hydrolysis of ester linkages (Palmquist *et al*., 2005). Hydrolysis refers to the cleavage of the ester bonds found between FA and the glycerol backbone present in triglycerides, glycolipids, and phospholipids, which is carried out extracellularly mainly by ruminal bacteria, although plant lipases may also play a role (Lourenço *et al*., 2010). Although the rate and extent of hydrolysis is normally high (> 85%; Doreau and Ferlay, 1994; Beam *et al*., 2000), it can be negatively affected by low ruminal pH in high concentrate diets, (Gerson *et al*., 1985), with very little hydrolysis occurring at pH levels less than 6.0 (Van Nevel and Demeyer, 1996). In addition, hydrolysis is decreased as melting point (Palmquist *et al*., 2005) and dietary fat concentration (Beam *et al*., 2000) are increased.

Figure 2-1. Metabolism of dietary lipids in the rumen.



Abbreviations: Triglycerides (TG), glycolipids (GL), phospholipids (PL), trans fatty acids (trans FA), mixture of fatty acids (FAs), and volatile fatty acids (VFA). Adapted from Lock *et al*., 2006.

Following hydrolysis, saturated fatty acids (SFA) remain unmodified, whereas unsaturated fatty acids (UFA) such as LA rapidly undergo BH by ruminal microbes. BH is a multi-step process that, when completed, results in the formation of SFA, such as stearic acid (C18:0; removal of double bonds by hydrogenation), and involves several isomerization and reduction steps (Harfoot and Hazlewood, 1997). Numerous intermediates are formed during this process, including positional *trans* 18:1 isomers and conjugated linoleic acid (CLA) isomers (Lock and Bauman, 2004; Chilliard *et al*., 2007). It has been suggested that both BH and *cis-trans* isomerization of

dietary UFA are processes that occur as part of the response mechanism of ruminal bacteria to deal with the toxic effects of UFA (Maia *et al*., 2007 and Heipieper *et al*., 2010). UFA disrupt cell integrity and limit bacterial growth, and these toxic effects increase as unsaturation increases (LN > LA; Maia *et al*., 2007, Maia *et al*., 2010). Consistent with this, in general, the rates of rumen BH of FA are typically faster with increasing unsaturation, and for most diets LA and LN are hydrogenated to the extent of 70 to 95% and 85 to 100%, respectively, which results in C18:0 being the major FA reaching the duodenum (Lock *et al*., 2006). SFA are thought to be less detrimental to rumen fermentation because they do not negatively influence bacterial plasma membrane function to the same extent as UFA (Jenkins, 1993).

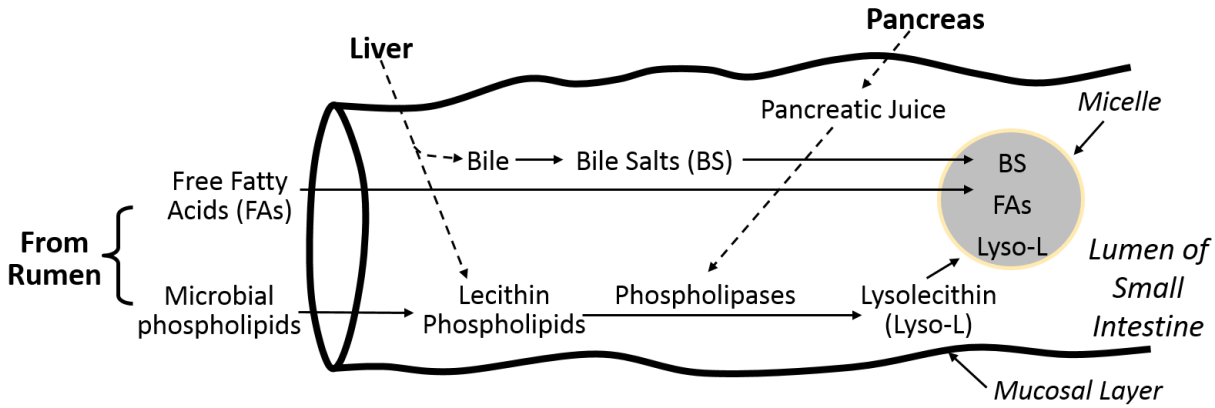
Digestion and Absorption of Dietary FA

No significant absorption of long-chain FA or further modifications occur to them during transit through the omasum and abomasum (Moore and Christie, 1984). Approximately 80 to 90% of duodenal FA flow corresponds to free FA and typically 65% of them are SFA (C16:0 and C18:0; Lock *et al*., 2006). The remaining lipid components are microbial phospholipids plus small amounts of triglycerides and glycolipids from residual feed material, which are hydrolyzed by intestinal and pancreatic lipases (Doreau and Ferlay, 1994). Because of the low pH in the abomasum and duodenum (2.0 to 2.5) the free FA occur in the protonated state, which facilitates their adsorption to the surface of feed particles (Drackley, 2005).

Absorption of FA takes place predominantly in the jejunum portion of the small intestine. However, this process cannot take place unless the lipids are first effectively solubilized into the aqueous environment. To this effect, and as in other species, micelle formation occurs to

allow efficient FA absorption (Davis 1990), and it is facilitated by the activity of both bile and pancreatic juice, which are secreted into the upper duodenum, where FA digestion begins. Bile contains bile salts and lecithin (phosphatidylcholine), and pancreatic juice provides the pancreatic phospholipase A_2, to convert lecithin to lysolecithin (lysophosphatidylcholine) and the bicarbonate to raise the pH (Figure 2-2). Lysolecithin, together with bile salts, desorbs the FA from feed particles and bacteria, and facilitates transfer of lipids to a soluble micellar phase, being absolutely required for FA absorption to occur (Moore and Christie, 1984). The newly formed water-soluble micelles can then facilitate transfer of FA contained in their inner core across the unstirred water layer of intestinal epithelial cells of the jejunum, where the FA and lysolecithin are absorbed by diffusion across the lipid bilayer (Lock *et al.*, 2006). Although several putative transporter proteins of long-chain FA have been identified in other species, to date, no data are available on the presence and role of these proteins in ruminants (Drackley, 2005).

Figure 2-2. Fat Digestion in the small intestine of ruminants.



Adapted from Lock *et al.*, 2006.

The higher comparative ability of ruminants *vs.* non-ruminants to absorb dietary FA was previously pointed out by Noble (1981). FA digestibility is usually higher in ruminant animals, particularly for SFA such as C16:0 and C18:0 (Moore and Christie, 1984). Since C18:0 is the predominant FA reaching the small intestine, it has been proposed that the higher capability of ruminants to absorb SFA is due to the reliance on lysolecithin as the major micelle stabilizer. Lysolecithin is one of several swelling amphiphiles, substances that expands the volume of bile salt micelles and their hydrophobic interior in aqueous environment, and it is the most efficient at increasing the solubility of C18:0. Freeman (1984) showed that, compared to other amphiphiles, lysolecithin was the only one that significantly increased the distribution of C18:0 into the micellar phase and away from the particulate phase. Other remarkable adaptations of ruminants to deal with high intestinal flow of SFA have been discussed by Drackley (2005) and Lock et al. (2006) and include: a) lower pH in duodenum that minimizes formation of calcium soaps of palmitate and stearate (known to be poorly absorbed in non-ruminants), and b) the presence of taurocholate, of lower pKa (2.0) than glycocholate (4.7), as the major bile salt, that is less likely to become insoluble in the more acidic conditions of the ruminant small intestine.

FA digestibility varies greatly in non-ruminants (Freeman, 1984) and it is decreased as chain length increases, and increased as the degree of unsaturation increases (Lessire *et al*., 1992), which results in very poor absorption of free C16:0 and C18:0 in non-ruminants (Noble, 1981). In ruminants, Lock *et al*. (2006) compiled results from 20 different independent studies and calculated total and individual FA digestibility for lactating dairy cattle. The authors reported total FA digestibility to average 74%, ranging from 58% to 86%, and emphasized that individual FA digestibility follows a similar pattern to that observed in non-ruminants, however, relative

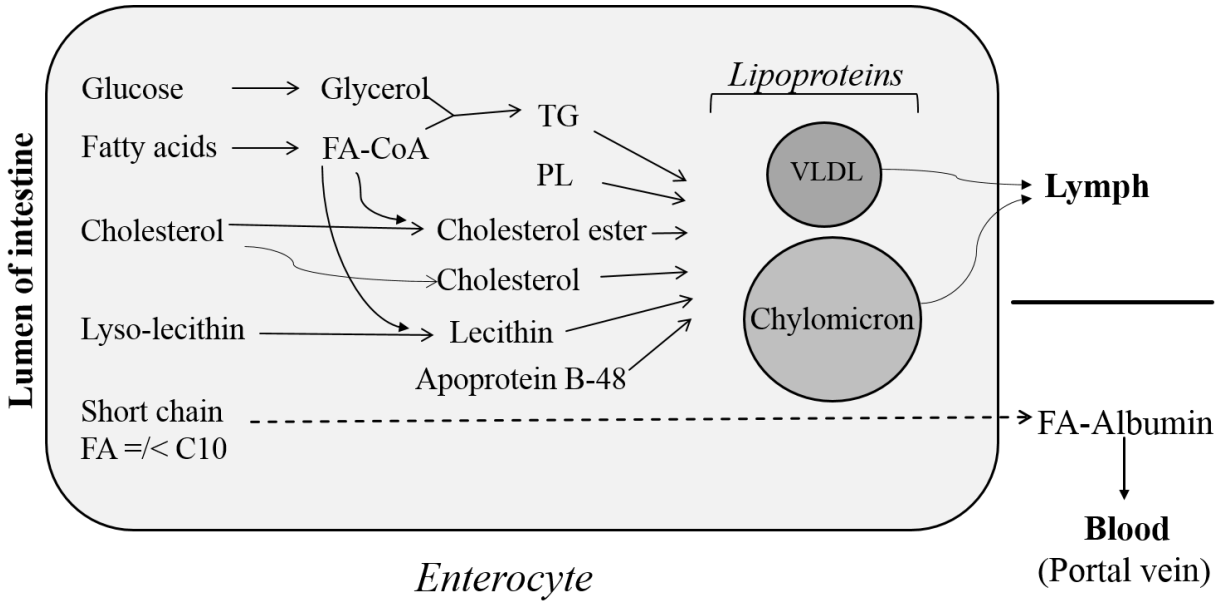
differences in the digestibility of individual FA were modest; mean digestibility values for 16:0, 18:0, 18:1, 18:2, 18:3 were 75, 72, 80, 78, and 77%, respectively (Lock *et al*., 2006).

Based on results by Palmquist (1991), true digestibility of FA may decline as their supply is increased. As discussed in the review by Bauchart (1993), the decrease in digestibility suggests that pancreatic phopholipase A_2 activity and bile lipids (phospholipids and bile salts) may become limiting for absorption of large loads (elevated flow) of long-chain FA. Differences in absorption of C16:0 and C18:0 have been suggested; Ferlay *et al*., (1993) reported that C16:0 was more digestible in the small intestine than was C18:0 (72.5 *vs*. 54.6%), however, at a similar duodenal flow (126 g/d of C16:0 vs. 169 g/d for C18:0), absorption of the two SFA was numerically similar (70% digestibility). In contrast, when feeding Ca-soaps of FA from palm oil or rapeseed at the same inclusion in the ration, Enjalbert *et al*. (1997) reported no significant differences on digestibility of C16:0 compared with total 18-carbon FA. It seems important that comparisons in digestibility of these two FA would also take duodenal flow differences into consideration, particularly because FA flow to the duodenum is usually much higher for C18:0 than for C16:0.

Upon entry to the enterocytes, the absorbed FA with chain length higher than 10 carbons are converted to their coenzyme A derivatives by acyl-CoA synthetase, and re-esterified into triglycerides (TG) by the α-glycerolphosphate pathway (Bach and Babayan, 1982). Importantly, no 2-monoglycerides are absorbed in functioning ruminants, and consequently the monoglyceride pathway is not active. In addition, it has been previously shown in sheep that 7 to 9% of the SA that enter the enterocyte is desaturated to oleic acid (OL, *cis*-9 C18:1) in the intestinal mucosa (Bickerstaffe *et al*., 1969). Following activation and re-esterification, TG, in conjunction with other lipid components (*i.e.* phospholipids, cholesterol and cholesterol esters), are packaged along with apoproteins synthesized in the enterocyte, into chylomicrons and VLDL (predominant in

ruminants; Bauchart, 1993), and leave the cell by pinocytosis, being discharged into lymph and then to the venous system by way of the intestinal and thoracic lymph ducts (Moore and Christie, 1984). On the other hand, if present, shorter chain FA (< / = 10 carbons), which are not easily esterified, nor incorporated into lipoproteins, leave the enterocyte mostly unmodified to enter the venous portal system bound to albumin (Bach and Babayan, 1982).

Figure 2-3. Fat Metabolism of lipids in the enterocytes of ruminants.



Abbreviations: Fatty acids (FA); Tryglycerides (TG); Phospholipids (PL); fatty acid CoA (FA CoA), Very low density lipoprotein (VLDL).

Tissue Utilization of Circulating TG

Following intestinal absorption, TG in chylomicrons and VLDL are rapidly hydrolyzed by lipoprotein lipase (LPL) that is secreted by parenchymal cells in peripheral tissues (Christie *et al*., 1986). This hydrolysis of TG leads to high amounts of free FA being available to extra-hepatic

tissues, such as the mammary gland, for milk fat synthesis. LPL is associated with vascular endothelial surfaces, bound by heparin sulfate chains, and its release responds rapidly to the secretion to the presence of TG-rich lipoproteins. Products of the LPL reaction are free FA (*i.e*. NEFA) and monoglycerides (likely 2-monoglycerol; Palmquist, 2006), which are in turn hydrolyzed by non-specific lipases associated with peripheral tissues (Drackley, 2005), to finally yield more free FA and glycerol. As a result of LPL activity on circulating TG, NEFA concentration is locally increased, which in turn increases the likelihood for NEFA uptake by the cells, however, not all NEFA are taken up by the tissue.

The ruminant liver does not actively secrete VLDL to supply TG to peripheral organs, nor does it exhibit significant LPL activity (Cordle *et al*., 1983); this could be related to the allocation of energy sources into different tissues. Compared with non-ruminants, LPL activity in ruminants does not change in order to compensate for the decreased energy supply under energy restriction situations. This mechanism, in non-ruminants, helps maintaining TG concentrations (from liver VLDL) to supply energy to organs such as heart and muscle. In ruminants, during feed restriction, the energy needs of these organs are met via NEFA (from adipose tissue) and ketones, and less from TG (Drackley, 2005). However, LPL activity can be modified in a tissue-specific manner, as part of physiological regulatory mechanisms. This is illustrated by the work of Shirley *et al*. (1973), who reported mammary LPL activity to increase markedly immediately prior to parturition, while it was simultaneously decreased in adipose tissue. This homeorhetic mechanism of regulation would prevent adipose tissue uptake of FA, while favoring the flow of FA into the mammary gland to support milk synthesis.

Another implication of the low secretion of VLDL from the liver is that the TG supply to the mammary gland during lactation would mostly come from lipoproteins synthesized in the

enterocytes of the small intestine. This is in agreement with Palmquist and Conrad (1971), who performed a curve analysis of labelled milk fat secretion and reanalysis of previously reported data, and concluded that 88% of long-chain FA in milk were derived directly from TG of intestinal lipoproteins, the remainder (12%) being derived from TG of endogenous origin.

The mechanisms for the transfer of FA from the circulation into mammary cells are not well documented. Palmquist (2006) discussed the different possible mechanisms for FA transport into mammary cells proposed in the literature. Proteins such as the FA translocator (FAT, CD 36) at the mammary epithelial cell, and the intracellular FA binding protein (FABP) have been suggested to play a role in transport. Similarly, a "flip flop" model, implying carrier mediated transport, has been proposed; however, this mechanism may be too slow to account for FA uptake (Palmquist, 2006). An alternative model is that described for the diffusion of amphipatic FA through the amphipatic microvillus of the small intestine (Thompson *et al*., 1983). Under this model, diffusion would be too slow to allow effective uptake of albumin-bound NEFA, however it could account for the uptake from TG-rich lipoproteins associated with the capillary epithelium. In addition, longer-chain and more saturated FA would diffuse through the membrane more rapidly because they are more hydrophobic, consistent with the order of uptake of FA in the bovine mammary gland described by Thompson and Christie (1991). Palmquist (2006) also suggested that because the concentration of Coenzyme A (CoASH) is very low and well below saturation in the cytosol, it could well be that the limiting steps in the rate of FA uptake are those that determine the rate of fatty acyl-CoA incorporation into TG which, in turn, frees up CoASH for acyl CoA synthetase, thereby freeing up a site for binding of a new FA to otherwise saturated FABP and subsequent removal of another FA from plasma; e.g., if there are no intracellular binding sites available for FA from TG hydrolysis, they are lost from the mammary glands as NEFA. This

hypothesis is in agreement with the increased concentration of plasma NEFA commonly observed when lipids are supplemented to the diet of lactating dairy cows (Choi and Palmquist, 1996; Piantoni *et al.*, 2013) and suggests that a limitation in the rate of FA uptake by the mammary gland, after TG hydrolysis by LPL, can result in elevated circulating plasma NEFA.

Milk Fat Synthesis

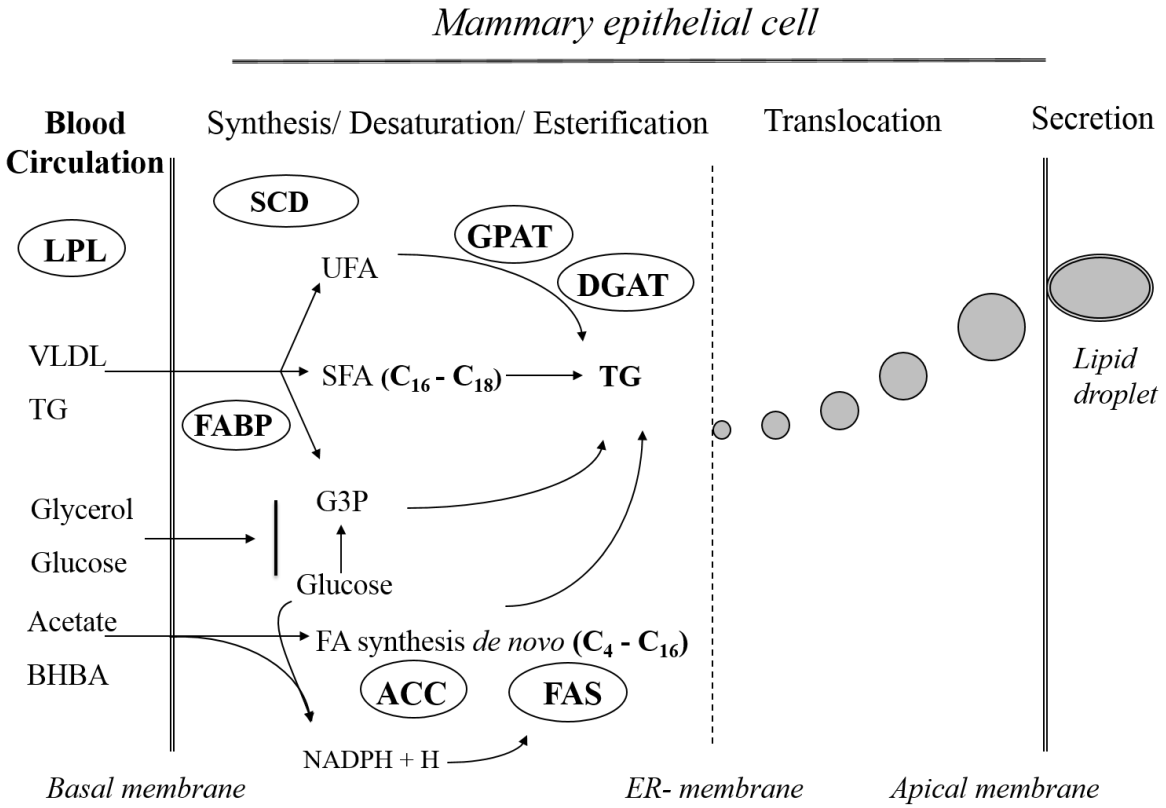
Fat is an important component of milk, and the extent of its synthesis is important since it represents about half of the energy content of milk (Emery, 1973). Milk fat is the most variable component of milk and it varies by many fold across species, ranging from ~1.9% in the horse to ~53% in the harp seal (Dils, 1986). In addition, the FA profile of milk fat is also highly variable between species (Jensen *et al*., 1991). Bovine milk fat concentration typically ranges from 3.7% to 4.1% and it is predominantly composed of triglycerides (98%), with the remainder being phospholipids, diglycerides, and cholesterol (Jensen, 2002). Arguably, bovine milk fat is the most complex of all natural fats, as its FA composition is remarkably diverse, containing more than 400 different FA, differing primarily in chain length, degree of unsaturation, and configuration of double bonds (Jensen, 2002).

