The Pennsylvania State University

The Graduate School

Department of Animal Science

RECOVERY OF NORMAL RUMINAL BIOHYDROGENATION AND DE NOVO FATTY ACID SYNTHESIS FOLLOWING INDUCTION OF MILK FAT DEPRESSION IN DAIRY COWS

A Dissertation in

Animal Science

by

Daniel E. Rico Navarrete

Submitted in Partial Fulfillment
of the Requirements
for the Degree of

Doctor of Philosophy

May 2013

The dissertation of Daniel E. Rico Navarrete was reviewed and approved* by the following:

Kevin J. Harvatine
Assistant Professor of Nutritional Physiology
Dissertation Advisor
Chair of Committee

Alexander Hristov
Associate Professor of Dairy Nutrition

Arlyn J. Heinrichs
Professor of Dairy Science

Michael Green
Professor of Nutrition Science and Physiology

Terry D. Etherton
Distinguished Professor of Animal Nutrition
Head of the Department of Animal Science

*Signatures are on file in the Graduate School

ABSTRACT

The opportunity exists to improve production efficiency on dairy farms by implementation of strategies conducive to maximal milk fat yield. Diet-induced milk fat depression (MFD) was first recognized over 150 years ago, and represents an important economic loss to dairy farms because it results in milk fat production bellow the genetic potential of animals. This condition is the result of altered ruminal fermentation that leads to the formation of specific intermediates of biohydrogenation (BH) of unsaturated fatty acids that reduce mammary lipid synthesis. The observation of time course of recovery of milk fat synthesis and the timing of associated changes following an episode of MFD provides insight into the mechanism. Furthermore this knowledge is of great importance to management of the condition, as field nutritionists would benefit from understanding the time required to observe recovery after a dietary correction. The set of studies in the present dissertation characterized the time course of induction and recovery from MFD to be between 7 to 13 days and between 11 to 15 days, respectively. In addition, we identified the reduction in dietary concentration of polyunsaturated FA as the most important factor to correct in order promote restoration of normal BH and recover milk fat synthesis. Ruminal adaptation was corroborated as the rate-limiting step in the rate of recovery from MFD and the potential for dietary probiotics use to accelerate recovery of milk de novo fatty synthesis was demonstrated. Together, these experiments provide a set of key observations in regards to dietary factors associated to the recovery of milk fat synthesis and the restoration of normal ruminal biohydrogenation pathways.

Keywords: Biohydrogenation, dairy cows, milk fat depression, recovery.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

ACC	Acetyl-CoA carboxylase
ALA	Alpha linolenic acid
AR(1)	Autoregressive
ARH(1)	Heterogeneous autoregressive
ßHBA	Beta-hydroxybutyric acid
BH	Biohydrogenation
BW	Body weight
CLA	Conjugated linoleic acid
CP	Crude protein
DIM	Days in milk
DM	Dry matter
DMI	Dry matter intake
ECM	Energy corrected milk
FA	Fatty acids
FASN	Fatty acid synthase
FCM	Fat corrected milk
FID	Flame ionization detector
FCM	Fat corrected milk
GC	Gas chromatography
GL	Glycolipids
HO	High oil
INOC	Inoculation
LA	Linoleic acid

LCFA	Long chain fatty acids
LF	Low forage
LF/HO	Low forage, high oil
MFD	Milk fat depression
MN	Monensin
MUFA	Monounsaturated fatty acids
MUN	Milk urea nitrogen
NDF	Neutral detergent fiber
NEFA	Non esterified fatty acids
NEL	Net energy of lactation
NFC	Non fiber carbohydrates
PA	Palmitic acid
PL	Phospholipid
PUFA	Polyunsaturated fatty acids
SCD	Stearoyl-CoA desaturase
SEM	Standard error of the mean
SFA	Saturated fatty acids
SREBP	Sterol regulatory element binding protein
TG	Triglycerides
TMR	Total mixed ration
UFA	Unsaturated fatty acids
VFA	Volatile fatty acids

ACKNOWLEDGEMENTS

First of all, I would like to thank Dr. Harvatine for this dedicated guidance and advisory throughout my entire Ph.D. program. Dr. Harvatine is an extremely brilliant and disciplined scientist, who has been paramount to my training in science, helping me improve in skills ranging from critical thinking, to experimental design, to statistics, to scientific writing. In addition, he is a very kind person and great role model of a successful, yet modest scientist.

I would also like to thank the members of my graduate committee Dr. Hristov, Dr. Heinrichs, and Dr. Green because of their valuable guidance during my graduate program. Through interactions in the classroom, journal club, and casual conversations, I have had the opportunity to learn a lot from them in diverse areas of science. I greatly value their insight and technical contributions to make this a better document.

To Dr. Harvatine's technicians Jackie Ying and Andrew Clarke, for their valuable technical assistance during numerous experiments. To my fellow lab mates Adam Niu, Whitney Rottman, Natalie Urrutia, Dr. Lying Ma, and Dr. Dimas de Oliveira, and to my fellow graduate students Javier Suarez, Alanna Kmicikewycz, Dr. Gustavo Lascano, and Dr. Chan He Lee for their friendship and valuable help with sampling during some of our studies.

In the same way, I would like to thank the staff at the PSU dairy barns staff for the continuous care of our animals as well as for their technical assistance during all of these studies.

To my brother Eduardo, who is a bright scientist with whom I been lucky to share the same level of interest in science, and whose insight and encouragement have been very important during my time in graduate school. Lastly, to my parents, the hardest working people I have ever met, I owe all of my accomplishments to their support and love.

To my parents.…

Chapter 1

Introduction

Fat is an important component of milk and other dairy products [See Bauman and Griinari (2001)]. Briefly, milk fat influences the physical properties, manufacturing characteristics and sensory attributes of dairy products. Moreover, milk fat is an important source of energy in human diets, although limited consumption has traditionally been recommended because of the concentration of saturated FA (SFA) and low concentration of unsaturated FA (UFA) normally found in milk fat (Jensen et al., 1991)]. Interestingly, new studies and re-evaluation of previous research have challenged the dogma of the association between saturated fat intake and cardiovascular disease (Mensink et al., 2003; Siri-Tarino et al., 2010). Furthermore, the presence of potentially health promoting bioactive FA unique to ruminant milk fat, have improved the appreciation of milk as a functional food (Lock and Bauman, 2004). Lastly, milk fat plays a fundamental part in production efficiency and profitability of dairy farms.

Of all of the major components of milk, fat is the most variable and is affected by several factors including diet, physiological state, and genetics [See review by Palmquist (2006)]. In particular, milk fat synthesis and composition are very responsive to nutritional factors such as dietary fiber and PUFA concentration. First described over a century and a half ago, diet-induced milk fat depression (MFD) represents a challenge to the efficiency and profitability of dairy production systems. Classical MFD is characterized by a reduction of up to 50% in milk fat yield with no concomitant reduction

in the synthesis of other milk components, and is often observed in ruminants fed low fiber or high oil diets (Bauman and Griinari, 2001).

Extensive research spanning decades provides a clear understanding of the cause of MFD, however, MFD is still commonly observed on dairy farms mainly because of the intent to maximize milk production by increased use of non-forage energy sources. For decades, the mechanism behind MFD eluded scientists as several theories were proposed. However, Bauman and Griinari (2001) postulated the Biohydrogenation Theory, which provides a unifying concept explaining how dietary factors can cause shifts in ruminal microbes and result in increased formation of specific intermediates of biohydrogenation (BH) that reduce mammary fat synthesis. Interestingly, recovery from MFD has received little attention and has not been directly investigated.

The main objective of this dissertation was to provide strategies to improve recovery milk fat synthesis following MFD. Milk fat concentration and yield, milk fat de novo synthesized FA concentration, and changes in milk trans isomer profile were the predominant response variables utilized to asses recovery because of their well characterized association with MFD. First, we conducted a high-resolution time course experiment and determined that both induction of and recovery from MFD occur progressively and are complete 14 to 18 d following changes in both dietary fiber and PUFA concentration. Additionally, the importance of ruminal adaptation as the rate-limiting step in both induction of and recovery from MFD was corroborated. Secondly, the specific role of correction of dietary fiber and PUFA on the rate of recovery from MFD was investigated and results demonstrated that dietary PUFA concentration is the

predominant factor impacting the rate of recovery. Thirdly, the impact of monensin on the rate of recovery from MFD was investigated. Monensin decreased the recovery of preformed FA, but had only slight effects on the recovery of normal rumen biohydrogenation and de novo FA synthesis. Lastly, a modest acceleration of recovery of de novo FA synthesis and of recovery of normal BH pathways was observed in response to ruminal inoculation with rumen digesta from non-MFD cows. Collectively, this series of experiments provides key insight into the timing of the response of milk fat to changes in diet and has identified key factors important to accelerate recovery of normal ruminal BH and milk fat synthesis.

Chapter 2

Literature Review

Milk Fat Synthesis and its Regulation

Lactation is a demanding physiological process that requires a series of coordinated changes in nutrient partitioning for its support (Bauman and Currie, 1980). Milk fat synthesis is of particular importance during lactation since milk fat represents about half of the energy content of milk (Emery, 1973). Compared to other components, milk fat is the most variable between 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 (Dils, 1986; Jensen et al., 1991). Bovine milk fat concentration ranges from 3.7 to 4.1% (wt/vol) and is predominantly composed of triglycerides (98%), with remainder being comprised phospholipids, diglycerides, and cholesterol (Jensen, 2002). Milk fat is the most complex of all natural fats, as more than 400 FA, differing primarily in level of unsaturation and chain length, have been detected in bovine milk (Jensen, 2002).

Milk lipids arise from de novo synthesis in mammary epithelial cells and from the uptake of preformed long chain fatty acids (LCFA) from blood (Emery, 1973). De novo FA synthesis accounts for most of the 4 to 14-carbon and about half of the 16-carbon FA in milk fat. Mammary synthesis of FA is carried out from acetate and β-hydroxybutyrate by acetyl CoA carboxylase (ACC) and fatty acid synthase [FASN; (Emery, 1973; Harvatine et al., 2009a)]. These and other key lipogenic enzymes are regulated by

transcription factors such as the sterol regulatory binding-element protein (SREBP) family (Eberle et al., 2004). Work in both the cow and mouse have highlighted the importance of SREBP 1 in the regulation of milk fat synthesis (Harvatine and Bauman, 2006; Rudolph et al., 2010), although other transcription factors are likely involved [See Review by Harvatine et al. (2009a)]. Both the gastrointestinal absorption of dietary FA and the FA mobilization from adipose tissue contribute to the circulating preformed FA pool, and account for the remaining 16-carbon and LCFA in milk fat (Emery, 1973). Subsequently, both de novo and preformed FA are esterified into triglycerides in the mammary epithelial cell and secreted as membrane coated droplets known as milk fat globules (Heid and Keenan, 2005).

Ruminal Metabolism of Dietary Lipids

The ruminal metabolism of dietary lipids has major implications on the profile of FA available for absorption and subsequent secretion in milk fat. Typically, diets fed to ruminants include forages and cereal grains, which contain fats mainly in the form of triglycerides and galactolipids, respectively. In addition, these dietary lipids are abundant in unsaturated FA such as linolenic acid (LA; *cis*-9, *cis*-12, C18:2) and α-linolenic acid (ALA; *cis*-9, *cis*-12, *cis*-15, C18:3). However, milk FA are mostly saturated [~70%; (Jensen, 2002)], which illustrates the extensive metabolism of lipids in the rumen.

Dietary lipids entering the rumen are subjected to a series of metabolic reactions (Illustrated in Error! Reference source not found.**)**. Specifically, ingested dietary lipids ndergo two sequential processes, lipolysis and biohydrogenation [BH; see reviews by

Jenkins (1993) and Harfoot and Hazlewood (1997)]. Lipolysis refers to the hydrolysis of the ester bonds found 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). In particular, lipase activity on triglycerides has been reported for the bacterium *Anareovibrio lipolytica*, but hydrolysis of phospholipids (PL) and glycolipids (GL) seems to be carried out by *Butyrivibrio*-like species (Lourenço et al., 2010). In addition, although the rate and extent of lipolysis 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; Van Nevel and Demeyer, 1996) and by increasing dietary fat concentration (Beam et al., 2000).

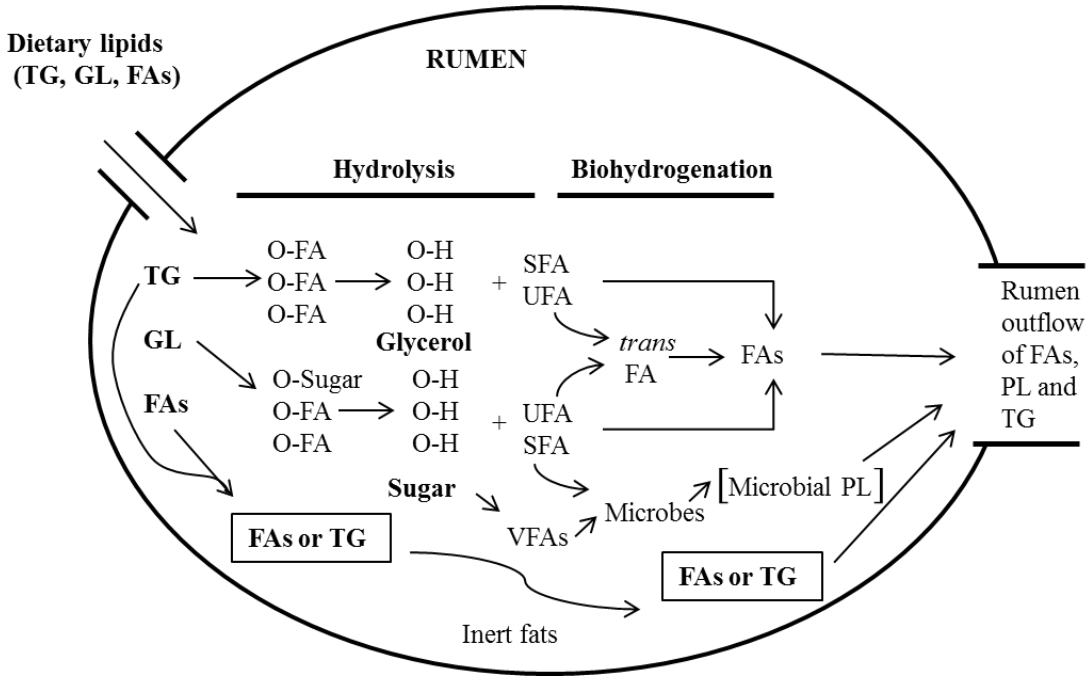


Figure 2-1. Metabolism of dietary lipids in the rumen. Abbreviations: Triglycerides (TG), glycolipids (GL), phospholipids (PL), trans fatty acids (trans FA), mixture of fatty acids (FAs), saturated fatty acids (SFA), unsaturated fatty acids (UFA), and volatile fatty acids (VFA). Adapted from Davis (1990).

Following lipolysis, saturated fatty acids (SFA) remain unmodified, whereas unsaturated fatty acids (UFA) such as LA (C18:2) rapidly undergo BH by ruminal microbes. Biohydrogenation is a multi-step process that results in formation of stearic acid (C18:0; removal of double bonds by hydrogenation). This process produces involves several isomerization and reduction steps and numerous intermediates, including positional *trans* 18:1 FA and conjugated linoleic acid (CLA) isomers, are formed (Harfoot and Hazlewood, 1997; Lock and Bauman, 2004; Chilliard et al., 2007). The predominant pathways of ruminal BH of LA (*cis*-9, *cis*-12, C18:2) and ALA (*cis*-9, *cis*-12, *cis*-15, C18:3) are show in Figure 2-2, although other pathways are clearly utilized. Currently, the predominant pathway of BH is thought to be dependent on the active microbial population, which is altered by dietary factors such as dietary concentration of fiber and PUFA (Chilliard et al., 2007; Jenkins et al., 2008; Lourenço et al., 2010).

Bovine milk fat may contains more than 20 CLA isomers, although *cis*-9, *trans*-11 CLA (also known as rumenic acid) is the predominant isomer and accounts for ~75-90% of the total CLA (Lock and Bauman, 2004). *Cis*-9, *trans*-11 CLA has been the focus of significant research efforts for nearly two decades because of its potential beneficial effects on human health, including cardiovascular disease and cancer (Kritchevsky, 2000; Lock and Bauman, 2004; Pariza, 2004). Other CLA isomers, such as *trans*-10, *cis*-12 CLA and *trans*-9, *cis*-11 CLA, have received interest because of their ability to specifically inhibit fat synthesis, and especially because of their effectiveness in inhibiting milk fat synthesis (Baumgard et al., 2000; Perfield et al., 2007). In particular, *trans*-10, *cis*-12 CLA has been extensively studied, and its potent anti-lipogenic effects

have been observed across several animal models (Foote et al., 2010; Kennedy et al., 2010; Harvatine and Bauman, 2011).

The geometric isomers of 10,12 CLA are synthesized ruminally by different enzymatic mechanisms and bacterial species compared to 9,11 CLA isomers (Wallace et al., 2007), and changes in dietary composition such as low fiber and or/ high oil diets have been shown to elicit a progressive shift in BH pathways towards increased *trans*-10, *cis*-12 CLA and *trans*-10 18:1 (Bauman and Griinari, 2003; Shingfield et al., 2006). *Trans*-11 and *trans*-10 18:1 are downstream intermediates in the BH of *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA, respectively (Figure 2-2), as they result from the hydrogenation of their parent isomers. The fore mentioned *trans* C18:1 isomers are often used as indicators of the shift in BH pathways as their concentration is higher and provides a more accurate analytical assessment. Lastly, *trans* 18:1 isomers are hydrogenated to yield stearic acid (C18:0). However, the high concentration of *trans* 18:1 isomers has been interpreted as a limitation in the capacity of the final reaction, however, Harvatine and Allen (2006) determined that the rate of the hydrogenation of *trans* isomers was high, but the large synthesis of *trans* isomers provides a substantial amount of substrate for the reaction.

Similarly to lipolysis, the rates of BH are also negatively affected by excess dietary fat and low ruminal pH (Van Nevel and Demeyer, 1996; Beam et al., 2000). The mechanism for the negative impact of dietary fat on lipid metabolism is not well understood, but it has been proposed it could be the result of increased coating of feed particles or direct toxic effects of UFA on ruminal microorganism (Jenkins, 1993; Maia et al., 2007). Linoleic acid (18:2) negatively impacts the membrane integrity of several

ruminal bacteria species and their growth (Maia et al., 2007; Maia et al., 2010), and the fore mentioned shift in BH pathways is the reflection of the changes in sensitive ruminal bacteria species (Lourenço et al., 2010; Weimer et al., 2010b).

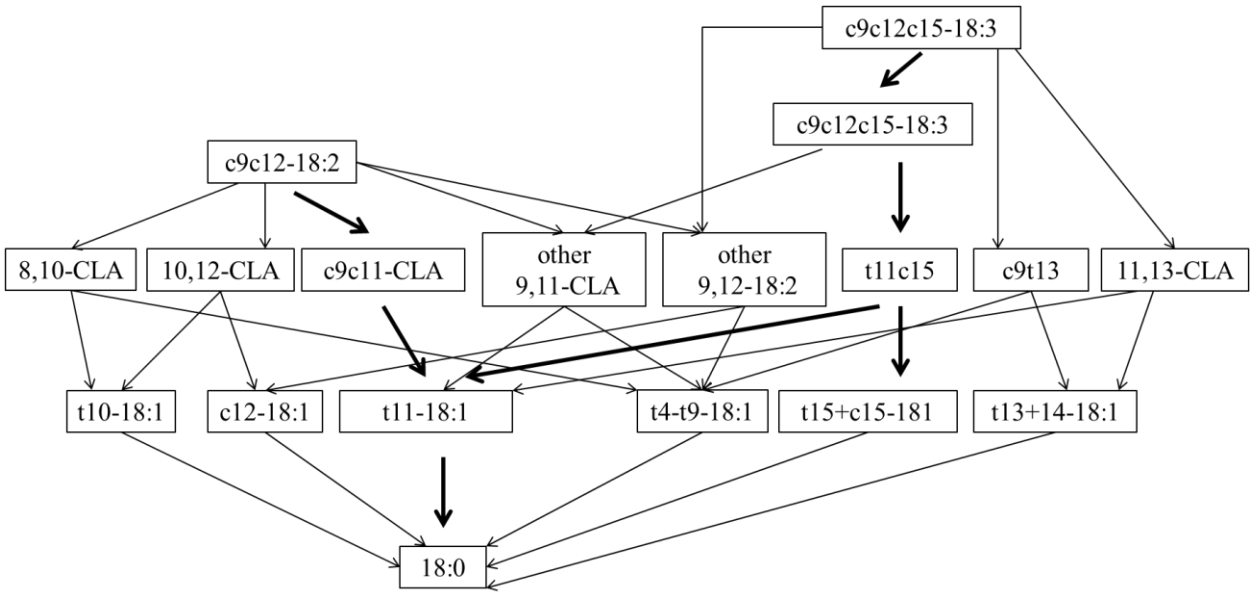


Figure 2-2. Pathways of ruminal biohydrogenation of LA (*cis*-9, *cis*-12, C18:2) and ALA (*cis*-9, *cis*-12, *cis*-15, C18:3). Thick arrows represent the predominant pathways. Lack of *cis* or *trans* configuration indicates that various *cis-cis, cis-trans,* and *trans-trans* configurations are possible. Adapted from Chilliard et al. (2007).

Historical Theories of MFD

Diet-induced MFD results from the interaction between dietary nutrients, ruminal fermentation, and mammary tissue metabolism. Davis and Brown (1970) grouped MFD diets into two main clusters: 1) high grain/low roughage diets that provide large amounts of highly fermentable carbohydrates and low amounts of fibrous feeds and 2) Diets that provide high amounts of unsaturated FA (e.g. oilseeds, high oil byproducts, etc.). However, the dietary effects of these components cannot be easily separated, since both can modify the ruminal environment, interfere with ruminal microbial processes, and cause MFD (Bauman and Griinari, 2001). The numerous theories proposed over the years to explain MFD have been extensively reviewed and discussed by others (Bauman and Griinari, 2001; 2003; Shingfield and Griinari, 2007) and the following section briefly highlights their main points.

The low milk fat syndrome was documented by Boussingault in 1845, who observed MFD in cows fed beets and proposed that a deficient supply of dietary fat was responsible (Van Soest, 1994). However, later work showed that although a limited supply of dietary FA can reduce milk yield, it has little impact on milk fat concentration (Bauman and Griinari, 2001). Given the importance of acetate for mammary synthesis of fat in ruminants (Bauman et al., 1970) and the observed reduction in the molar ratio of acetate to propionate in animals fed highly fermentable diets, acetate deficiency was proposed as an explanation for MFD (Davis and Brown, 1970). However, kinetic studies using isotope dilutions showed that ruminal production of acetate is not changed when feeding high grain diets that induced MFD, and that the observed reduction in molar

proportions of acetate is due to an increase in propionate synthesis (Bauman and Griinari, 2003). Furthermore, ruminal infusion of acetate had minimal effects on milk fat yield (Davis and Brown, 1970). Interestingly, changes in the ruminal VFA profile are insignificant when high oil diets are fed, although an increase in milk *trans* FA is observed (Bauman and Griinari, 2001; 2003), suggesting a common denominator in MFD between high oil and low forage diets.