In addition to the long-chain FA derived from plasma, the other major nutrients utilized for milk fat synthesis include glucose, acetate and β-hydroxybutyrate (BHBA). Glucose is absolutely required for milk synthesis, being a precursor for lactose or other carbohydrates in mammals (Oftedal and Iverson, 1995) and is taken up *via* the facilitative transport systems GLUT-1, and probably, the Na+ -dependent glucose transporter (Palmquist, 2006). Uptake of glucose, BHBA, and TG can be described by Michaelis-Menten kinetics, whereas acetate uptake is strongly linear

(Baldwin *et al*, 1980). However, it must be considered that factors other than arterial concentration of these metabolites can govern their concentration and utilization (Baldwin, 1985). This is demonstrated by the results of Cant *et al*. (1993), who reported increased mammary BHBA uptake, as FA uptake from plasma TG, as well as glucose uptake increased, as a result of feeding a high fat diet, that included yellow grease.

As discussed previously, the mammary gland is supplied with long-chain FA from blood, with more than 95% of 18-carbon and longer-chain FA coming from TG-rich lipoproteins. Although NEFA are also taken up, their contribution to milk fat is typically low (Grummer, 1991). However, it can supply significant amounts of FA in the early weeks of lactation, when mobilization of body fat reserves is high and insulin is low (Corl *et al*., 2006). Blood-derived long-chain FA are used almost exclusively for incorporation into milk fat, as other processes like oxidative degradation of FA are negligible in the mammary gland (Annison *et al*., 1967). The remainder of the FA found in milk arise from *de novo* synthesis in mammary epithelial cells (Emery, 1973) and they account for most of the 4 to 14-carbon and about half of the 16-carbon FA found in milk fat. The main processes involved in milk fat synthesis and secretion are shown in Figure 2-4.

Figure 2-4. Main processes related to lipid metabolism in mammary epithelial cells.



FA uptake from blood circulation, desaturation of long-chain FA, de novo synthesis of FA and milk fat globule secretion are shown. Abbreviations: Lipoprotein lipase (LPL); Stearoyl CoA desaturase (SCD); Fatty acid binding protein (FABP); Acetyl CoA carboxylase (ACC); Fatty acid synthase (FAS); acyl CoA:glycerol-sn-3-phosphate acyl transferase (GPAT), diacylglycerol acyltransferase (DGAT); tryglicerides (TG).

De Novo Synthesis of FA in Mammary Gland

De novo mammary synthesis of FA in ruminants is carried out by the catalytic action of acetyl CoA carboxylase (ACC) and fatty acid synthase (FAS; Emery, 1973) through a series of cyclic steps that ends when the nascent FA reaches 12 to 16 carbons in length, and a chain-specific thioesterase (Thioesterase I) releases the FA to terminate the cycle (Palmquist, 2006). The coordinated actions of acyl transferase and β-ketoacylsynthase (two catalytic subunits of FAS) are

important during the synthesis *de novo* of FA. Because the acyl transferase releases intermediates during this process, some of the intermediates can escape transfer to the β-ketoacylsynthase subunit, which results in short-chain and medium-chain FA being incorporated into milk fat (Palmquist, 2006). Hansen and Knudsen (1980) postulated that the more relaxed specificity of the acyl transferase in ruminants, relative to non-ruminants, was responsible for the release of significant amounts of short and medium-chain FA.

ACC and FAS, as well as other key lipogenic enzymes are regulated by transcription factors such as the sterol regulatory binding-element protein (SREBP) family (Eberle *et al*., 2004). SREBP 1, in particular, is important in the regulation of milk fat synthesis, as it has been established from work in the cow and mouse (Harvatine and Bauman, 2006; Rudolph *et al.*, 2010).

Requirements for FA synthesis are a carbon source and reducing equivalents in the form of NADPH +H. In ruminants, acetate (later converted to acetyl-CoA) and BHBA (later converted to butyryl-CoA) are the primary carbon sources utilized, while glucose and acetate are the primary sources of reducing equivalents (Bauman and Davis, 1974). Both BHBA and acetate are used by FAS as a "primer" for the synthesis of FA, however, BHBA is used more efficiently than acetate by FAS in the mammary gland of lactating cows (Nandedkar *et al*., 1969; Lin and Kumar, 1972) and it is incorporated as the methyl terminal 4-carbon moiety of up to 50 to 60% of the FA synthesized *de novo* (Palmquist *et al*., 1969). Bauman *et al*., (1970) demonstrated that acetate is an important source of NAPH +H in ruminants, which is the result of elevated activity the cytosolic enzyme NADP-isocitrate dehydrogenase in ruminants, as compared to other mammals. This mechanism for the production of reducing equivalents that does not involve the oxidation of glucose and accounts for 50 to 60% of the total NADPH +H used by FAS (Mellenberger and Bauman, 1974). The production of reducing equivalents by the oxidation of glucose *via* the pentose

phosphate pathway (PPP) is an equally important source of NADPH +H in ruminants and it also provides glycerol-3-phosphate (G3P) for FA esterification as an alternative to the glycolytic pathway (10 to 40% of glucose is oxidized through PPP in ruminant mammary gland; Palmquist, 2006).

Modifications to FA and Incorporation into Milk TG

Desaturation of FA in the mammary gland is another important modification to FA taken up from blood and it explains the presence of most of the monounsaturated FA (MUFA) found in milk fat (Kinsella, 1970). The enzyme responsible for this process is Stearoyl-CoA desaturase (SCD), which has a high activity in mammary and adipose tissue, and somewhat lower in intestinal tissue (Palmquist 2006). SCD is located in the endoplasmic reticulum and its primary substrates are the activated FA stearoyl-CoA and pamitoyl CoA, whereas considerably lower activity is observed with myristoyl-CoA as substrate (Bickerstaffe and Annison, 1970).

The last part of the milk fat synthetic process in the mammary epithelial cell is TG formation, which involves the esterification of FA to a G3P moiety. G3P is mostly generated by glycolysis (Bickerstaffe and Annison, 1971) or by phosphorylation of free glycerol by glycerol kinase (Palmquist, 2006). Before esterification can occur, FA must be activated to their coenzyme A derivatives by the action of acyl-CoA synthetase. The first committed step in TG synthesis is the acylation of G3P by acyl CoA:glycerol-*sn*-3-phosphate acyl transferase (GPAT) whose regulatory role was suggested by Coleman *et al*. (2004).

Cooper and Grigor (1980) reported that acylation at *sn*-1 in rat mammary TG favors OL, whereas C16:0 predominates at *sn*-2. This pattern is true in most species, although in the cow,

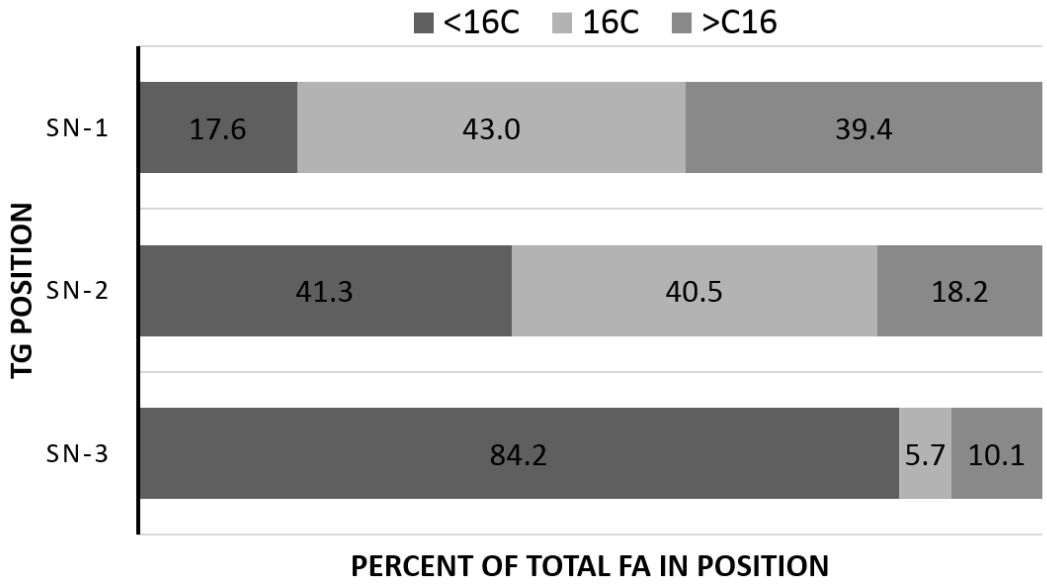
C16:0 is found nearly equally at the *sn*-1 and *sn*-2 positions (Breckenridge and Kuksis, 1968). A more detailed discussion on the esterification specificity of individual FA is presented in Chapter 3.

Diacylglycerol acyltransferase (DGAT; *sn*-1,2-diacylglycerol transacylase) esterifies both long-chain and short-chain FA at the *sn*-3 position. Regulation of total activity has been reported in liver and adipose tissues, but little information is available for the mammary gland (Palmquist, 2006). Because it is up-regulated with increasing abundance of FA, most likely it is in a highly active state in lactating mammary tissue. Mice that lack both copies of the gene for DGAT 1 are unable to secrete milk (Smith *et al*., 2000).

The *sn*-3 position is characterized by high percentage of short-chain FA, whereas the opposite is observed with the *sn*-1 position, which is esterified with a large majority of long-chain FA (16-carbon and 18-carbon). The non-randomness of the distribution of FA in bovine milk TG was first reported by Kumar *et al*. (1960) and McCarthy *et al*. (1960), who used pancreatic hydrolysis to distinguish between FA at *sn*-1- and 3-positions and those at 2-position of the glycerol molecule. Breckenridge and Kuksis (1968) reported a complete characterization of the distribution of FA in milk TG (Figure 2-5). The specificity of FA esterification, that explains their distribution in TG, is likely related to a mechanism regulating milk fat synthesis. In a recent meta-analysis, Glasser *et al*. 2008, pointed out the interdependence between short/medium-chain FA and long-chain FA, and suggested that milk fat synthesis is dependent upon the simultaneous supply of these FA for the esterification step of milk fat synthesis. I a series of studies, Knudsen *et al*. (Hansen and Knudsen, 1980; Marshall and Knudsen, 1980; Grunnet and Knudsen, 1981; Hansen *et al*., 1984a, b) examined TG synthesis and the specific incorporation of short-chain and medium-chain FA into TG in goat mammary glands. The synthesis of medium-chain FA is

dependent on simultaneous removal of the acyl-CoA produced by FAS, whereas long-chain FA are released as free FA by thioesterase I (Palmquist 2006). As described above, long-chain FA are esterified preferentially at positions *sn*-1 and *sn*-2 (Figure 2-5); the ready supply of diacylglycerols allows short-chain and medium-chain FA to be esterified rapidly at *sn*-3, facilitating their removal from FAS. These studies have shown the importance of the rate of activation of FA in the mammary gland relative to the rate of *de novo* synthesis and the supply of G3P for milk fat synthesis (Palmquist, 2006). If the supply of exogenous FA were low, the relative concentration of short-chain and medium-chain FA could be increased, even though total synthesis (yield) was not increased. Conversely, with an increasing supply of exogenous long-chain FA, *de novo* synthesis may be reduced because they compete for DGAT. Limiting the supply of G3P similarly would limit diacylglycerol supply, also causing *de novo* synthesis to be reduced. These observations demonstrate also that regulation of the relative proportions of short-chain, medium-chain, and long-chain FA is much more complex than simply by regulation of ACC (Palmquist, 2006).

Figure 2-5. Distribution of FA groups in TG by source.



De novo fatty acids originate from mammary de novo synthesis (< 16C), preformed fatty acids originate from extraction from plasma (> 16C), and mixed fatty acids originate from both sources (16C). Adapted from data by Breckenridge and Kuksis, 1968.

Effects of Fat Supplementation

In the following section the general effects of lipid supplementation in lactating dairy cows will be discussed briefly. The reviews by Palmquist and Jenkins (1980), Grummer (1991), and Coppock and Wilks (1991), and the recent meta-analysis by Rabiee *et al*. (2012) are recommended for further information.

The addition of supplemental fat sources to diets is a common practice in dairy nutrition, which has the advantage of providing more energy per unit of DM than other nutrients, while simultaneously supplying essential liposoluble vitamins and FA. Supplemental fat is typically added to lactating dairy rations in order to increase dietary energy density and to support milk

production. However, there is increasing interest in the potential to provide specific FA to the diet for non-caloric purposes.

Although in general fat supplementation has been shown to increase milk yield, milk fat yield, and the efficiency of milk production, negative effects have also often been observed such as reduced DMI and reduced concentration of milk fat and milk protein (Allen, 2000, Rabiee *et al*., 2012). The extent of these simultaneous changes will ultimately determine the overall effect of the supplemental fat, and the associated decision regarding their inclusion in diets for lactating dairy cows. An important consideration is the fact that not all fat supplements induce the same responses. Great variation has been reported in production performance for different fat types, and indeed the same supplement across different diets and studies. This is demonstrated by the meta-analysis by Rabiee *et al*., (2012), which reported the range in responses for different fat supplements being as much as 5 standard deviations from the mean, with the direction of responses (positive or negative) differing between fats.

Effects on DMI

The meta-analysis by Allen (2000), discusses the effects of fats and fat supplementation on DMI and it is recommended for an extended reading. Overall, fat supplementation reduces DMI, possibly through effects on fiber digestion in the reticulo-rumen, as well as by triggering signals that affect gut motility and induce satiety (Allen, 2000).

Some factors have been proposed to determine the effects of fat addition to diet on DMI, including type and form of fat (*e.g*. free oil, oilseeds, unprocessed animal fats, Ca-salts of FA, prilled fats, free FA or TG), acceptability, chain length and FA profile. Allen (2000) reported

decreased DMI in 11 out of 24 comparisons when calcium salts of palm FA were supplemented to lactating dairy cows, no effect of adding hydrogenated fats, and decreased DMI with the addition of unprocessed animal fats. Calcium salts of palm FA had the strongest effect on DMI, as the relative depression per unit of fat added was approximately twice of that observed for the supplementation of unprocessed animal fats.

There is high variation in the ratio of 16-carbon to 18-carbon FA of diets fed to lactating dairy cows. Although calcium salts of palm FA have a high content of C16:0 relative to other FA sources, its greater hypophagic effects are probably not related to higher C16:0 content, because there is no evidence that 16-carbon FA are more hypophagic than 18-carbon FA (Allen, 2000), and thus no effect of chain length on DMI is to be expected from currently available studies. In addition, a regression analysis of pooled data reported in the literature did not detect a significant effect of 16-carbon to 18-carbon FA ratio on DMI of lactating cows (Firkins and Eastridge, 1994).

Another important factor determining DMI response to fat supplementation is the FA profile of the fat supplement. UFA decrease DMI (Allen, 2000), which occurs with simultaneous increases in the gut peptides cholecystokinin-8 (CCK), as well as glucagon-like peptide amide 1 (GLP 1; Christensen *et al*., 1994). This hypophagic effect becomes more pronounced as the degree of unsaturation increases, and it has been implicated in a decreased milk yield response to UFA supplementation (Christensen *et al*., 1994). As discussed previously, UFA are toxic to rumen bacteria, particularly in species responsible for cellulolytic degradation (Maia *et al*., 2007), potentially affecting rumen digestion of nutrients and milk production. In addition, rumen metabolism of UFA can result in some situations to increased production of specific BH intermediates such as *trans*-10, *cis*-12 CLA, which reduces mammary milk fat synthesis, and is involved in milk fat depression (MFD; Bauman *et al.*, 2011). SFA, as found in greater

concentrations in tallow or commercially available SFA supplements, may be particularly useful as a supplemental fat source because these FA have minimal effects on rumen microbial activity (Palmquist and Jenkins, 1980).

Milk Production and Composition

Fat supplementation usually results in increased milk yield. In a recent meta-analysis, using 68 comparisons from literature, Rabiee *et al.* (2012) reported an average increase in milk yield of 1.05 kg of milk/cow/d ($P = 0.006$) by fat supplementation. Overall, fat supplementation results in no change in milk fat concentration or milk protein yield, it slightly decreases milk protein concentration (-0.077%, $P < 0.001$) and tends to increase milk fat yield (0.027 kg/d, $P = 0.064$). An important consideration however, is that milk yield and milk components responses to supplemental fat across studies were highly heterogeneous, and this variation was associated with the type of fat supplement used, being high for tallow, Megalac and oilseeds, and low for comparisons that included prilled fats.

Data from other studies suggests that degree of saturation may be an important explanatory factor if the variation in milk yield and milk components. Although the effects of degree of saturation of the fat supplement were not specifically discussed in the meta-analysis by Rabiee *et al*. (2012), it might help explain the reported differences between the types of fats included in their analysis. As mentioned previously, DMI is decreased as unsaturation of the fat supplement is increased. Similarly, the concentration and yield of milk components can be affected by the degree of saturation. Harvatine and Allen (2006) reported that milk fat concentration and yield were linearly decreased as the degree of unsaturation of a fat supplement was increased. This effect

seems to be of greater importance in high producing cows (Rico and Harvatine, 2011), probably because of the higher passage rates of digesta out of the rumen in high producing cows, relative to lower producing cows, which can result in the accumulation of specific BH intermediates associated with reduced milk fat synthesis in the mammary gland (Bauman *et al.*, 2011).

Moreover, positive effects of supplementing SFA have been reported in the literature, such as increased milk yield, milk fat yield (Christensen *et al*., 1994; Relling and Reynolds, 2007), and milk protein yield (Harvatine and Allen, 2006; Wang *et al*., 2007). For example, as shown in Table 2-1, feeding a mixture of SFA at 3.5% of ration DM resulted in an increase in milk fat percentage and yield compared to polyunsaturated (Ca-salts of soybean FA) and monounsaturated (Ca-salts of palm FA distillate) fat treatments. Furthermore, the SFA treatment increased milk fat compared to the non-fat supplemented control treatment (Relling and Reynolds, 2007). Although the overall effects of feeding SFA to lactating dairy cows has been extensively reported, the role of individual specific FA has received only limited attention, particularly in regards to the effects of the long-chain SFA C16:0 and C18:0.

Table 2-1. Production responses of lactating dairy cows to supplementation of fat supplements with varying degree of saturation.

	Diet					*P* - value
Item	Control	SFA[1]	MUFA[2]	PUFA[3]	SEM	SFA *vs*. UFA
DMI, kg/d	23.8	23.1	22.1	22	1.1	0.12
Milk, kg/d	36.9	37.3	35.8	34.8	1.4	0.11
Fat, %	3.37	3.86	3.32	2.61	0.25	0.03
Fat, g/d	1,249	1,436	1,184	911	0.1	0.02

[1]SFA = Saturated free fatty acid supplement; [2]MUFA = Monounsaturated FA from Ca-salts of palm fatty acid distillate; [3]PUFA = Polyunsaturated FA from Ca-salts of soybean oil fatty acids.

Adapted from Relling and Reynolds, 2007.

Conclusion

Dietary lipids undergo extensive modifications once the cow ingests them. The many processes that take place in the rumen and the subsequent adaptations of the animal to maximize FA digestion, as well as the homeorhetic mechanisms that regulate the use of metabolic substrates according to physiologic needs, is proof of the remarkable adaptations of ruminants to specific dietary conditions, that allow them to utilize feeds not usable by other animal species. The potential to maximize animal responses during lactation by the use of saturated fat justifies the interest in understanding the effects of specific SFA, as they can impact overall animal performance and milk composition, and are thus closely related to farm profitability. The objectives of this thesis therefore were to investigate the effect of supplementing the effects of the long-chain SFA C16:0 and C18:0 to the diet of lactating dairy cows, on digestion, metabolism, and cow performance.

Chapter 3

EFFECT OF PALMITIC AND STEARIC ACID ON MILK YIELD, MILK COMPONENTS, AND FEED EFFICIENCY ACROSS PRODUCTION LEVEL OF COWS

Abstract

The impacts of dietary palmitic and stearic acids on feed intake, yield of milk and milk components, and feed efficiency of dairy cows with a wide range of milk production were evaluated in an experiment with a crossover arrangement of treatments with a covariate period. A wide range of milk production (38 to 65 kg/d) was used to determine if response to fat supplementation varied according to production level. Thirty-two Holstein cows (143 ± 61 DIM) were assigned randomly to treatment sequence within level of milk yield. Treatments were diets supplemented (2% of diet DM) with palmitic acid (PA; 99% C16:0) or stearic acid (SA; 98% C18:0). Treatment periods were 21 d with the final 4 d used for sample and data collection. The corn silage and alfalfa haylage based diets were formulated to contain 29% NDF, 17% CP, and 5.7% crude fat. The statistical model included the random effect of cow and the fixed effect of treatment and period. No interactions were detected between treatment and level of milk production when all cows received a common diet during the covariate period for any response variable. Compared with SA, the PA treatment increased milk fat concentration (3.55 vs. 3.66%, $P < 0.01$) and yield (1.59 vs. 1.68 kg/d), and 3.5% fat-corrected milk yield (45.6 vs. 47.5 kg/d). Treatment did not affect DMI, milk yield, milk protein yield, body weight, or body condition score. Milk protein concentration was lower for PA compared with SA treatment (3.24 vs. 3.29%). The PA treatment increased feed efficiency (3.5% fat-corrected milk yield/DMI) compared with SA (1.48 vs. 1.40). Results demonstrate that palmitic acid is more effective than stearic acid in

improving milk fat concentration and yield as well as efficiency of feed conversion to milk. Responses were independent of production level and without change in body condition score or body weight. Further studies are required to test the consistency of these responses across different types of diets.

Introduction

The effect of supplementing fats in lactating dairy cow rations has been widely studied. Fat supplements are often used because they increase dietary energy density of the diet and can improve energy balance, feed efficiency, and the yield of milk and milk fat (Rabiee *et al*., 2012). However, the effects of individual fatty acids (FA) have not yet been adequately studied to successfully allow for the identification of specific FA that maximize the yield of milk and milk components. The concentration and yield of milk fat can be increased by feeding SFA supplements to dairy cows, compared with both control (non-added fat diets) and unsaturated FA (UFA) supplements (Relling and Reynolds, 2007). Even though the effects of SFA supplements have been reported in numerous studies, it is still unclear what effects individual SFA have, with only a limited number of studies available that used pure sources of individual SFA such as palmitic (C16:0) and stearic (C18:0) acids (*e.g*. Steele and Moore, 1968; Enjalbert *et al*., 1998).

Recent research has focused on studying the effects of C16:0, using C16:0-enriched fat supplements. This FA has been reported to increase milk yield, milk fat concentration and yield, as well as the efficiency of milk production (Mosley *et al*., 2007; Lock *et al*., 2013; Piantoni *et al*., 2013). Information on the effects of feeding stearic acid (C18:0) particularly when compared to C16:0 is much more limited, with only a few studies available. Steele and co-workers performed a series of studies in the 1960´s feeding relatively pure sources of SFA to low producing dairy cows and reported that both C16:0 and C18:0 increased milk fat concentration and yield relative to control. The responses to C16:0 feeding, however, were higher and more consistent across studies, compared with C18:0 (Steele and Moore, 1968; Steele, 1969). These results are supported by more recent studies which used duodenal infusions C16:0 and C18:0 that reported higher milk fat concentration and yield in cows infused with these FA, relative to control (Enjalbert *et al*.,

1998). Moreover, C16:0 had a higher uptake efficiency by the mammary gland when compared with C18:0 in that study. In our recent work, the increased milk fat concentration and yield in response to C16:0 supplementation was explained entirely by the increase in 16-carbon FA secreted into milk (Lock *et al*., 2013 and Piantoni *et al*., 2013). The aforementioned results suggest that C16:0 feeding will increase milk fat content and yield as compared with C18:0 due to a greater incorporation of C16:0 into milk fat.

There are a limited number of studies that have evaluated the effect of milk production of cows on their response to fat feeding. Harvatine and Allen (2005) reported a greater increase in milk protein yield in high producing cows relative to lower producing cows, in response to a saturated fat supplement compared with an unsaturated fat supplement. In a field study, Warntjes *et al*. (2008) showed that cows with a lower milk yield had a higher milk production increase in response to 16:0-enriched fat supplementation relative to cows with higher milk production. However, Piantoni *et al*. (2013) reported that production responses to a pure C16:0 fat supplement compared with soyhulls did not differ across production level of cows.

To our knowledge, no previous studies have directly compared the effects of C16:0 and C18:0 across a wide range of milk production levels in dairy cows. Our objective was to compare the effects of C16:0 and C18:0 supplementation on feed intake and production responses of dairy cows with a wide range of milk production. We hypothesized that C16:0 feeding would increase milk fat content and yield as compared with C18:0 due to a greater incorporation of C16:0 into milk fat.

Materials and Methods

Design and Treatments

Experimental procedures were approved by the Institutional Animal Care and Use Committee at Michigan State University. Thirty-two multi and primiparous Holstein cows averaging 143 ± 61 DIM (mean ± SD) and with a milk production ranging from 38 to 65 kg/d from the Michigan State University Dairy Field Laboratory were randomly assigned to treatments within level of milk production. At the beginning of the experiment, BW of cows was 657 ± 45 kg and milk yield was 33.1 ± 3.3 kg/d (mean ± SD).

The experiment used a crossover arrangement of treatments in two consecutive 21 d periods, preceded by a 14 d covariate period. Animals received a common diet with no fat supplementation during the covariate period to obtain baseline values, and were then randomly allocated to either a C16:0-enriched fat supplemented (**PA** treatment) or C18:0-enriched fat supplemented (**SA** treatment) diet for period one and switched to the other diet during period two. The C16:0 supplement (Emery Oleochemicals, Selangor, Malaysia) and C18:0 supplement (Acidchem International Sdn Bhd, Penang, Malaysia) were both free FA products of high purity and contained more than 97% of either C16:0 or C18:0, respectively, and 99 % total SFA.

Diets were based on corn silage as the major forage component and corn grain as the major concentrate component of the diet (Table 1). The C16:0-enriched fat supplement and the C18:0-enriched fat supplement were included in the diet at 2% DM (Table 1). Diets were formulated to contain 30% NDF and 16% CP, and mineral and vitamins were formulated according to NRC (2001) recommendations. DM concentration was determined twice weekly for forages, and diets were adjusted when necessary.

Data and Sample Collection

Throughout the experiment cows were housed in individual tie stalls. Access to feed was blocked from 0800 to 1000 h to allow for orts and feed offered to be weighed daily. Cows were fed 115% of expected intake at 1000 h daily and feed intake was recorded and adjusted daily. Cows were milked twice daily at 0430 and 1730, with milk yield recorded electronically at each milking throughout the experiment. Water was available *ad libitum* in each stall and stalls were bedded with sawdust and cleaned twice per day.

Response variables were averaged for d 11 to 14 of the covariate period and for d 18 to 21 of each of the treatment periods. Diet ingredients and orts were sampled during d 18 to 21 and composited for each period. Milk was sampled at each milking from d 11 to 14 for the covariate and period from d 18 to 21 of the treatment periods. Two samples were taken from each cow at each milking. One aliquot was collected in a sealed tube with preservative (Bronopol Tablet; D&F Control Systems, San Ramon, CA) and stored at 4^{o} C for milk component analysis. The second aliquot was stored without preservative at -20^{o} C until analyzed for FA composition. Blood samples were taken every 15 hours during last 4 d of each period to obtain a 24 h composite sample, representing intervals of 3 hours between samples. Blood was collected by coccygeal venipuncture into three evacuated tubes; two contained potassium EDTA as an anticoagulant and the other contained potassium oxalate as an anticoagulant and sodium fluoride as a glycolytic inhibitor. Blood was stored on ice until centrifugation at 2,000 x g for 15 min at 4˚C (within 30 min of sample collection). Body weights were measured on the last 2 d of the covariate period as well as on d 20 and 21 of each of the treatment periods. BCS was determined by three trained investigators on a 5-point scale (Wildman *et al*., 1982) on the same days as body weights were measured.

Sample Analysis

Diet ingredients and orts were dried at 55° C in a forced-air oven for 72 h for DM determination. Dried samples were ground with a Wiley mill (1-2 mm screen; Arthur H. Thomas, Philadelphia, PA). Diet ingredients were analyzed for NDF, CP, and starch according to the methods described by Harvatine and Allen (2005). FA concentrations of feed ingredients were determined using a modification of the one step transesterification method developed by Sukhija and Palmquist (1988) as described previously by Lock *et al*. (2013).

Individual milk samples were analyzed for fat, true protein, and lactose concentration by mid-infrared spectroscopy (AOAC, 1990, method 972.160) by the Michigan Herd Improvement Association (Universal Lab Services, Lansing MI). Yields of 3.5% FCM, ECM, and milk components were calculated using milk yield and component concentrations for each milking, summed for a daily total, and averaged for each collection period (Reist et al., 2002).

Milk samples used for analysis of FA composition were composited based on milk fat yield (d 18 to 21). Lipids were extracted, methylated, and FA composition determined by GLC, according to the methods described by Lock *et al*. (2013). Short chain FAME were corrected for mass discrepancy using the response factors published by Ulberth and Schrammmel (1995). Yields of individual FA (g/d) were calculated using milk fat yield and FA concentration to determine yield on a mass basis using the molecular weight of each FA while correcting for glycerol and other milk lipid classes (Shauff *et al*., 1992; Glasser *et al*., 2007).

Plasma samples were analyzed in duplicate using commercial kits to determine the plasma concentrations of glucose (glucose oxidase method, PGO Enzyme product No. P7119, Sigma Chemical Co., St. Louis, MO; inter-assay CV: 1.1%, NEFA (NEFA-HR kit, Wako Chemicals USA, Richmond, VA; inter-assay CV 2.7%), BHBA (procedure #2440, Stanbio Laboratory,

Boerne, TX; inter-assay 3.1%), and insulin (Coat-A-Count RIA kit, Siemens Healthcare Diagnostics, inter-assay CV: 9.0%, Los Angeles, CA).

Statistical Analysis

All data were analyzed using the fit model procedure of JMP (Version 9.0.2, SAS Institute, Cary, NC) according to the following model:

$$Y_{ijk} = \mu + C_i + P_j + T_k + P_j \times T_k + pMY + pMY \times T_k + pMY^2 + pMY^2 \times T_k + e_{ijk}$$

where Y_{ijk} = dependent variable, μ = overall mean, C_i = random effect of cow (i = 1 to 32), P_j = fixed effect of period (j = 1 to 2), T_k = fixed effect of treatment (k = 1 to 2), pMY = preliminary milk yield used as covariate, pMY x T_k = interaction between treatment and preliminary milk yield, pMY^2 = preliminary milk yield squared, pMY^2 x T_k = interaction between treatment and preliminary milk yield squared, and e_{ijk} = residual error. Normality of the residuals was checked with normal probability and box plots and homogeneity of variances with plots of residuals versus predicted values. When necessary, data was transformed and this was noted in the tables. Main effects were declared significant at $P \leq 0.05$, and trends were declared at $P \leq 0.10$. Interactions were declared significant at $P \leq 0.10$, and trends were declared at $P \leq 0.15$. Interactions were evaluated, but removed from the statistical model when not significant ($P > 0.15$). In general, period by treatment interaction was not significant, but variables with significant interactions are noted in the tables. All data was expressed as least square means and standard error of the means, unless otherwise specified.

Results

Production Responses

There were no differences in the concentrations of CP, forage NDF or starch between PA and SA diets (Table 3-1). The concentration of C16:0 and C18:0 was 2.35 and 2.01% of total ration DM, for PA and SA, respectively (Table 3-1). Relative to SA, the PA treatment increased total daily C16:0 intake ~ 4 fold. The SA treatment increased total daily C18:0 intake ~ 16 fold. Total FA intake and intake of other FA was similar across diets (Table 2).

There was no interaction between preliminary milk yield and treatment for any of the production responses evaluated (Table 3). Thus, the remainder of the production results will concentrate on the main effects of the PA and SA treatments. Compared with SA, PA increased milk fat concentration by 3.1%, from 3.55 to 3.66% ($P < 0.01$), and milk fat yield by 5.6%, from 1.59 to 1.68 kg/d ($P < 0.0001$; Table 3). Consequently, PA increased 3.5 % FCM yield relative to SA by 1.9 kg/d (4.2%, $P < 0.01$). There were no differences between the treatments for the yields of milk, milk protein, or milk lactose (all $P > 0.11$). PA slightly decreased milk protein concentration relative to SA ($P < 0.01$) from 3.29 to 3.24%. DMI was not different between treatments ($P = 0.39$), however the increase in 3.5% FCM with the PA treatment resulted in a 5.7% improvement in feed efficiency (3.5% FCM/DMI) compared with SA ($P < 0.0001$; Table 3). BW and BCS were not affected by treatment (both $P > 0.11$).

Plasma Metabolites and Hormones

Plasma glucose concentration was slightly increased by PA, relative to SA (1.5%, P = 0.041). Similarly, relative to SA, PA increased NEFA concentration (+9.1%, P = 0.008), and the response to treatment was affected by preliminary milk yield (pMY x treatment interaction, P < 0.01). There were no effects of treatment on plasma insulin or BHBA (P > 0.5, Table 4).

Milk Fatty Acid Concentrations and Yields

Concentrations and yields of selected FA are shown in Tables 3-5 and 3-6, respectively. Relative to SA, PA increased the concentration of C16:0 and C16:1 in milk fat by 17.7 and 11.7%, respectively (both P < 0.0001) and decreased the concentrations of most other milk FA (Table 5). On the contrary, relative to PA, SA increased the concentration of C18:0 and C18:1 in milk fat by 18% and 7.1%, respectively (both P < 0.0001, Table 5).

The concentration of FA in milk fat according to source (<16 carbon FA from *de novo* synthesis in the mammary gland, >16 carbon FA originating from extraction from plasma, and 16-carbon FA originating from mixed sources) was altered by the treatments. For PA and SA the FA profile of milk fat was 28.0 and 30.5 *de novo* (<16 carbon; P < 0.0001) FA, 38.6 and 32.8 16-carbon FA (P < 0.0001), and 34.8 and 35.3 preformed (>16 carbon; P < 0.001) g/100 g FA, respectively (Table 5).

The increase in milk fat yield with PA (90 g/d) corresponded to a 24% increase in the yield of 16-carbon FA relative to SA (117 g/d increase, P < 0.0001; Table 6). There was a simultaneous decrease in the yield of *de novo* FA (<16 carbon FA; 3.2%, P = 0.04) and preformed FA (>16 carbon FA; 3.0%, P = 0.04) relative to SA. The yield of *de novo* FA in milk fat from C8:0 to C14:0

were all decreased by PA relative to SA (all $P < 0.05$), whereas C6:0 was unchanged ($P = 0.53$). The yield of C4:0, was increased by PA relative to SA ($P < 0.001$), following a different trend than all other *de novo* FA in milk fat. As the chain length of these *de novo* FA increased their reduction in yield in PA vs. SA increased linearly (Figure 1). The yield of C18:0 was increased by SA relative to PA (10.4%, $P < 0.0001$). However, this increase of 13 g/d of C18:0 in the SA treatment was much less than the 113 g/d increase in C16:0 with the PA treatment. There was no effect of treatments on the yield of *cis*-9 C18:1 ($P = 0.47$) or total *trans* C18:1 ($P = 0.75$).

Discussion

The use of purified sources of C16:0 and C18:0 in the present experiment allowed us to directly compare the effects of these FA while avoiding the potential confounding effects of other FA in the fat supplements. Therefore, the diets differed only in the inclusion of either C16:0 or C18:0. Previous research has shown that the production and metabolic responses to fat supplements can differ in cows at different levels of milk production (Harvatine and Allen, 2005; Warntjes *et al*., 2008). Preliminary milk yield was chosen as a covariate in the present experiment in order to determine any interactions between treatment and cow production level. Additionally, this information is readily available to the dairy producer and can be easily used for grouping and feeding cows. Cows in the present study responded similarly to treatment (PA or SA), as evidenced by a lack of interaction between treatment and preliminary milk yield for all production variables and most other variables evaluated, with the exception of plasma NEFA and the concentration and yield of some FA in milk. These results are similar to Piantoni *et al*. (2013), who reported no interaction between preliminary milk yield and production responses to C16:0 feeding when compared with a soyhulls control diet.

Fat supplements including mostly SFA can increase both milk fat concentration and yield (Christensen *et al*., 1994; Relling and Reynolds, 2007). In our study, with high producing cows, fat concentration and yield increased by 3.1% and 5.7%, respectively, for PA relative to SA, which was completely accounted for by an increase in 16-carbon FA secretion into milk. We have reported similar results when C16:0-enriched supplements have been fed to dairy cows at 2% of ration DM, compared with non-added fat controls (Lock *et al*., 2013, Piantoni *et al*., 2013). Importantly, the extent of the milk fat response in our experiment was very similar to that reported in those studies (100g/d and 80g/d, for Lock *et al*., 2013 and Piantoni *et al*., 2013, respectively), even though the treatment comparison used in our experiment was iso-caloric (PA *vs.* SA). Steele and Moore (1968) and Steele (1969) evaluated the effects of feeding C16:0 or C18:0 at ~ 4% of ration DM, to low producing dairy cows. Relative to control, C16:0 increased milk fat concentration and yield consistently across the two studies. Responses to C18:0, however were inconsistent as C18:0 did not increase milk fat concentration and it increased fat yield only in one of the two studies. Moreover, C18:0 increased milk fat yield to a lesser extent compared with C16:0.