The glucogenic-insulin theory postulated that MFD is caused by a shortage of nutrients for mammary lipid synthesis caused by elevated insulin (McClymont and Vallance, 1962; Annison et al., 1974). Propionate is the main source of carbon for glucose in ruminants (Seal and Reynolds, 1993), and the increased absorption of propionate while feeding highly fermentable diets results in increased plasma glucose and increased insulin secretion (Annison et al., 1974). According to glucogenic-insulin theory, there is a shortage of nutrients available to the mammary gland because of insulin-induced decrease in adipose tissue lipolysis and increased adipose tissue uptake and use of acetate, β-hydroxybutyric acid (BHBA), and dietary LCFA (Rigout et al., 2002; Bauman and Griinari, 2003). Davis and Brown (1970) summarized 13 propionate infusion experiments that reported milk fat yield reductions of 0 to 14%. Likewise, a summary of 24 glucose infusion experiments reported similar variability in milk fat yield [+4 to -16%; (Bauman and Griinari, 2001)]. In a recent meta-analysis, Maxin et al. (2011) observed negative associations between milk fat yield and post-ruminal infusions of glucose and propionate, suggesting a role of these nutrients in explaining milk fat yield. However, propionate infusions decreased the yield of milk C4:0, C6:0, C8:0, and C18:0 only, whereas glucose infusions decreased the yield of milk C4:0, C6:0, and

preformed FA, and increased the yield of other de novo synthesized FA (Maxin et al., 2011). These observations are not in agreement with classical diet-induced MFD in which the phenotype is characterized by reductions in all de novo FA (Bauman and Griinari, 2003). Importantly, in the Maxin et al. (2011) meta-analysis, the studies included in the propionate and glucose response data sets used animals in early lactation (115 ± 48 and 73 ± 42 DIM, respectively). Bauman and Griinari (2003) proposed that the energy status of the cow was an important factor in the response to propionate and glucose infusions during early lactation (negative energy balance). Specifically, insulin is expected to have a larger effect on cows in a negative energy balance with higher level of mobilization because of the large decrease in circulating lipids (Bauman et al., 1988). This is supported by the observation that hyperinsulinemic-euglycemic clamp experiments using post-peak cows reported an average milk fat yield reduction of 5% (Griinari et al., 1997; Mackle et al., 1999), whereas in cows in early lactation, a reduction between 27 and 35% was observed (Bauman and Griinari, 2003; Corl et al., 2006). Lastly, the reductions in milk fat yield observed in these experiments were due to reduced preformed FA, which is opposite from classical MFD which results in a greater reduction in de novo synthesized FA (Bauman and Griinari, 2003; Corl et al., 2006).

Trans FA are formed in the rumen incomplete BH of UFA as discussed above (Error! Reference source not found.). Davis and Brown (1970) observed that total *trans* 18:1 FA were elevated in milk fat of MFD cows, and proposed a possible association between total *trans* 18:1 FA and MFD. Although several studies observed the direct relationship between MFD and milk total *trans* C18:1 FA, inconsistencies were reported, as others showed no corresponding reductions in milk fat yield (Bauman and Griinari,

2003). Griinari et al. (1998) showed that MFD was specifically associated to increased milk *trans*-10 C18:1 rather than to all *trans* C18:1 isomers, and later proposed the Biohydrogenation Theory (Bauman and Griinari, 2001). According to this theory, under some dietary conditions, there is an alteration of the normal pathways of ruminal BH, resulting in the production of unique FA intermediates such as *trans*-10, *cis*-12 CLA that are potent inhibitors of milk fat synthesis (Bauman and Griinari, 2001). Although *trans*-10 C18:1 concentration is strongly associated with milk fat yield, it has not been definitively demonstrated to cause MFD (Lock et al., 2007; Shingfield et al., 2009). Harvatine et al. (2009a) summarized 5 studies on the effect of different doses of pure preparations *trans* C18:1 isomers on milk fat yield, and reported no effect of *trans*-9, *trans*-10, *trans*-11 or *trans*-12 C18:1, although this remains an area of controversy and is hindered by the availability of pure isomer preparation [e.g. (Shingfield et al., 2009). However, studies have clearly identified that other *trans* FA, such as *trans*-10, *cis*-12 CLA, are potent inhibitors of mammary lipid synthesis in dairy cows (Baumgard et al., 2000; Baumgard et al., 2001; Peterson et al., 2003). de Veth et al. (2004) studied the effects of increasing doses of abomasally infused *trans*-10, *cis*-12 CLA across studies and found a strong curvilinear relationship between the CLA dose and the reduction of milk fat yield. Interestingly, the maximum reduction observed with CLA infusion was ~50%, although much higher doses appear to be toxic and inhibit lactation (Bell and Kennelly, 2003). The reduction in milk fat during diet-induced MFD is not fully explained by *trans*-10, *cis*-12 CLA, and it has been suggested that other still unidentified bioactive FA may be involved in MFD (Shingfield and Griinari, 2007; Harvatine et al., 2009a). Although several conjugated C18:3 (Saebo et al., 2005a; Gervais and Chouinard, 2008) and CLA

isomers (Harvatine et al., 2009a) have been tested, only *cis*-10, *trans*-12 and *trans*-9, *cis*-11 CLA have been shown to cause MFD in dairy cows (Saebo et al., 2005b; Perfield et al., 2007).

Phenotype, Time Course, and Mechanism of MFD

Milk fat depression is a specific reduction in milk fat yield, with no concurrent change in milk yield or yields of other milk components (Bauman and Griinari, 2001). Under other circumstances a reduction in milk fat percent is observed when milk yield is increased, but fat yield remains unchanged; however, this is not considered MFD since the amount of milk fat secreted is unaffected (Bauman and Griinari, 2001). Beyond a marked reduction in milk fat yield, classical MFD is characterized by a concomitant change in the FA profile of milk fat. During classical diet-induced MFD the yield of all FA is decreased, however the yield of milk de novo FA is reduced to a greater extent than preformed FA (Banks et al., 1984; Loor and Herbein, 1998; Harvatine and Bauman, 2011). Additionally, as previously noted, a concurrent increase in the concentration of specific *trans* FA, including *trans*-10 C18:1 and *trans*-10, *cis*-12 CLA is observed. This well described phenotype of diet-induced MFD provides multiple response variables to observe the magnitude and timing of the condition.

Although not well characterized, the study of the time course of induction of and recovery from MFD is important to understanding the mechanism of MFD. The described changes in milk FA composition occur progressively, and the time course of events leading to MFD differs between diet-induced MFD and abomasal infusions of

trans-10, *cis*-12 CLA (CLA-induced MFD). Abomasal and intravenous infusions of *trans*-10, *cis*-12 CLA have been shown to induce a progressive decrease in milk fat concentration and yield that is maximal in 3 to 4 d (Baumgard et al., 2000; Gervais et al., 2009; Harvatine and Bauman, 2011). Interestingly, recovery of milk fat is also rapid and occurs within 4 d following cessation of abomasal infusion of *trans*-10, *cis*-12 CLA (Baumgard et al., 2000), demonstrating the ability of the mammary gland to respond rapidly to this CLA isomer. On the contrary, the near maximal reduction of milk fat yield during MFD takes between 13 and 19 d (Roy et al., 2006; Shingfield et al., 2006), and there is evidence that ruminal adaptation plays an important role in the delayed mammary response during MFD. Satter and Bringe (1969) demonstrated that both the induction of and the recovery from MFD were accelerated when in addition to changing between a high and low foarage diet rumen contents exchanged with cows already adapted to the new diet. A progressive shift of ruminal BH from the normal to the altered pathways occurs during MFD (Shingfield et al., 2006). Briefly, milk concentations of FA isomers of the normal BH pathway (*trans*-11 C18:1 and *cis*-9, *trans*-11 CLA) increase rapidly and peak within 5 to 7 d, whereas isomers of the altered BH pathway (*trans*-10 C18:1 and *trans*-10, *cis*-12 CLA) increase more slowly and reach peak concetrations within 18 to 21 d (Roy et al., 2006; Shingfield et al., 2006; O'Donnell-Megaro et al., 2012). These observations support the theory that changes in rumen enviroment and flora are the rate limiting factor in the onset of MFD.

Both the post-absorptive mechanism of CLA and diet-induced MFD have been extensively studied. In particular, CLA-induced MFD studies are of special value since they allow for investigation of the mechanism without the confounding effects of other

nutrients and metabolites resulting from milk fat depressing diets. Induction of MFD by CLA has no short or long term effects on circulating levels of glucose, NEFA, BHBA, insulin, IGF-1, or leptin [Reviewed by Harvatine et al. (2009a)]. Using tissue explant culture, CLA reduced mammary tissue acetate incorporation into fat and oxidation to CO_2 by 82% and 61%, respectively, demonstrating a functional role of CLA in the mammary gland (Baumgard et al., 2002). Further studies of changes in mammary gene expression have provided key insight into the mechanism of MFD. Consistent with decreased de novo FA synthesis during MFD, both diet- and CLA-induced MFD cause a coordinated downregulation of key mammary gland lipogenic enzymes including ACC and FASN and enzymes involved in lipid uptake such as lipoprotein lipase (Piperova et al., 2000; Ahnadi et al., 2002; Peterson et al., 2003; Harvatine and Bauman, 2006). The expression of other genes such as stearoyl-CoA desaturase (SCD; Δ^9-desaturase) has been shown to decrease during MFD, however, numerous studies have shown inhibition of SCD without concomitant reductions in milk fat yield [reviewed by Harvatine et al. (2009a)]. Milk fat desaturase index is commonly calculated as the product to precursor relationship of the SCD enzyme (Perfield et al., 2006), and will be further discussed in Chapter 3.

Gene expression is regulated by transcription factors, and as a central regulator of lipogenic enzymes, the Sterol Response Element Binding Protein 1 (SREBP1) transcription factor has been demonstrated to be an important regulator of lipid synthesis in the mammary gland [See review by Bauman et al. (2011)]. Expression of SREBP1 is downregulated by *trans*-10, *cis*-12 CLA both in vitro and in vivo (Peterson et al., 2004; Harvatine and Bauman, 2006). Furthermore, the lipid synthesis enzymes downregulated

during CLA-induced and MFD have a sterol response element in their promoter and are regulated by SREBP1 (Harvatine and Bauman, 2006).

Rumen Modifiers and MFD

Dietary components impact the ruminal environment and the predominant microbial population, which in turn affect the predominant pathways of ruminal BH and the onset of MFD (Jenkins et al., 2008; Lourenço et al., 2010). Several dietary interventions such as buffers, Vitamin E, direct fed microbials, and monensin have been shown to affect ruminal fermentation and have the potential to modify the incidence and recovery of MFD.

High concentrate, low forage diets are typically highly fermentable and can result in reductions in ruminal pH and changes in ruminal BH causing MFD (Erdman, 1988; Kalscheur et al., 1997; Khorasani and Kennelly, 2001). The use of buffers such as $NaHCO_3$, MgO, $KHCO_{3,}$ and K_2CO_3 may stabilize rumen pH and minimize the shifts in ruminal fermentation. Decreased ruminal pH has been shown to elicit changes in the microbial population (specifically cellulolytic bacteria) and BH pathways resulting in increased formation of *trans*-10 C18:1 and *trans*-10, *cis*-12 CLA (Qiu et al., 2004; Fuentes et al., 2009). Furthermore, Choi et al. (2005) showed that *cis*-9, *trans*-11 CLA is produced at higher ruminal pH compared to *trans*-10, *cis*-12 CLA, suggesting that bacteria in the alternative BH pathway are more tolerant to acidic conditions. The positive effect of buffers on ruminal fermentation is in line with MFD prevention by

mitigation of the negative effects of low forage diets on ruminal BH [See review by Bauman and Griinari (2001).

Yeast supplementation may also prevent MFD similar to buffers by stabilization of the ruminal environment under some conditions. In general, yeast supplementation increases cellulolytic bacterial concentration and stimulates lactate-utilizing bacteria, resulting in improved fiber digestibility, dry matter intake, and fermentation stability [i.e. ruminal pH, lactate concentration and acetate to propionate molar ratios (Williams et al., 1991; Erasmus et al., 1992; Wallace, 1994)]. A recent meta-analysis indicated that yeast supplementation increased energy corrected milk and modestly increased yield of milk fat and protein (Poppy et al., 2012). Longuski et al. (2009) reported that yeast culture supplementation prevented induction of MFD during a short-term high fermentable diet challenge (2 d of complete replacement of dry ground corn by high moisture corn). Briefly, the fermentable carbohydrate challenge decreased milk fat yield from 1.42 to 1.30 kg/d in the control diet, while yeast supplemented cows increased milk fat yield from 1.40 to 1.47 kg/d. Interestingly, yeast supplementation did not prevent the decrease in ruminal pH caused by the fermentable carbohydrate challenge. In addition, milk de novo or *trans* FA were not affected by the challenge or yeast supplementation. It is possible that a longer dietary intervention was needed to see changes in milk FA profile. However, yeast supplementation had no effect on milk fat yield or milk fat BH intermediates in cows fed higher fiber diets (Hristov et al., 2010 4507), suggesting that the potential effect of yeast on MFD prevention is through the stabilization of ruminal fermentation in highly fermentable diets, however, the mechanism behind this is not known.

Although dietary sugar is rapidly degraded in the rumen and could cause a reduction of ruminal pH, it has been shown to have beneficial effects on ruminal fermentation and milk fat synthesis (Broderick et al., 2008; Martel et al., 2011). In MFD inducing diets (e.g. low fiber and moderate PUFA concentrations) increasing replacement of corn grain with molasses linearly increased milk concentration of de novo FA and linearly decreased *trans*-10 C18:1, although no effect on milk fat yield was observed (Martel et al., 2011). Similarly, in a separate experiment, Martel et al. (2011) observed that molasses feeding under MFD conditions favored the return to the normal BH pathway, as evidenced by decreased *trans*-10 and increased *trans*-11 C18:1 in milk fat. These observations support the idea that molasses inclusion in the diet has the potential to alleviate diet-induced MFD, possibly because of its high sucrose content. When sucrose replaced dietary starch, milk fat concentration and yield were also increased in dairy cows (Ordway et al., 2002; Broderick et al., 2008).

The ability of sugars to stabilize BH and increase milk fat synthesis is interesting, but the mechanism is not clear. Compared to starch, sucrose increases the molar proportion of butyrate at the expense of acetate and propionate (Strobel and Russell, 1986; Khalili and Huhtanen, 1991), and considering the important role of butyrate as a primer for de novo FA synthesis (Palmquist, 2006), increased ruminal production of butyrate could potentially help explain the increases in milk fat yield. However, little is known about the ability of butyrate to increase milk fat yield. In addition, replacement of high moisture corn with molasses increased fiber digestibility (Broderick and Radloff, 2004), perhaps due to positive effects on cellulolytic bacteria which are in turn associated with ruminal BH of PUFA (Harfoot and Hazlewood, 1997).

Monensin is an ionophore commonly fed to dairy cows to increase production efficiency (Ipharraguerre and Clark, 2003). Monensin targets gram-positive bacteria and is known to alter ruminal BH extent and pathways (Fellner et al., 1997; Jenkins et al., 2003; Russell and Houlihan, 2003). Using an *in vitro* system, Fellner et al. (1997) reported that monensin decreased the BH of linoleic acid (C18:2) to steric acid (18:0). Under normal conditions, increased ruminal outflow of BH intermediates is not problematic, however, under dietary conditions that promote a shift in BH pathways in favor of *trans*-10, *cis*-12 CLA formation monensin could increase the risk of MFD. Results from a meta-analysis showed monensin supplementation increased milk *trans* FA and decreased de novo FA (Duffield et al., 2008b). Additionally, monensin feeding has been shown to further increase the formation of *trans*-10, *cis*-12 CLA in high PUFA diets (Alzahal et al., 2008; He et al., 2012). Increasing feed efficiency is important to reduce feed costs, but the usefulness of monensin is severely compromised if MFD occurs.

Lastly, supplementation of vitamin E above the current NRC recommendation has been shown to mitigate the negative effect of high dietary oil on milk fat yield under some dietary conditions (Bell et al., 2006; Pottier et al., 2006; O'Donnell-Megaro et al., 2012). Pottier et al. (2006) observed that vitamin E prevented the *trans*-11 to *trans*-10 shift, however, this response seems to be dependent upon the timing of vitamin E supplementation, as return to the normal BH pathway was not observed once MFD was established (Pottier et al., 2006; Zened et al., 2012). Interestingly, the stimulatory effect of monensin on *trans*-10 C18:1 formation was eliminated by vitamin E supplementation (Bell et al., 2006).

Conclusion

Increasing milk fat yield is an important goal of dairy businesses in order to increase production efficiency and profitability. Milk fat depression is observed under dietary conditions that cause alterations of the ruminal environment and its microbial population, resulting in a progressive shift in BH pathways towards increased production of milk fat depressing intermediates. Importantly, although the time course of events occurring during recovery from MFD is of basic and applied importance for management of the condition, it has not been specifically investigated.

Chapter 3

Induction of and Recovery from Milk Fat Depression Occur Progressively in Dairy Cows Switched Between Diets that Differ in Fiber and Oil Concentration

Abstract

Milk fat depression (MFD) caused by intermediates of ruminal biohydrogenation commonly occurs in dairy cattle. The time course of recovery from MFD is important to mechanistic investigation and management of the condition. Nine cows were used in a repeated design allowing analysis of recovery from diet-induced milk fat depression (dMFD). A high fiber and low fat control diet was fed during the control and recovery periods. A low fiber and high oil (LF/HO) diet was fed during the induction period. Milk yield was not affected by treatment. Milk fat percentage and yield progressively decreased during induction and were lower by d 3 and 5 ($P < 0.05$), respectively. Milk fat concentration and yield progressively increased when fed the recovery diet and were not different from control on d 19 and 11, respectively. Yield of de novo synthesized fatty acids decreased progressively during the induction period and was lower than control by d 5 ($P < 0.01$). A biphasic response was seen for milk fat *trans* isomers, where *trans*-11 C18:1 and *cis*-9, *trans*-11 CLA were elevated initially and *trans*-10 C18:1 and *trans*-10, *cis*-12 CLA increased progressively during the induction period. Similarly, a biphasic response was seen during recovery from MFD with *trans*-10 C18:1 and *trans*-10, *cis*-12 rapidly decreasing initially and *trans*-11 C18:1 and *cis*-9, *trans*-11 CLA rising slightly

above control levels during the second phase. Recovery from diet induced MFD occurs progressively with a short lag when dietary fiber and oil concentrations are corrected.

Key words: Recovery, milk fat depression, biohydrogenation.

Introduction

Low fat syndrome, referred to as milk fat depression (MFD), was first documented more than 150 years ago (Van Soest, 1994) and represents a challenge for the efficiency and profitability of modern dairies. MFD is characterized by up to a 50% reduction in milk fat yield in response to high concentrate/low forage diets and/or diets supplemented with plant or fish oils (Bauman and Griinari, 2003). In ruminants, the dietary factors that cause MFD are associated with altered rumen fermentation and the production of unique bioactive fatty acids (FA) from metabolism of dietary polyunsatured FA (PUFA). Milk fat depression is a well-characterized example of the interaction of diet and alimentary canal microflora resulting in a change in tissue metabolism.

Davis and Brown (1970) first postulated the elevation in milk *trans* FA as a factor explaining MFD. *Trans*-10, *cis*-12 CLA is one of multiple BH intermediates known to inhibit milk fat synthesis in the mammary gland and its mechanism of action involves the down-regulation of genes related to FA transport and synthesis (Harvatine et al., 2009a). The mammary gland responds rapidly to abomasally infused *trans*-10, *cis*-12 CLA with

decreased milk fat yield by 14 h and maximal response by 60 h (Harvatine and Bauman, 2011). Additionally, milk fat yield is rapidly recovered approximately 2 d after cessation of abomasal infusion (Baumgard et al., 2000). Similarly, feeding rumen protected *trans*-10, *cis*-12 CLA has been shown to cause a maximal decrease of milk fat yield within a week of dietary intervention (Medeiros et al., 2010). Induction of MFD through dietary modification requires 11 to 19 d for complete induction (Shingfield et al., 2006; He et al., 2012) as changes in the rumen environment and a shift in the microbial population must occur to result in synthesis of sufficient quantities of milk fat depressing intermediates to affect mammary lipid synthesis.

The mechanism of MFD has been studied extensively, but the time course of recovery of milk fat yield and the associated changes in milk FA profile have not been specifically investigated. This information is of key scientific and applied importance. The time course of induction and recovery is essential for further mechanistic investigation of the causative events and methods to accelerate recovery. Furthermore, knowledge of the time required to induce and recover milk fat following dietary modification is crucial to determining causes of MFD in farms and setting expectations for recovery. Our objective was to characterize the time course of induction and recovery of diet induced MFD in dairy cows and the associated changes in milk FA profile. We hypothesized that induction of recovery and recovery from MFD would follow a similar time course.

Materials and Methods

Experimental Design and Treatments

All experimental procedures were approved by the Pennsylvania State University Institutional Animal Care and Use Committee. Nine ruminally cannulated Holstein cows (254 ± 81 d postpartum; mean ± SD) from the Pennsylvania State University Dairy Production Research and Teaching Center were used in this study. The experiment was conducted from October to December of 2009. Treatments were: 1) Control [36.9% neutral detergent fiber (NDF) and no PUFA supplementation], 2) induction of MFD (Induction) by feeding a low forage/high oil diet (LF/HO; 29.5% NDF and 3.7% PUFA; Table 3-1; Appendix table 7-1), and 3) recovery from MFD by feeding the control diet (Recovery). All diets were balanced to provide adequate MP and ME for 650 kg lactating dairy cows producing 38 kg milk/d assuming a DMI of 25 kg/d using the Cornell-Penn-Miner System (CPM Dairy version 3.07a, Cornell University, Ithaca, NY; University of Pennsylvania, Kennett Square, PA; and William H. Miner Agricultural Research Institute, Chazy, NY). Cows were randomly assigned to a treatment sequence in a repeated design that allowed analysis of recovery from a MFD diet (Table 3-2). In each sequence, induction followed control and recovery followed induction. A pretrial period was necessary to achieve this sequence. Each treatment period was 21 d in length. Cows were treated with Posilac (Elanco Animal Health, Greenfield, IN) on d 1 and 11 of each period.

Cows were fed once daily (0800 h) at 110% of expected intake and milked twice daily. Intake and milk yield were recorded daily. Each total mixed ration (TMR) was

sampled once per week and stored at -20°C, thawed at room temperature, dried in a forced-air oven for 72 h at 55°C, and ground in a Wiley mill through a 1-mm screen (A. H. Thomas, Philadelphia, PA). Samples of TMR were composited by period and analyzed for dry matter, crude protein, and select minerals by wet chemistry procedures according to AOAC (2000) and for NDF and acid detergent insoluble fiber according to Van Soest [(1991); Cumberland Valley Analytical Services Inc., Maugansville, MD; Table 3-1].

Milk was sampled every other d at both milkings. One subsample was stored at 4°C with preservative (Bronolab-WII) until analyzed for fat (Filter B) and protein by infrared spectroscopy [Fossomatic 4000 Milko-Scan and 400 Fossomatic, Foss Electric, Hillerød, Denmark; AOAC (2000) method 972.160, Dairy One Lab, State College, PA]. Another subsample was stored at -20°C without preservative until analyzed for FA composition.

Fatty Acid Analysis

Stored milk samples from the morning and afternoon milking were thawed in room temperature water and pooled by d according to fat yield at each milking. Milk fat was extracted with hexane:isopropanol according to Hara and Radin (1978), and FA methyl esters were prepared by base-catalized transmethylation according to Chouinard et al. (1999). FA methyl esters were quantified by gas chromatography using an Agilent 6890A gas chromatograph (Agilent Technologies, Palo Alto, CA) equipped with a fused-silica capillary column [SP-2560; 100 m x 0.25 mm (i.d.) with 0.2-μm film thickness;

Supelco Inc. Bellefonte, PA], and a flame ionization detector with hydrogen as the carrier gas. Initial oven temperature was 80°C and ramped 2°C/min to 190°C and held for 15 minutes. Inlet and detector temperatures were 250°C with a 100:1 split ratio. Gas constant flows were held at Hydrogen carrier 1 ml/min and detector hydrogen 25 ml/min, airflow 400 ml/min, and nitrogen plus carrier at 40 ml/min.

Fatty acid peaks were identified in the gas chromatographic analysis using pure methyl ester standards (GLC 60; NuChek Prep Inc., Elysian, MN). An equal weight reference standard (GLC 74; NuChek) was used to determine correction factors for individual FA. Milk FA yield was calculated as described by Glasser et al. (2007), however, the coefficient to estimate the proportion of FA in milk TG was calculated for each sample rather than using the suggested fixed factor of 0.944. The mean proportion of FA in total milk lipids was calculated for each sample by multiplying by 0.98885 as a correction for other milk lipid fractions based on the difference between the mean proportion of FA in milk TG and in total milk lipids (Glasser et al., 2007). The milk FA desaturase indexes were calculated for each sample as the ratio of product to substrate + product for the stearoyl Co-A desaturase enzyme. Total FA concentration and FA profile of the TMR was determined by gas chromatography after direct methylation (Sukhija and Palmquist, 1988).

Statistical Analysis

Data were statistically analyzed as a replicated design using the MIXED procedure of SAS with repeated measures (version 9.3, SAS Institute Inc., Cary, NC)).