Fat supplements can sometimes, but not always, reduce DMI and the response usually depends on the type of fat being fed (Allen, 2000; Relling and Reynolds, 2007). The hypophagic effects of fats seem to be more pronounced for UFA than for SFA supplements (Drackley *et al*., 1992). Previous studies that compared C16:0-enriched supplements *vs.* non-added fat controls have reported either no effect (Warntjes *et al*., 2008; Piantoni *et al*., 2013) or a decrease in DMI (Rico and Harvatine, 2011; Lock *et al*., 2013). The lack of difference between PA and SA on DMI in our study suggests that the effects of C16:0 and C18:0 on DMI are similar. Feed efficiency, however, was affected by treatment and this is explained by an increase in 3.5% FCM yield for

PA, relative to SA, with similar DMI across treatments (Table 3). This is similar to Piantoni *et al*. (2013), who also reported increased feed efficiency as a result of an increase in 3.5% FCM and no change in DMI when C16:0 was supplemented at 2% ration DM.

The observed lack of effects of treatment on milk yield is in line with the results from Steele (1969) who reported that C16:0 and C18:0 increased milk yield in a similar manner when compared with a control diet that did not include added fat. When individually compared with non-added fat control diets, both C16:0-enriched and C18:0-enriched supplements have increased (Steele, 1969; Mosley *et al.*, 2007; Piantoni *et al*., 2013) or had no effect on milk yield (Steele and Moore 1968; Warntjes *et al*, 200; Lock *et al*., 2013). These differences may suggest differential partitioning of the energy supplied through the supplemental FA, sometimes more efficiently directed towards milk fat and in some cases more available as energy for milk synthesis, or other uses in different tissues. C16:0 and C18:0 may have partially spared glucose that could be used for milk lactose synthesis in those experiments that reported positive milk yield responses, relative to controls.

Lowered plasma insulin concentrations in lactating cows fed fat supplements has been previously reported by Choi *et al*. (1996, 2000) when a calcium soap of long chain FA was included in the diet to replace starch. Carbohydrate supply in our experiment was the same across treatments, which may explain the lack of difference in insulin concentration between PA and SA. Similarly, this may also indicate that C16:0 and C18:0 have similar effects on insulin secretion. Cant *et al*. (1993) concluded that an increased mammary supply of FA from fat supplementation spared acetate for oxidation by extra-mammary and extra-hepatic tissues, thus increasing the efficiency of glucose use for lactose synthesis. In our experiment, the slight increase in plasma

glucose in PA, relative to SA, was not high enough to drive increased concentrations of either plasma insulin or milk lactose yield, consistent with the lack of difference in milk yield.

Plasma NEFA have been reported previously to increase as a result of adding saturated fat to diets (Drackley *et al*., 1992; Choi *et al*., 1996, 2000) and specifically for C16:0 supplemented cows (Piantoni *et al*., 2013). Additionally, there is evidence that mammary lipid secretion depends moderately on the availability of preformed FA, such as plasma circulating NEFA, which in turn are regulated by insulin (Rigout *et al*., 2002; Bauman and Griinari, 2003; Corl *et al*., 2006). The moderate increase (9.2%) in plasma NEFA observed in the present study may have contributed to a higher supply of preformed FA, particularly of C16:0, to the mammary gland, explaining part of the observed increase in milk fat secretion with the PA treatment, relative to SA. Moreover, the lack of differences in BW and BCS between treatments suggests that the changes in plasma NEFA are not due to effects of treatment on the mobilization of body fat reserves.

Extensive work has been done in evaluating the effects of dietary fat on milk fat composition and yield (see reviews by Grummer, 1991 and Palmquist, *et al*., 1993). It is widely recognized that lipid supplementation can decrease *de novo* FA concentration and yield (Glasser, *et al*., 2008). Our results are in agreement with the aforementioned reviews as the concentration and yield of *de novo* FA decreased with PA, relative to the SA treatment. However, the yield of C4:0 was higher in PA relative to SA (Figure 1 and Table 6), suggesting that incorporation of this FA was favored by additional C16:0 entering the mammary gland, contrary to the effects observed for other *de novo* FA. The increase in C4:0 yield in our experiment is in agreement with previous reports from studies that supplemented C16:0-enriched fats (Mosley *et al*., 2007; Lock *et al*., 2013, Piantoni *et al*., 2013). Barbano and Sherbon (1980) suggested that an increased secretion of C4:0 into milk fat might be part of a mechanism to help maintain milk fat fluidity at body temperature

as long-chain FA of high melting point are taken up from plasma when long chain FA are fed. In addition, it has been reported that C16:0 can have a strong stimulatory effect on the synthesis of C4:0, as well as on its incorporation into triglycerides in dispersed bovine mammary gland cells, and to smaller extent, an increased synthesis and incorporation of C16:0 itself into triglycerides (Hansen and Knudsen, 1987a, b). The increased yield of C4:0 and C16:0 in our study is in agreement with the proposed stimulatory effects of C16:0 on incorporation on these FA into milk TG.

The observed decrease in the yield of other *de novo* synthesized FA, however could be due to a number of reasons. Lock *et al*. (2013) discussed possible mechanisms implicated in the reduction of *de novo* FA synthesis which may include an inhibitory effect of C16:0 supplementation on activity of acetyl-CoA carboxylase and/or fatty acid synthase due to allosteric inhibition by C16:0 (Wright *et al*., 2002), or the inhibition of mammary gland acetyl-CoA carboxylase by palmitoyl-CoA derived from C16:0 (Miller *et al*., 1970). In this context, the observed increase in C4:0 yield in milk fat with PA observed in our study, might indicate reduced activity of either ACC or FAS, and thus lead to reduced *de novo* FA synthesis. In addition, Hansen and Knudsen (1987a) proposed that the most likely explanation for the inhibitory effect of certain FA on the rate of *de novo* synthesis is that they compete with newly synthesized medium-chain acyl-CoA for the *sn*-2 and *sn*-3 positions of the TAG backbone during the esterification steps of mammary lipid synthesis. Long chain acyl-CoA compete effectively with butyryl CoA for the *sn*-3 position in ruminant mammary gland (Marshall and Knudsen, 1979). Breckenridge and Kuksis (1968) reported that approximately 90% of the FA from C4:0 to C10:0 were incorporated into the *sn*-3 position and that the placement of C4:0 into this position in particular is highly selective. Additionally, the placement of some 18-carbon FA and C14:0 is also selective, with C18:0 and

C18:1 occurring preferentially in *sn*-1 position, 14:0 in *sn*-2 and 16:0 equally distributed in *sn*-1 and *sn*-2, but poorly incorporated into *sn*-3 (Breckenridge and Kuksis, 1968). In our study, an increased supply and subsequent esterification of C16:0 at *sn*-1 and *sn*-2 sites would potentially displace 14:0 and, to a lesser degree, FA from C8:0 to C12:0, effectively decreasing their secretion into milk (Figure 1). This competitive mechanism is likely to be predominant when long chain SFA are fed, as opposed to any direct inhibition of lipogenic enzymes, as that exhibited by specific FA such as *trans*-10, *cis*-12 CLA (Bauman *et al*., 2011).

The increase in both concentration and yield of preformed FA (>16-carbon) for SA, relative to PA, can in the same way be attributed to a greater availability of C18:0 for milk fat synthesis in the mammary gland with the SA treatment. However, compared with SA, PA increased the secretion of C16:0 (113 g/d) and its desaturation product, *cis*-9 C16:1, (4.5 g/d) into milk, whereas SA only modestly increased C18:0 secretion into milk (13 g/d) and did not have an effect on its desaturation product, *cis*-9 C18:1. These results would suggest that efficiency of incorporation of C16:0 into milk fat is higher than that of C18:0, which is in agreement with Enjalbert *et al*. (1998), whose results show a limited mammary uptake of C18:0, compared with C16:0. However, it is likely that other factors could have influenced the response, such as FA digestibility differences (not evaluated in this experiment) or even a faster incorporation/utilization of C18:0 into other tissues, the latter leading to differential partitioning of C16:0 and C18:0 in tissues. There are some indications that digestibility of supplemented C18:0 may be negatively affected, especially when it is fed at high doses (Steele, 1969) and its duodenal flow is increased. Ferlay *et al*. (1993) reported that digestibility of C18:0 decreased substantially from 73% to 45% when the duodenal flow of C18:0 increased from 170 to 445 g/d. However is has also been recently shown that digestibility of C16:0 can be decreased when fed at 2% of ration DM when compared with a non-added fat diet

(Piantoni *et al*., 2013). In our experiment, particle size of the C18:0-enriched fat supplement was larger than that of the C16:0-enriched supplement; this could have potentially limited surface area available for digestion, decreasing its overall digestibility. Specific studies aimed to compare FA digestibility when feeding C16:0 and C18:0 relative to control, while taking particle size differences into account, are required in order to clarify potential differences in digestion between these FA.

Conclusion

Results from this experiment demonstrate that a dietary C16:0-enriched fat supplement improves milk fat concentration and yield as well as the efficiency of feed conversion into milk relative to a C18:0-enriched fat supplement. Further studies are required that investigate the effects of other dietary factors such as fat, fiber, and starch content of the diet have on the response during both early and late lactation. The determination of optimal dose to be fed under practical conditions is also required.

Table 3-1. Ingredients and nutrient composition of PA and SA diets fed during the treatment periods

	Treatment[1]	
Ingredients, % of DM	**PA**	**SA**
Alfalfa hay	6.6	6.6
Alfalfa haylage	7.4	7.4
Corn silage	26.1	26.1
Cottonseed, whole with lint	3.6	3.6
Ground Corn	25.9	25.9
Soybean Meal	12.8	12.8
Vitamin and mineral mix[2]	8.6	8.6
Soy Hulls	7.0	7.0
Fat Supplement[3]	2.0	2.0
Nutrient Composition, % of DM		
DM, % of as fed	58.3	58.3
NDF	29.7	29.7
Forage NDF,	19.1	19.1
Forage NDF, % of NDF	64.2	64.2
CP	15.0	15.0
Starch	27.1	27.1
Total FA	4.42	4.42
C16:0, % DM	2.35	0.45
C18:0, % DM	0.12	2.01

[1] Dietary treatments PA (palmitic acid) and SA (stearic acid)

[2] Vitamin and mineral mix contained 58.3% dry ground shell corn, 12.3% limestone, 7.9% sodium bicarbonate, 6.8% di-calcium phosphate, 3.2% urea, 3.2% magnesium, 3.2% tallow, 3.0% white salt, and < 1% of each of the following: trace minerals, biotin vitamin E, vitamin A, vitamin D and selenium.

[3] PA supplement contained 97.9% C16:0 of total FA. SA supplement contained 97.4% C18:0 of total FA.

Table 3-2. Fatty acid concentration and intake of PA and SA diets fed during the treatment periods.

Fatty Acid Concentration, g/100 g[1]	**PA**[2]	**SA**[3]
C16:0	53.2	10.2
C18:0	2.6	45.6
C18:1 9c	12.2	12.2
C18:2 9c,12c (n-6)	26.2	26.2
C18:3 9c,12c,15c (n-3)	2.6	2.6
Σ Others	3.1	3.3
Σ SFA	57.0	57.1
Σ MUFA	13.9	13.9
Σ PUFA	29.1	29.0
Fatty Acid Intake, g/d[4]		
Total	1412	1421
C16:0	752	144
C18:0	37	648
C18:1 9c	173	173
C18:2 9c,12c (n-6)	370	372
C18:3 9c,12c,15c (n-3)	37	37
Σ Others	44	47
Σ SFA	805	811
Σ MUFA	197	197
Σ PUFA	411	413

[1] Fatty acid concentration is expressed as g/100 g of total fatty acids.

[2] PA supplement contained 97.9% C16:0 of total FA.

[3] SA supplement contained 97.4% C18:0 of total FA.

[4] Fatty acid intake is expressed as g/d of individual fatty acids.

Table 3-3. Dry matter intake, milk production and composition, feed efficiency, BW, and BCS for cows fed treatment diets.

	Treatment			Treatment
Item	**PA**	**SA**	**SEM**	***P*-value**
DMI, kg/d	32.2	32.9	0.71	0.188
Milk Yield, kg/d				
Milk	46.6	45.8	2.02	0.223
3.5% FCM[1]	47.5	45.6	1.64	<0.01
ECM[2]	47.7	46.1	1.63	<0.01
Feed Efficiency[3]	1.48	1.40	0.05	<0.0001
Milk Composition				
Fat,%	3.66	3.55	0.09	0.013
Protein,%	3.24	3.29	0.05	<0.01
Lactose, %	4.75	4.76	0.04	0.71
Milk solids yield, kg/d				
Fat	1.68	1.59	0.05	<0.0001
Protein	1.50	1.49	0.05	0.703
Lactose	2.22	2.18	0.1	0.242
BW, kg	719.8	722.7	13.62	0.121
BCS	2.93	2.99	0.11	0.106

[1] 3.5% Fat-corrected milk.

[2] Energy-corrected milk.

[3] kg 3.5% Fat corrected milk/kg DMI.

Table 3-4. Plasma metabolites and hormones of cows fed treatment diets.

Item	Treatment		SEM	Treatment *P*-value
	PA	SA		
Glucose, mg/dL	56.6	55.7	0.84	0.041
NEFA, μEq/L[1]	96.3	88.2	4.10	0.008
Insulin, μIU/mL	9.60	9.10	0.89	0.904
BHBA, mg/dL	5.20	5.00	0.38	0.881

[1] pMY (premilinary milk yield): $P = 0.767$; pMYx Treatment $P = 0.03$

Table 3-5. Milk fatty acid concentrations of cows fed treatment diets.

	Treatment			Treatment
Item (g/100 g)	**PA**	**SA**	**SEM**	***P*-value**
Selected Individual Fatty Acids				
4:0	3.03	3.02	0.06	0.81
6:0	2.11	2.21	0.04	<0.0001
8:0	1.27	1.39	0.03	<0.0001
10:0	3.25	3.67	0.10	<0.0001
12:0	3.85	4.38	0.13	<0.0001
14:0	11.07	12.14	0.16	<0.0001
14:1	0.94	0.97	0.05	0.026
16:0	36.74	31.22	0.41	<0.0001
16:1	1.81	1.62	0.06	<0.0001
18:0[4]	7.39	8.72	0.24	<0.0001
18:1 *trans*[3]	1.86	1.97	0.10	<0.01
18:1 9 *c*[4]	16.67	17.86	0.27	<0.0001
18:1 11*c*[5]	0.57	0.60	0.02	<0.01
18:2 9*c*, 12*c* (n-6)	2.63	2.87	0.05	<0.0001
18:3 9*c*, 12*c*, 15*c* (n-3)	0.32	0.36	0.01	<0.0001
CLA 9*c*, 11*t*	0.33	0.34	0.02	0.32
Σ CLA	0.34	0.35	0.02	0.32
Unknown	0.62	0.64	0.02	0.011
Others[1]	6.15	6.62	0.09	<0.0001
Summations[2]				
Σ Saturated FA	71.1	69.4	0.45	<0.0001
Σ MUFA *cis*	21.3	22.4	0.35	<0.0001
Σ PUFA *cis*	3.44	3.75	0.06	<0.0001
Σ branched chain FA	1.20	1.31	0.02	<0.0001
De novo	28.0	30.5	0.40	<0.0001
Mixed	38.6	32.8	0.43	<0.0001
Preformed	34.8	35.3	0.43	<0.0001

[1] Consists of 44 identified fatty acids ranging from C5:0 to C22:5

[2] *De novo* fatty acids originate from mammary *de novo* synthesis (< 16 carbons), preformed fatty acids originate from extraction from plasma (> 16 carbons), and mixed fatty acids originate from both sources (C16:0 + *cis*-9 C16:1).

[3] Total 18:1 *trans* fatty acids.

[4] pMY (preliminary milk yield) $P = 0.205$ and pMY x treatment interaction $P = 0.018$

[5] pMY (preliminary milk yield) $P = 0.036$ and pMY x Treatment interaction $P = 0.075$

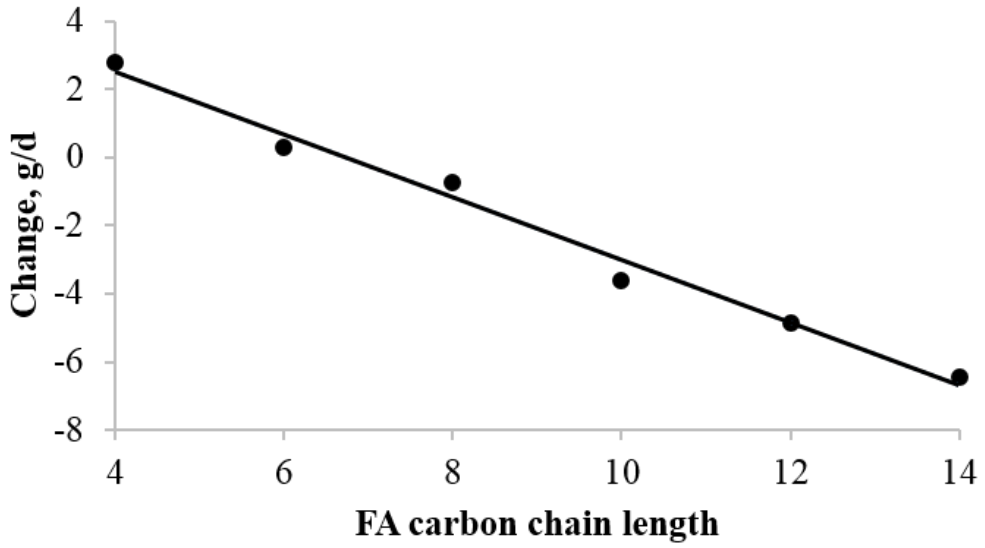
Table 3-6. Milk fatty acid yields of cows fed treatment diets.

Item (g/d)	Treatment		SEM	Treatment *P*-value
	PA	SA		
Summations[1]				
De novo	441	455	17.0	0.0392
Mixed	608	491	21.0	<0.0001
Preformed	524	539	16.0	0.0363
Selected Individual Fatty Acids				
4:0	47.9	45.1	1.94	0.0004
6:0	33.4	33.1	1.40	0.536
8:0	20.1	20.8	0.88	0.0346
10:0	51.2	54.8	2.51	0.0006
12:0	60.4	65.3	2.92	0.0002
14:0	175	181	6.92	0.0183
14:1	14.6	14.3	0.16	0.40
16:0	580	467	20.2	<0.0001
16:1 *cis*-9	28.3	23.8	1.08	<0.0001
18:0	116	129	5.20	<0.0001
18:1 *trans*[2]	29.3	29.0	0.39	0.75
18:1 *cis*-9	260	262	7.48	0.47
18:2 *cis*-9, *cis*-12	41.4	42.6	1.61	0.0899
18:3 *cis*-9, *cis*-12, *cis*-15	5.07	5.35	0.21	0.0016

[1] *De novo* fatty acids originate from mammary *de novo* synthesis (< 16 carbons), preformed fatty acids originate from extraction from plasma (> 16 carbons), and mixed fatty acids originate from both sources (C16:0 + *cis*-9 C16:1).

[2] Total 18:1 *trans* fatty acids.

Figure 3-1. Change in milk FA yield (PA - SA) in relation to FA carbon chain length.