The model was $Y_{ijklm} = \mu + S_i + P_j + C_k(S_i) + T_l + D_m + T_l \times D_m + e_{ijklm}$, where Y_{ijklm} is the variable of interest, μ is the overall mean, S_i is the random effect of sequence (i = 1 to 3), P_j is the random effect of period (j = 1 to 3), $C_k(S_j)$ is the random effect of cow nested in sequence (k = 1 to 9), T_l is the fixed effect of treatment (l = 1 to 3), D_m is the fixed effect of time (m = 1 to 12), $T_l \times D_m$ is the interaction of treatment and time, and e_{ijklm} is the residual error. The ARH(1) or AR(1) covariance structures were used based on model fit, time was the repeated variable, and cow by treatment was the subject. Denominator degrees of freedom were adjusted by the Kenward-Rogers method. The preplanned contrasts were control vs induction and control vs recovery at each time point. Data were log transformed when appropriate and back-transformed data are reported. Data points with Studentized Residuals > 3.0 were considered outliers and excluded from analysis, typically less than 3 data points per time point. Only a few points were excluded from the analysis and rarely more than one per response variable.

Results

Diet Description

The LF/HO diet used to induce milk fat depression had a lower fiber and a higher FA concentration relative to control (29.5 vs. 36.9% NDF and 6.9 vs. 2.6% FA, respectively; Table 3-1). Dietary monounsaturated FA and PUFA concentrations were higher in the LF/HO compared to the control diet (17.5 vs. 7.0 and 37.3 vs. 11.6 g/kg diet DM, respectively; Appendix table 7-1).

Dry Matter Intake and Milk Production and Composition

There was a treatment by time interaction for DM intake ($P < 0.01$; Table 3-3). Intake decreased progressively from d 1 of induction and was on average 3.1 kg/d lower that control from d 6 to 21 (Appendix figure 7-1). During recovery dry matter intake increased progressively and was not different from control by d 11.

There was no main effect of treatment or treatment by time interaction for milk yield (Table 3-3). However, on d 20 of induction milk yield was lower than control ($P < 0.01$; Figure 3-1a). Milk fat content and yield decreased progressively from d 1 of induction and were lower from control by d 3 and 5 ($P < 0.05$), respectively. Milk fat content and yield reached a nadir around d 9 (Figures 3-1b and 3-1c). During induction milk fat concentration was reduced 34 ± 3.6% (Mean ± SD) relative to control from d 9 to 21. During recovery milk fat concentration progressively increased from d 1 and was not different from control on d 11 and from d 17 to 21 ($P > 0.09$). Similarly, milk fat yield recovered progressively and was not different from control after d 11 ($P > 0.07$).

Milk protein percentage increased progressively during induction, was higher than control after d 11 ($P < 0.01$ from d 11 to 21), and reached a plateau on d 13 (Appendix figure 7-2). Milk protein percentage was 6.1 ± 0.6% (Mean ± SD) higher during MFD induction compared to control from d 11 and 21. Milk protein yield was not affected by treatment.

Milk de novo and Preformed FA

Concentration of milk FA less than 16 carbons (de novo synthesized) decreased progressively during induction, tended to be lower than control on d 3 ($P = 0.09$), and was lower on d 5 when it reached a nadir ($P < 0.001$; Figure 3-2a). During recovery, the concentration of milk FA less than 16 carbons increased progressively. In contrast, the concentration of milk FA greater than 16 carbons (preformed FA) increased during induction, plateaued on d 5, and decreased during recovery (Figure 3-2b). Changes in individual de novo and preformed FA paralleled these patterns (Appendix table 7-2). Similar to milk fat concentration, yield of FA less than 16 carbons decreased progressively during induction and was lower than control by d 5 ($P < 0.001$; Figure 3-2c). Interestingly, yield of FA greater than 16 carbons started to decrease only after d 5 of induction and was lower than control on d 13, 17, and 21 ($P < 0.05$; Figure 3-2d). During recovery, yield of FA greater than 16 carbons was lower than control only on d 0 and 3 ($P < 0.05$) and was higher than the control on d 21 ($P < 0.05$).

Milk Trans Fatty Acids

Induction caused a rapid increase in milk fat *trans*-11 C18:1 and *cis*-9, *trans*-11 CLA concentration with maximum concentrations of 2.5 and 1.7% of FA reached on d 3 and 5, respectively (Figure 3-3a and 3-3c). After this peak both FA progressively decreased with *trans*-11 C18:1 decreasing below control levels ($P < 0.05$ on d 15, 19, and 21). Milk fat *trans*-10 C18:1 and *trans*-10, *cis*-12 CLA content increased progressively during induction and were higher than control on d 1 and 3 of the induction period, respectively ($P < 0.05$; Figure 3-3b and 3-3d). A near-peak concentration of *trans*-10

C18:1 (4.85% of FA) was achieved on d 9 of the induction period, while *trans*-10, *cis*-12 CLA progressively increased for the entire 21 d observation period (d 21 = 0.09% of FA).

During recovery, *trans*-10 C18:1 and *trans*-10, *cis*-12 CLA content followed a similar trend with both decreasing progressively to control levels by d 11 and 7, respectively. Milk fat *trans*-11 C18:1 increased progressively to control levels on d 7 and then increased above control from d 11 to 21 of recovery [31 ± 9% (Mean ± SD); $P < 0.05$]. Interestingly, *cis*-9, *trans*-11 CLA content decreased initially and was the not different from control on d 3, but increased after d 7 and was higher than control from d 13 to 21 of recovery [33 ± 19% (Mean ± SD); $P < 0.05$].

Milk Δ9-desaturase Indexes

During induction, milk C14 and C16 desaturase indexes increased progressively and reached a plateau around d 9 (Figures 3-4a and 3-4b). During recovery, both indexes decreased progressively reaching a nadir at d 7.

Discussion

The replicated design allowed investigation of the time course of induction and recovery from MFD while accounting for cow and period variation. The low NDF and high oil diet was selected to mimic conditions that commonly lead to reduced milk fat on

farms. These two factors are known to modify the rumen environment and the microbial population leading to ruminal synthesis and outflow of biohydrogenation intermediates that depress milk fat synthesis (Bauman and Griinari, 2003). To achieve the difference in diet fermentability and PUFA content, several ingredients differed between the two rations. Importantly, conclusions are not drawn from individual ingredients but from induction of and recovery from MFD. Even though rations are not formulated to be deficient in fiber or to provide excess PUFA, our model serves to test the mechanism of induction by exemplifying a scenario where a well formulated ration causes MFD due to changes in feed DM, feed nutrient composition, or mixing errors. The experimental design was successful in induction of MFD and recovery within the experimental periods. The diet fed during the control and recovery represents a near best-case scenario diet where fiber is adequate and PUFA are minimized.

All animals received bST, and there is no reason to expect any impact of this hormone on the rates of induction of and recovery from MFD. As reviewed by Bauman (1992), bST treatment has minimal impact on milk components, whereas milk yield is gradually increased with a maximal response observed 6 days after dosing. In the present study, the patterns of both induction of and recovery from MFD were not affected following bST treatment.

The LF/HO diet reduced milk fat by 34%, which is near the maximal 50% reduction observed for diet induced MFD (Harvatine et al., 2009a). The extent of MFD in the current experiment is a higher degree than normally observed on farms (Bailey et al., 2005). However, the model used serves to test the time course of induction of and recovery from diet induced MFD. Considering that dietary induction of MFD requires

changes in rumen microbial populations and ruminal metabolism of FA (Bauman and Griinari, 2001), it was expected that onset of MFD would take longer than the acute response seen during abomasal infusion of *trans*-10, *cis*-12 CLA (Baumgard et al., 2000; Harvatine and Bauman, 2011). Shingfield et al. (2006) reported that feeding a corn silage-based ration supplemented with an oil supplement [4.5% of DM; fish and sunflower oil (1:2 w/w)] resulted in a progressive decrease and a near-maximal milk fat yield reduction of approximately 46% by d 19. Similarly, addition of a high linoleic acid oil blend in alfalfa haylage based rations resulted in a marked decrease in milk fat yield reaching a nadir on d 11 (He et al., 2012). Other non-time course experiments have reported substantial reductions in milk fat within 14 d. For example, Harvatine and Bauman (2006) achieved a 38% reduction in milk fat concentration by 14 d with a low forage/high oil diet. Similarly, feeding a high concentrate/low forage diet, Peterson et al. (2003) reported a 25% decrease in milk fat concentration by day 21. In the current experiment, near-maximal reductions in milk fat concentration and yield were observed around d 9 and 13, respectively.

The time course of recovery from dMFD has not been well investigated or reported in the literature. Gama et al. (2008) observed milk fat concentration and profile for 12 d following fish oil induced MFD. A progressive increase in milk fat concentration was reported; however, complete recovery of milk fat was not observed at the end of 12 d despite a decrease in *trans*-10 *cis*-12 CLA and other *trans* isomers (Gama et al., 2008). Similarly, a low resolution experiment showed a progressive recovery of milk fat concentration that was near complete 2 weeks after the correction of dietary NDF (Weiss, 2012). In the current experiment the time course of recovery was similar to the time

course of induction. Recovery of milk fat was achieved around d 9 after restoration of adequate dietary fiber and minimizing dietary PUFA concentration. The delay between diet switch and a significant response in milk fat during both induction and recovery clearly shows that the period of ruminal adaptation to the new diet is much slower than the response of the mammary gland to CLA, which occurs between 14 to 48 h after abomasal infusion (Harvatine and Bauman, 2011). Satter and Bringe (1969) experimentally demonstrated ruminal adaptation as a rate limiting factor by simultaneously switching rumen contents of dairy cows fed high and low forage diets and reported that 70% of the maximal reduction in milk fat was achieved by d 3 and complete MFD within 5 to 6 d.

Two distinct pathways of BH of linoleic acid (C18:2 *cis*-9, *cis*-12) have been previously described (Griinari et al., 1998). The normal pathway of BH is characterized by formation of *trans*-11 C18:1 and *cis*-9, *trans*-11 CLA as intermediates, while under altered ruminal conditions changes in the microbial communities of the rumen result in increased formation of *trans*-10 C18:1 and *trans*-10, *cis*-12 CLA (Jenkins et al., 2008). Absorbed FA are rapidly incorporated into milk fat within approximately 6 h (Harvatine and Bauman, 2011) allowing use of milk fat to characterize the time course of adaptation in rumen biohydrogenation. In the present experiment, the LF/HO diet resulted in increased formation of several *trans* FA, and a biphasic response was observed for the BH pathways during both induction and recovery from dMFD. During the first phase of induction, *trans*-11 C18:1 and *cis*-9, *trans*-11 CLA rapidly increased and peaked on d 3 and 5 at concentrations 2 and 3 fold higher than control, respectively. Presumably, this is due to a decreased rate of the final steps of the normal biohydrogenation pathway. The

observed lag between substrate provision (PUFA) and peak concentrations of *trans*-11 C18:1 suggests that modification of the microbial population has a greater impact on the rate of BH than the increased supply of PUFA.

During the second phase of induction *trans*-11 C18:1 and *cis*-9, *trans*-11 CLA declined while *trans*-10 C18:1 and *trans*-10, *cis*-12 CLA progressively increased 2 and 1.6 fold, respectively. The second phase represents a shift in the predominant pathway for biohydrogenation, presumably due to a major change in the microbial populations. Previous studies have reported a similar time course of changes in BH intermediates in milk (Shingfield et al., 2006) and in the rumen (Zened et al., 2013) in response to high oil and high oil, low fiber diets. Shingfield et al. (2006) showed that concentrations of *trans*-11 C18:1 and *cis*-9, *trans*-11 CLA in milk fat peaked at a concentration that was greater than 5-fold higher than control at d 6 and then declined to 2-fold higher than control by d 16, whereas *trans*-10 C18:1 and *trans*-10, *cis*-12 CLA increased progressively and reached stable concentrations on d 16. The long lag between substrate provision (PUFA) and peak concentrations of *trans*-10 C18:1 suggests that modification of the microbial population has a greater impact on the predominant pathway of biohydrogenation than increased supply of PUFA.

In the present experiment a biphasic response was also observed during recovery. During the first phase the alternate pathway isomers including *trans*-10 C18:1 and *trans*-10, *cis*-12 CLA rapidly declined 3 and 4.2 fold, respectively, between d 0 and 3. Concurrently, *trans*-11 C18:1 progressively increased while *cis*-9, *trans*-11 CLA decreased to control levels. During the second phase of recovery both *trans*-11 C18:1 and *cis*-9, *trans*-11 CLA increased 1.3 fold above control levels from d 11 and 13 to 21,

respectively, while the alternate isomers remained low. Interestingly, the control and recovery diet were the same and differences in milk FA may represent further adaptations in the microbial population occurring after the alternate isomers have subsided. This second phase of recovery may represent a period of decreased activity of the last steps of biohydrogenation similar to the first phase of induction and may be a period of increased susceptibility for relapse into MFD. Similar to our results, *trans*-10 C18:1 and *trans*-10 *cis*-12 CLA decreased approximately 4.8 and 3.8 fold, respectively, 12 d after switching a MFD diet was fed (Gama et al., 2008), but the time course of the normal BH pathway isomers was not reported.

Milk content and yield of de novo FA (less than 16 carbons) were substantially decreased during MFD and were inversely related to milk content of *trans*-10 C18:1 and *trans*-10, cis-12 CLA. This has been observed in several studies where MFD was induced by fiber deficiency and or by increasing the dietary PUFA content [i.e. (Peterson et al., 2003; Shingfield et al., 2006; Gama et al., 2008)]. Conversely, during the recovery period milk content and yield of de novo FA increased progressively in concurrence with the restoration of the normal BH pathway. This is in agreement with the larger decrease in de novo synthesized FA commonly observed during more extensive MFD (Bauman et al., 2011). During abomasal infusion of CLA the initial decrease in milk fat that occurred between 14 and 36 h was an equal proportion of de novo and preformed FA, while a larger decrease in de novo synthesized FA was observed after 36 h (Harvatine and Bauman, 2011). The resolution of the current experiment precluded the ability to observe a similar two-stage response.

The milk fat desaturase indexes are commonly used as an indication of stearoyl-CoA desaturase (SCD1) enzyme activity (Perfield et al., 2006; Jacobs et al., 2011). In the current experiment, the reduction in milk fat was inversely related to the desaturase indexes as these indexes increased during induction and decreased during recovery. The desaturase indexes gradually changed during both induction and recovery and the timing was similar to the changes observed in the concentration of *trans*-10 C18:1 and milk fat yield. Jacobs et al. (2011) reported a reduction in both SCD1 activity and C16 and C18 desaturase indexes when feeding 2.7% of the diet (dry matter basis) as soybean oil. However, unlike the present study, the elevated dietary content of PUFA did not result in MFD (Jacobs et al., 2011). In agreement with our results, others have reported increased desaturase index during dMFD (Gama et al., 2008). Interestingly, abomasal infusion of near maximal effective *trans*-10 *cis*-12 CLA doses rapidly and robustly decreased the desaturase index (Perfield et al., 2006; Harvatine and Bauman, 2011), but infusions of low doses of *trans*-10 *cis*-12 CLA did not alter the desaturase index (Baumgard et al., 2001; Peterson et al., 2002). The discrepancy between diet induced MFD experiments may be due to differences in the amounts of *trans*-10 *cis*-12 CLA reaching the mammary gland. Additionally, it is possible that the observed elevation in the desaturase index in the current experiment is related to the production of other bioactive isomers, some of which may increase desaturase activity. Lastly, this provides further evidence that inhibition of FA desaturation is not a functional mechanism of diet induced MFD (Harvatine et al., 2009b).

Milk fat depression is commonly encountered when feeding high-energy diets and some byproduct feeds. Based on the time-course of induction, the causative dietary

change resulting in MFD is expected to have occurs 7 to 14 d prior to MFD and provides a time window for further investigation. After identification of MFD, dietary adjustments that will increase milk fat are expected to do so in 7 to 14 d allowing setting a realistic expectation to determine success of an intervention. Mechanistically, the time-course of induction and recovery demonstrates the importance of modification of the rumen microbial population as a key limiting step to induction of and recovery from MFD. Lastly, this study represents a best-case scenario of a rather drastic change in diet fermentability and PUFA concentration. The experimental design validated in this study provides a method to investigate the ability of individual macronutrients and feed additives to accelerate recovery from MFD.

Conclusions

Induction of and recovery from MFD occurred progressively after a short lag. During induction ruminal biohydrogenation follows a two-phase response with an initial slowing of the normal pathway followed by a progressive increased utilization of the alternate pathway. Biohydrogenation also appears to follow a two-phase response during recovery. Modification of ruminal biohydrogenation pathways is a rate-limiting step of induction and recovery of diet induced MFD as the time course is much slower than previously observed for abomasal CLA infusion. In addition, modification of ruminal microbial populations, rather than supply of PUFA, appears to be the rate-limiting step modification of the rate of BH and synthesis of alternate biohydrogenation intermediates

during induction of MFD. However, decreasing dietary PUFA may be more important during recovery. The characterization of the temporal changes during the recovery from diet induced MFD provides useful insight into the regulation of milk fat synthesis and the framework to further study ways to accelerate recovery from MFD.

Table 3-1. Ingredient and chemical composition of experimental diets.

Item	LF/HO[1]	Control
Ingredients, g/100 of DM		
Corn silage[2]	30.0	31.6
Alfalfa haylage[3]	3.5	25.0
Ground corn	26.8	13.7
Roasted soybeans	12.9	-
Canola meal	8.6	10.8
Cookie meal[4]	4.7	5.3
Grass hay/straw[5]	4.3	3.3
Soybean oil	2.9	-
Aminoplus[6]	-	4.2
Cottonseed hulls	3.0	2.9
Minerals and vitamins mix[7]	3.3	3.1
Chemical composition[8] (g/100 g DM unless otherwise stated; n=3)		
CP	15.5	16.6
NDF	29.5	36.9
ADF	18.3	24.6
Fatty acids	6.9	2.6
Starch	27.0	18.0
Ash	5.7	7.5

[1]LF/HO = low fiber high soy oil diet.
[2]Contained 34.2% dry matter (DM)
[3]Contained 37.7% DM
[4]Cookie byproduct (Bakery Feeds, Honey Brook, PA) contained (% of DM) 12.8% CP, 14% NDF, 49.7% starch, and 9.2% ether extract
[5]Contained 88.3% DM
[6]Aminoplus is a soybean meal based protein source (51% CP, DM basis), Archer Daniels Midland Co. (Decatur, IL)
[7]Contained (%, as fed basis): 45.8 dried corn distillers grains with solubles; 35.8 limestone (38% Ca); 8.3 magnesium oxide (54% Mg); 6.4 salt; 1.73 vitamin ADE premix; 1.09 selenium premix (0.06% selenium); and 0.88 trace mineral mix. Composition (DM basis): 11% CP; 18% NDF; 5.2% fat; 14.9% Ca; 0.35% P; 4.58% Mg; 0.41% K; 0.31% S; 357 mg/kg Cu; 1,085 mg/kg Zn; 181 mg/kg Fe; 6.67 mg/kg Se; 125,875 IU/kg vitamin A (retinyl acetate); 31,418 IU/kg vitamin D (Activated 7-dehydrocholesterol); and 946 IU/kg vitamin E (dl-alpha tocopheryl acetate).
[8]Analyzed by Cumberland Valley Analytical Services (Maugansville, MD; n = 3 per diet)

Table 3-2. Treatment assignments of a repeated measures design to study the recovery from diet induced MFD.

Assignments[1]	Pre-experiment	Period 1	Period 2	Period 3
1	Control	Control	Induction	Recovery
2	Induction	Recovery	Control	Induction
3	Control	Induction	Recovery	Control

[1]All assignments followed a sequence in which MFD Induction was followed by Recovery

Table 3-3. Effect of milk fat depression induction and recovery on production and milk fatty acid profile.

					P value[1]		
Item	Control	LF/HO	Recovery	SEM	Trt	Time	Trt x time
DMI, kg/d	26.9	24.2	24.7	1.3	<0.001	<0.01	<0.01
Milk yield, kg/d	32.9	32.0	32.8	2.1	0.33	0.15	0.33
Fat, %	3.93	2.99	3.33	0.123	<0.001	0.21	<0.001
Fat yield, kg/d	1.3	1.0	1.1	0.059	<0.001	0.37	<0.001
De novo FA yield, g/d[2]	333	193	241	15	<0.001	0.12	<0.001
Preformed FA yield, g/d	430	408	414	25	0.38	0.04	<0.001
-- % of total FA ---							
De novo FA	28.1	21.1	23.6	0.6	<0.001	<0.01	<0.001
Preformed FA	36.3	46.4	41.6	0.8	<0.001	<0.001	<0.001
Trans-11 C18:1	0.97	1.11	0.97	0.061	<0.05	<0.001	<0.001
Trans-10 C18:1	0.53	2.54	1.04	0.262	<0.001	<0.001	<0.001
Cis-9, *trans*-11 CLA[3]	0.61	0.99	0.73	0.048	<0.001	<0.001	<0.001
Trans-10, *cis*-12 CLA	0.01	0.05	0.02	0.003	<0.001	<0.001	<0.001
C14 desaturase index	0.08	0.12	0.10	0.008	<0.001	0.36	<0.001
C16 desaturase index	0.05	0.08	0.07	0.004	<0.001	0.06	<0.001

[1]Trt = Treatment effect; Time = effect of day in treatment.

[2]FA < 16 C originate from de novo synthesis in the mammary gland; FA > 16 C originate from extraction from plasma.

[3]Conjugated Linoleic acid.

[4]C14 desaturase index = C14:1/(C14:1 + C14:0); C16 desaturase index = C16:1/(C16:1 + C16:0).

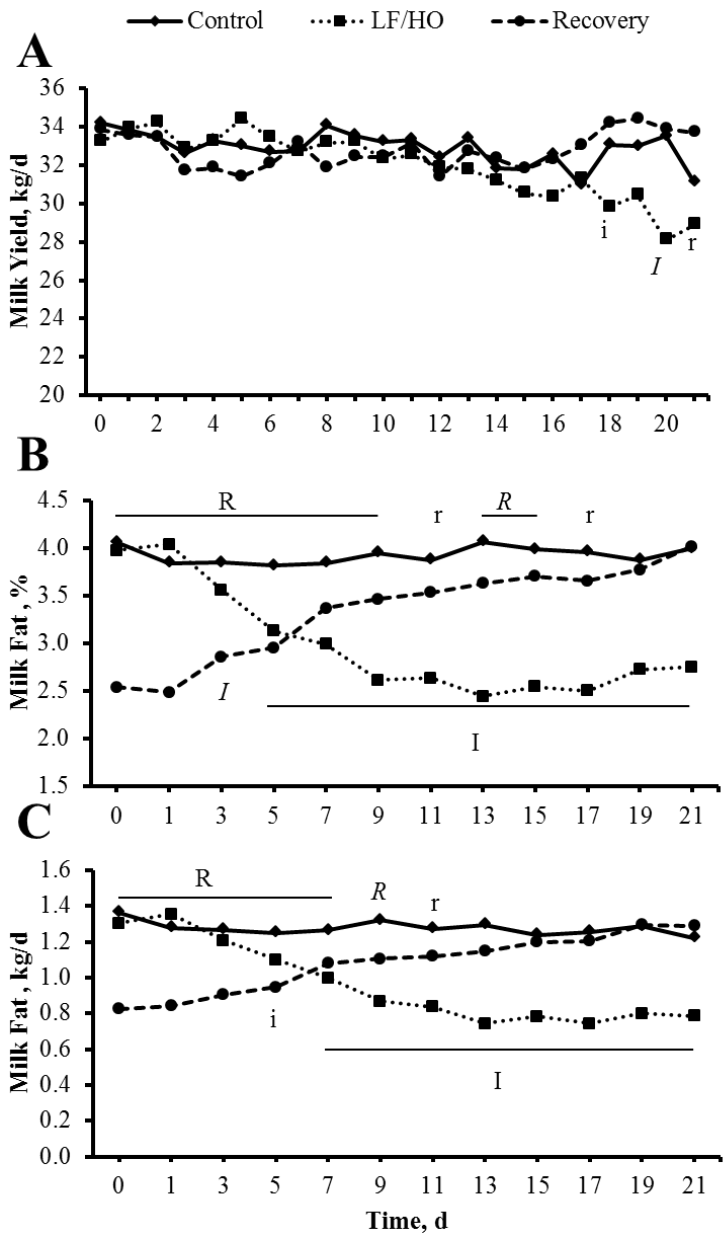


Figure 3-1. Time course of milk production and milk fat during induction and recovery from diet-induced milk fat depression.

Shown are milk yield (Panel A; kg/d), milk fat percentage (Panel B), and milk fat yield (Panel C; kg/d) of cows fed a low fiber, high oil diet (Induction; LF/HO), a high fiber diet, low oil (Control), or a high fiber, low oil diet after LF/HO (Recovery). Preplanned contrasts tested the difference between Control and Induction (I = $P < 0.01$, *I* = $P < 0.05$, and i = $P < 0.1$) and between Control and Recovery (R = $P < 0.01$, *R* = $P < 0.05$, and r = $P < 0.1$; SEM = 2.48, 0.18 and 0.09 for milk yield, fat yield, and fat percent, respectively).

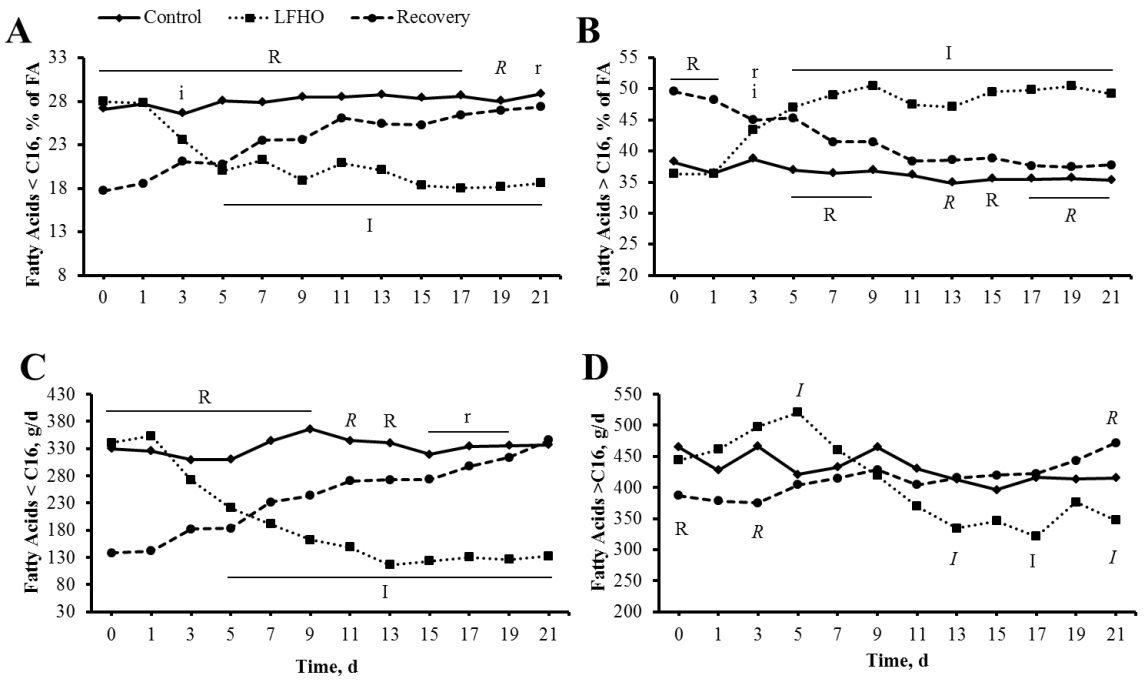


Figure 3-2. Time course of de novo and preformed milk FA during induction and recovery from diet-induced milk fat depression.

Shown are milk fat concentration (% of total milk FA) and yield (g/d) of de novo (Panel A and C, respectively) and preformed (Panel B and D) FA of cows fed a low fiber, high oil diet (Induction; LF/HO), a high fiber diet, low oil (Control), or a high fiber, low oil diet after LF/HO (Recovery). Preplanned contrasts tested the difference between Control and Induction (I = $P < 0.01$, *I* = $P < 0.05$, and i = $P < 0.1$) and between Control and Recovery (R = $P < 0.01$, *R* = $P < 0.05$, and r = $P < 0.1$; SEM = 1.34, 1.90, 27.7 and 46.3 for de novo and preformed FA concentrations, and de novo and preformed yields, respectively).

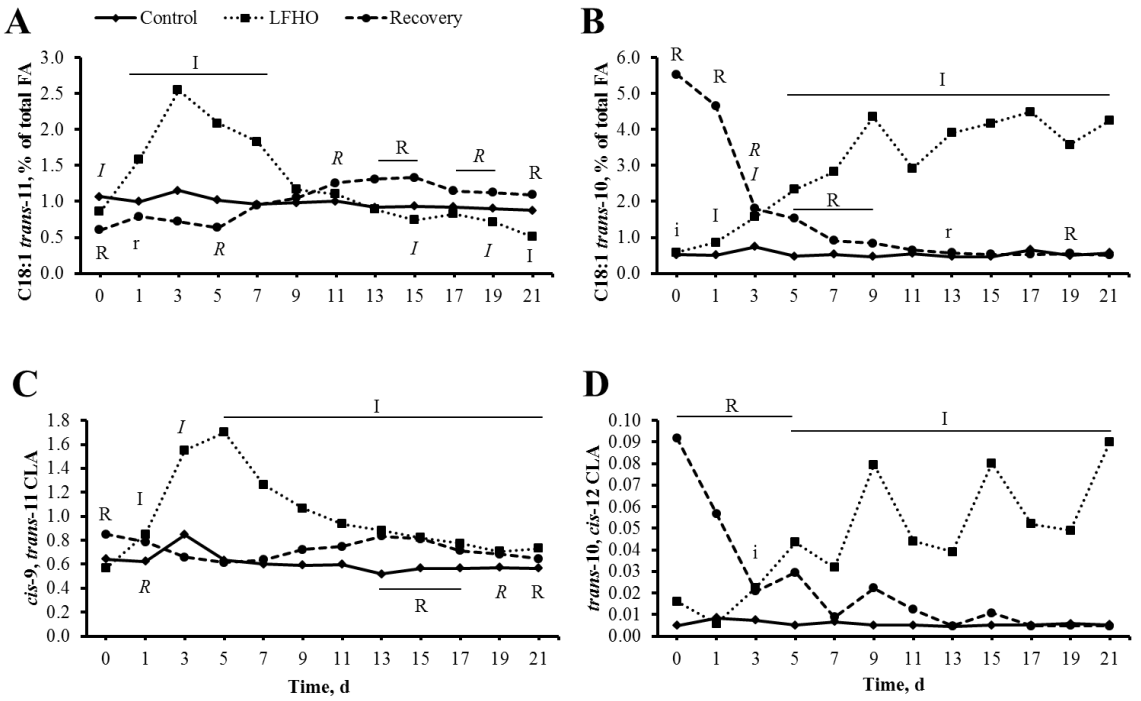


Figure 3-3. Time course of milk *trans* FA concentration during induction and recovery from diet-induced milk fat depression.

Shown are the predominant isomers of the normal biohydrogenation pathway [*trans*-11 C18:1 (Panel A) and *cis*-9, *trans*-11 CLA (Panel C).and the predominant isomers of the alternate biohydrogenation pathway [*trans*-10 C18:1 (Panel B) and *trans*-10, *cis*-12 CLA (Panel D)] of cows fed a low fiber, high oil diet (Induction; LF/HO), a high fiber diet, low oil (Control), or a high fiber, low oil diet after LF/HO (Recovery). Preplanned contrasts tested the difference between Control and Induction (I = $P < 0.01$, *I* = $P < 0.05$, and i = $P < 0.1$) and between Control and Recovery (R = $P < 0.01$, *R* = $P < 0.05$, and r = $P < 0.1$; SEM = 0.27, 0.38, 0.11 and 0.011 for C18:1 trans-11, trans-10, cis-9, trans-11 and trans-10, cis-12 CLA).

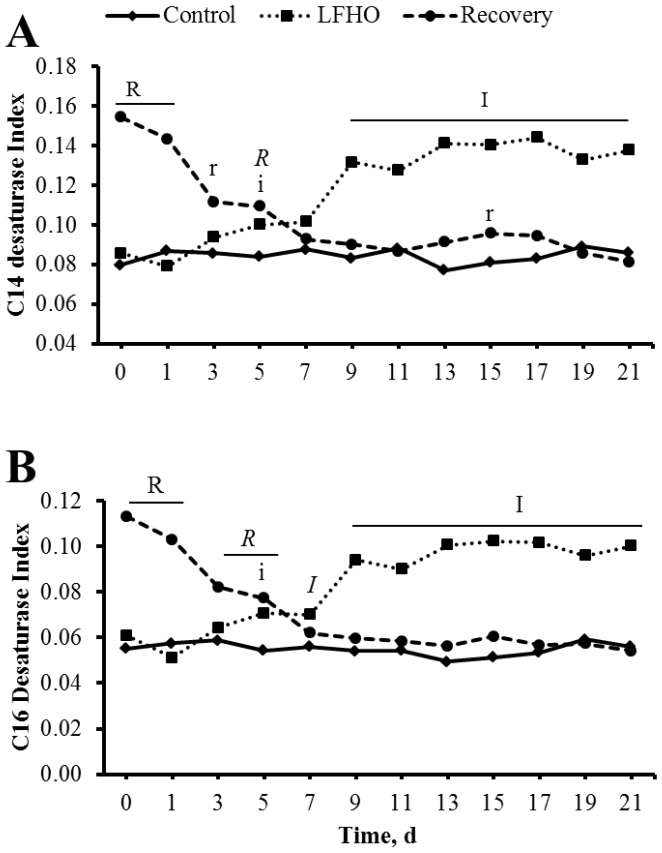


Figure 3-4. Time course of C14 and C16 desaturase indexes during induction and recovery from diet-induced milk fat depression.

Shown are the C14 desaturase index (Panel A) and the C16 desaturase index (Panel B) of cows fed a low fiber, high oil diet (Induction; LF/HO), a high fiber diet, low oil (Control), or a high fiber, low oil diet after LF/HO (Recovery). Preplanned contrasts tested the difference between Control and Induction (I = $P < 0.01$, *I* = $P < 0.05$, and i = $P < 0.1$) and between Control and Recovery (R = $P < 0.01$, *R* = $P < 0.05$, and r = $P < 0.1$; SEM = 0.011 and 0.008 for C14 and C16 desaturase indexes, respectively).

Chapter 4

Effect of Diet Fiber and Polyunsaturated Fatty Acid Concentration on Recovery from Diet-Induced Milk Fat Depression in Monensin Supplemented Dairy Cows

Abstract

The effects of primarily correcting dietary fiber or polyunsaturated fatty acid (PUFA) concentration on the time-course of recovery from diet-induced milk fat depression (MFD) in monensin supplemented diets was investigated. Eight ruminally cannulated and 9 non-cannulated multiparous Holstein cows were randomly assigned to treatment sequences in a 3 x 3 Latin Square design. Each period was divided into a MFD induction and a recovery phase (10 d and 18 d, respectively). Milk fat depression was induced by feeding a low fiber, high oil diet (25.9% NDF, 1.6% soybean oil and 7.5 % whole soybeans). Following the induction period, cows were switched to recovery treatments designed to correct dietary fiber, PUFA, or both fiber and PUFA concentration. Treatments during recovery were: 1) High forage, low PUFA diet (control; 31.8% NDF, no added oil), 2) Low forage, low PUFA diet (LF; 28.4% NDF, no added oil), and 3) High forage, high PUFA diet (HO; 31.5% NDF, 1.5% soybean oil and 7.8% whole soybeans). Milk and milk component yield, milk FA profile, and ruminal pH were measured on every third day during the recovery phase. Milk yield decreased progressively in HO and control, whereas it was maintained in the LF diet and was higher than control on d 15. Milk fat concentration increased progressively during recovery in all treatments, but was on average 9% lower in LF than in control from d 12 to 18. Milk

fat yield increased progressively in all treatments and was not different between control and LF at any time point, but was lower in HO compared to control on d 15. Similar to milk fat yield, yield of de novo synthesized and 16 carbons FA increased progressively and were not different between control and LF, but did not recover in HO. Conversely, yield of preformed FA was not different between LF and control, but was increased by HO compared to control on d 9 and 18. Milk *trans*-10 C18:1 decreased progressively in all treatments, but was higher in both HO and LF compared to control from d 3 to 18 and d 6 to 18, respectively. Correcting dietary PUFA concentration is the predominant factor impacting the rate of recovery from diet-induced MFD.

Key words: Monensin, milk fat depression, dairy cows.

Introduction

Milk fat synthesis is highly variable and responsive to nutritional factors. Milk fat depression is a specific reduction in the fat content of milk often observed in dairy cows fed low forage or high oil diets (Bauman and Griinari, 2003). Specific *trans* FA, such as *trans*-10, *cis*-12 conjugated linoleic acid (CLA), formed as intermediaries during altered ruminal biohydrogenation of polyunsaturated FA have been identified as the causative factors of diet-induced MFD (Harvatine et al., 2009a). The alternate biohydrogenation pathway is considered a result of a change in rumen environment, altered rumen microbial population, or a large amount of substrate for biohydrogenation (Chilliard et

al., 2007; Lourenço et al., 2010; Weimer et al., 2010b). Dietary risk factors associated with MFD and the mechanisms underlying the condition have been extensively studied (Bauman et al., 2011), however, recovery from MFD and mechanisms to accelerate recovery have not been well characterized.

Dietary fiber and PUFA concentration and monensin (MN) are considered risk factors for MFD. Monensin is commonly used in diets for dairy cows to increase feed efficiency, however, it has been associated to lower milk fat concentration concomitantly with increased milk *trans* FA and reduced milk de novo synthesized FA (Duffield et al., 2008b). In addition, an interaction dietary PUFA concentration and MN has been previously observed (Alzahal et al., 2008; He et al., 2012). For example, MN supplementation in high PUFA diets increased the concentration of intermediates of the alternate BH pathway in rumen fluid and milk (Jenkins et al., 2003; Alzahal et al., 2008; He et al., 2012).

We have recently reported a high-resolution time course of the induction of and recovery from diet-induced MFD. Briefly, MFD was induced to near maximal levels in 9 d by feeding a low fiber and high PUFA diet (29.5% NDF and 6.9% FA; Chapter 3). After correction of both dietary fiber and PUFA concentration recovery from MFD occurred progressively, was not different from control on d 11, and was complete by d 19 (Chapter 3). This correction of both dietary fiber and PUFA concentration represents a best-case scenario for recovery of milk fat, but results in a substantial decrease in diet energy density and risks loss of milk yield. Furthermore, increasing dietary NDF increased milk fat yield and decreased milk fat *trans*-10, *cis*-12 CLA concentration in fiber deficient diets (Weiss, 2012), but had no effect in diets with higher levels of fiber

(Alzahal et al., 2009). Our objective was to study the effect of correction of dietary NDF and PUFA concentration on milk fat synthesis and re-establishment of normal FA biohydrogenation pathways in diets supplemented with monensin. We hypothesized that compared to control, correcting dietary PUFA primarily would have a greater impact on recovery from diet-induced MFD than correction of dietary NDF alone, because PUFA are the substrates for formation of *trans* FA and modify rumen microbial populations.

Materials and Methods

Experimental Design and Treatments

The experiment was conducted from August to November of 2011. All experimental procedures were approved by the Pennsylvania State University Institutional Animal Care and Use Committee. Eight ruminally cannulated and 9 non-cannulated multiparous Holstein cows (127 ± 37 d postpartum; mean ± SD) were randomly assigned to 1 of 6 treatment sequences in a 3 x 3 Latin Square design. Sequences 1 through 5 were allocated 3 times each, and sequence 5 was allocated 2 times. Cows were housed in a tie-stall barn located at the Pennsylvania State University Dairy Production Research and Teaching Center. Each period was divided into a 10 d induction phase and an 18 d recovery phase. First, MFD was induced by feeding a low fiber, high oil diet (Induction; 25.9% NDF, 1.6% soybean oil, and 7.5% whole soybeans; Table 4-1). The recovery treatments that followed were designed to correct dietary fiber, PUFA, or both fiber and PUFA. Treatments during recovery were: 1) high fiber, low oil

diet (Control; 31.8% NDF and no added oil), 2) low fiber, low oil diet (LF; 28.4% NDF and no added oil), and 3) high fiber, high oil diet (HO; 31.5% NDF, 1.5% soybean oil and 7.8% whole soybeans; Table 4-1). The LF and HO represent lower fiber and higher oil relative to the control, respectively. Monensin (Rumensin 90; Elanco Animal Health, Greenfield, IN) was top-dressed to all cows at a rate of 450 mg/cow per day in 1.0 kg (DM Basis) of cookie meal for the duration of the experiment. All cows received bST (Posilac; Elanco Animal Health) every 14 d.

Feed Sampling and Analysis

Cows were fed individually once daily (0800 h) at 110% of expected intake and intake was recorded daily. Forage and base diet DM concentration was determined weekly for diet adjustment and DMI determination (72 h at 55°C). All individual feed ingredients were sampled weekly and stored at -20°C, thawed at room temperature, and dried in a forced-air oven for 72 h at 55°C for determination of DM content. Individual feeds were ground in a Wiley mill through a 1-mm screen (A. H. Thomas Co., Philadelphia, PA). Samples of all ingredients were composited by period and individual forages and a representative mixture of concentrate feeds were analyzed for nutrient composition by wet chemistry procedures (Cumberland Valley Analytical Services Inc., Maugansville, MD). Briefly, assays conducted were DM and CP according to AOAC (2000), NDF and ADF according to Van Soest (1991) using heat stable amylase and sodium sulfite, and starch according to Hall [(2009); Table 4-1]. Monensin concentration

in the top dress was determined by HPLC with tandem mass spectrometry (Covance laboratories, Greenfield, IN).

Milk Sampling and Analysis

Cows were milked twice daily at 0500 and 1700 h and milk yield determined by an integrated milk meter (AfiMilk; SAE Afikim, Israel). Milk was sampled at both milkings on d 0, 3, 6, 9, 12, 15, and 18 of each recovery phase. One subsample was stored at 4°C with preservative (Bronolab-WII) until analyzed for fat (Filter B) and protein by infrared spectroscopy [Fossomatic 4000 Milko-Scan and 400 Fossomatic, Foss Electric; AOAC (2000) method 972.160, Dairy One Lab]. A second subsample was immediately spun at 3,000 x g at 4°C and fat cake stored at -20°C. Samples were thawed in room temperature water and pooled within day by milk fat yield, and analyzed for FA composition as described in Chapter 3. Briefly, milk lipids were extracted with hexane:isopropanol according to Hara and Radin (1978), and transmethylated as described by Christie (1982)) and modified by Chouinard et al. (1999). Quantification of FA methyl esters was performed by gas chromatography with a flame ionization detector. Milk FA yields were calculated similarly to Glasser et al. (2007) as described in Chapter 3. The milk FA desaturase indexes were calculated as an estimation of the activity of the stearoyl Co-A desaturase enzyme [product/(substrate + product)].

Rumen pH Observation and Analysis

Ruminal pH was monitored in 8 ruminally cannulated cows for 24 h on d 0, 3, 6, 9, 12, 15, and 18 of each recovery phase using indwelling ruminal pH probes (Kahne Ltd., Auckland, New Zealand). Probes were programmed to record pH every 5 min during each 24 h period. A two-point calibration (pH 4 and 7) was conducted before insertion and after removal. Data were disregarded if either point was outside of ± 0.1 units from calibration at the time of removal. Mean pH, standard deviation, and time under pH 5.8 and 5.6 were determined for each observation day using Igor Pro 6.2.2.2 (WaveMetrics, Inc., Lake Oswego, OR) as described by Oba and Allen (2000).

Statistical Analysis

Data were analyzed using the MIXED procedure of SAS with repeated measures (version 9.3, SAS Institute, Cary, NC). The model was $Y_{ijklm} = \mu + S_i + P_j + C_k(S_i) + X_l + T_m + D_n + T_m \times D_n + e_{ijklmn}$, where Y_{ijklmn} is the variable of interest, μ is the overall mean, S_i is the random effect of sequence (i = 1 to 3), P_j is the random effect of period (j = 1 to 3), $C_k(S_i)$ is the random effect of cow nested in sequence (k = 1 to 17), X_l is the fixed effect of d 0 of the recovery phase as covariate, T_m is the fixed effect of treatment (m = 1 to 3), D_n is the fixed effect of time (n = 1 to 7), $T_m \times D_n$ is the interaction of treatment and time, and e_{ijklmn} is the residual error. The ARH(1) or AR(1) covariance structures were used based on model fit, time was the repeated variable, and cow by treatment was the subject. The preplanned contrasts were control vs HO and control vs LF at each time point. Significance and tendencies of main effects and

preplanned contrasts were declared at $P < 0.05$ and $P < 0.10$, respectively, and interactions at $P < 0.10$ and $P < 0.15$, respectively.

Secondly, a random regression analysis was performed on milk fat concentration and yield and on concentration of select milk FA (MIXED procedure of SAS) according to the following model: $Y_{ijklm} = \mu + C_i + P_j + T_k + D_l + D_l^2 + T_k \times D_l + T_k \times D_l^2 + e_{ijkl}$, where μ is the overall mean, C_i is the random effect of cow (i = 1 to 17), P_j is the random effect of period (j = 1 to 3), T_k is the fixed effect of treatment (k = 1 to 3), D_l is the linear effect of time (l = 1 to 7), D_l^2 is the quadratic effect of time (l = 1 to 7), $T_k \times D_l$ is the interaction of treatment and time, $T_k \times D_l^2$ is the interaction of treatment and the quadratic effect of time, and e_{ijkl} is the residual error. A reduced model was used that excluded the interaction of treatment by the quadratic effect of time when not significant. The ARH(1) and UN covariance structures were used based on model fit.

In both analyses the denominator degrees of freedom were calculated by the Kenward and Rogers method. Data were log transformed when appropriate and back-transformed data are reported. Data points with Studentized Residuals outside of ± 3.0 were considered outliers and excluded from the analysis, typically less than 3 data points per time point.

Results

Induction of milk fat depression

The induction diet reduced milk fat concentration by 27± 6% (mean ± SD) in all periods. Average milk fat concentrations at the start and end of each MFD induction phase were averaged 3.11 ± 0.11% and 2.26 ± 0.11%, respectively (mean ± SEM). Chemical composition of all diets followed closely expected values and monensin concentration in the premix topdress was 87% of target value.

Dry Matter Intake and Milk Production and Composition

There was a treatment by time interaction for DMI (Table 4-2). Dry matter intake sharply decreased in all treatments between d 0 and 1 of the recovery phase, and progressively increased thereafter reaching a plateau on approximately d 7 (Figure 4-1a). Dry matter intake was higher in LF compared to control on 16 of 18 d and HO was lower than control on d 16. There was no treatment by time interaction for milk yield; although on d 15 LF was higher than control (4.7 kg/d; $P < 0.01$). There was an effect of time ($P = 0.04$) and a tendency ($P = 0.09$) for an effect of treatment on milk yield (Table 4-2). Milk yield decreased over time and overall tended to be higher in LF compared to control (42.3 vs. 44.2 kg/d).

There were no treatment by time interactions for milk fat concentration and yield. Milk fat concentration and yield increased progressively in all treatments during the recovery phase (Time $P < 0.001$; Table 4-2; Figure 4-1c and 4-1d). Overall, milk fat

concentration was lower in LF compared to control ($P < 0.05$). Specifically, LF decreased ($P = 0.02$) milk fat concentration compared to control from d 12 to 18 by 9.0 ± 1.8% (Mean ± SD); however, LF did not affect milk fat yield at any time point. Milk fat concentration and yield were not different between control and HO except on d 15, when HO decreased milk fat yield by 11% relative to control ($P < 0.03$).

There was an effect of time on milk protein yield ($P = 0.04$), but no treatment or treatment by time interaction. Compared to control, LF increased milk protein yield by 11.0 ± 1.5% (Mean ± SD) from d 15 to 18 ($P < 0.05$). However, there was a treatment by time interaction for milk protein concentration ($P < 0.05$). Concentration of milk protein progressively decreased from d 3 to 18 in all treatments (Time effect: $P < 0.001$; Table 4-2), however, from d 6 to 18 it was 2.9 ± 0.6% (Mean ± SD) higher in HO compared to control ($P < 0.05$; not shown).

Milk de novo and Preformed FA

There was a treatment by time interaction ($P < 0.001$; Table 4-2) for the yield of milk FA less than 16 C (de novo synthesized) and 16 C FA and the proportion of milk de novo, 16 C FA, and greater than 16 C (preformed FA; Table 4-3).

The concentration of milk de novo FA increased progressively for the control and LF treatments, but was marginally decreased in LF compared to control from d 15 to 18 [4.3 ± 0.38% (Mean ± SD); $P < 0.05$; Figure 4-2a]. Similarly, yield of de novo synthesized FA increased for both control and LF, but was not different between control and LF at any time point (Figure 4-2b). In contrast, concentration and yield of de novo

synthesized FA remained relatively constant in HO treatment and were decreased compared to control from d 3 to 18 [$P < 0.01$; 27.4 ± 1.2% and 34.0 ± 3.4% (Mean ± SD) on d 15 and 18, respectively].