$y = 0.92x + 6.21$

$R^2 = 0.99$

Chain length calculated as the difference in the yield of each FA, PA minus SA treatment.

Chapter 4

MILK YIELD AND MILK FAT RESPONSES TO INCREASING LEVELS OF PALMITIC ACID SUPPLEMENTATION OF DAIRY COWS RECEIVING LOW AND HIGH-FAT DIETS

Abstract

Dose-dependent effects of a palmitic acid (C16:0)-enriched fat supplement on feed intake and production responses of dairy cows were evaluated. Basal dietary fat concentration (2.7% or 4.2% ether extract) was used as a split-plot to determine relationships between basal dietary fat concentration and fat supplement dose. A covariate period with a common diet (3.5% ether extract) was included to evaluate treatment interactions. Sixteen Holstein cows (149 ± 56 DIM) were assigned randomly to treatment sequence within basal fat group (n = 8 cows/group). A C16:0-enriched fat (PA; Bergafat F100; 87% C16:0) was supplemented at 0, 0.75, 1.50, or 2.25% of ration DM in a 4x4 Latin Square design within each basal fat group. Periods were 14 d with the final 4 d used for data collection. Corn silage and alfalfa silage-based diets were formulated to contain 30% NDF and 16.5% CP. The statistical model included the random effect of cow and the fixed effects of basal group, PA dose, period, and their interactions. 3.5% FCM was used as a covariate in the model to account for effects of pre-trial milk and fat yield. The PA dose increased milk fat concentration (3.78, 3.88, 4.01, and 4.03%), fat yield (1.62, 1.68, 1.78, and 1.70 kg/d), and 3.5% FCM yield (45.3, 46.1, 47.9, and 45.8 kg/d) for 0, 0.75, 1.50, and 2.25% PA, respectively. PA dose had no effect on milk protein and lactose concentration, DMI, BW, or BCS, but tended to increase yields of milk, milk protein, and milk lactose. There were no main effects of basal fat group on the yield of milk or milk components, but feed efficiency (3.5% FCM/DMI) was higher for the high-fat relative to the low-fat basal group (1.74 vs. 1.51). There was an

interaction of basal fat group with PA dose for yields of milk and milk protein, and a trend for yields of milk fat and 3.5% FCM. Results demonstrate that response to PA varies with dose, and under the dietary conditions tested, the yield of 3.5% FCM and milk fat were optimal when PA was fed at 1.5% of ration DM.

Introduction

There is a growing interest in understanding the effects of different fat sources on milk production parameters, and special attention has recently been given to determining the effects of specific saturated FA (**SFA**). We have recently focused on palmitic acid (**C16:0**) supplementation because of its potential effects on fat concentration and yield, 3.5% FCM, and feed efficiency of lactating dairy cows, when compared with non-fat supplemented diets (Lock *et al*., 2013; Piantoni *et al*., 2013) and other SFA (see Chapter 3). Determining the optimal level of any fat supplement in a diet is key in order to maximize production efficiency and farm profitability. To our knowledge, however, only one study has directly evaluated production responses to increasing dietary levels of a C16:0-enriched fat supplement (85% of total FA) fed at 0%, ~2% (500 g/d), ~4% (1000 g/d), or ~6% (1500 g/d) of dietary DM (Mosley *et al*., 2007). These authors reported increased yield of milk and milk fat as the dietary inclusion of the C16:0-enriched fat supplement increased from 0% to ~2% of ration DM, and these responses did not increase further at the higher doses. Our recent studies have evaluated the impact of C16:0 and C16:0-enriched fats when supplemented at 2% of ration DM. Considering that most of the dairy farms that currently use supplemental fat would include it the diets within the range of 0.5 to 2% of ration DM, and that there is no evidence for higher doses to be beneficial, the determination of an optimal dose within this range would have important practical relevance.

Although there is strong evidence that C16:0 can increase milk fat yield (*e.g*. Steele, 1969, Lock *et al*., 2013 and Piantoni *et al*., 2013), some studies have reported no beneficial effect of feeding this FA to dairy cows (Storry *et al*, 1967; Warntjes *et al*, 2008; Rico and Harvatine, 2011). These differences could be due to a series of factors related to the animal and the diet (*e.g*. fiber, starch, ruminal fermentability of carbohydrates, fat content). Virtanen (1966) and Banks *et al*.

(1976a) demonstrated that low fat diets might limit the yield of milk and milk fat. Along these lines, a recent meta-analysis highlighted the interdependence of the yield of 18-carbon and 4 to 16-carbon FA yield in milk, and suggested that milk lipid synthesis in the mammary gland is dependent upon the simultaneous supply of short/medium-chain FA and long-chain FA for the esterification step of milk fat synthesis (Glasser *et al*., 2008). This implies that low-fat diets may limit the amount of fat secreted into milk and thus an increased supply of dietary long-chain FA, such as C16:0, could potentially increase milk fat yield. To our knowledge, no previous studies have directly tested the effects of an enriched C16:0-enriched fat supplement in diets with a low or high basal fat content in lactating dairy cows. Therefore, our objectives were two-fold: first, to determine the dose-dependent effects of a C16:0-enriched fat supplement on feed intake, and production responses of dairy cows; and second, to establish if the basal fat content of the diet impacted the response to C16:0-enriched fat supplementation.

Materials and Methods

Design and Treatments

Experimental procedures were approved by the Institutional Animal Care and Use Committee at Michigan State University. Sixteen mid-lactation, multiparous Holstein cows, averaging 149 ±56 DIM (mean ± SD) from the Michigan State University Dairy Field Laboratory, were used in a 4x4 Latin square experiment with a split plot. All animals received a common diet with no fat supplementation during a 14 d covariate period to obtain baseline values and were then randomly allocated to either a low fat basal diet (**LOW FAT**, 2.7% ether extract, n = 8) or a high fat basal diet (**HIGH FAT**, 4.2% ether extract, n = 8) that was fed to each group throughout the

remainder of the experiment. Within each plot a 4x4 Latin square arrangement of treatments was used in four consecutive 14 d periods. The four treatments were increasing doses of a C16:0-enriched fat supplement (**PA**; Bergafat F100, Berg+Schmidt, Germany). Treatments were: 1) 0% PA (**D-0%**), 0.75% PA (**D-0.75%**), 1.50% PA (**D-1.5%**), and 2.25% PA (**D-2.25%**). The C16:0-enriched fat supplement was a FFA product of high purity and contained more than 87% C16:0 and 99% total SFA.

Diets were based on corn silage and alfalfa-silage as the main forage components and corn grain as the major concentrate component, and were formulated to contain 30% NDF and 16.5% CP (Table 1). Mineral and vitamins were formulated to meet NRC (2001) recommendations. Dry matter concentration was determined twice weekly for forages, and diets were adjusted when necessary. Throughout the experiment cows were housed in individual tie stalls. Access to feed was blocked from 0800 to 1000 h to allow for orts and feed offered to be weighed daily. Cows were fed 115% of expected intake at 1000 h daily and feed intake was recorded and adjusted daily. Water was available *ad libitum* in each stall and stalls were bedded with sawdust and cleaned twice per day. Cows were milked twice daily and milk yield was recorded at each milking throughout the experiment. BW of cows was 680 ± 73 kg and both milk yield (48.9 kg/d) and 3.5 FCM (49.1 kg/d) were not different between the basal fat groups at the beginning of the experiment ($P = 0.58$ and $P = 0.51$, respectively).

Data and Sample Collection

Response variables were averaged for d 11 to 14 of the covariate period and for each of the treatment periods. Diet ingredients and orts were sampled daily during d 11 to 14 and composited for each period. Milk was sampled at each milking from d 11 to 14. Two samples were taken from

each cow at each milking. One aliquot was collected in a sealed tube with preservative (Bronopol Tablet; D&F Control Systems, San Ramon, CA) and stored at 4ºC for milk component analysis. The second aliquot was stored without preservative at -20ºC until analyzed for FA composition. On the last day of each period BCS was determined by three trained investigators on a 5-point scale (Wildman *et al*., 1982).

Sample Analysis

Diet ingredients and orts were dried at 55°C in a forced-air oven for 72 h for DM determination. Dried samples were ground with a Wiley mill (1-mm screen; Arthur H. Thomas, Philadelphia, PA). All samples were analyzed for NDF, CP, and starch according to the methods described by Harvatine and Allen (2005). All nutrients are expressed as percentages of DM determined by drying at 105°C in a forced air oven for more than 8 h. FA concentrations of feed ingredients and orts were determined using a one-step transesterification method using GLC analysis as described previously (Lock *et al*., 2013).

Individual milk samples were analyzed for fat, true protein, and lactose concentration by mid-infrared spectroscopy (AOAC, 1990, method 972.160) by the Michigan Herd Improvement Association (Universal Lab Services, Lansing MI). Yields of 3.5% fat corrected milk (**3.5% FCM**), energy corrected milk (**ECM**), and milk components were calculated using milk yield and component concentrations for each milking, summed for a daily total, and averaged for each collection period (Reist *et al*., 2002).

Milk samples used for analysis of FA composition were composited based on milk fat yield (d 11 to 14). For each period, FA composite samples (~ 40mL) were skimmed by centrifugation at 17,800 x *g* for 30 min at 4ºC, and the fat cake collected. Lipids were extracted, methylated, and

FA composition determined by GLC according to the methods described by Lock *et al*. (2013). Short chain FAME were corrected for mass discrepancy using the response factors published by Ulberth and Schrammmel (1995). Yields of individual FA (g/d) were calculated using milk fat yield and FA concentration to determine yield on a mass basis using the molecular weight of each FA while correcting for glycerol and other milk lipid classes (Shauff *et al*., 1992; Glasser et al., 2007).

Statistical Analysis

All data were analyzed using the mixed model procedure of SAS (Version 9.0.2, SAS Institute, Cary, NC) according to the following model:

$$Y_{ijkl} = \mu + P_j + F_k + D_l + F_k \times D_l + P_j \times F_k + P_j \times D_l + P_j \times F_k + P_j \times D_l \times F_k + pMY + C_m(F_k) + e_{ijkl}$$

where Y_{ijkl} = dependent variable, μ = overall mean, P_j = fixed effect of period (j = 1 to 4), F_k = fixed effect of basal fat group (k = 2), D_l = fixed effect of dose (l = 1 to 4), pMY = preliminary milk yield used as covariate, $C_m(F_k)$ = random effect of cow nested in basal fat group (i = 1 to 16),and e_{ijkl} = residual error. Normality of the residuals was checked with normal probability and box plots and homogeneity of variances with plots of residuals versus predicted values. Main effects were declared significant at $P \leq 0.05$, and tendencies were declared at $P \leq 0.10$. Interactions were declared significant at $P \leq 0.10$, and tendencies were declared at $P \leq 0.15$. Interactions were evaluated, but removed from the statistical model when not significant ($P > 0.15$). Linear, quadratic and cubic contrasts were done in order to evaluate interactions between dose and basal

fat group. A contrast between PA dose D0% and D0.75% for LOW FAT and HIGH FAT was done in order to directly test the hypothesis that the addition of long-chain FA to a low fat diet could impact animal responses. All data was expressed as least square means and standard error of the means, unless otherwise specified.

Results

The effects of treatments on production variables, as well as milk fat composition and milk FA yield are shown in Tables 2, 3, and 4, respectively. Specific effects of treatments on the yield of milk and milk fat, and the yield of milk FA sources is displayed in Figures 1 and 2, respectively. The contrast between D-0% and D-0.75%, as well as those describing the specific effects of dose in LOW FAT and HIGH FAT are not shown in the Tables, but will be described in the following section.

Production Responses

The main effect of basal fat on production variables was not significant (Table 2), with the exception of feed efficiency, which was 15% higher for HIGH FAT, relative to LOW FAT (1.74 *vs*. 1.51, $P = 0.039$). Although the main effect of basal fat was not significant for production responses, there were a number of basal fat by PA dose interactions (Table 4-2, Figures 4-1 and 4-2).

Milk Fat concentration and yield were affected by PA dose ($P < 0.01$). Maximal milk fat response occurred at PA dose D-1.5% for both basal fat groups, and was increased 140g/d (+9%) and 170g/d (+10%), relative to D0% for LOW FAT and HIGH FAT, respectively. Milk fat

concentration increased linearly as PA dose increased (P <0.001) and fat yield tended to increase cubicly (P = 0.078) as dose increased. There was a tendency for a linear interaction between PA dose and basal fat for milk fat yield (P = 0.138), explained by a quadratic increase in LOW FAT and a cubic increase in HIGH FAT (Figure 4-1). The quadratic increase in LOW FAT corresponded to an increase in milk fat yield for D-0.75% relative to D-0% (110 g/d, P = 0.027), whereas the cubic effect in HIGH FAT was a result of the lack of difference between these two doses (P = 0.82). There were no interactions between basal fat and PA dose for milk fat concentration (P = 0.84).

DMI was not affected by basal fat or PA dose (P = 0.13 and P = 0.33, respectively, Table 2). Milk yield was not affected by basal fat, however, it tended to increase cubicly (P = 0.064) as PA dose increased with a significant quadratic interaction between basal fat and PA dose (P = 0.068). PA dose increased milk yield quadratically in LOW FAT FAT and cubicly in HIGH FAT diets (Figure 4-2). Both 3.5% FCM and ECM were affected by PA dose (P = 0.021 and P = 0.028, respectively; Table 2). 3.5% FCM tended to increase cubicly as PA dose increased, with a trend for a cubic interaction between basal fat and PA dose (P = 0.116)

Milk protein yield tended to increase cubicly as PA dose increased (P = 0.075, Table 2), with a linear interaction between basal fat and PA dose (P = 0.030). Similar to milk yield, lactose yield tended to be increased cubicly by PA dose (P =0.099), however, no interaction between basal fat and PA dose was detected (P = 0.25). Concentrations of protein and lactose, as well as body weight and body condition score, were not affected by treatments (all P > 0.20).

Milk Fatty Acid Concentrations and Yields

PA dose linearly increased the concentrations of C16:0 and C16:1 in milk fat (both $P < 0.0001$; Table 4-3). This corresponded to a relative increase of 18 and 13% in C16:0 and C16:1 concentration, respectively at the highest PA DOSE (D-2.25%), compared with D-0%. The concentration of C18:0 in milk fat decreased linearly as PA dose increased ($P < 0.0001$) and the change corresponded to a 15% decrease when comparing D-2.25% to D-0%. Basal fat affected the concentration of C18:0 in milk fat and was always higher in HIGH FAT compared with LOW FAT diets ($P < 0.001$)

FA composition of milk fat according to source (< 16 carbon FA from *de novo* synthesis in the mammary gland, > 16-carbon FA originating from extraction from plasma, and 16-carbon FA originating from mixed sources) was affected by treatments (Table 3). Concentration of *de novo* FA was higher in LOW FAT relative to HIGH FAT ($P < 0.01$) and it was decreased quadratically in LOW FAT ($P < 0.01$) and cubicly in HIGH FAT ($P < 0.001$) as PA dose increased, which corresponded with a cubic interaction between basal fat and PA dose ($P < 0.001$). Similar to the production responses, the cubic effect of dose in HIGH FAT was due to the lack of difference between D-0% and D0.75% ($P > 0.05$). The concentration of mixed FA was increased linearly by PA DOSE ($P < 0.0001$) and was not affected by basal fat ($P = 0.13$). The concentration of preformed FA was 15% higher in HIGH FAT relative to LOW FAT ($P < 0.01$) and was linearly decreased by PA DOSE ($P < 0.001$). The concentration of SFA and MUFA in milk fat was unaffected by treatments ($P > 0.05$), whereas concentration of PUFA decreased linearly as PA DOSE increased ($P = 0.0002$; data not shown).

The effects of treatments on the yield of FA in milk fat derived from *de novo* (<16 carbon FA), preformed (>16 carbon FA), or mixed (16-carbon FA) sources are shown in Table 4-4 and

Figure 4-3. The yield of 16-carbon FA increased linearly as PA DOSE increased ($P < 0.0001$), and it responded quadratically in LOW FAT ($P = 0.027$) and linearly in HIGH FAT ($P < 0.0001$), with a significant linear interaction between basal fat and PA dose ($P = 0.063$). At the D-1.5% PA dose, the yield of 16-carbon FA was 99 g/d (+18%) and 119 g/d (+21%) higher than D-0% for LOW FAT and HIGH FAT, respectively. These changes in 16-carbon secretion into milk explain most of the maximal milk fat yield response observed at PA dose D1.5%. There was a significant cubic interaction between basal fat and PA dose for the yield of *de novo* FA ($P < 0.01$) and a quadratic increase was observed in LOW FAT, whereas HIGH FAT responded cubicly (Figure 4-3). *De novo* FA yield, however, was not different for D-0% relative to D-0.75% ($P > 0.27$). Yield of C18:0 was 52% higher for HIGH FAT, relative to LOW FAT ($P < 0.01$) and decreased linearly as PA dose increased ($P = 0.013$). The yield of *cis*-9 C18:1 was higher for HIGH FAT relative to LOW FAT (+ 22%, $P = 0.018$) and a tendency for an interaction between basal fat and dose was detected ($P = 0.143$). There was no effect of PA dose on the yield of preformed FA ($P = 0.29$) whereas there was a main effect of basal fat ($P = 0.007$) with HIGH FAT diets having higher yields of preformed FA compared with LOW fat diets.

Discussion

Our experimental design allowed the evaluation of the effects of feeding incremental doses of C16:0 in diets with low or high basal fat concentrations. Importantly, both low and high basal fat diets in our study represented rations that can typically be fed to high producing cows under commercial conditions (Table 1), including ingredients that are widely available to dairy farmers. Although the main effect of basal fat was non-significant for most production variables, responses

to PA dose were affected by the basal fat content of the diet (interaction between treatments, Table 2).

When fed at up to 3% of dietary DM, saturated FA supplementation mostly results in no change in DMI (Palmquist and Jenkins, 1980). In a meta-analysis of 29 treatment means reported in the literature, Allen (2000) showed that hydrogenated FA did not affect DMI. In agreement with this observation, we detected no effect of PA dose on DMI. However, some studies that fed C16:0-enriched supplements at similar doses have reported decreased (Lock *et al*., 2013) or increased (Mosley *et al*., 2007) DMI, when compared to a control diet with no supplemental fat. Reasons for these differences across studies require further investigation.