Milk 16 C FA concentration approached near maximal values by d 3 in both control and LF, but tended to be higher in LF than control on d 3 and 9 ($P = 0.09$). However milk 16 C FA remained constant in HO and was lower than control from d 3 to 18 [$P < 0.001$; 12.4 ± 3.4% (Mean ± SD); Figure 4-2c]. Similarly, yield of 16 C FA increased progressively in control and LF, and tended ($P < 0.1$) to be lower in LF than in control on d 12, however, it was maintained relatively constant in HO and was lower than control from d 3 to 18 [$P < 0.001$; 21.2 ± 4.5% (Mean ± SD); Figure 4-2d].

Concentration of milk preformed FA decreased progressively in control and LF and tended to be lower in LF compared to control on d 6 and 9 ($P < 0.1$; Figure 4-2e). In contrast, concentration of milk preformed FA remained nearly constant in HO and was on average 20.5 ± 5.3% (Mean ± SD) higher than control from d 3 to 18 ($P < 0.01$). Yield of milk preformed FA remained constant in control and LF, but modestly increased over time in HO and was higher in HO compared to control on d 9 and 18 (16 and 12%, respectively; $P < 0.05$; Figure 4-2f).

Milk *Trans* Fatty Acids

There was a treatment by time interaction for the concentration of milk *trans*-10 and *trans*-11 C18:1 and *trans*-10, *cis*-12 and *cis*-9, *trans*-11 CLA ($P < 0.01$; Table 4-3). Concentration of milk *trans*-10 C18:1 and *trans*-10, *cis*-12 CLA decreased progressively

in all treatments (Figure 4-3a and 4-3c). However, both FA were considerably higher in HO compared to control from d 3 to 18 [*trans*-10 C18:1: 135.8 ± 50.1% and *trans*-10, *cis*-12 CLA: 187.8 ± 57.3% (Mean ± SD)], whereas in the LF treatment both FA were marginally higher than control, but near baseline concentration, from d 9 to 18 [*trans*-10 C18:1: 67.4 ± 24.7% and *trans*-10, *cis*-12 CLA: 89.7 ± 22.4% (Mean ± SD)].

The concentration of milk *trans*-11 C18:1 decreased modestly over time in control and LF, but was lower in LF relative to control from d 6 to 18 [30.2 ± 2.7% (Mean ± SD); Figure 4-3b]. However, HO increased *trans*-11 C18:1 progressively and was higher than control from d 3 to 18 [96.0 ± 5.5% (Mean ± SD) from d 15 to 18]. *Cis*-9, *trans*-11 CLA followed a similar pattern to *trans*-11 C18:1 (Figure 4-3d). Briefly, *cis*-9, *trans*-11 CLA decreased progressively in both control and LF and was not different at any time point. In contrast, HO increased progressively and was higher than control from d 3 to 18 [90.0± 3.7% (Mean ± SD) from d 15 to 18].

Milk Δ9-desaturase Indexes

There was a treatment by time interaction for C14 and C16 desaturase indexes (Table 4-3; Figure 4-4). Both indexes decreased progressively over time, but the C14 index was higher in HO and LF than control from d 6 to 9. Similarly, the C16 index was higher in HO than control from d 9 to 18, whereas it was higher in LF compared to control on d 6 and from d 12 to 18 ($P < 0.05$).

Ruminal pH

There was no interaction of treatment and time or overall effect of treatment on rumen average pH, standard deviation (SD), and time below pH 5.8 or pH 5.6. However, there was an effect of time for all variables (Table 4-5; Figure 4-5). Average rumen pH rapidly increased during the recovery phase in all treatments, peaked on d 3, and modestly decreased thereafter (Figure 4-5a). The SD of rumen pH decreased initially (d 0 to 3) in all treatments and remained relatively constant thereafter, and tended to be higher in HO compared to control only on d 3 (P = 0.09). The time below pH 5.8 and 5.6 decreased rapidly, reached their minimal values on d 3 in all treatments, and progressively increased from d 3 to 18 (Figure 4-5c and 4-5d). However, the day prior to recovery (d 0) animals in the LF group spent more time below pH 5.6 (P = 0.04) and tended to spend more time below pH 5.8 (P = 0.09).

Discussion

Alteration of ruminal fermentation in the presence of dietary PUFA results in MFD, although basal levels of PUFA are sufficient for the condition to occur. Milk fat depression can be induced by feeding high concentrate, low fiber diets (Peterson et al., 2003), low fiber, high oil diets (Griinari et al., 1998; Harvatine and Bauman, 2006; Gama et al., 2008), or high fiber, high oil diets (Shingfield et al., 2006; Alzahal et al., 2008). In the present study, cows were fed a low fiber, high oil diet for 10 d to induce MFD. This is representative of a high fermentability, high PUFA diet using ingredients typically fed

and represents diets sometimes fed on dairy farms due to changes in forage DM, nutrient composition, and mixing errors that lead to diet-induced MFD. Additionally, it is also representative of smaller decreases in milk fat observed when diet are marginally high in PUFA or fermentability. Previously, induction of MFD was achieved in 10 d using a similar high corn silage, low fiber, and high PUFA diet (Chapter 3). The observation of recovery from MFD requires a reduction in milk fat synthesis, although a maximal reduction is not necessary. In the present study, milk fat concentration was lower that typically observed in Holstein cows and it is possible that some degree of MFD was present, however, MFD induction was successful, as milk fat concentration was reduced by approximately half of the 50% maximal decrease possible with classical MFD (Bauman and Griinari, 2001).

The recovery diets were designed to represent correction of diet fermentability, PUFA level, or both fermentability and PUFA level. Correction of PUFA was achieved by removal of soybean oil and substitution of expeller soybean meal for whole roasted soybeans resulting in a 1.6 and 7.5 percentage units (DM basis) decrease in soy oil and soybeans, respectively, in the control diet relative to the MFD induction diet. Diet fermentability was corrected in the control diet by increasing dietary NDF by 5.9 percentage units relative to the MFD induction diet. Recovery diets were balanced relative to the control and not relative to the induction diet, which was simply used to achieve MFD. Compared to control, the effect of dietary fat was tested by increasing dietary soy oil and soybeans by 1.5 and 7.8 percentage units (DM basis), respectively, in the HO diet; whereas the effect of fiber was tested by a forage to grain substitution that

decreased dietary NDF 3.4% units (DM basis) in LF relative to control. Altered ruminal fermentation that results in absorption of bioactive FA is a result of the rumen environment, carbohydrate and FA profile and availability, and many complex interactions. It is important to recognize that treatments are expected to have an impact on the rumen environment, microbial populations, and amount of substrate available for biohydrogenation. A 7 d washout period was used between periods in the current experiment because correction of only fiber or PUFA was expected to limit recovery. Additionally, complete rescue was not necessary entering the subsequent induction period.

All recovery diets contained lower NDF compared to the induction diet, which may explain the observed initial reduction in intake at the start of the recovery phase. The LF diet was lower in NDF and forage NDF than the control diet and a change in DMI is expected depending on ruminal fill and energy balance (Allen, 2000). The increased intake observed with LF may indicate physical fill limitation of intake in the higher forage NDF control and HO diets, and may have allowed for the observed numerically higher milk yield and FCM in LF treatment. Rumen pH did not differ between LF and control and milk *trans* isomer profile was quite similar indicating little difference in the rumen environment and microbial populations. Although not determined, there is little indication of a difference in diet digestibility. Dry matter intake was unaffected by HO compared to control, despite the higher dietary oil concentration and energy density in the HO diet. This difference may have been too small to observe a change in intake or the higher FA concentration may have altered the ruminal bacteria populations and decreased fiber digestibility because of the negative effects of PUFA on fibrolytic bacteria (Jenkins,

1993; Maia et al., 2007). Rumen pH was similar between HO and control, but milk *trans* isomer profile clearly indicated altered fermentation with HO, which is associated with decreased fiber digestibility (Jenkins, 1993).

Previously, we have reported that correction of both dietary fiber and PUFA concentration recovered milk fat concentration and yield within 19 d (Chapter 3). Similarly, Weiss et al. (2012) reported that milk fat concentration was rescued gradually within 2 weeks when dietary NDF was increased from 28 to 33% of diet DM by feeding a corn milling by-product. In the current study, a similar progressive increase in milk fat yield was observed over 18 d by increasing fiber, minimizing PUFA, or increasing fiber and minimizing PUFA. However, maintaining high PUFA decreased the recovery of de novo lipid synthesis and normalization of ruminal biohydrogenation.

Correction of both dietary fiber and oil concentration resulted in complete recovery of milk fat yield and de milk novo FA yield in 19 and 21 d, respectively, in our previous work (Chapter 3). In the current experiment, milk fat concentration was lower in the LF diet from d 12 to 18; however, milk yield was numerically increased during the same period resulting in no change in milk fat yield. Differences in the concentration and yield of de novo synthesized FA was minimal between control and LF and both followed a similar trend; increasing progressively during recovery from MFD and plateauing around d 15. In contrast, the HO treatment resulted in lower milk fat yield on d 15 and maintained a milk FA profile consistent with classical diet-induced MFD (Bauman and Griinari, 2003). Specifically, de novo FA concentration and yield did not recover in the HO treatment and resulted in a decrease in milk fat yield despite the small increase in yield of preformed FA. The decreased recovery of de novo FA synthesis is likely related

to the higher milk *trans*-10 18:1 and *trans*-10, *cis*-12 CLA concentrations in the HO treatment, which despite decreasing progressively during recovery were considerably higher than control. The progressive increase in milk *trans*-11 C18:1 and *cis*-9, *trans*-11 CLA indicated a gradual return to normal ruminal BH, although with a limited capacity. The observed increase in milk preformed FA concentration and yield in the HO group was likely the result of increased absorbed FA from soy oil and whole soybeans and indicated little inhibition of the uptake of preformed FA in the mammary gland.

Monensin has been shown to act synergistically with dietary unsaturated FA to further increase *trans*-10 18:1 concentration in milk (Alzahal et al., 2008; He et al., 2012). In the current experiment all diets were supplemented with monensin. Recovery of fat yield, de novo FA yield, and normal *trans* FA in the control and LF group suggested complete or near complete recovery from MFD by d 18. However, it appears that some degree of MFD was still present by d 18 in the HO treatment as suggested by the higher milk *trans* FA content. This is likely a result of an altered microbial population due to dietary PUFA concentration or an interaction of monensin and PUFA (Jenkins et al., 2003; Alzahal et al., 2008; Weimer et al., 2010b).

Previously, a two-phase response has been observed for BH intermediates during recovery from MFD by correction of dietary fiber and PUFA (Chapters 3 and 5). Briefly, milk concentrations of intermediates in the altered BH pathway (*trans*-10 C18:1 and *trans*-10, *cis*-12 CLA) decreased during the first phase and presumably represent re-establishment of normal microbial populations. The second phase was characterized by a slight elevation of *trans* FA in the normal pathway (*trans*-11 C18:1 and *cis*-9, *trans*-11 CLA) suggesting a secondary phase of adaptation in ruminal microbes and BH capacity.

In the current experiment, FA in the altered pathway decreased rapidly during the first phase of recovery for control and LF treatments, but the isomers in the normal pathway were not elevated above baseline in a secondary phase. In the HO treatment the isomers in the altered pathway also decreased progressively in the first phase, although the decrease was delayed compared to control. However, a second phase was evident in HO as isomers in the normal pathway increased progressively indicating a gradual return to the normal BH pathway. It is possible that monensin supplementation prevented or delayed the second adaptation in BH intermediates observed previously (Chapters 3 and 5).

Milk FA desaturase indexes are used as proxies for the activity of stearoyl-CoA desaturase enzyme [SCD (Perfield et al., 2006)]. Milk desaturase indexes have been shown to decrease progressively and reach a nadir from d 7 to 12 during recovery from MFD when both dietary fiber and PUFA are corrected (Chapters 3 and 5). Similarly, in the present experiment, the C14 and C16 desaturase indexes decreased progressively in all treatments and reached a nadir around d 15, although they were consistently higher in LF and HO compared to control through d 18. Increased desaturase activity has been observed previously during diet-induced MFD [(Gama et al., 2008); Chapter 3], although *trans*-10, *cis*-12 CLA treatment decreases desaturase indexes. The desaturase enzyme is very responsive to dietary and environmental factors and may be influenced by other bioactive compounds during diet-induced MFD. The difference in desaturase index demonstrates metabolic difference in mammary metabolism between control and LF and HO, even though smaller differences in de novo synthesis and bioactive FA were observed between control and LF.

Diet-induced MFD is correlated with changes in the rumen environment, microbial populations, and amount and type of FA and carbohydrate substrate available. Low rumen pH has been classically proposed as a driving factor in the change in the microbial population and rates and pathway of BH (Choi et al., 2005; Fuentes et al., 2009). However, rumen pH was corrected rapidly when fed the recovery diet, but the change in BH occurred progressively. Additionally, rumen pH was corrected in the HO treatment, but recovery of normal BH was inhibited. The lag between improvement in rumen pH and recovery of normal BH may demonstrate the lag required for re-establishment of the microbial populations, or may represent a smaller role of pH in diet-induced MFD. Additionally, rumen pH was not different from control, but PUFA concentration alone reduced the rate of recovery.

PUFA are known to have direct toxic effects on ruminal bacteria (Maia et al., 2007; Maia et al., 2010) resulting in a shift in the ruminal microbial population (Lourenço et al., 2010). The HO treatment slowed the reduction in intermediates of the altered BH pathway, although a progressive return to the normal BH pathway was observed. In addition to changes in BH, excess dietary PUFA are known to inhibit fiber digestibility (Jenkins, 1993). Importantly, as indicated for the lack of effect on DMI and milk yield, it is likely that the negative effects of the excess dietary PUFA in HO did not greatly affect fiber digestibility.

Conclusions

Recovery of milk fat synthesis and normal ruminal BH can most effectively be achieved through correction of dietary PUFA concentration. Decreasing diet fermentability provides additional benefits, but may result in reduced milk yield. Increasing dietary fiber while maintaining a high PUFA concentration resulted in reduced milk fat yield and milk de novo FA, and higher milk *trans* FA, all of which are consistent with classical diet-induced MFD. Additionally, milk fat synthesis was rapidly rescued by correcting diet fermentability and PUFA concentration while maintaining monensin supplementation and provides support for focusing on dietary PUFA and other risk factors when designing strategies to recover from diet-induced MFD.

Table 4-1. Ingredient and chemical composition of experimental diets.

	Diet[1]			
	Induction	Control	HO	LF
Ingredient, % of DM				
Corn silage[2]	36.3	41.1	39.9	35.8
Alfalfa haylage[3]	7.7	18.5	19.3	15.6
Canola meal	11.2	11.1	10.5	10.6
Ground corn	18.9	6.6	3.9	15.3
Roasted soybeans	7.5	-	7.8	-
Expeller soybean meal[4]	0.8	8.8	2.6	6.7
Cottonseed hulls	3.5	3.8	4.8	4.1
Cookie meal	4.6	1.9	1.8	4.3
Grass hay/straw[5]	3.1	3.1	3.1	2.6
Minerals and vitamins mix[6]	2.4	3.0	2.8	3.0
Sugar cane molasses	2.1	1.5	1.4	1.4
Soybean oil	1.6	-	1.5	-
Optigen[7]	0.4	0.5	0.5	0.5
Nutrient[8], % of DM				
CP	16.3	19.2	19.0	18.0
NDF	25.9	31.8	31.3	28.4
ADF	16.3	21.6	21.6	19.0
Starch	31.3	24.0	21.6	29.0
Mean particle size[9], mm	4.32	3.81	4.19	3.81

[1]Induction is a lower forage and higher oil diet fed during the induction phase of each period; Control is correction of fiber and oil concentration; HO is correction of fiber concentration, but not oil concentration; LF is correction of oil concentration, but not fiber concentration.

[2]Contained 38.5% DM, 8.1% CP, 31.1% NDF, and 17.3% ADF.

[3]Contained 46.4% DM, 20.8% CP, 40.9% NDF, and 35.4% ADF.

[4]SoyPLUS (West Central Cooperative, Ralston, IA).

[5]Contained 88.9% DM, 8.6% CP, 68.2% NDF, and 41.3% ADF.

[6]Contained (%, as fed basis): 45.8 dried corn distillers grains with solubles; 35.8 limestone (38% Ca); 8.3 magnesium oxide (54% Mg); 6.4 salt; 1.73 vitamin ADE premix; 1.09 selenium premix (0.06% selenium); and 0.88 trace mineral mix. Composition (DM basis): 11% CP; 18% NDF; 5.2% fat; 14.9% Ca; 0.35% P; 4.58% Mg; 0.41% K; 0.31% S; 357 mg/kg Cu; 1,085 mg/kg Zn;

181 mg/kg Fe; 6.67 mg/kg Se; 125,875 IU/kg vitamin A (retinyl acetate); 31,418 IU/kg vitamin D (Activated 7-dehydrocholesterol); and 946 IU/kg vitamin E (dl-alpha tocopheryl acetate).

[7]Optigen is a non-protein N source (278% CP, DM basis), Alltech Inc. (Lexington, KY).

[8]Analyzed by Cumberland Valley Analytical Services (Maugansville, MD; n = 3 per diet).

[9]The particle size distribution was analyzed using the Penn State Particle Separator (n = 2 per diet). Particles remaining (% of total) in the upper sieve, middle sieve, lower sieve, and bottom pan were 3, 27, 47, and 24, respectively for the Induction diet; 3, 35, 41, and 22, respectively for the Control diet; 2, 31, 45, and 21, respectively for the HO diet, and 3, 28, 45, and 25, respectively for the LF diet.

Table 4-2. Effect of primarily correcting dietary fiber or PUFA concentration on milk production and composition during recovery from diet-induced milk fat depression in monensin supplemented diets.

	Treatment[1]				*P*-value[2]		
Item	Control	HO	LF	SEM	Trt	Time	Trt x Time
DMI, kg/d	25.4	25.8	27.3^{F}	0.40	<0.001	<0.001	0.07
Milk Yield, kg/d							
Milk	42.3	41.9	44.2 ^{f}	1.36	0.09	0.04	0.24
Fat	1.10	1.04	1.09	0.04	0.21	<0.001	0.18
Protein	1.33	1.35	1.40	0.04	0.16	0.04	0.20
Milk Composition, %							
Fat, %	2.65	2.57	2.50^{F}	0.09	0.02	<0.001	0.16
Protein, %	3.13	3.24	3.16	0.03	<0.001	<0.001	<0.05
Milk FA by source, g/d[3]							
< 16 C	240	177	233	8	<0.001	<0.001	<0.001
16 C	289	233	283	11	<0.001	<0.001	<0.001
> 16 C	446	482^{O}	429	16	0.01	0.01	0.11

[1]Control = high forage low oil diet; HO = high forage high oil diet; LF = low forage low oil diet during recovery from diet-induced milk fat depression.

[2]Trt = Treatment effect; Time = effect of day in recovery from milk fat depression.

[3]FA < 16 C originate from de novo synthesis in the mammary gland; FA > 16 C originate from extraction from plasma; 16 C FA originate from both sources.

Preplanned contrasts tested the difference between Control and LF (^{F} = $P < 0.05$, and ^{f} = $P < 0.1$), and between Control and HO (^{O} $P < 0.05$, and ^{o} $P < 0.1$).

Table 4-3. Effect of primarily correcting dietary fiber or PUFA concentration on milk FA composition during recovery from diet-induced milk fat depression in monensin supplemented diets.

	Treatment[1]				*P*-value[2]		
FA, % of FA	Control	HO	LF	SEM	Trt	Time	Trt x Time
trans-10 C18:1	2.62	4.55	3.23	0.25	<0.001	<0.001	<0.001
trans-11 C18:1	1.47	2.28	1.19	0.12	<0.001	<0.001	<0.001
cis-9, *trans*-11 CLA[3]	0.99	1.47	0.89	0.06	<0.001	<0.001	<0.001
trans-10, cis-12 CLA	0.019	0.038	0.025	0.002	<0.001	<0.001	<0.001
FA < 16 C[4]	22.6	17.8	22.1	0.21	<0.001	<0.001	<0.001
16 C	26.8	23.9	27.4	0.32	<0.001	<0.001	<0.001
FA > 16 C	42.7	49.9	42.3	0.49	<0.001	<0.001	<0.001
Sum of *trans* C18:1	5.97	9.93	6.33	0.20	<0.001	<0.001	<0.001
C14 desaturase index[5]	0.13	0.14	0.14	0.005	<0.001	<0.001	<0.001
C16 desaturase index	0.07	0.08	0.08	0.003	<0.001	<0.001	<0.001

[1]Control = high forage low oil diet; HO = high forage high oil diet; LF = low forage low oil diet during recovery from diet-induced milk fat depression.

[2]Trt = Treatment effect; Time = effect of day in recovery from milk fat depression.

[3]Conjugated linoleic acid.

[4]FA < 16 C originate from de novo synthesis in the mammary gland; FA > 16 C originate from extraction from plasma; 16 C FA originate from both sources.

[5]C14 desaturase index = C14:1/(C14:1 + C14:0); C16 desaturase index = C16:1/(C16:1 + C16:0).

Table 4-4. Effect of primarily correcting dietary fiber or PUFA concentration on the rate of recovery of milk composition and FA profile determined by random regression.

Item	Treament[1]	Estimates[2]			*P*-value[3]		
		Int	L	Q	Int	L	Q
Milk Fat							
%	Control	2.23	0.057	-0.00087	<0.001	<0.001	<0.05
	HO	2.12	0.069	-0.00161			
	LF	2.24	0.046	-0.00111			
kg/d	Control	1.03	0.015	-	<0.001	<0.001	-
	HO	0.95	0.011	-			
	LF	0.98	0.015	-			
Milk FA by Source, % of Total FA[4]							
< 16 C	Control	18.50	0.550	-0.0124	<0.001	<0.001	<0.001
	HO	18.33	-0.234	0.0122			
	LF	19.50	0.513	-0.0142			
16 C	Control	24.53	0.427	-0.0136	<0.001	<0.001	<0.001
	HO	24.36	-0.092	0.0036			
	LF	24.52	0.598	-0.0234			
> 16 C	Control	48.36	-0.893	0.0246	<0.001	<0.001	<0.001
	HO	48.56	0.379	-0.0170			
	LF	47.49	-1.002	0.0338			
Milk *Trans* FA, % of Total FA							
trans-10 C18:1							
	Control	5.56	-0.710	0.0247	<0.001	<0.001	<0.001
	HO	5.79	-0.307	0.0069			
	LF	4.94	-0.533	0.0185			
trans-11 C18:1							
	Control	1.51	-0.023	0.0010	<0.001	<0.001	<0.001
	HO	1.66	0.066	-0.0002			
	LF	1.80	-0.127	0.0049			
cis-9, *trans*-11 CLA[5]							
	Control	1.24	-0.051	0.0017	<0.001	<0.001	<0.001
	HO	1.26	0.002	0.0010			
	LF	1.34	-0.091	0.0033			
trans-10, *cis*-12 CLA							
	Control	0.04	-0.006	0.0002	<0.001	<0.001	<0.001
	HO	0.05	-0.001	0.00002			
	LF	0.04	-0.004	0.0001			

[1]Trt = Control = high forage low oil diet; HO = high forage high oil diet; LF = low forage low oil diet during recovery from diet-induced milk fat depression.

[2] Intercept (Int) and slopes of the regression terms; L = linear and Q = quadratic effect of day in recovery from milk fat depression.

[3]Probability for effect of the regression term. The interaction of treatment and the linear (L) and quadratic (Q) effects of time.

[4]FA <16 C originate from de novo synthesis in the mammary gland; FA >16 C originate from extraction from plasma; 16 C FA originate from both sources.

[5]Conjugated linoleic acid.

Table 4-5. Effect of primarily correcting dietary fiber or PUFA concentration on the rate of recovery of ruminal pH parameters during recovery from milk fat depression.

	Treatment[1]				*P*-value[2]		
pH parameter	Control	HO	LF	SEM	Trt	Time	Trt x Time
Average	6.11	6.15	6.11	0.04	0.64	<0.001	0.84
Standard deviation	0.35	0.33	0.39	0.02	0.07	<0.01	0.68
Time below 5.8, h	5.03	4.60	5.41	0.81	0.76	<0.001	0.82
Time below 5.6, h	2.38	2.07	3.00	0.63	0.36	<0.001	0.71

[1]Control = high forage low oil diet; HO = high forage high oil diet; LF = low forage low oil diet during recovery from diet-induced milk fat depression.

[2] Trt = Treatment effect; Time = effect of day in recovery from milk fat depression.

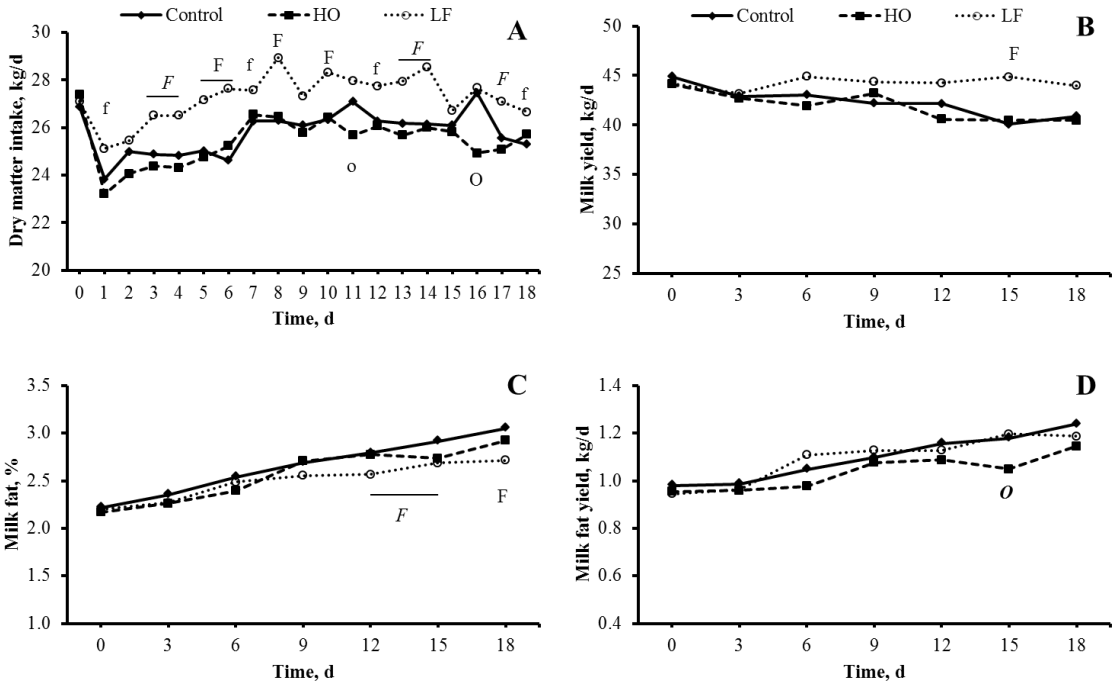


Figure 4-1. Effect of primarily correcting dietary fiber or PUFA concentration on the time course of intake, milk production, and milk fat during recovery from milk fat depression.

Shown are dry matter intake (Panel A), milk yield (Panel B), milk fat concentration (Panel C), and milk fat yield (Panel D). Cows were fed a high forage, low oil diet (Control; Diamond), a high forage, high oil diet (HO; Square) or a low forage, low oil diet (LF; open circle). Preplanned contrasts tested the difference between Control and LF ($^{F=}$ $P < 0.01$, ^{F} $= P < 0.05$, and ^{f} $= P < 0.1$), and between Control and HO (^{O} $P < 0.01$, ^{O} $P < 0.05$, and ^{o} $P < 0.1$; SEM = 0.40, 1.36, 0.09, and 0.04, for dry matter intake, milk yield, milk fat concentration, and milk fat yield, respectively).

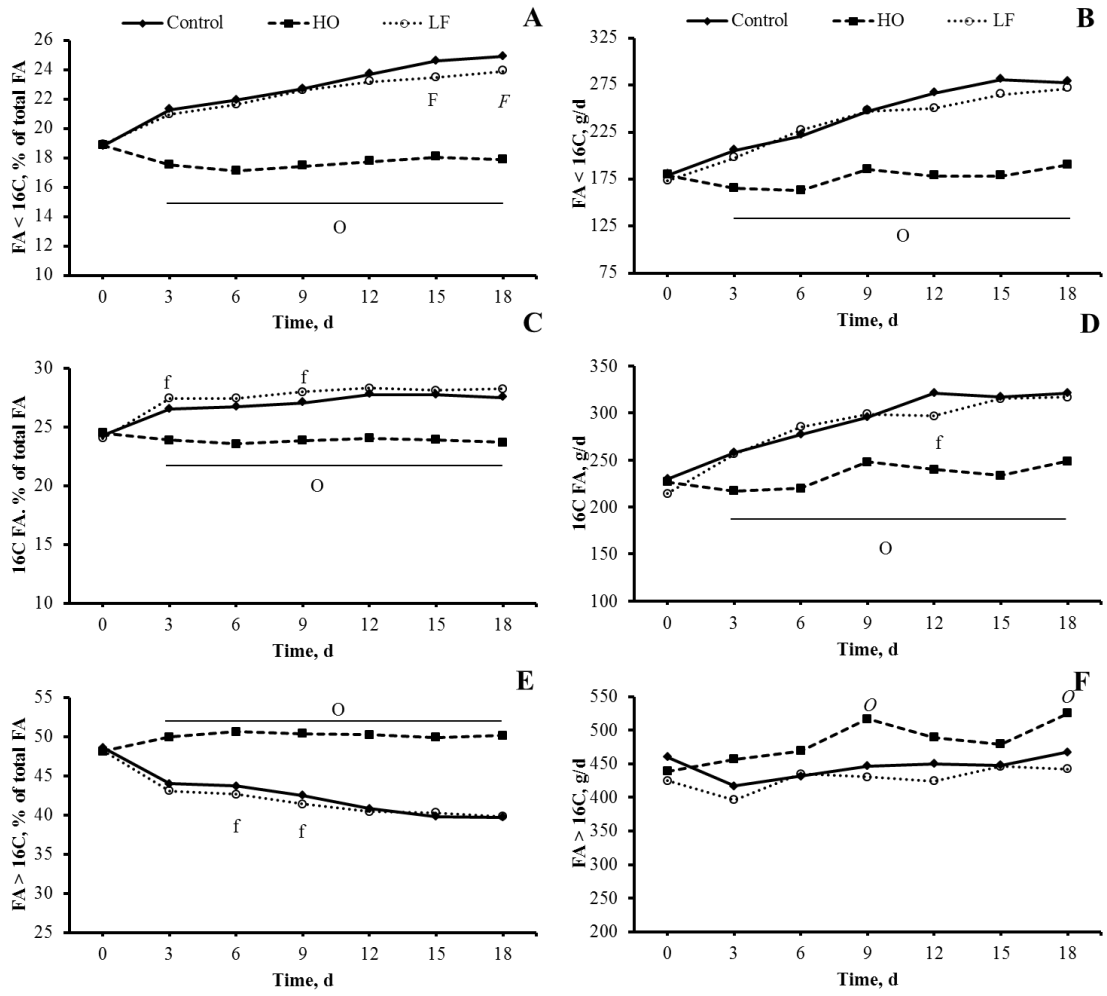


Figure 4-2. Effect of primarily correcting dietary fiber or PUFA concentration on the time course of concentration and yield of milk FA by source during recovery from milk fat depression.

Shown are the milk fat concentration and yield of de novo (Panel A and B, respectively), 16 C (Panel C and D, respectively), and preformed (Panel E and F, respectively) FA. Cows were fed a high forage, low oil diet (Control; Diamond), a high forage, high oil diet (HO; Square) or a low forage, low oil diet (LF; open circle). Preplanned contrasts tested the difference between Control and LF ($^{F=}$ $P < 0.01$, ^{F} = $P < 0.05$, and ^{f} = $P < 0.1$), and between Control and HO (^{O} $P < 0.01$, ^{O} $P < 0.05$, and ^{o} $P < 0.1$; SEM = 0.21, 8, 0.32, 11, 0.49, and 16 for the concentrations and yields of de novo, C16, and preformed FA, respectively).

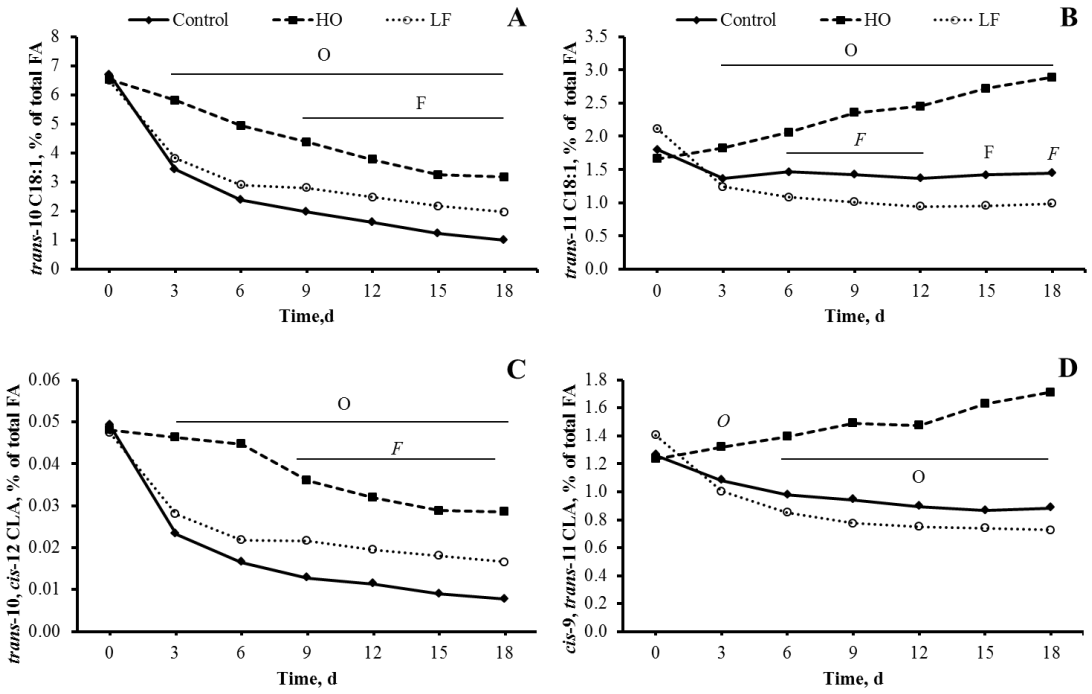


Figure 4-3. Effect of primarily correcting dietary fiber or PUFA concentration on the time course of milk trans FA concentration during recovery from milk fat depression.

Shown are the predominant isomers of the alternate biohydrogenation pathway [*trans*-10 C18:1 (Panel A) and *trans*-10, *cis*-12 CLA (Panel C)] and the predominant isomers of the normal biohydrogenation pathway [*trans*-11 C18:1 (Panel B) and *cis*-9, *trans*-11 CLA (Panel D). Cows were fed a high forage, low oil diet (Control; Diamond), a high forage, high oil diet (HO; Square) or a low forage, low oil diet (LF; open circle). Preplanned contrasts tested the difference between Control and LF ($^{F=}$ $P < 0.01$, ^{F} = $P < 0.05$, and ^{f} = $P < 0.1$), and between Control and HO (^{O} $P < 0.01$, ^{O} $P < 0.05$, and ^{o} $P < 0.1$; SEM = 0.25, 0.12, 0.002, and 0.06 for the concentrations of *trans*-10 C18:1, *trans*-11 C18:1, *trans*-10, *cis*-12 CLA, and *cis*-9, *trans*-11 CLA, respectively).

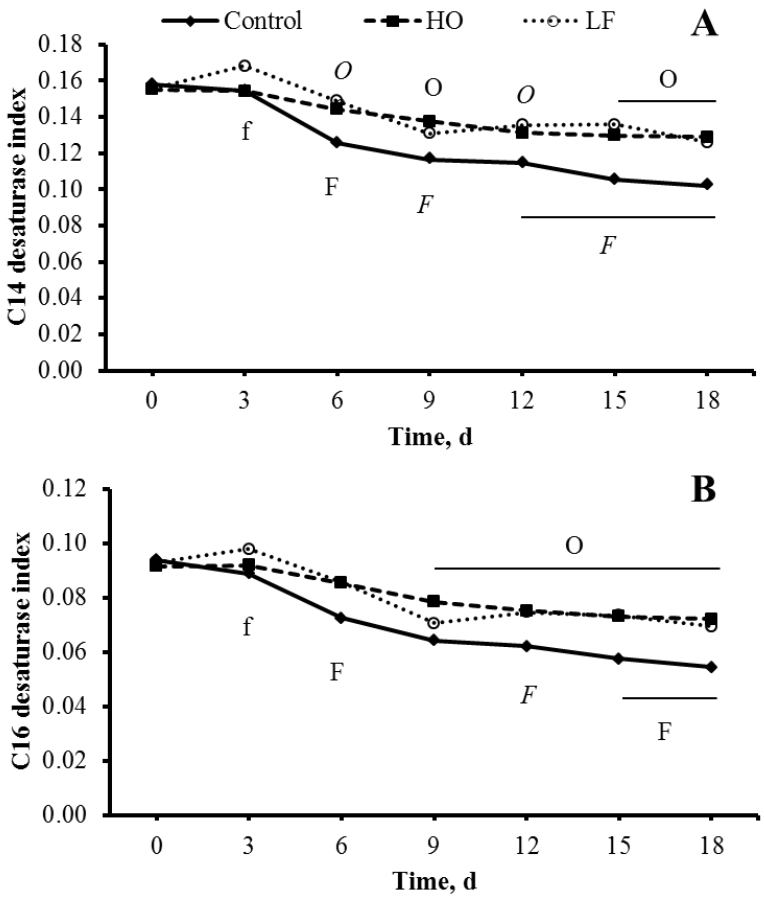


Figure 4-4. Effect of primarily correcting dietary fiber or PUFA concentration on the time course of C14 and C16 desaturase indexes.

Representative indexes shown include the C14 desaturase index (Panel A) and the C16 desaturase index (Panel B). Cows were fed a high forage, low oil diet (Control; Diamond), a high forage, high oil diet (HO; Square) or a low forage, low oil diet (LF; open circle). Preplanned contrasts tested the difference between Control and LF ($^{F=}$ $P < 0.01$, ^{F} $= P < 0.05$, and ^{f} $= P < 0.1$), and between Control and HO (^{O} $P < 0.01$, ^{O} $P < 0.05$, and ^{o} $P < 0.1$; SEM = 0.005 and 0.003 for the C14 and C16 desaturase indexes, respectively).

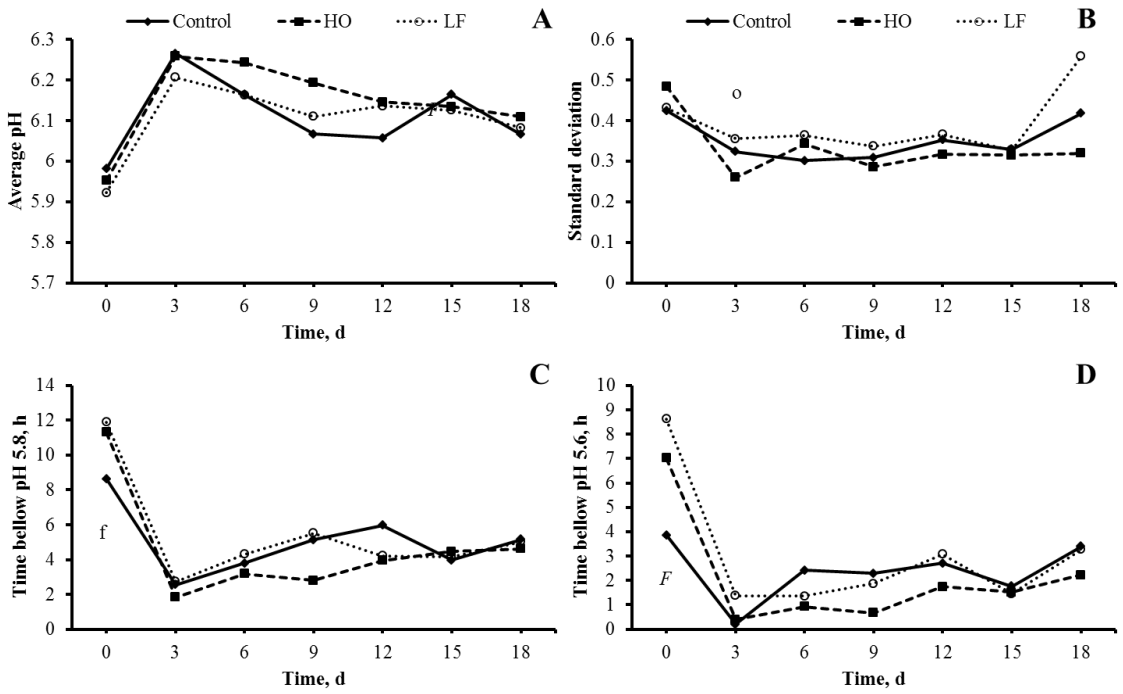


Figure 4-5. Effect of primarily correcting dietary fiber or PUFA concentration on the time course of ruminal pH parameters during recovery from milk fat depression.

Shown are average pH (Panel A), standard deviation (Panel B), time below pH 5.8 (Panel C), and time below pH 5.6 (Panel 5.6). Cows were fed a high forage, low oil diet (Control; Diamond), a high forage, high oil diet (HO; Square) or a low forage, low oil diet (LF; open circle). Preplanned contrasts tested the difference between Control and LF ($^{F=}$ $P < 0.01$, ^{F} $= P < 0.05$, and ^{f} $= P < 0.1$), and between Control and HO (^{O} $P < 0.01$, ^{O} $P < 0.05$, and ^{o} $P < 0.1$; SEM = 0.04, 0.02, 0.81, and 0.63 for average pH, standard deviation, and time bellow pH 5.8 and 5.6, respectively).

Chapter 5

Effect of Monensin on Recovery from Diet Induced Milk Fat Depression in Dairy Cows

Abstract

Sixteen Holstein cows were arranged in a crossover design that investigated the effect of monensin on the recovery from diet-induced milk fat depression (MFD). Milk fat depression was induced in the first phase of each period by feeding a low fiber, high oil diet (25.3% NDF, 4% whole soybeans, and 2.1% soybean oil) with monensin for 10 d. A recovery phase of 18 d followed where cows were switched to a high forage, low oil diet (31.2% NDF and no supplemental oil). Treatments during recovery were 1) control (no monensin supplementation) or 2) monensin administered as a topdress at a rate of 450 mg/cow per d. Dry matter intake was observed daily and milk yield and composition and milk FA profile were measured every 3 d during recovery. There was no effect of monensin on DMI or yield of milk or milk protein and lactose. Milk fat concentration and yield increased progressively during recovery in both treatments. However, there was no treatment by time interaction for milk fat yield. Monensin decreased milk fat yield from d 6 to 15, but it was the same as control on d 18. There was a treatment by time interaction for milk fat concentration, which was decreased by monensin only on d 3 and 6. The yield of milk de novo synthesized FA increased progressively in both treatments and was not affected by treatment. Similarly, yield of 16 C FA increased progressively, but was decreased by monensin on d 6 and 9. Preformed FA yield was lower in the monensin group from d 6 to 15, but was not different from control on d 18. Importantly,

milk FA concentration of *trans*-10 C18:1 and *trans*-10 *cis*-12 conjugated linoleic acid rapidly decreased in both groups, however, monensin slightly increased *trans*-10 C18:1 concentration above baseline on d 15 and 18. Monensin feeding slightly reduced the rate of recovery from diet-induced MFD predominantly through a delayed recovery of preformed FA, although a similar level of recovery was achieved by d 18. In conclusion, monensin supplementation has minimal impact on recovery of normal rumen biohydrogenation and de novo FA synthesis during recovery from milk fat depression by correction of dietary NDF and PUFA concentration.

Key words: Monensin, milk fat depression, dairy cows.

Introduction

Milk fat depression is characterized by a specific reduction in milk fat synthesis, and is caused by bioactive *trans* FA arising from altered ruminal fermentation (Bauman and Griinari, 2001). Many dietary factors impact the formation and ruminal outflow of bioactive FA including dietary concentration of highly fermentable feeds, PUFA, and effective fiber, feeding methods and ionophores. The interaction of these risk factors sometimes results in modification of the rumen environment, the microbial population, and the pathways of FA biohydrogentation, which leads to increased formation of the bioactive FA that cause MFD (Jenkins et al., 2003; Weimer et al., 2010b).

Ionophores are lipophilic molecules toxic to many bacteria, protozoa, and fungi (Russell and Strobel, 1989). Monensin is an ionophore that has been shown to increase milk yield and feed efficiency in lactating dairy cows (Ipharraguerre and Clark, 2003; Duffield et al., 2008b). Monensin alters the ion flux and ATPase systems of sensitive bacteria resulting in an increase in maintenance energy expenditure and compromised growth and reproduction (Ipharraguerre and Clark, 2003). Monensin supplementation results in selection of ruminal bacteria that produce less H_2 and acetate and more propionate and ATP (Russell and Strobel, 1989). In addition, in some circumstances monensin leads to reduced biohydrogenation capacity and utilization of pathways resulting in bioactive FA formation (Fellner et al., 1997; Jenkins et al., 2003).

The effect of monensin on milk fat yield and concentration is not consistent across studies as some have reported reductions (Phipps et al., 2000; Odongo et al., 2007; Alzahal et al., 2008), while other have reported no effect (Lean et al., 1994; Duffield et al., 1999; He et al., 2012). It is likely that the variation in response to monensin feeding is related to interactions with diet type. For example, starch source and level of dietary PUFA have been previously shown to reduce the rates of FA biohydrogenation [BH; (Fellner et al., 1997; Duffield et al., 2008b)] and increase the concentration of alternate BH intermediates, such as *trans*-10 C18:1, in rumen fluid and milk fat (Jenkins et al., 2003; Alzahal et al., 2008; He et al., 2012). In addition, monensin and PUFA synergistically increased *trans*-10 C18:1 formation in vitro (Jenkins et al., 2003) and in vivo (Alzahal et al., 2008; He et al., 2012).

Strategies to rescue milk fat synthesis after MFD has occurred are important to reduce the duration of the condition. We have previously validated the time course of

MFD induction and recovery, which provides the basis for the experimental design used (Chapter 3). Briefly, during induction of MFD milk fat yield was near nadir by 11 d and a higher fiber and low PUFA diet completely recovered occurred milk fat yield by d 19. Previous investigations of monensin have predominantly focused on the long-term or steady state impacts of supplementation; however, long-term changes in the microbial population after termination of monensin have been reported (Weimer et al., 2008). The objective of this study was to investigate the effect of monensin on the rate of recovery of milk fat synthesis and milk FA profile after induction of MFD while supplementing monensin in dairy cows. The hypothesis was that monensin would minimally affect the rate of recovery of milk fat synthesis.

Materials and Methods

Experimental Design and Treatments

The experiment was conducted from February to May of 2012. All experimental procedures were approved by the Pennsylvania State University Institutional Animal Care and Use Committee. Sixteen multiparous Holstein cows (183 ± 21 d postpartum; mean ± SD) were randomly assigned to treatment sequences in a crossover design balanced for residual effects. Cows were housed in a tie-stall barn located at the Pennsylvania State University Dairy Production Research and Teaching Center. All

cows received bST (Posilac; Elanco Animal Health, Greenfield, IN) administered every 14 d for the duration of the experiment.

A 21 d pretrial and washout period was used to allow adaptation to monensin. Commercially sourced monensin (Rumensin 90; Elanco Animal Health) was topdressed at a rate of 280 mg/cow per day (in 0.6 kg of cookie meal, DM basis) from d 1 to 10 d and at 450 mg/cow per day (in 1.0 kg of cookie meal, DM basis) from d 11 to 21 of the pretrial and washout periods. Each experimental period was divided into a MFD induction phase (10 d) and a recovery phase (18 d). During induction all cows were fed a low fiber, high oil diet with monensin (Induction; 25.3% NDF, 7.4% whole soybeans, and 1.6% soybean oil; 450 mg/cow per d monensin; Table 5-1). During recovery cows were fed a higher forage, low oil diet (31.2% NDF; no whole soybeans or added oil). Treatments were applied during the recovery phase only and were control or supplementation with monensin topdress (MN; 450 mg/cow per day).

Cows were fed individually once daily (0800 h) at 110% of expected intake. Intake was observed daily. Forage and base diet DM was determined weekly for diet adjustment and DMI determination (72 h at 55°C). All individual feed ingredients were sampled weekly and stored at -20°C, thawed at room temperature, and dried in a forced-air oven for 72 h at 55°C for determination of DM content. Individual feeds were ground in a Wiley mill through a 1-mm screen (A. H. Thomas Co., Philadelphia, PA). Samples of all ingredients were composited by period and individual forages and a representative mixture of concentrate feeds were analyzed for nutrient composition by wet chemistry procedures (Cumberland Valley Analytical Services Inc., Maugansville, MD). Briefly, assay conducted were DM and CP according to AOAC (2000), NDF and ADF according

to Van Soest (1991) using heat stable amylase and sodium sulfite, and starch according to Hall (2009). Monensin concentration in the top dress was measured by HPLC with tandem mass spectrometry (Covance laboratories, Greenfield, IN).