There are only a limited number of studies that have specifically evaluated the effect of basal fat concentration of the diet on milk production responses to dietary fat. Using purified diets, Virtanen (1966) characterized the effect of feeding diets containing a very low fat content (37 g/d from vegetable oils) and reported milk production to increase by 40% and milk fat yield by 56% as dietary FA supply went from 37 to 130 g/d. Banks *et al*. (1976a) reported similar results and suggested that a diet that supplied only 81 g/d of FA was fat-deficient, as milk yield and milk fat yield were both increased when this diet was supplemented with different fats. The aforementioned studies, however, used low producing cows (~7 and 12 kg/d, respectively) and determination of treatment effects, as well as responses measured were limited. On the other hand, the recent dose response studies by Mosley *et al*. (2007) and Drackley *et al*. (2007) used high producing dairy cows and provided similar amounts of total FA in the base diets (~540 g/d and ~480 g/d, respectively) to those used in our experiment (~500 g/d of FA for LOW FAT at PA dose D0%). The basal fat content of the diet did not limit milk yield in our study, as evidence by the lack of difference between basal fat groups (Table 4-2). This difference is likely the result of an adequate

energy supply from other dietary ingredients to meet metabolic demands of animals in our experiment. The increased milk yield reported by Virtanen (1966) and Banks *et al*. (1976a) might be the result of improved energy balance in cows fed fat supplements (as the low fat diets might have restricted energy supply), rather than evidence of induced fat deficiency by feeding the basal diets, as it was concluded by the authors. Although basal fat did not have any effect on milk yield in our study, a significant interaction between PA dose and basal fat was detected, which indicates that the effect of feeding increasing levels of C16:0 on milk yield was affected by basal fat content of the diet (quadratic response in LOW FAT ad cubic tendency in HIGH FAT, Figure 4-2). Kronfeld *et al*. (1982) suggested that the increased milk yield that occurs as a consequence of feeding fats to dairy cows is probably the result of an improved efficiency of milk fat synthesis. In agreement with this idea, Cant *et al*. (1993) concluded that an increased mammary supply of FA from fat supplementation reduced lipogenesis from acetate, which can in turn be used for oxidation by other tissues, and increased BHBA uptake, thus increasing the overall efficiency of glucose use for lactose synthesis and increasing milk yield. Other authors have suggested that the suppression of *de novo* synthesis of FA in the mammary gland that occurs when long-chain FA are fed, can decrease the oxidative use of glucose to generate reducing equivalents for milk fat synthesis (Storry *et al*., 1973). As discussed by Palmquist and Jenkins (1980), long chain FA inhibition of acetyl CoA carboxylase (ACC) would reduce the metabolism of glucose through the pentose phosphate pathway to yield NADPH, allowing its use in other milk synthetic processes. These explanations are in line with our previous observation that plasma glucose is elevated when a C16:0 supplement is fed (Chapter 3) and the trend for increases in both milk yield and protein yield observed in the present study that occurred with the simultaneous decrease in the yield of *de novo* FA with both low and high basal fat diets as PA dose increased. Consistent with our results,

Wang *et al*. (2010) reported increased milk production, as well as increased milk fat and protein yield when a saturated fat supplement containing C16:0 and C18:0 (54% and 34% of total FA, respectively) was fed to lactating dairy cows under heat stress conditions.

Milk fat yield tended to increase as PA dose increased and responded differently in LOW FAT and HIGH FAT, probably driven by milk yield that was affected in a similar manner. The quadratic increase in LOW FAT and cubic in HIGH FAT as PA dose was increased, may indicate that the LOW FAT diet was limiting milk fat yield. This is supported by the increase in milk fat yield (+110 g/d or +7%) in D-0.75%, relative to D-0%, that occurred in LOW FAT only. The lack of difference in milk fat yield between the aforementioned PA doses in HIGH FAT suggests that this treatment did not limit fat secretion into milk. In combination, differences in response to PA dose between basal fat treatments at the lowest PA dose support the hypothesis that a low long-chain FA supply can limit milk fat synthesis as previously suggested by Glasser *et al*. (2008). The limitation to fat yield from a reduced supply of long-chain FA to the mammary gland in LOW FAT is also consistent with the observed increase in concentrations of *de novo* FA in LOW FAT and increased concentration and yield of preformed FA (> 16 carbon) in HIGH FAT (+15% and +25%, respectively). A limited supply of long chain FA in LOW FAT would have increased the concentration of *de novo* FA in milk TG. This is in agreement with Glasser *et al.* (2008) which showed that the proportion of short and medium-chain FA is higher in milk TG when diets supply limited amounts of long-chain FA, which would, if present, compete effectively for esterification to the glycerol backbone during milk fat synthesis (Hansen and Knudsen, 1987). Furthermore, this explains the increased concentration and yield of preformed FA in HIGH FAT, relative to LOW FAT in our study.

The effects of PA dose on the concentration and yield of *de novo* synthesized FA were similar across basal fat diets, as they were decreased quadratically in LOW FAT and cubicly in HIGH FAT, as PA dose increased (Figure 4-3). Fat supplementation usually decreases *de novo* FA concentration and yield (Grummer, 1991), however, response to C16:0 supplementation may be more inconsistent, as evidenced by a negative effect on *de novo* synthesis reported in some studies (Banks *et al*., 1976b; Mosley *et al*., 2007; Piantoni *et al*., 2013), but not in others (Lock *et al*., 2013). According to the meta-analysis by Glasser *et al.* (2008), a positive relationship between *de novo* synthesized and long-chain FA can be expected in low lipid diets; however, when fats are supplemented to the diet, a simultaneous decrease in *de novo* FA and an increase in long-chain FA occurs, corresponding to an inverse relationship between the two FA sources (Enjalbert *et al*., 1998; Glasser *et al*., 2008). In our experiment, the lack of difference between D-0% and D-0.75% with a quadratic increase response to PA dose in the LOW FAT treatment seems to be in agreement with the observations of these authors, indicating that supplementing C16:0 at low doses (D-0.75%) to a low fat diet does not affect *de novo* FA synthesis, however, when the long-chain FA limitation to fat synthesis is overcome, a decrease in *de novo* synthesis occurs (corresponding to the overall quadratic decrease). As previously discussed in Chapter 3, the overall reduction in the synthesis *de novo* of FA that occurs from C16:0 feeding, could be a result of different mechanisms that include an inhibitory effect of C16:0 on activity of enzymes systems involved in lipogenesis, such as ACC and/or fatty acid synthase activity, or the inhibition of mammary gland ACC by palmitoyl-CoA derived from C16:0, as well as FA competition for esterification positions into milk TG during mammary lipid synthesis. As suggested previously (Chapter 3), the competitive mechanism during the esterification steps of fat synthesis is likely to be predominant when long

chain SFA are fed, in the absence of other bioactive FA, such as *trans*-10, *cis*-12 CLA, a potent inhibitor of lipogenesis in the mammary gland (Bauman *et al*., 2011).

The incorporation of C16:0 into milk fat (g/d) increased as PA dose increased, however, the response was quadratic in LOW FAT and linear in HIGH FAT (Figure 2). The reason for this difference, however, is not clear. Mosley et al. (2007) reported a linear increase in milk 16-carbon FA yield as the dose of the C16:0-enriched fat was increased up to 5.2% of dietary DM, more than two times the maximum amount targeted in our experiment. Maximum milk fat yield response occurred at PA dose D-1.5% in both basal fat diets, despite the fact that 16-carbon FA continued to increase. The lower milk fat yield at D-2.25%, seems to be the result of decreased *de novo* FA yields in both the LOW and HIGH fat basal diets, and decreased preformed FA yield in LOW FAT only. A reduction in the yield of preformed FA could be the result of reduced digestibility of long-chain FA at PA dose 2.25%, since there is no indication that mammary uptake of long-chain FA would have been reduced. Possible digestibility changes as a result of feeding increasing amounts of C16:0 need to be investigated in order to establish the extent at which they could explain the observed decreases in many of the responses evaluated at PA dose D-2.25% in the present study.

Conclusion

Results demonstrate that production responses to increasing doses of C16:0 supplementation are affected by the basal fat content of the basal diet. Importantly, the increased milk fat yield observed in this study for the low fat diet at a C16:0 dose of 0.75%, proves the hypothesis that a low fat supply may limit milk fat secretion into milk. Results also demonstrate that response to C16:0 varies with dose, and under the dietary conditions tested, the yield of 3.5% FCM and milk fat were optimal when C16:0 was fed at 1.5% of ration DM.

Table 4-1. Ingredient and nutrient composition of diets fed during the treatment periods.

Basal diets were either low or high in fat and supplemented with increasing doses of PA (0, 0.75, 1.5, and 2.25% DM)

	Low Fat Basal Diet				High Fat Basal Diet			
Ingredients, % of DM	**0%**	**0.75%**	**1.5%**	**2.25%**	**0%**	**0.75%**	**1.5%**	**2.25%**
Alfalfa Haylage	11.8	11.8	11.8	11.8	11.8	11.8	11.8	11.8
Corn silage	27.0	27.0	27.0	27.0	27.0	27.0	27.0	27.0
Wheat Straw	3.09	3.09	3.09	3.09	3.09	3.09	3.09	3.09
Ground Corn	15.5	15.5	15.5	15.5	15.5	15.5	15.5	15.5
Cottonseed	0.00	0.00	0.00	0.00	16.7	16.7	16.7	16.7
Soybean meal	14.7	14.7	14.7	14.7	14.7	14.7	14.7	14.7
Limestone	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54
Vitamin and mineral mix[1]	1.85	1.85	1.85	1.85	1.85	1.85	1.85	1.85
Sodium Bicarbonate	0.62	0.62	0.62	0.62	0.62	0.62	0.62	0.62
Soyhulls	25.0	24.2	23.5	22.7	8.32	7.57	6.82	6.07
Bergafat F-100	0.00	0.75	1.50	2.25	0.00	0.75	1.50	2.25
Nutrient Composition, % of DM								
DM, % of as fed	55.7	55.8	55.8	55.8	55.8	55.8	55.8	55.8
NDF	32.6	32.1	31.7	31.2	31.3	30.8	30.4	29.9
CP	16.9	16.8	16.7	16.5	17.5	17.4	17.3	17.2
EE	2.7	3.4	4.2	4.9	4.2	4.9	5.6	6.4
FA	1.7	2.5	3.2	3.9	3.2	3.9	4.7	5.4

[1] Vitamin and mineral mix contained 58.3% dry ground shell corn, 12.3% limestone, 7.9% sodium bicarbonate, 6.8% di-calcium phosphate, 3.2% urea, 3.2% magnesium, 3.2% tallow, 3.0% white salt, and < 1% of each of the following: trace minerals, biotin vitamin E, vitamin A, vitamin D and selenium.

Table 4-2. Dry matter intake, milk production and composition, feed efficiency, BW, and BCS for cows fed treatment diets.

							P-value				
		PA dose, % dietary DM					Main Effects			Contrasts[2]	
Variable	**Basal[1]**	**0%**	**0.75%**	**1.5%**	**2.25%**	**SEM**	**Basal**	**Dose**	**Basal x Dose**	**Dose**	**Basal x Dose**
DMI, kg/d	LOW	29.8	29.3	29.9	28.7	0.88	0.131	0.33	0.92	NS	-
	HIGH	27.6	27.9	28.0	27.0	0.97					
Milk Yield, kg/d											
Milk	LOW	41.9	42.6	42.7	39.7	1.73	0.17	0.059	0.093	0.064C	0.068Q
	HIGH	45.4	44.4	46.2	45.2	1.8					
FCM[3]	LOW	43.4	45.2	45.9	42.9	1.88	0.17	0.021	0.134	0.066C	0.116Q
	HIGH	47.1	47.0	50.0	48.8	1.97					
ECM[4]	LOW	43.5	45.0	45.6	42.4	1.85	0.19	0.028	0.102	0.060C	0.123Q
	HIGH	46.7	46.5	49.4	48.2	1.93					
Feed Efficiency[5]	LOW	1.45	1.55	1.53	1.5	0.07	0.039	0.21	0.15	NS	0.072Q
	HIGH	1.71	1.67	1.77	1.8	0.08					
Milk Composition, %											
Fat	LOW	3.73	3.87	3.95	4	0.17	0.78	<0.01	0.84	<0.001L	-
	HIGH	3.83	3.89	4.07	4.06	0.18					
Protein	LOW	3.23	3.2	3.21	3.19	0.07	0.43	0.32	0.35	0.070C	-
	HIGH	3.11	3.1	3.15	3.13	0.07					
Lactose	LOW	4.77	4.78	4.77	4.75	0.05	0.56	0.84	0.97	NS	-
	HIGH	4.78	4.82	4.81	4.79	0.06					

[1] Basal: Basal fat group, includes HIGH FAT and LOW FAT treatments; [2] Contrasts for PA dose were L: Linear; Q: Quadratic; C: Cubic. Contrasts declared significant at $P < 0.05$ and at $P < 0.1$ for basal x dose interaction. Trends declared significant at $P < 0.1$ for dose and at $P < 0.15$ for basal x dose interaction; [3] 3.5% Fat-corrected milk. [4] Energy-corrected milk. 5 3.5% Fat-corrected milk/DMI.

Table 4-2. (Cont'd)

							P-value				
		PA dose, % dietary DM					Main Effects			Contrasts[2]	
Variable	**Basal[1]**	**0%**	**0.75%**	**1.5%**	**2.25%**	**SEM**	**Basal**	**Dose**	**Basal x Dose**	**Dose**	**Basal x Dose**
Milk components, kg/d											
Fat	LOW	1.55	1.66	1.69	1.59	0.09	0.24	<0.01	0.147	0.078C	0.138L
	HIGH	1.7	1.71	1.87	1.81	0.09					
Protein	LOW	1.34	1.35	1.35	1.24	0.06	0.37	0.083	0.101	0.075C	0.030L
	HIGH	1.38	1.36	1.44	1.4	0.06					
Lactose	LOW	2.01	2.04	2.05	1.9	0.09	0.19	0.084	0.25	0.099C	-
	HIGH	2.19	2.15	2.24	2.17	0.1					
Body Weight, kg	LOW	723	722	717	720	25.45	0.35	0.76	0.83	NS	-
	HIGH	682	687	684	682	25.66					
Body Condition Score	LOW	2.85	2.57	2.84	3.02	0.23	0.20	0.41	0.96	NS	-
	HIGH	2.46	2.39	2.57	2.66	0.26					

[1] Basal: Basal fat group, includes HIGH FAT and LOW FAT treatments; [2] Contrasts for PA dose were L: Linear; Q: Quadratic; C: Cubic. Contrasts declared significant at $P < 0.05$ and at $P < 0.1$ for basal x dose interaction. Trends declared significant at $P < 0.1$ for dose and at $P < 0.15$ for basal x dose interaction; [3] 3.5% Fat-corrected milk. [4] Energy-corrected milk. [5] 3.5% Fat-corrected milk/DMI.

Table 4-3. Milk fatty acid concentrations of cows fed treatment diets.

							P - value				
		PA Dose, % of dietary DM					Main Effects			Contrasts[2]	
Item (g/100 g)	Basal[1]	0%	0.75%	1.50%	2.25%	SEM	Basal	Dose	Basal x Dose	Dose	Basal x Dose
4:0	LOW	2.70	2.73	2.76	2.64	0.10	0.020	0.076	0.079	0.097Q	0.022C
	HIGH	3.12	3.20	3.03	3.06	0.10					
6:0	LOW	2.09	2.07	2.02	1.94	0.05	0.54	<0.0001	0.28	<0.0001L	-
	HIGH	2.19	2.11	2.07	1.93	0.06					
8:0	LOW	1.34	1.30	1.24	1.19	0.03	0.088	<0.0001	0.026	0.041C	0.013C
	HIGH	1.28	1.20	1.20	1.05	0.04					
10:0	LOW	3.82	3.59	3.30	3.21	0.13	0.009	<0.0001	<0.001	0.015C	<0.001C
	HIGH	3.12	2.87	3.08	2.46	0.14					
12:0	LOW	4.76	4.39	3.98	3.91	0.15	<0.001	<0.0001	<0.0001	<0.01C	< 0.001C
	HIGH	3.51	3.24	3.64	2.74	0.16					
14:0	LOW	13.4	12.7	11.9	11.6	0.25	<0.01	<0.0001	0.001	0.043C	<0.01C
	HIGH	11.6	11.0	11.3	9.83	0.26					
14:1	LOW	1.09	1.04	1.01	0.99	0.08	0.046	<0.01	0.60	<0.0001L	-
	HIGH	0.82	0.77	0.78	0.70	0.08					
16:0	LOW	35.8	37.9	39.2	41.2	0.98	0.19	<0.0001	0.40	<0.0001L	-
	HIGH	33.0	36.	37.4	40.1	1.04					
16:1	LOW	1.83	1.88	1.98	2.02	0.09	0.019	<0.001	0.93	<0.0001L	-
	HIGH	1.45	1.50	1.63	1.69	0.10					

[1] Basal: Basal fat group, includes HIGH FAT and LOW FAT treatments.

[2] Contrasts for PA dose were L: Linear; Q: Quadratic; C: Cubic. Contrasts were declared significant at $P < 0.05$ and at $P < 0.1$ for basal x dose interaction. Trends declared significant at $P < 0.1$ for dose and at $P < 0.15$ for basal x dose interaction.

[3] *De novo* FA originate from mammary *de novo* synthesis (< 16 carbons), preformed FA originate from extraction from plasma (> 16 carbons), and mixed FA originate from both sources (C16:0 + *cis*-9 C16:1).

Table 4-3. (Cont'd).

Item (g/100 g)	Basal[1]	PA Dose, % of dietary DM				SEM	*P* - value				
							Main Effects			Contrasts[2]	
		0%	0.75%	1.50%	2.25%		Basal	Dose	Basal x Dose	Dose	Basal x Dose
18:0	LOW	6.72	6.45	6.29	5.82	0.29	<0.001	<0.001	0.102	<0.0001L	0.062L
	HIGH	10.2	9.41	8.61	8.56	0.32					
18:1 9c	LOW	15.5	15.6	16.3	15.8	0.67	0.073	0.90	0.094	NS	0.081L
	HIGH	18.2	17.8	17.0	17.4	0.73					
Total 18:1 *t*	LOW	1.65	1.55	1.59	1.55	0.15	0.018	<0.0001	<0.0001	0.004C	<0.001C
	HIGH	2.39	2.27	1.84	2.39	0.16					
Summations[3]											
De novo	LOW	29.3	27.9	26.3	25.6	0.53	<0.01	<0.0001	<0.001	0.011C	<0.001
	HIGH	25.6	24.3	25.0	21.7	0.56					
Mixed	LOW	37.6	39.7	41.1	43.2	1.01	0.14	<0.0001	0.37	<0.0001L	-
	HIGH	34.5	37.6	39.0	41.8	1.07					
Preformed	LOW	33.3	32.6	32.8	31.5	1.03	<0.01	<0.01	0.067	<0.0001L	0.089L
	HIGH	39.6	37.9	35.9	36.3	1.10					

[1] Basal: Basal fat group, includes HIGH FAT and LOW FAT treatments.

[2] Contrasts for PA dose were L: Linear; Q: Quadratic; C: Cubic. Contrasts were declared significant at $P < 0.05$ and at $P < 0.1$ for basal x dose interaction. Trends declared significant at $P < 0.1$ for dose and at $P < 0.15$ for basal x dose interaction.

[3] *De novo* FA originate from mammary *de novo* synthesis (< 16 carbons), preformed FA originate from extraction from plasma (> 16 carbons), and mixed FA originate from both sources (C16:0 + *cis*-9 C16:1).

Table 4-4. Milk fatty acid yields (g/d) for cows fed treatment diets.