Cows were milked twice per day at 0500 and 1700 h and milk yield was determined by an integrated milk meter (AfiMilk; SAE Afikim, Israel). Milk was sampled at both milkings every 3 d during recovery phases. One subsample was stored at 4°C with preservative (Bronolab-WII) until analyzed for fat (Filter B) and protein by infrared spectroscopy [Fossomatic 4000 Milko-Scan and 400 Fossomatic, Foss Electric; AOAC (2000) method 972.160, Dairy One Lab]. A second subsample was immediately spun at 3,000 x g at 4°C and fat cake stored at -20°C. Samples were thawed in room temperature water, pooled within day by milk fat yield, and analyzed for FA composition as described in Chapter 3. Briefly, milk lipids were extracted with hexane: isopropanol according to Hara and Radin (1978) and transmethylated as described by Christie (1982)) and modified by Chouinard et al. (1999). Quantification of FA methyl esters was performed by gas chromatography with a flame ionization detector. Milk FA yields were calculated similarly to Glasser et al. (2007) as described in Chapter 3. The milk FA desaturase indexes were calculated for each sample as an estimation of the activity of the stearoyl Co-A desaturase enzyme [product/(substrate + product)].

Statistical Analysis

Data were analyzed using the MIXED procedure of SAS with repeated measures (version 9.3, SAS Institute, Cary, NC) according to the following model: $Y_{ijklm} = \mu + S_i +$

$P_j + C_k(S_i) + X_l\ T_m + D_n + T_m \times D_n + e_{ijklmn}$, where Y_{ijklmn} is the variable of interest, μ is the overall mean, S_i is the random effect of sequence (i = 1 to 2), P_j is the random effect of period (j = 1 to 2), $C_k(S_i)$ is the random effect of cow nested in sequence (k = 1 to 16), X_l is the fixed effect of d 0 of the recovery phase as covariate, T_m is the fixed effect of treatment (l = 1 to 2), D_n is the fixed effect of time (m = 1 to 7), $T_m \times D_n$ is the interaction of treatment and time, and e_{ijklmn} is the residual error. The ARH (1) or AR(1) covariance structures were used based on model fit, time was the repeated variable, and cow by treatment was the subject. Preplanned contrasts tested the effect of monensin at each time point. Significant and tendencies of main effects and preplanned contrasts were declared at $P < 0.05$ and $P < 0.10$, respectively, and interactions at $P < 0.10$ and $P < 0.15$, respectively. Secondly, a random regression analysis was performed on milk fat concentration and yield and concentration of select FA (MIXED procedure of SAS; version 9.3, SAS Institute) according to the following model: $Y_{ijklm} = \mu + C_i + P_j + T_k + D_l + D_l^2 + T_k \times D_l + T_k \times D_l^2 + e_{ijklm}$, where μ is the overall mean, C_i is the random effect of cow (i = 1 to 16), P_j is the random effect of period (j = 1 to 2), T_k is the fixed effect of treatment (k = 1 to 1), D_l is the linear effect of time (l = 1 to 7), D_l^2 is the quadratic effect of time (l = 1 to 7), $T_k \times D_l$ is the interaction of treatment and time, $T_t \times D_l^2$ is the interaction of treatment and quadratic effect of time, and e_{ijklm} is the residual error. A reduced model was used when higher level interaction terms did not improve model fit. The ARH (1) or UN covariance structures were used based on model fit. In both analyses, denominator degrees of freedom were adjusted by the Kenward-Rogers method. Data were log transformed when appropriate, but back-transformed for reporting. Data

points with Studentized Residuals outside of ± 3.0 were considered outliers and excluded from the analysis.

Results

Induction of milk fat depression

The induction diet was successful in reducing milk fat to an extent that allowed observation of recovery in both periods (-23 ± 2.25%; mean ± SD). Average milk fat concentrations at the start and end of each MFD induction phase averaged 3.69 ± 0.16% and 2.84 ± 0.16%, respectively (mean ± SEM). Both experimental diets fed were similar to those balanced and monensin concentration in the premix was 102% of the targeted dose.

Dry Matter Intake and Milk Production and Composition

There was a significant effect of time, but no effect of treatment or treatment by time interaction on DMI (Table 5-1; Figure 5-1). Intake decreased initially, but progressively increased during recovery. Milk yield decreased progressively in both treatments (Time effect $P = 0.01$), but there was no effect of treatment or treatment by time interaction (Table 5-2). There was a tendency for lower milk yield in monensin compared to control on d 9 ($P < 0.09$; Figure 5-1). Milk fat concentration and yield increased progressively for both treatments during recovery (Time effect $P < 0.001$).

There was a treatment by time interaction for milk fat concentration. Monensin decreased milk fat percent on d 3 and 6 and tended to decrease milk fat percent on d 9 and 15 ($P < 0.1$), however, monensin was the same as control on d 18 (Figure 5-1). There was no treatment by time interaction on milk fat yield, but there was a treatment effect where monensin decreased milk fat yield by 7% ($P = 0.02$; Table 5-2). Specifically, milk fat yield was decreased by monensin from d 6 to 15 ($P < 0.05$), but it was the same as control on d 18. By regression analysis the rate of recovery of milk fat concentration was decreased by monensin ($P < 0.001$), but there was no effect of monensin on the rate of recovery of milk fat yield ($P > 0.1$). There was no treatment by time interaction for milk protein concentration and yield, which decreased progressively in both treatments (Time effect $P < 0.01$; Table 5-2) and was not different between treatments overall or at any time point (Data not shown).

Milk FA by Source

There was an effect of time ($P < 0.01$), but no effect of treatment or treatment by time interaction for milk fat concentration of FA less than 16 C (de novo synthesized) and 16 C FA (16C; Table 5-3). However, there was a tendency for a treatment by time interaction for the concentration of FA greater than 16 C ($P = 0.13$; preformed FA; Table 5-3). The concentration of both milk de novo and 16 C FA increased progressively in both treatments during recovery and was not different between treatments at any time point. In addition, by the regression analysis the rate of increase in de novo and 16 C FA was slightly decreased by monensin ($P < 0.01$; Figure 5-2; Table 5-4). In contrast, the

concentration of preformed FA decreased progressively in both treatments, tended to be lower on d 3 ($P < 0.1$), and was lower for monensin on d 15 and 18 (3.1 ± 0.06%; Mean ± SD; $P < 0.05$). Accordingly, the rate of decrease of milk preformed FA concentration was modestly higher in monensin ($P < 0.01$; Table 5-4).

Yield of milk de novo FA increased progressively during recovery in both groups, but tended to be decreased by monensin on d 6 and 9 ($P < 0.1$). However, there was no main effect of treatment or treatment by time interaction ($P > 0.2$; Table 5-2; Figure 5-2). Yield of 16 C FA was affected by time and treatment and was decreased by monensin on d 6 and 9 ($P < 0.05$) and tended to be lower on d 15 ($P = 0.06$; Table 5-2; Figure 5-2). There was a treatment by time interaction for yield of milk preformed FA. Control maintained a near constant yield of preformed FA, while monensin progressively decreased preformed FA from d 0 to 6 and was lower than control from d 6 to 15 [13.9 ± 2.0% (Mean ± SD); Table 5-2; Figure 5-2]. Concentration of steric acid (C18:0) in milk was not affected by treatment or time and averaged 10.5 ± 0.15% (Mean ± SD; $P > 0.2$; data not shown).

Milk Trans Isomers

There was an effect of time ($P < 0.01$), but no effect of treatment or treatment by time interaction for milk *trans* FA isomers (Table 5-3). Milk *trans*-10 C18:1 concentration rapidly decreased in both treatments and tended to be higher in monensin on d 12 ($P < 0.1$), and was increased 21 ± 5.5% (Mean ± SD; $P < 0.01$), but near baseline concentrations, on d 15 and 18 when it reached a nadir (Figure 5-3). Similarly, milk

trans-10, *cis*-12 conjugated linoleic acid (CLA) concentration rapidly decreased reaching a nadir on d 15 and was not different between treatments at any time point. Accordingly, the rate of decrease of both *trans*-10 C18:1 and *trans*-10, *cis*-12 CLA was lower in the monensin group (Table 5-4). Milk *trans*-11 C18:1 and *cis*-9, *trans*-11 CLA concentrations decreased progressively through d 18 in both treatments and on d 3 tended to be 28 and 18% higher, respectively, in monensin ($P < 0.1$; Figure 5-3). However, the rate of decrease of both FA by regression analysis was slightly higher in monensin (Table 5-4).

Milk Δ9-desaturase indexes

There was no treatment by time interaction for the desaturase indexes (Table 5-3). The C14 and C16 desaturase indexes decreased progressively, reached a nadir around d 9, and were not affected by treatment at any time point (Figure 5-4). The CLA index showed a similar time course and was not affected by treatment (Data not shown).

Discussion

During each experimental period, MFD was first induced by feeding a low fiber, high oil diet and recovery was subsequently promoted by feeding a higher fiber, low oil diet similarly to Chapters 3 and 4. The absolute level of recovery was just below milk fat concentration normally observed in commercial herds (3.34% ± 0.07). Importantly, the goal of the project was to investigate the rate of recovery, which does not require

complete rescue. The length of the recovery phase was based on a previous study where recovery from diet-induced MFD was complete in 19 d when a high fiber, low oil diet was fed (Chapter 3). In addition, a washout period of 21 d was utilized to allow adequate time for establishment of the effect of monensin on the rumen.

Weimer et al. (2010b) suggested that experimental designs such as Latin Squares were not properly suited for microbiological studies using monensin. This was based on the observation that following monensin withdrawal some bacterial taxa and the whole bacterial community composition do not return to the same status as previous to monensin supplementation (Weimer et al., 2008; Weimer et al., 2010b). Importantly, our experimental design was not investigating the steady state effect of monensin, but aimed to test the impact of immediate withdrawal of monensin when MFD occurs compared to continual feeding on the rate of recovery.

In a recent meta-analysis of 77 studies, monensin supplementation decreased DMI by 0.3 kg/d and increased milk yield by 0.7 kg/d and feed efficiency by 2.5% (Duffield et al., 2008b). Under the conditions of the present experiment, monensin did not change DMI and milk yield, although there was a numerical decrease in milk yield with monensin. Similarly, Alzahal et al. (2008) and He et al. (2012) observed no effect of monensin on DMI and milk yield. In the current experiment a carry-over effect of monensin may explain the lack of a difference in DMI and milk yield.

The meta-analysis by Duffield et al. (2008b) also reported that milk fat concentration decreased by 0.13 percentage units and fat yield was unchanged during monensin supplementation. In addition, milk *trans* FA were increased and de novo FA

were decreased (Duffield et al., 2008b). To our knowledge, no previous study has evaluated the effect of monensin on the rate of recovery from MFD. The reduced rate of recovery of milk fat yield was predominantly explained by a lower yield of preformed FA, although yield of 16 C FA was also reduced to a lesser extent and yield of de novo FA was numerically decreased. During classical diet-induced MFD the reduction in de novo FA synthesis is greater than the reduction in preformed FA secretion resulting in an increased proportion of preformed FA, especially during more severe MFD [Chapter 3 (Baumgard et al., 2001; Harvatine and Bauman, 2011)].

Previous studies on the time course of recovery from diet-induced MFD have shown progressive increases in milk fat synthesis mainly related to recovery of de novo FA, but also to a smaller recovery of preformed FA secretion (Chapters 3 and 4). Interestingly, when monensin was supplemented during recovery from diet-induced MFD, a progressive increase in milk de novo FA secretion was observed, whereas preformed FA were maintained (Chapter 5), similarly to the control group in the present experiment. Although not measured in the current experiment, it is possible that monensin feeding resulted in a more glucogenic fermentation pattern (McGuffey et al., 2001), which may have caused an increase in insulin secretion and a subsequent increase adipose uptake of preformed FA and decreased FA mobilization (Duffield et al., 2008a). This may have reduced the availability of circulating FA to the mammary gland(Corl et al., 2006). A recent meta-analysis reported a negative association between milk fat yield and propionate and glucose infusions in early lactation cows, primarily caused by reduction of preformed FA (Maxin et al., 2011). Similarly to the present experiment, hyperinsulinemic-euglycemic clamp studies using post-peak cows in positive energy

balance reported a similar magnitude of reduction in milk fat yield [i.e. 5% (Griinari et al., 1997; Mackle et al., 1999)]. Despite the decreased rate of recovery of milk fat synthesis, there was no difference between control and monensin by d 18 of the recovery phase, indicating that correction of diet fermentability and PUFA concentration provide rescue of milk fat synthesis regardless of monensin supplementation.

The predominant BH pathway during normal fermentation results in formation of *trans*-11 C18:1 and *cis*-9, *trans*-11 CLA as intermediates. However, diet-induced MFD is associated with an increase in alternate BH isomers including *trans*-10, *cis*-12 CLA that is a potent inhibitor of milk fat synthesis (Baumgard et al., 2000; Perfield et al., 2006) and *trans*-10 C18:1 that is highly correlated with MFD, but may not be a bioactive FA (Lock et al., 2007). In the current experiment, both *trans*-10 C18:1 and *trans*-10, *cis*-12 CLA decreased rapidly during recovery from MFD in both treatments indicating little difference in the recovery of BH by monensin. This is in agreement with the similar recovery of de novo synthesized FA and is indicative of near equal recovery from classical diet-induced MFD.

During recovery from diet-induced MFD two distinct phases of changes in milk *trans* FA have been previously observed and referred to as a biphasic response, (Chapters 3, 4, and 6). In agreement, in the present experiment during the first phase of recovery FA in the altered BH pathway including *trans*-10 C18:1 and *trans*-10, *cis*-12 CLA rapidly decreased and reached near minimal levels by d 6. During the second phase FA in the normal pathway including *trans*-11 C18:1 and *cis*-9, *trans*-11 CLA were predominant. A brief and slight elevation of FA in the normal BH pathway has been reported during recovery from diet-induced MFD (Chapters 3 and 6). However, FA in the normal

pathway were not elevated above baseline in the secondary phase, in agreement with other reports of recovery in monensin supplemented diets (Chapter 5) and suggest an effect of monensin on BH rates (Fellner et al., 1997; Weimer et al., 2010b).

The meta-analysis by Duffield et al. (2008b) reported that monensin decreased milk C18:0 by 7.8% and increased *trans* C18:1 FA and CLA by 22% indicating reduced ruminal BH. In the present experiment there was no significant effect of monensin on milk concentration of C18:0, but there was tendency for higher *trans*-11 C18:1 and *cis*-9, *trans*-11 CLA on d 3. Ruminal bacteria that carry out biohydrogenation are classified into groups A and B. Group A bacteria can hydrogenate linoleic acid (*cis*-9, *cis*-12 18:2) into *trans*-11 C18:1, whereas group B bacteria (gram-positive) form C18:0 from C18:1 FA such as *trans*-11 (Harfoot and Hazlewood, 1997). The observed tendency for higher *trans*-11 C18:1 and *cis*-9, *trans*-11 CLA may be the result of ruminal accumulation of *trans*-11 C18:1 by inhibition of gram positive bacteria (Russell and Houlihan, 2003) and subsequent endogenous desaturation into *cis*-9, *trans*-11 CLA by steroyl CoA desaturase (SCD1) (Griinari et al., 2000).

The C14 and C16 desaturase indexes were used as markers for SCD1 activity (Perfield et al., 2006). Both indexes were unaffected by monensin and decreased progressively during recovery and reaching a nadir around d 9, similarly to previous reports (Chapters 3, 4, and 6).

Conclusions

The rate of recovery of milk fat synthesis was marginally reduced by monensin predominantly due to decreased preformed FA incorporation into milk fat. However, the re-establishment of normal rumen biohydrogenation occurred rapidly and de novo milk fat synthesis increased nearly equally with or without monensin withdrawal. Importantly, by the end of the recovery period milk fat yield was recovered in both groups. Therefore, classical diet-induced milk fat depression can be rescued in monensin supplemented diets by correcting diet fermentability and PUFA concentration.

Table 5-1. Ingredient and chemical composition of diets.

	Induction[1]	Control
Ingredient, % of DM		
Corn silage[2]	29.6	36.5
Alfalfa haylage[3]	7.1	17.9
Canola meal	16.3	13.9
Ground corn	22.5	9.9
Roasted soybeans	4.0	-
Expeller soybean meal[4]	2.0	4.5
Cottonseed hulls	2.6	2.2
Cookie meal	3.1	3.3
Grass hay/straw[5]	3.2	4.5
Minerals & vitamins mix[6]	3.4	3.3
Sugar cane molasses	3.6	3.9
Soybean oil	2.1	-
NPN supplement[7]	0.4	0.2
Nutrient, % of DM		
CP	19.1	17.6
NDF	25.3	31.2
ADF	16.5	20.6
Starch	30.6	24.6

[1]Induction is a low forage, higher oil diet fed during the induction phase of each period; Recovery is a high forage, low fat diet fed during the recovery phase of each period.

[2]Contained 40% DM, 8% CP, 34% NDF, and 19.6% ADF (DM basis)

[3]Contained 39.4% DM, 21.4% CP, 41.9% NDF, and 36.6% ADF (DM basis)

[4]Mechanically extracted soybean meal (Turbomeal; J. L. Moyer and Sons Inc., Turbotville, PA)

[5]Contained 89.4% DM, 8.9% CP, 69.2% NDF and 42.4% ADF (DM basis)

[6]Contained (%, as fed basis): 45.8 dried corn distillers grains with solubles; 35.8 limestone (38% Ca); 8.3 magnesium oxide (54% Mg); 6.4 salt; 1.73 vitamin ADE premix; 1.09 selenium premix (0.06% selenium); and 0.88 trace mineral mix. Composition (DM basis): 11% CP; 18% NDF; 5.2% fat; 14.9% Ca; 0.35% P; 4.58% Mg; 0.41% K; 0.31% S; 357 mg/kg Cu; 1,085 mg/kg Zn; 181 mg/kg Fe; 6.67 mg/kg Se; 125,875 IU/kg vitamin A (retinyl acetate); 31,418 IU/kg vitamin D (Activated 7-dehydrocholesterol); and 946 IU/kg vitamin E (dl-alpha tocopheryl acetate).

[7]Controlled release urea (Optigen, Alltech Inc.,Lexington, KY; 278% CP, DM basis)

Table 5-2. Effect of monensin supplementation on DMI and milk yield and composition during recovery from diet-induced milk fat depression.

	Treatment[1]			*P*-value[2]		
Item	Control	MN	SEM	Trt	Time	Trt x time
DMI, kg/d	27.3	27.1	0.85	0.52	<0.001	0.91
Milk Yield, kg/d						
Milk	40.7	39.6	0.70	0.25	0.01	0.60
Fat	1.26	1.17	0.03	0.02	<0.001	0.15
Protein	1.28	1.26	0.02	0.49	<0.001	0.52
Milk Composition, %						
Fat, %	3.15	3.01	0.06	0.01	<0.001	0.05
Protein, %	3.17	3.18	0.04	0.67	<0.001	0.98
Milk FA by source, g/d[3]						
< 16 C	287	275	12.5	0.23	<0.001	0.46
16 C	312	292	7.8	0.03	<0.001	0.26
> 16 C	486	439	12.2	0.01	0.04	0.04

[1]Control = no monensin; MN = monensin fed at 450 mg/cow per day during recovery from diet-induced milk fat depression.

[2] Trt = Treatment effect; Time = effect of day in recovery from milk fat depression.

[3]FA < 16 C originate from de novo synthesis in the mammary gland; FA > 16 C originate from extraction from plasma;16 C FA originate from both sources.

Table 5-3. Effect of monensin supplementation on milk FA composition during recovery from diet-induced milk fat depression.

	Treatment[1]			*P*-value[2]		
FA, % of total FA	Control	MN	SEM	Trt	Time	Trt x Time
Trans-10 C18:1	1.39	1.50	0.21	0.69	<0.001	0.13
Trans-11 C18:1	1.41	1.55	0.10	0.26	<0.001	0.59
Cis-9, *trans*-11 CLA[3]	0.84	0.91	0.05	0.24	<0.001	0.58
Trans-10, cis-12 CLA	0.010	0.011	0.001	0.66	<0.001	0.77
FA < 16 C[4]	24.3	24.6	0.33	0.48	<0.001	0.80
16 C	26.4	26.6	0.36	0.71	<0.001	0.89
FA > 16 C	41.4	40.7	0.51	0.08	<0.001	0.13
C14 desaturase index[5]	0.10	0.11	0.004	0.44	<0.001	0.74
C16 desaturase index	0.06	0.06	0.002	0.74	<0.001	0.45

[1]Control = no monensin; MN = monensin fed at 450 mg/cow per day during recovery from diet induced milk fat depression.

[2] Trt = Treatment effect; Time = effect of day in recovery from milk fat depression.

[3]Conjugated linoleic acid.

[4]FA < 16 C originate from de novo synthesis in the mammary gland; FA > 16 C originate from extraction from plasma; 16 C FA originate from both sources.

[5]C14 desaturase index = C14:1/(C14:1 + C14:0); C16 desaturase index = C16:1/(C16:1 + C16:0).

Table 5-4. Effect of monensin supplementation on the rate of recovery of milk composition and FA profile determined by random regression

		Regression term[2]			*P* value[3]		
Item	Trt[1]	Int	L	Q	Int	L	Q
Milk Fat							
%	Control	2.71	0.079	-0.003	<0.001	<0.001	<0.01
	MN	2.85	0.017	0.001			
kg/d	Control	1.11	0.01	-	<0.001	0.19	-
	MN	1.16	0.005	-			
Milk FA by Source, % of Total FA[4]							
< 16 C	Control	20.92	0.667	-0.02	<0.001	<0.001	<0.001
	MN	21.31	0.635	-0.019			
16 C	Control	23.76	0.463	-0.016	<0.001	<0.001	<0.001
	MN	23.67	0.523	-0.017			
> 16 C	Control	45.82	-0.945	0.029	<0.001	<0.001	<0.001
	MN	45.94	-0.968	0.027			
Milk Trans FA, % of Total FA							
trans-10 C18:1	Control	3.38	-0.475	0.018	<0.001	<0.001	<0.001
	MN	3.35	-0.421	0.015			
trans-11 C18:1	Control	1.84	-0.046	-	<0.001	<0.001	-
	MN	1.93	-0.047	-			
cis-9, *trans*-11 CLA[5]	Control	1.15	-0.048	0.001	<0.001	<0.001	<0.001
	MN	1.23	-0.059	0.001			
trans-10, cis-12 CLA	Control	0.03	-0.004	0.0001	<0.001	<0.001	<0.001
	MN	0.02	-0.003	0.0001			

[1]Control = no monensin; MN = monensin fed at 450 mg/cow per day during recovery from diet induced milk fat depression.

[2] Intercept (Int) and slopes of the regression terms; L = linear and Q = quadratic effect of day in recovery from milk fat depression.

[3]Probability for effect of the regression term. The interaction of treatment and the linear (L) and quadratic (Q) effects of time.

[4]FA < 16 C originate from de novo synthesis in the mammary gland; FA > 16 C originate from extraction from plasma; 16 C FA originate from both sources.