							P - value				
		PA Dose, % of dietary DM					Main Effects			Contrasts[2]	
Item (g/d)	**Basal[1]**	**0%**	**0.75%**	**1.50%**	**2.25%**	**SEM**	**Basal**	**Dose**	**Basal x Dose**	**Dose**	**Basal x dose**
Summations[3]											
De novo	LOW	433	436	420	383	9.40	0.68	<0.001	0.018	<0.01Q	<0.01C
	HIGH	410	394	445	371	10.7					
Mixed	LOW	553	618	652	643	41.9	0.65	<0.0001	0.21	<0.0001L	-
	HIGH	562	618	682	713	43.5					
Preformed	LOW	481	501	520	467	25.1	0.007	0.29	0.129	NS	0.021Q
	HIGH	626	603	615	615	26.8					
Selected Fatty Acids											
4:0	LOW	39.8	42.7	44.6	39.4	3.45	0.077	0.006	0.127	<0.01L	0.053Q
	HIGH	49.9	52.0	52.5	52.2	3.52					
6:0	LOW	31.1	32.6	32.9	29.2	2.08	0.30	<0.001	0.33	0.018C	NS
	HIGH	35.1	34.4	36.3	33.0	2.13					
8:0	LOW	20.1	20.5	20.2	18.1	1.08	0.96	<0.0001	0.060	<0.01C	NS
	HIGH	20.5	19.4	21.3	17.9	1.12					
10:0	LOW	57.2	56.6	53.4	48.8	3.01	0.20	<0.0001	<0.01	<0.01C	<0.01Q
	HIGH	50.0	46.4	55.4	42.2	3.18					
12:0	LOW	70.7	68.8	63.6	58.9	3.52	0.064	<0.0001	<0.001	<0.001C	<0.001Q
	HIGH	56.7	52.5	66.3	47.1	3.76					

[1] Basal: Basal fat group, includes HIGH FAT and LOW FAT treatments.

[2] Contrasts for PA dose were L: Linear; Q: Quadratic; C: Cubic. Contrasts were declared significant at $P < 0.05$ and at $P < 0.1$ for basal x dose interaction. Trends declared significant at $P < 0.1$ for dose and at $P < 0.15$ for basal x dose interaction.

[3] *De novo* FA originate from mammary *de novo* synthesis (< 16 carbons), preformed FA originate from extraction from plasma (> 16 carbons), and mixed FA originate from both sources (C16:0 + *cis*-9 C16:1).

Table 4-4. (Cont'd).

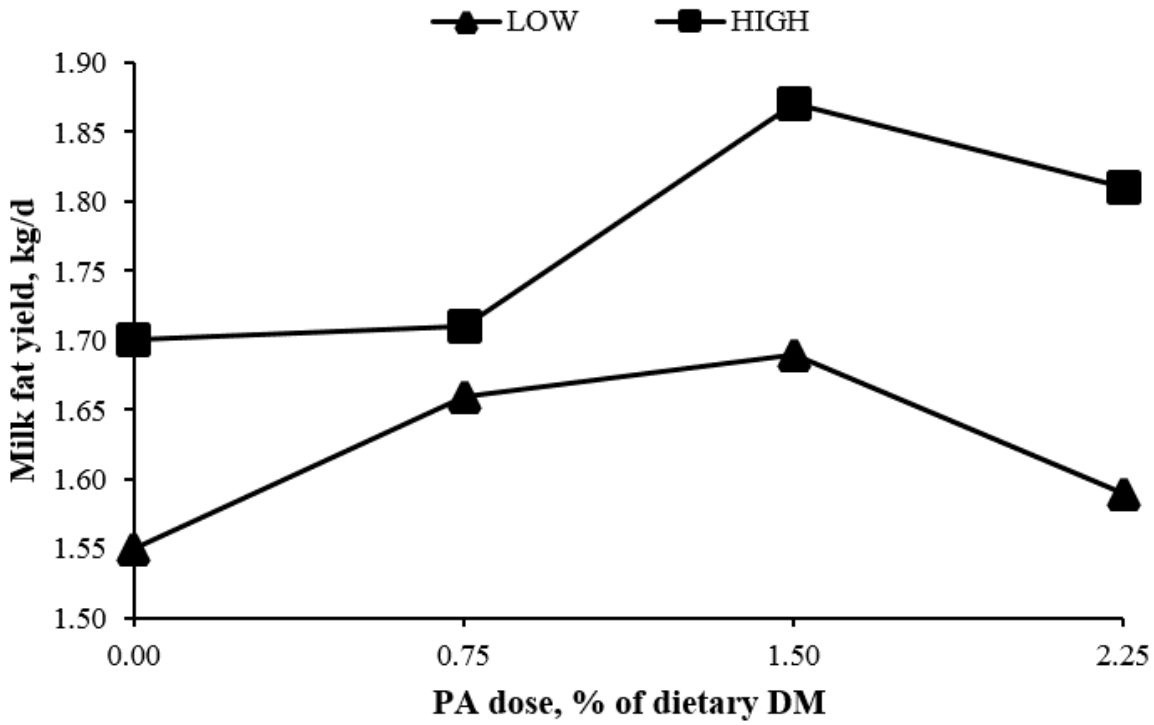
							P - value				
		PA Dose, % of dietary DM					Main Effects			Contrasts[2]	
Item (g/d)	**Basal[1]**	**0%**	**0.75%**	**1.50%**	**2.25%**	**SEM**	**Basal**	**Dose**	**Basal x Dose**	**Dose**	**Basal x dose**
Selected Fatty Acids											
14:0	LOW	198	199	189	174	10.5	0.56	<0.01	0.039	0.023C	0.013Q
	HIGH	185	177	200	167	11.1					
14:1 9*c*	LOW	15.9	15.9	15.8	14.6	1.27	0.16	0.015	0.56	0.019L	-
	HIGH	13.2	12.5	13.6	11.8	1.31					
16:0	LOW	527	590	621	613	40.2	0.61	<0.0001	0.22	<.0001L	-
	HIGH	538	593	652	684	41.8					
16:1 9c	LOW	26.5	29.0	31.4	30.0	2.24	0.46	<0.001	0.47	<.0001L	-
	HIGH	24.1	25.0	29.2	29.1	2.34					
18:0	LOW	98.5	101	102	87.7	8.41	<0.01	0.056	0.30	0.013L	-
	HIGH	156	147	146	142	8.90					
18:1 9c	LOW	221	238	258	233	13.6	0.018	0.16	0.143	NS	0.036Q
	HIGH	289	283	289	294	14.6					
Total 18:1 *t*	LOW	23.9	23.6	25.0	22.6	2.12	0.004	0.031	<0.001	0.013Q	0.003Q
	HIGH	37.8	36.5	31.3	41.0	2.27					

[1] Basal: Basal fat group, includes HIGH FAT and LOW FAT treatments.

[2] Contrasts for PA dose were L: Linear; Q: Quadratic; C: Cubic. Contrasts were declared significant at $P < 0.05$ and at $P < 0.1$ for basal x dose interaction. Trends declared significant at $P < 0.1$ for dose and at $P < 0.15$ for basal x dose interaction.

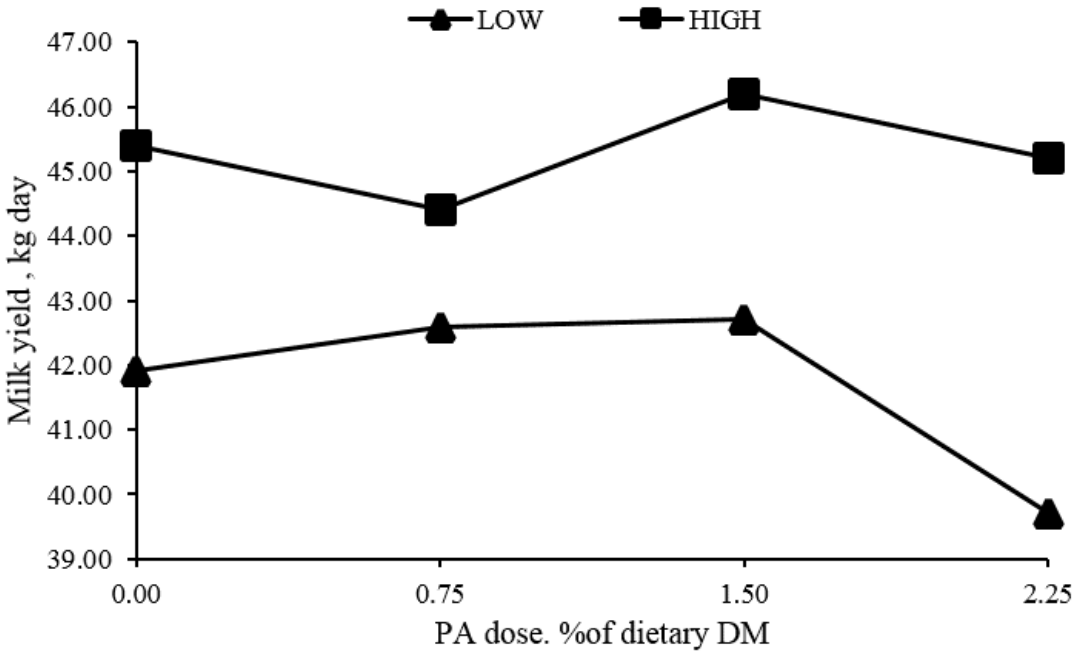
[3] *De novo* FA originate from mammary *de novo* synthesis (< 16 carbons), preformed FA originate from extraction from plasma (> 16 carbons), and mixed FA originate from both sources (C16:0 + *cis*-9 C16:1).

Figure 4-1. Effect of PA dose on milk fat yield in LOW FAT and HIGH FAT diets.



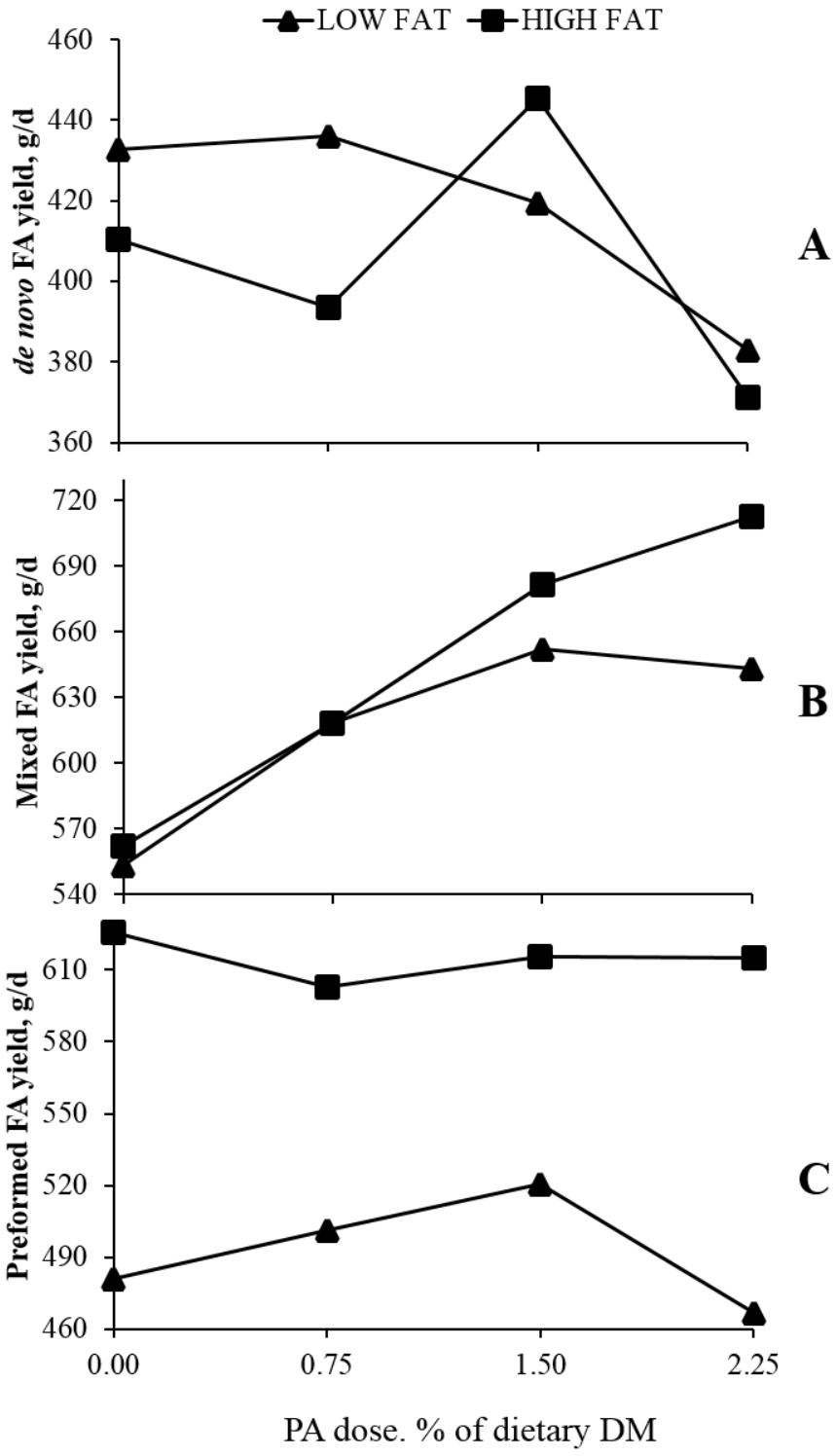
Basal fat group and PA dose tended to interact linearly (P = 0.13). Milk fat yield increased quadratically in LOW FAT (P < 0.01, SEM = 0.17) and was cubic in HIGH FAT (P = 0.05, SEM = 0.18). Milk fat yield was higher for D-0.75% relative to D-0% in LOW FAT (110 g/d, P = 0.027), and was not different in HIGH FAT (P = 0.82).

Figure 4-2. Effect of PA dose on milk yield in LOW FAT and HIGH FAT diets.



A significant quadratic interaction between basal fat group and PA dose was detected ($P = 0.068$). Milk yield increased quadratically in LOW FAT ($P < 0.01$, SEM = 1.73) and tended to be cubic in HIGH FAT ($P = 0.103$, SEM = 1.80)

Figure 4-3. Effect of PA dose on the yield of milk FA in LOW FAT and HIGH FAT diets.



Milk FA by source are *de novo* synthesized (< 16-carbon; Panel A), mixed (16-carbon; Panel B), and preformed (>16-carbon; Panel C) and basal fat group are shown. LOW FAT (2.7% ether extract); HIGH FAT (4.2% ether extract). SEM = 10.7, 43.5 and 26.8 for *de novo*, mixed and preformed FA, respectively. *P* values for the main effects are shown in Table 4. Panel A: *de novo* FA quadratic ($P = 0.04$) for LOW FAT and cubic for HIGH FAT ($P < 0.001$). Panel B: Mixed quadratic for LOW FAT (P = 0.03) and linear for HIGH FAT ($P = <0.001$). Panel C: Preformed quadratic for LOW FAT ($P < 0.01$) and not significant for HIGH FAT ($P = 0.47$).

Chapter 5

OVERALL CONCLUSIONS

Since milk yield and its composition, as well the efficiency of milk production, are key factors that impact farm profitability, the identification of dietary factors that can optimize these parameters is highly relevant. The use of fat supplements can help in achieving this goal. Our objectives included the characterization of the responses to the supplementation of individual SFA and the identification of potential interactions between these FA and other dietary components and animal factors. C16:0 supplementation resulted in improved yield of milk and milk components, while having no impact on DMI or milk protein yield, compared with C18:0. In addition, milk production level did not affect the response to the supplementation of C16:0. This implies that the response to these FA can be expected to be similar for all cows, regardless of their production level. Production responses to C16:0 varied with dose, and maximal 3.5% FCM yield and milk fat yield were observed when a C16:0-enriched fat supplement was fed at 1.5% of ration DM. In addition, the important role of an adequate fat content in the diet was examined; it was established that this factor can affect the response to C16:0 supplementation, as indicated by the quadratic increases in the low fat group, and the cubic increases in the high fat group for yield of milk and milk components. Collectively, this series of experiments provides evidence for the differential effects of feeding individual SFA on production responses, and it establishes the role of other factors related to the animal and the diet in the response to individual dietary SFA. Our work also provides information that can be potentially used to guide feeding decisions aimed at maximizing cow lactation performance and increase milk price and farm income. Further work is required to

characterize the effects of supplementing these SFA to cows in critical physiological periods, such as early and late lactation, as well as other dietary factors that may impact production responses.

REFERENCES

REFERENCES

Allen, M. S. 2000. Effects of diet on short term regulation of feed intake by lactating dairy cattle. J. Dairy Sci. 83:1598-1624.

Annison E. F., J. L. Linzell, S. Fazakerly, and B. W. Nichols. 1967. The oxidation and utilization of palmitate, stearate, oleate and acetate by the mammary gland of the fed goat in relation to their overall metabolism, and the role of plasma phospholipids and neutral lipids in milk fat synthesis. Biochem. J. 102:637-647.

AOAC, 1990. Official methods of analysis (15th ed.). AOAC Int, Arlington, VA. Vol 2.
Bach, A. C., & Babayan, V. K. (1982). Medium-chain triglycerides: an update. Am. J. Clin. Nutr. 36:950-962.

Baldwin, R.L., Smith, N.E., Taylor, J., Sharp, M. 1980. Manipulating metabolic parameters to improve growth rate and milk secretion. J. Anim. Sci. 51:1416–1428.

Banks, W., J. L. Clapperton., M. E. Ferrie and A. G. Wilson. 1976a. Effect of feeding fat to dairy cows receiving a fat-deficient basal diet. I. Milk yield and composition. J. Dairy Res. 43: 213-218.

Banks, W., J. L. Clapperton., M. E. Ferrie and A. G. Wilson. 1976b. Effect of feeding fat to dairy cows receiving a fat-deficient basal diet. II. Fatty acid composition of the milk fat. J. Dairy Res. 43: 219-227.

Barbano, D. M. and J. W. Sherbon, 1980. Polyunsaturated protected lipid: effect on triglyceride molecular weight distribution. J. Dairy Sci. 63:731–740.

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

Bauman, D.E., Brown, R.E., Davis, C.L. 1970. Pathways of fatty acid synthesis and reducing equivalent generation in mammary gland of rat, sow, and cow. Arch. Biochem. Biophys. 140: 237–244.

Bauman, D.E., and C.L Davis. 1974. Biosynthesis of milk fat. In: Lactation, Vol. II (B.L. Larson, V.R. Smith, eds.), pp. 31–75, Academic Press, NewYork.

Bauman, D. E., and J. M. Griinari. 2003. Nutritional regulation of milk fat synthesis. Annu. Rev. Nutr. 23:203–227.

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

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

Bickerstaffe R. and E. F. Annison. 1969. Glycerokinase and desaturase activity in pig, chicken and sheep intestinal epithelium. Comp. Biochem. Physiol. 31:47-54.

Bickerstaffe, R., and E.F. Annison. 1970. The desaturase activity of goat and sow mammary tissue. Comp. Biochem. Physiol. 35:653–665.

Bickerstaffe, R., and E.F. Annison. 1971. Triglyceride synthesis in goat and sow mammary tissue. Int. J. Biochem. 2:153–162.

Breckenridge, W. C. and A. Kuksis. 1968. Specific distribution of short-chain fatty acids in molecular distillates of bovine milk fat. J. Lipid Research 9:388-393.

Cant, J. P., DePeters, E. J. & Baldwin, R. L. 1993. Mammary uptake of energy metabolites in dairy cows fed fat and its relationship to milk protein depression. J. Dairy Sci. 76:2254–2265.

Chilliard, Y., F. Glasser, A. Ferlay, L. Bernard, J. Rouel, and M. Doreau. 2007. Diet, rumen biohydrogenation and nutritional quality of cow and goat milk fat. Eur. J. Lipid Sci. Technol. 109:828-855.

Choi, B. R. & Palmquist, D. L. (1996) High fat diets increase plasma cholecystokinin and pancreatic polypeptide, and decrease plasma insulin and feed intake in lactating cows. J. Nutr. 126:2913–2919.

Choi, B., D. Palmquist, and M. Allen. 2000. Cholecystokinin mediates depression of feed intake in dairy cattle fed high fat diets. Domest. Anim. Endocrinol. 19:159–175.

Christensen, R. A., J. K. Dracklev, D. W. Lacount, and J. H. Clark. 1994. Infusion of four long-chain fatty acid mixtures into the abomasum of lactating dairy cows. J. Dairy Sci. 77:1052-1069.

Christie, W.W., Noble, R.C., Clegg, R.A., 1986. The hydrolysis of very low-density lipoproteins and chylomicrons of intestinal origin by lipoprotein lipase in ruminants. Lipids. 21:252–253.

Coleman, R.A., Lee, D.P. 2004. Enzymes of triacylglycerol synthesis and their regulation. Prog. Lipid Res. 43:134–176.

Cooper, S.M., Grigor, M.R. 1980. Fatty acid specificities of microsomal acyltransferases esterifying positions-1 and -2 of acylglycerols in mammary glands from lactating rats. Biochem. J. 187:289–295.

Coppock, C. E., and D. L. Wilks. 1991. Supplemental fat in high- energy rations for lactating cows: Effects on intake, digestion, milk yield and composition. J. Anim. Sci. 69:3826–3837.

Cordle, S. R., Yeaman, S. J., and R.A. Clegg. 1983. Salt-resistant (hepatic) lipase - Evidence for its presence in bovine liver and adrenal cortex. Biochem. Biophys. Acta 753: 213-219.

Corl, B. A., S. T. Butler, W. R. Butler, and D. E. Bauman. Short Communication: Regulation of Milk Fat Yield and Fatty Acid Composition by Insulin. 2006. J. Dairy Sci. 89:4172–4175.

Davis, C. L. 1990. Fats in Animal Feeds. Barnaby Inc., Sycamore, IL.

Dils, R. R. 1986. Comparative aspects of milk fat synthesis. J. Dairy Sci. 69:904-910.

Doreau, M. and A. Ferlay. 1994. Digestion and utilization of fatty acids by ruminants. Anim. Feed Sci. Technol. 45:379-396.

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

Drackley, J. K. 2005. Interorgan lipid and fatty acid metabolism in growing ruminants. Biology of Growing Animals. 3:323-350.

Drackley, J. K., T. R. Overton, G. Ortiz-Gonzalez, A. D. Beaulieu, D. M. Barbano, J. M. Lynch, and E. G. Perkins. 2007. Responses to increasing amounts of high-oleic sunflower fatty acids infused into the abomasum of lactating dairy cows. J. Dairy Sci. 90:5165–5175.

Eberle, D., B. Hegarty, P. Bossard, P. Ferre, and F. Foufelle. 2004. SREBP transcription factors: master regulators of lipid homeostasis. Biochimie. 86:839-848.

Emery, R. S. 1973. Biosynthesis of milk fat. J. Dairy Sci. 56:1187-1195.

Enjalbert, F., Nicot, M. C., Bayourthe, C., Vernay, M. & Moncoulon, R. 1997. Effects of dietary calcium soaps of unsaturated fatty acids on digestion, milk composition and physical properties of butter. J. Dairy Res. 64:181–195.

Enjalbert, F., Nicot, M. C., Bayourthe, C., and Moncoulon, R. 1998. Duodenal infusions of palmitic, stearic or oleic acids differently affect mammary gland metabolism of fatty acids in lactating dairy cows. J. Nutr. 128:1525-1532.

Ferlay, A., Chabrot, J., Elmeddah, Y. & Doreau, M. (1993) Ruminal lipid balance and intestinal digestion by dairy cows fed calcium salts of rapeseed oil fatty acids or rapeseed oil. J. Anim. Sci. 71:2237–2245.

Firkins, J. L., and M. L. Eastridge. 1994. Assessment of the effects of iodine value on fatty acid digestibility, feed intake, and milk production. J. Dairy Sci. 77:2357–2366

Freeman, C. P. 1984. The digestion, absorption and transport of fats- non-ruminants. In: J. Wiseman (Ed.) Fats in Animal Nutrition. pp. 105-121. Butterworths, London, UK.

Gerson, T., A. John, and A. S. D. King. 1985. The effects of dietary starch and fibre on the in vitro rates of lipolysis and hydrogenation by sheep rumen digesta. J. Agric. Sci. 105:27-30.

Glasser, F., M. Doreau, A. Ferlay, and Y. Chilliard. 2007. Technical note: Estimation of milk fatty acid yield from milk fat data. J. Dairy Sci. 90:2302–2304.

Glasser, F., A. Ferlay, M. Doreau, P. Schmidely, D. Sauvant and Y. Chilliard. 2008. Long-chain fatty acid metabolism in dairy cows: A meta-analysis of milk fatty acid yield in relation to duodenal flows and de novo synthesis. J. Dairy Sci. 88:2771–2785.

Grummer, R. R. 1991. Effect of feed on the composition of milk fat. 1991 J. Dairy Sci. 74:3244-3257.

Grunnet, I., Knudsen, J. 1981. Direct transfer of fatty acids synthesized 'de novo' from fatty acid synthetase into triacylglycerols without activation. Biochem. Biophys. Res. Com. 100:629–636.

Hansen, J.K., and J. Knudsen. 1980. Transacylation as a chain-termination mechanism in fatty acid synthesis by mammalian fatty acid synthetase. Synthesis of butyrate and hexanoate by lactating cow mammary gland fatty acid synthetase. Biochem. J. 186: 287–294.

Hansen, H.O., I. Grunnet, and J. Knudsen. 1984a. Triacylglycerol synthesis in goat mammary gland. The effect of ATP, Mg^{2+} and glycerol 3-phosphate on the esterification of fatty acids synthesized de novo. Biochem J. 220:513–519.

Hansen, H.O., Grunnet, I., and J. Knudsen. 1984b. Triacylglycerol synthesis in goat mammary gland. Factors influencing the esterification of fatty acids synthesized de novo. Biochem J. 220:521–527.

Hansen, H. O. and J. Knudsen. 1987a. Effect of exogenous long-chain fatty acids on lipid biosynthesis in dispersed ruminant mammary gland epithelial cells: Esterification of long-chain exogenous fatty acids. J. Dairy Sci. 70:1344 – 1349.

Hansen, H. O. and J. Knudsen. 1987b. Effect of exogenous long-chain fatty acids on individual fatty acid synthesis by dispersed ruminant mammary gland cells. J. Dairy Sci. 70:1350-1354.

Harfoot, C. G. and G. P. Hazlewood. 1997. Lipid metabolism in the rumen. Pages 382-426 in The Rumen Microbial Ecosystem (Second Edition). P. N. Hobson and D. S. Stewart, ed. Chapman & Hall, London.

Harvatine, K. J. and D. E. Bauman. 2006. SREBP1 and thyroid hormone responsive spot 14 (S14) are involved in the regulation of bovine mammary lipid synthesis during diet-induced milk fat depression and treatment with CLA. J. Nutr. 136:2468-2474.

Harvatine, K. J., and M. S. Allen. 2005. The effect of production level on feed intake, milk yield, and endocrine responses to two fatty acid supplements in lactating cows. J. Dairy Sci. 88:4018–4027.

Harvatine, K. J. and M. S. Allen 2006. Effects of fatty acid supplements on milk yield and energy balance of lactating dairy cows. J. Dairy Sci. 89:1092-1103.

Harvatine, K. J., Y. R. Boisclair, and D. E. Bauman. 2009. Recent advances in the regulation of milk fat synthesis. Animal 3:40-54.

Heipieper, H.J., J. Fisher, and F. Meinhardt. 2010. *cis-trans* isomerase of unsaturated fatty acids: An immediate bacterial adaptive mechanism to cope with emerging membrane perturbation caused by toxic hydrocarbons. Pages 1605–1614 in Handbook of Hydrocarbon and Lipid Microbiology K. N. Timmis, ed. Springer-Verlag Berlin Heidelberg.

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

Jensen, R. G., A. M. Ferris, and C. J. Lammi-Keefe. 1991. The composition of milk fat. J. Dairy Sci. 74:3228-3243.

Jensen, R.G. 2002. Invited Review: The composition of bovine milk lipids: January 1995 to December 2000. J. Dairy Sci. 85:295–350.

Kinsella, J.E. 1970. Stearic acid metabolism by mammary cells. J. Dairy Sci. 53:1757–1765.

Kronfeld, D. S. 1976. The potential importance of the proportions of glucogenic, lipogenic, and aminogenic nutrients in regard to the health and productivity of dairy cows. Beihefte zur Zietschrift fuer Tierphysiologie, Tierernaehrung und Futtermittelkunde. 7:3–26.

Kronfeld, D.D. 1982. Major metabolic determinants of milk volume, mammary efficiency. and spontaneous ketosis in dairy cows. J. Dairy Sci. 65:2204–2212.

Kumar, S., T. I. Pynadath, and K. Lalka. 1960. Location of butyric acid in bovine milk triglycerides. Biochim. Biophys. Acta. 42:373–374.

Lessire, M., M. Doreau, and A. Aumaitre. 1992. Digestion and metabolism of fats in domestic animals. In: Manuel des Corps Gras. pp. 683-694. Lavoisier, Paris.

Lin, C.Y., Kumar, S. 1972. Pathway for the synthesis of fatty acids in mammalian tissues. J. Biol. Chem. 247:604–606.

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

Lock, A. L., C. L. Preseault, J. E. Rico, K. E. DeLand, and M. S. Allen. 2013. Feeding a C16:0-enriched fat supplement increased the yield of milk fat and improved feed efficiency. J. Dairy Sci. Submitted.

Lock, A.L., K.J. Harvatine, J.K. Drackley, and D.E. Bauman. 2006. Concepts in fat and fatty acid digestion in ruminants. Proc. Intermountain Nutr. Conf. pp. 85-100.

Lourenço, M., E. Ramos-Morales, and R. J. Wallace. 2010. The role of microbes in rumen lipolysis and biohydrogenation and their manipulation. Animal 4:1008-1023.

Maia, M. R. G., L. C. Chaudhary, L. Figueres, and R. J. Wallace. 2007. Metabolism of polyunsaturated fatty acids and their toxicity to the microflora of the rumen. Antonie van Leeuwenhoek 91:303–314.

Maia, M. R. G., L. C. Chaudhary, C. S. Bestwick, A. J. Richardson, N. McKain, T. R. Larson, I. A. Graham, and R. J. Wallace. 2010. Toxicity of unsaturated fatty acids to the biohydrogenating ruminal bacterium, Butyrivibrio fibrisolvens. BMC Microbiology 10:52.

Marshall, M. O., and J. Knudsen. 1979. Specificity of diacylglycerol acyltransferase from bovine mammary gland, liver and adipose tissue towards acyl-CoA esters. Eur. J. Biochem. 94:93-98.

Marshall, M.O., and J. Knudsen. 1980. Factors influencing the in vitro activity of diacylglycerol acyltransferase from bovine mammary gland and liver towards butyryl-CoA and palmitoyl-CoA. Biochim. Biophys. Acta 617:393–397.

McCarthy, R. D., S. Patton, and L. Evans. 1960. Structure and synthesis of milk fat. II. Fatty acid distribution in the triglycerides of milk and other animal fats. J. Dairy Sci. 43: 1196–1201.

Mellenberger, R.W., Bauman, D.E. 1974. Fatty acid synthesis in rabbit mammary tissue during pregnancy and lactation. Biochem. J. 138:373–379.

Miller, A. L., M. E. Geroch, and H.R. Levy. 1970. Rat mammary-gland acetyl-coenzyme A carboxylase. Interaction with milk fatty acids. Biochem. J. 118:645-657.

Moore, J. H. and W. W. Christie. 1984. Digestion, absorption and transport of fats in ruminant animals. In: J. Wiseman (Ed.) Fats in Animal Nutrition. pp. 123-149. Butterworths, London, UK.

Mosley, S. A.,E. E., B. Hatch, J. I. Szasz, A. Corato, N. Zacharias, D. Howes, M. A. McGuire. 2007. Effect of varying levels of fatty acids from palm oil on feed intake and milk production in Holstein cows. J. Dairy Sci. 90:987-93.

Nandedkar, A.K.N., E.W. Schirmer, T.I. Pynadath, and S. Kumar. 1969. Biosynthesis of fatty acid in mammary tissue. I. PuriWcation and properties of fatty acid synthetase from lactating- goat mammary tissue. Arch. Biochem. Biophys. 134:554–562.

Noble, R. C. 1981. Digestion, transport and absorption of lipids. In: W. W. Christie (Ed.) Lipid metabolism in ruminant animals. pp. 57-93. Pergamon Press Ltd., Oxford, UK.

NRC. 2001. Nutritional Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Sci., Washington, DC.

Oftedal, O.T., Iverson, S.J. 1995. Comparative analysis of nonhuman milks. A. Phylogenetic variation in the gross composition of milks. In: Handbook of Milk Composition (R.G. Jensen, ed.), pp. 749–789, Academic Press, San Diego.

Palmquist, D.L., C.L. Davis, R.E. Brown, and D.S. Sachan, 1969. Availability and metabolism of various substrates in ruminants. V. Entry rate into the body and incorporation into milk fat of D(-)β-hydroxybutyrate. J. Dairy Sci. 52:633–638.

Palmquist, D.L., Conrad. H.R. 1971. Origin of plasma fatty acids in lactating cows fed high grain or high fat diets. J. Dairy Sci. 54:1025–1033.

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

Palmquist, D.L., Beaulieu, A.D., Barbano, D.M. 1993. Feed and animal factors influencing milk fat composition. J. Dairy Sci. 76:1753–1771.

Palmquist, D. L., A. L. Lock, K. J. Shingfield, and D. E. Bauman. 2005. Biosynthesis of conjugated linoleic acid in ruminants and humans. Adv. Food Nutr. Res. 50:179-217.

Palmquist, D. L. 2006. Milk fat: Origin of fatty acids and influence of nutritional factors thereon. In Advanced Dairy Chemistry Volume 2 Lipids (pp. 43-92). Springer US.

Piantoni, P., A. L. Lock, and M. S. Allen. 2013. Palmitic acid increased yields of milk and milk fat and nutrient digestibility across production level of lactating cows. J. Dairy Sci. (submitted).

Rabiee, A. R., K. Breinhild, W. Scott, H. M. Golder, E. Block, and I. J. Lean. 2012. Effect of fat additions to diets of dairy cattle on milk production and components: a meta-analysis and meta-regression. J. Dairy Sci. 95:3225-3247.

Reist, M., D. Erdin, D. von Euw, K. Tschuemperlin, H. Leuenberger, Y. Chilliard, H.M. Hammon, C. Morel, C. Philipona, Y. Zbinden, N. Kuenzi, and J.W. Blum. 2002. Estimation of energy balance at the individual and herd level using blood and milk traits in high-yielding dairy cows. J. Dairy Sci. 85:3314–3327.

Relling, A. E., and C. K. Reynolds. 2007. Feeding rumen-inert fats differing in degree of saturation decreases intake and increases plasma concentrations of gut peptides in lactating dairy cows. J. Dairy Sci. 90:1506–1515.

Rico, D. E., and K. J. Harvatine. 2011. Effect of high palmitic acid fat supplement on ruminal fermentation and milk production in high- and low-producing dairy cows. J. Dairy Sci. 94 (E-Suppl. 1):202 (Abstr.).

Rigout, S., Lemosquet, S., Bach A., Blum, J.W., and H. Rulquin. 2002. Duodenal infusion of glucose decreases milk fat production in grass silage-fed dairy cows. J. Dairy Sci. 85: 2541–2550.

Rudolph, M. C., J. Monks, V. Burns, M. Phistry, R. Marians, M. R. Foote, D. E. Bauman, S. M. Anderson, and M. C. Neville. 2010. Sterol Regulatory Element Binding Protein (Srebf-1) and Dietary Lipid Regulation of Fatty Acid Synthesis in the Mammary Epithelium. Am. J. Physiol. Endocrinol. Metab. 299:E918–E927.

Schauff, D., J. Clark, and J. Drackley. 1992. Effects of feeding lactating dairy cows diets containing extruded soybeans and calcium salts of long-chain fatty acids. J Dairy Sci. 75:3003–3019.

Shirley, J.F., Emery, R.S., Convey, E.M., Oxender, W.D. 1973. Enzymic changes in bovine adipose and mammary tissue, serum and mammary tissue hormonal changes with initiation of lactation. J. Dairy Sci. 56:569–574.

Smith, S.J., Cases, S., Jensen, D.R., Chen, H.C., Sande, E., Tow, B., Sanan, D.A., Raber, J., Eckel, R.H., Farese, R.V. Jr. 2000. Obesity resistance and multiple mechanisms of trigly- ceride synthesis in mice lacking DGAT 1. Nat. Genet. 25:87–90.

Steele, W. J. 1969.The effects of dietary palmitic and stearic acids on milk yield and composition in the cow. J. Dairy Res. 36:369-373.

Steele, W., and J. H. Moore. 1968. The effects of a series of saturated fatty acids in the diet on milk-fat secretion in the cow. J. Dairy Res. 35:361-370.

Storry, J. E., J. A. Rook, A. J. Hall. 1967. The effect of the amount and type of dietary fat on milk fat secretion in the cow. Br. J. Nutr. 21:425-438.

Storry, J. E., A. J. Hail, and V. W. Johnson. 1973. The effects of increasing amounts of dietary tallow on milk-fat secretion in the cow. J. Dairy Res. 40:293-299.

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

Thomson, A.B.R., Hotke, C.A., O'Brien, B.D., Weinstein, W.M. 1983. Intestinal uptake of fatty acids and cholesterol in four animals species and man: Role of unstirred water layer and bile salt micelle. Comp. Biochem. Physiol. 75A:221–232.

Thompson, G.E., Christie, W.W. 1991. Extraction of plasma triacylglycerols by the mammary gland of the lactating cow. J. Dairy Res. 58:251–255.

Ulberth, F., and F. Schrammel. 1995. Accurate quantitation of short-, medium-, and long-chain fatty acid methyl esters by split-injection capillary gas-liquid chromatography. J. Chromatogr. A. 704:455–463.

Van Nevel, C. J. and D. I. Demeyer. 1996. Influence of pH on lipolysis and biohydrogenation of soybean oil by rumen contents in vitro. Reprod. Nutr. Dev. 36:53-63.

Virtanen, A.I. 1966. Milk production of cows on protein-free feed. Studies of the use of urea and ammonium salts as the sole nitrogen source open new important perspectives. J. Dairy Sci. 153:1603–1614.

Wang J. P., Bu, D. P., Wang, J. Q., Huo, X. K., Guo T. J., Wei, H. Y., Zhou, L . Y., Rastani, R. R., Baumgard, L. H., and F. D. Li. 2010. Effect of saturated fatty acid supplementation on production and metabolism indices in heat-stressed mid-lactation dairy cows. J. Dairy Sci. 93: 4121–4127.

Warntjes, J. L., P. H. Robinson, E. Galo, E. J. DePeters, and D. Howes. 2008. Effects of feeding supplemental palmitic acid (C16:0) on performance and milk fatty acid profile of lactating dairy cows under summer heat. Anim. Feed Sci. Technol. 140:241–257.

Wildman, E. E., G. M. Jones, P. E. Wagner, R. L. Boman, H. F. Troutt Jr., T. N. Lesch. 1982. A dairy cow body condition scoring system and its relationship to selected production characteristics. J. Dairy Sci. 65:495-501.

Wright T.C., J.P. Cant, B. W. McBride. 2002. Inhibition of fatty acid synthesis in bovine mammary homogenate by palmitic acid is not a detergent effect. J. Dairy Sci. 85:642-647.