[5]Conjugated linoleic acid

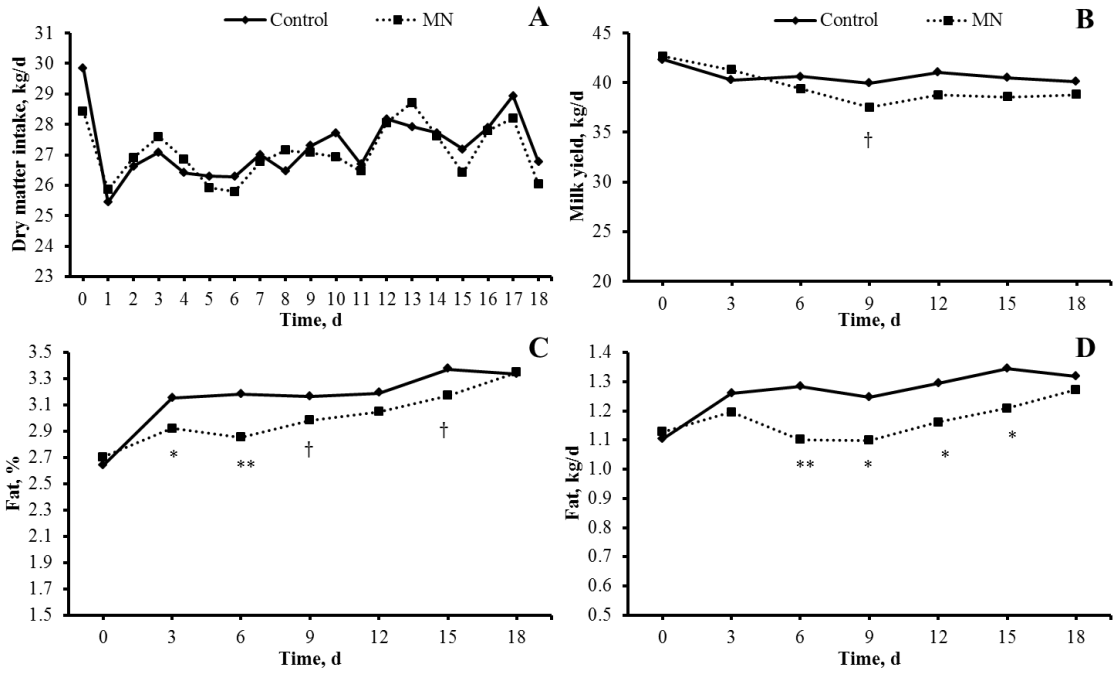


Figure 5-1. Effect of monensin supplementation on intake, milk production, and milk fat during recovery from milk fat depression.

Shown are dry matter intake (Panel A), milk yield (Panel B), milk fat concentration (Panel C), and milk fat yield (Panel D). Milk fat depression was first induced by feeding a low forage and high oil diet. Milk fat depression was recovered by feeding a moderate fiber and low fat diet alone (control) or with monensin supplementation (MN) for 18 d. Preplanned contrasts tested the difference between control and monensin (** $P < 0.01$; * $P < 0.05$; † $P < 0.1$ SEM = 0.85, 0.70, 0.06, and 0.03, for dry matter intake, milk yield, milk fat concentration, and milk fat yield, respectively).

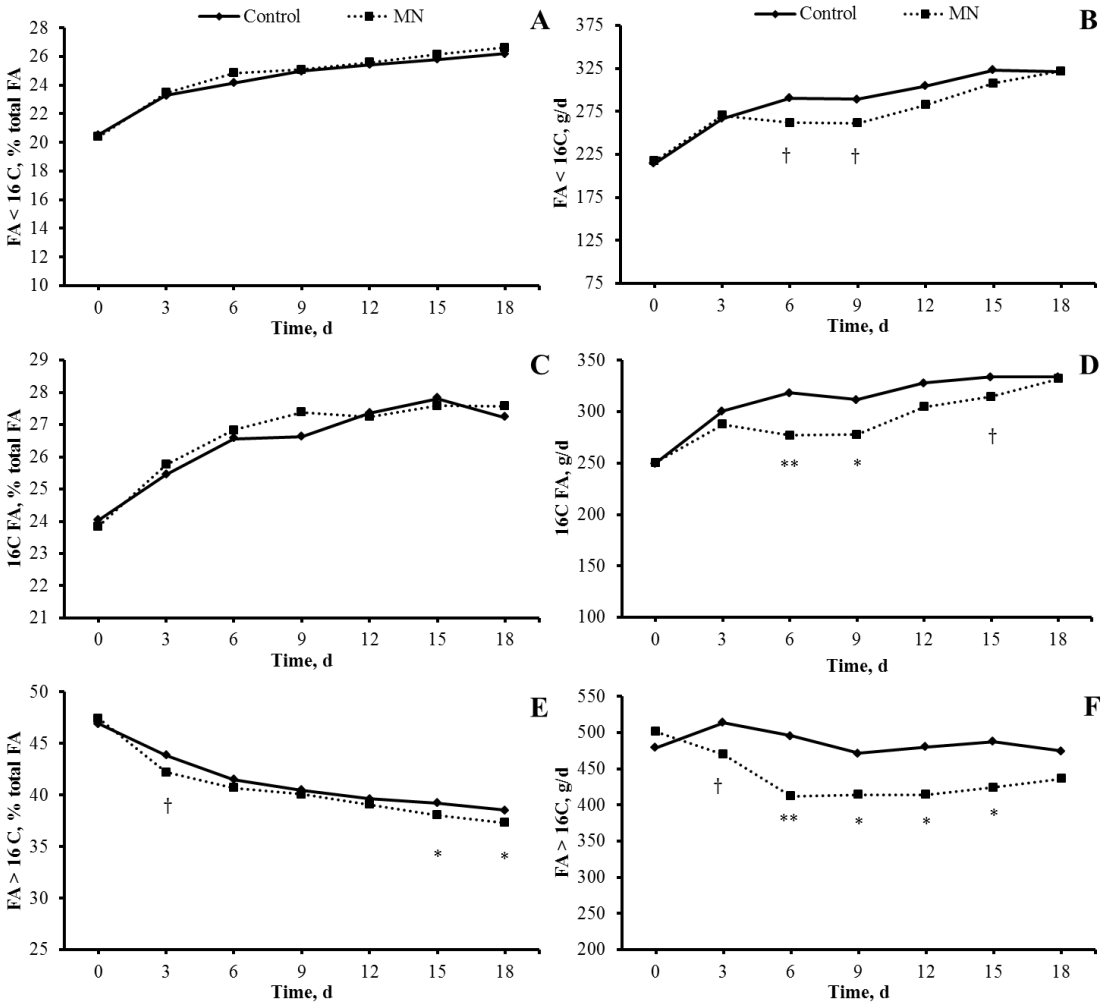


Figure 5-2. Effect of monensin supplementation on the concentration and yield of milk FA by source during recovery from diet induced milk fat depression.

Shown are the milk fat concentration and yield of de novo (Panel A and B, respectively), 16C (Panel C and D, respectively), and preformed (Panel E and F, respectively) FA. Milk fat depression was first induced by feeding a low forage and high oil diet. Milk fat depression was recovered by feeding a moderate fiber and low fat diet alone (control) or with monensin supplementation (MN) for 18 d. Preplanned contrasts tested the difference between control and monensin (** $P < 0.01$; * $P < 0.05$; † $P < 0.1$ SEM = 0.21, 8, 0.32, 11, 0.49, and 16 for the concentrations and yields of de novo, C16, and preformed FA, respectively; SEM = 0.33, 12.5, 0.36, 7.8, and 0.51 for the concentrations and yields of de novo, C16, and preformed FA, respectively).

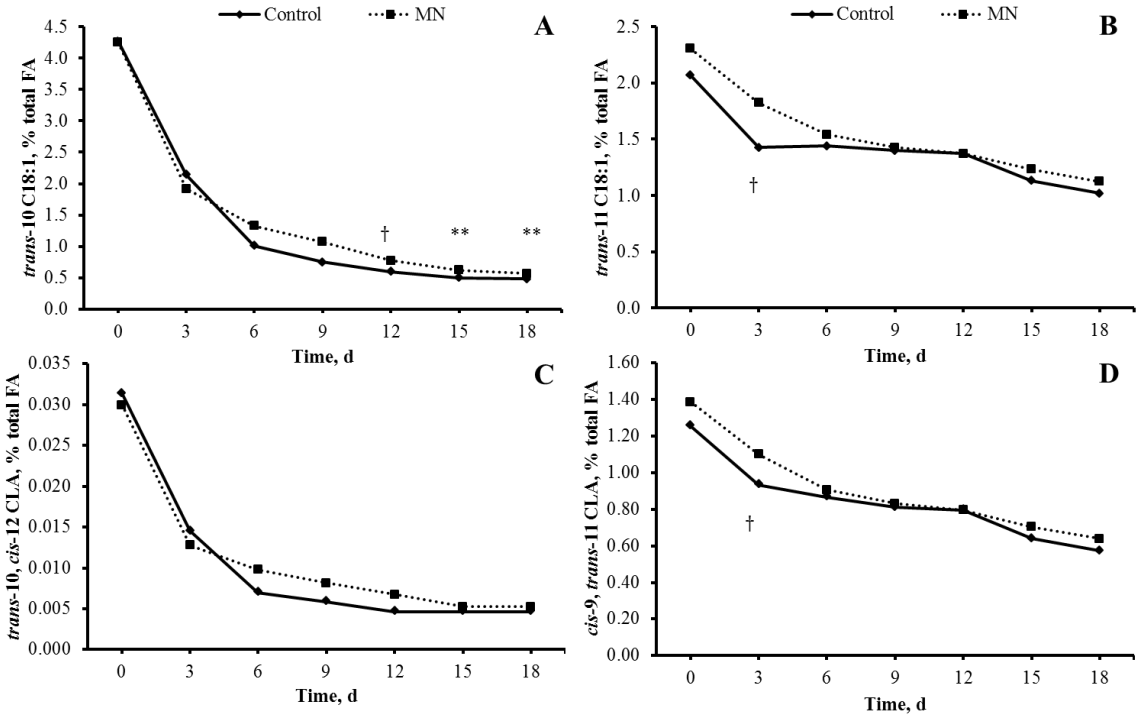


Figure 5-3. Effect of monensin supplementation on milk *trans* FA concentration during recovery from diet induced milk fat depression.

Shown are the predominant isomers of the alternate biohydrogenation pathway [*trans*-10 C18:1 (Panel A) and *trans*-10, *cis*-12 CLA (Panel C)] and the predominant isomers of the normal biohydrogenation pathway [*trans*-11 C18:1 (Panel B) and *cis*-9, *trans*-11 CLA (Panel D). Milk fat depression was first induced by feeding a low forage and high oil diet. Milk fat depression was recovered by feeding a moderate fiber and low fat diet alone (control) or with monensin supplementation (MN) for 18 d. Preplanned contrasts tested the difference between control and monensin (** $P < 0.01$; * $P < 0.05$; † $P < 0.1$); SEM = 0.21, 0.10, 0.001, and 0.05 for the concentrations of *trans*-10 C18:1, *trans*-11 C18:1, *trans*-10, *cis*-12 CLA, and *cis*-9, *trans*-11 CLA, respectively).

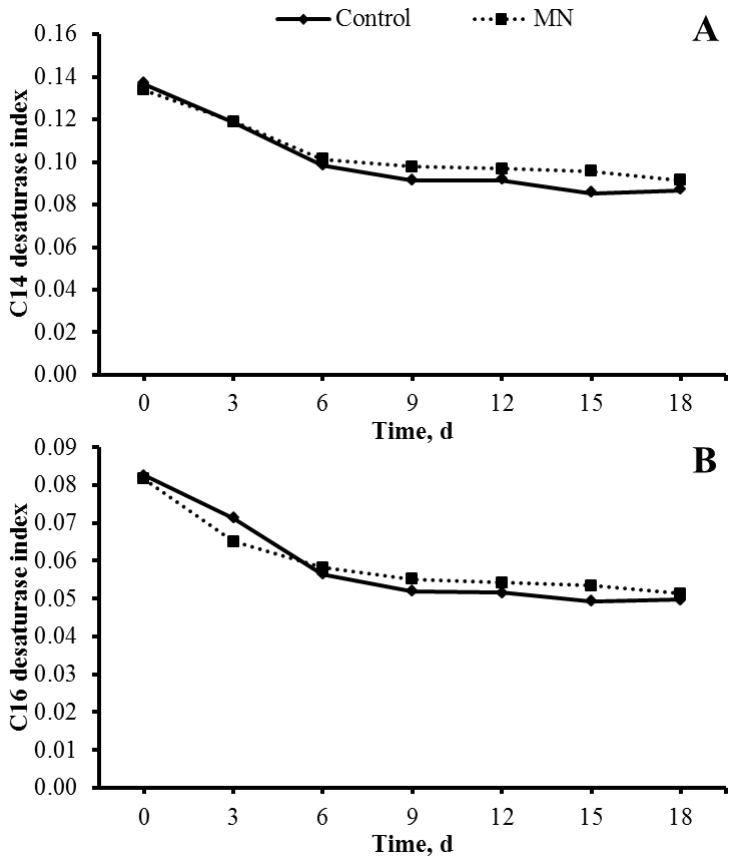


Figure 5-4. Effect of monensin supplementation on milk FA desaturase indexes during recovery from diet induced milk fat depression.

Representative indexes shown include the C14 desaturase index (Panel A) and the C16 desaturase index (Panel B). Milk fat depression was first induced by feeding a low forage and high oil diet. Milk fat depression was recovered by feeding a moderate fiber and low fat diet alone (control) or with monensin supplementation (MN) for 18 d. Preplanned contrasts tested the difference between control and monensin (** $P < 0.01$; * $P < 0.05$; † $P < 0.1$; SEM = 0.004 and 0.002 for the C14 and C16 desaturase indexes, respectively).

Chapter 6

The Effect of Rumen Digesta Inoculation on the Time Course of Recovery from Diet-induced Milk Fat Depression in Dairy Cows

Abstract

Ten ruminally-cannulated cows were used in a crossover design that investigated the effect of rumen digesta inoculation from non-milk fat depressed cows on recovery from diet-induced milk fat depression (MFD). Two additional cows fed a high forage, low oil diet (31.8% NDF, no added soybean oil, and 5.2% whole soybeans) were used as rumen digesta donors. Milk fat depression was induced during the first 10 d of each period by feeding a low fiber and high oil diet (Induction; 26.1% NDF, 1.6% soybean oil, and 9.7% whole soybeans). A recovery phase followed where all cows were switched to the high forage, low oil diet, and were allocated to 1) control (no inoculation) or 2) ruminal inoculation with donor cow digesta (8 kg/d for 6 d). Milk yield and composition were measured every 3 d. Milk fat concentration increased progressively during the recovery phase and there was no effect of treatment at any time point. However, a random regression analysis identified a modest acceleration of the recovery of milk fat concentration with inoculation. The concentration of milk de novo fatty acids increased progressively during recovery for both treatments, and was higher for inoculated compared to control on d 6. In agreement, milk fat concentration of *trans*-10 C18:1 and *trans*-10 *cis*-12 CLA decreased progressively in both treatments, and were lower in inoculated cows on d 6. Ruminal inoculation from non-milk fat depressed cows modestly accelerated the rate of recovery of de novo FA synthesis and normal ruminal fatty acid

biohydrogenation, demonstrating the opportunity for other interventions that improve the ruminal environment to accelerate recovery from this condition.

Key words: milk fat depression, ruminal inoculation, dairy cows.

Introduction

Low fat syndrome in dairy cows, referred to as milk fat depression, was first documented more than 150 years ago, and is characterized by a dramatic reduction in milk fat yield in response to high concentrate/low forage diets or diets supplemented with plant or fish oils (Bauman and Griinari, 2003). In ruminants, the dietary factors that cause MFD are associated with altered rumen fermentation and the production of unique bioactive fatty acids (FA) from biohydrogenation (BH) of dietary polyunsaturated fatty acids (PUFA). Dietary factors such as PUFA content and diet fermentability impact both the extent of BH and the specific intermediates formed (Jenkins et al., 2008; Fuentes et al., 2009), presumably through modification of the rumen microbial population (Tajima et al., 2001; Weimer et al., 2010b). *Trans*-10, *cis*-12 conjugated linoleic acid (CLA) is one of multiple BH intermediates known to inhibit milk fat synthesis in the mammary gland (Perfield et al., 2007) and its mechanism of action involves the down regulation of genes related to FA uptake, transport, synthesis, and esterification in the mammary gland (Baumgard et al., 2002).

The mammary gland responds very rapidly to abomasal infusion of CLA with maximal response and recovery occurring in 2 to 3 d (Baumgard et al., 2000). However, induction and recovery from MFD caused by high fermentability and PUFA diets is

much slower. For example, feeding a low fiber and high oil (29.5% NDF and ~3% soybean oil) diet to lactating dairy cows caused MFD in 7 d and recovery was achieved after 11 d on a higher fiber and lower fat ration (Chapter 3). Interestingly, a two-phase adaptation has been observed during induction and recovery of diet-induced MFD. During induction *cis*-9, *trans*-11 CLA peaked on d 3 and then progressively decreased as *trans*-10, cis-12 CLA progressively increased. The delayed onset of diet-induced MFD and lag to peak concentrations of *trans* FA associated with MFD suggests that ruminal adaptation has a larger impact on the time course compared to substrate availability for BH. Partial or complete replacement of rumen contents have been previously studied in sheep (Cole, 1991; Gregg et al., 1998) and dairy cows (Satter and Bringe, 1969; Weimer et al., 2010a). Specifically, Satter and Bringe (1969) switched between a high and a low forage diet, with or without a simultaneous near-total rumen contents switch from cows fed the same diet and observe that both induction of and recovery from MFD were accelerated by rumen switch relative to diet switch alone. This suggests that microbial adaptation is a key limiting factor. Furthermore, Weimer et al. (2010b) reported extensive shifts in ruminal bacteria communities during diet-induced MFD. Our objective was to test the effect of ruminal inoculation from a non-milk fat depressed cow on the rate of recovery of milk fat yield and milk FA profile after a diet switch. We hypothesized that the recovery from diet-induced MFD would be accelerated by inoculation with the normal ruminal microflora.

Materials and Methods

Experimental Design and Treatments

All experimental procedures were approved by the Pennsylvania State University Institutional Animal Care and Use Committee. Twelve rumen cannulated Holstein cows were housed at the Pennsylvania State University Dairy Production Research and Teaching Center. Ten cows (197 ± 94 DIM, and 41 ± 9.8 kg milk/d; mean ± SD) were randomly assigned to treatment sequences in a cross-over design with 28 d periods, and balanced for residual effects. Two additional cows (195 ± 79 DIM and 39 ± 9.8 kg milk/d; mean ± SD) fed a high forage, low oil diet (31.8% NDF and no added oil; Table 6-1) were used as rumen digesta donors. Each treatment period was 28 d in length and included a 10 d MFD induction and 18 d MFD recovery phase. Cows were milked twice per day at 0500 and 1700 h and received Posilac (Elanco Animal Health, Greenfield, IN) every 14 d.

During the MFD induction phase (10 d) all cows were fed a low forage, high oil diet (Induction; 26.1% NDF and 1.6 % soybean oil, and 9.7% whole soy beans (% of DM); Table 6-1). During the subsequent MFD recovery phase cows were fed a high forage, low oil diet (Recovery; 31.8% NDF, no added soybean oil and 5.2% whole soybeans) with our without ruminal digesta inoculation. Rumen digesta inoculation included removal of 8 kg of digesta at 1300 h on d 1 to 6 and replacement with digesta from non-MFD donor cows. Similarly, 8 kg of rumen contents was evacuated and returned to control cows on the same days. The high forage, low oil diet was also fed during a 7 d washout between experimental periods.

Cows were fed once daily (0800 h) at 110% of expected intake. Forage and diet DM was determined weekly for diet adjustment and DMI determination (72 h at 55°C). All individual feed ingredients were sampled weekly and stored at -20°C, thawed at room temperature, and dried in a forced-air oven for 72 h at 55°C for determination of DM content. Individual feeds were ground in a Wiley mill through a 1-mm screen (A. H. Thomas Co., Philadelphia, PA). Samples of all ingredients were composited by period and individual forages and a representative mixture of concentrate feeds were analyzed for nutrient composition by wet chemistry procedures (Cumberland Valley Analytical Services Inc., Maugansville, MD). Briefly, assays conducted were, DM and CP according to AOAC (2000), NDF and ADF according to Van Soest (1991), using heat stable amylase and sodium sulfite, and starch according to Hall (2009).

Milk Sampling and Analysis

The time course of recovery from milk fat depression was observed by collection of milk samples every third day during the recovery phase of each period. One subsample was stored at 4°C with liquid preservative (Bronolab-WII; Dairy One Coop. Inc., State College, PA) until analyzed for fat and protein by infrared spectroscopy (Fossomatic 4000 Milko-Scan and 400 Fossomatic, Foss Electric, Hillerød, Denmark; AOAC, 2000, method 972.160, Dairy One). A second subsample was stored at -20°C without preservative until analyzed for FA composition.

Milk FA analysis was performed on samples from d 0, 6, 12, and 18 of the recovery phase as described in Chapter 3. Briefly, milk fat was composited by day based

on milk fat yield at each milking, lipids were extracted with hexane:isopropanol according to Hara and Radin (1978), and transmethylated as described by Christie (1982) and modified by Chouinard et al. (1999). Quantification of FA methyl esters was performed by gas chromatography using a fused-silica capillary column (SP-2560; 100 m x 0.25 mm (i.d.) with 0.2-μm film thickness; Sigma-Aldrich, St. Louis, MO) and a flame ionization detector with hydrogen as the carrier gas. Milk FA yields were calculated similarly to Glasser et al., (2007), as described in Chapter 3.

Statistical Analysis

Data were statistically analyzed as a crossover design using the MIXED procedure of SAS with repeated measures (version 9.3, SAS Institute, Cary, NC). The model was $Y_{ijklmn} = \mu + S_i + P_j + C_k(S_i) + X_l + T_m + D_n + T_m \times D_n + e_{ijklmn}$, where Y_{ijklmn} is the variable of interest, μ is the overall mean, S_i is the random effect of sequence (i = 1 to 2), P_j is the random effect of period (j = 1 to 2), $C_k(S_j)$ is the random effect of cow nested in sequence (k = 1 to 10), X_l is the fixed effect of d 0 of the recovery phase as a covariate, T_m is the fixed effect of treatment (m = 1 to 2), D_n is the fixed effect of time (n = 1 to 4), $T_m \times D_n$ is the interaction of treatment and time, and e_{ijklmn} is the residual error. Time was the repeated variable and cow by period was the subject. The ARH(1) or AR(1) covariance structures were utilized based on the Akaike and Bayesian information criteria. The Kenward-Rogers denominator degrees of freedom adjustment was used.

A secondary characterization of the data was performed by random regression analysis using the MIXED procedure of SAS (version 9.3, SAS Institute). The model was

$Y_{ijkl} = \mu + C_i + P_j + T_k + D_l + D^2_l + T_k \times D_l + T_k \times D^2_l\ e_{ijkl}$, where μ is the overall mean, C_i is the random effect of cow (i = 1 to 10), P_j is the random effect of period (j = 1 to 2), T_k is the fixed effect of treatment (k = 1 to 2), D_l is the linear effect of time (l = 1 to 4), D^2_l is the quadratic effect of time (l = 1 to 4), $T_k \times D_l$ is the interaction of treatment and time, $T_t \times D^2_l$ is the interaction of treatment and time^2, and e_{ijkl} is the residual error. The denominator degrees of freedom were calculated by the Satterwaite procedure.

Data for some variables were log transformed when appropriate and back-transformed data are reported. Data points with studentized residuals greater or less than 3.0 were considered outliers and excluded from analysis, typically less than 3 data points per time point. Cows that did not respond to MFD induction (less than a 15% decrease in milk fat concentration) were excluded from the analysis for that period.

Results

Induction of milk fat depression

The induction diet successfully reduced milk fat concentration in both periods (-27 ± 8.6%; mean ± SD). Average milk fat concentrations at the start and end of each MFD induction phase averaged 3.84 ± 0.17% and 2.79 ± 0.17%, respectively (mean ± SEM).

Dry Matter Intake and Milk Production and Composition

There was no effect of treatment or treatment by time interaction for DMI or milk yield (26.8 ± 1.27 and 33.9 ± 1.86 kg/d, mean ± SEM, respectively; Table 6-2). There was an effect of time on milk yield with both treatments progressively decreasing milk yield during the recovery phase; however, the biggest reduction occurred between d 0 and 3 (5.0 kg/d average reduction; Figure 6-1). Milk protein concentration and yield were not affected by rumen inoculation, but milk protein yield decreased over time (Table 6-2 and data not shown).

Milk fat concentration increased progressively after switching to the recovery diet; however, there was no difference between treatments at any time point (Figure 6-1). In contrast, milk fat yield remained constant during recovery from MFD and there was no effect of treatment at any time point (Figure 6-1). Using random regression analysis the rate of recovery from MFD was modestly higher in inoculated cows as indicated by a greater slope for milk fat concentration and yield in inoculated cows (Table 6-4; $P < 0.001$). There was no treatment or treatment by time interaction for the yield of milk de novo or preformed FA (< 16 C and > 16 C, respectively; Table 6-2). There was an effect of time on the yield of de novo FA (Table 6-2), where yield of de novo FA increased progressively, but was not different between treatments at any time point during recovery from MFD (Data not shown).

Milk FA Profile and Δ9-desaturase indexes

There was an effect of time, but no treatment or treatment by time interaction on milk fat concentration of de novo (< 16 C), 16 carbon (16 C), and preformed FA (> 16 C; Table 6-3). In both treatments, the proportion of de novo FA increased progressively, while the proportion of preformed FA decreased progressively during recovery from MFD (Figure 6-2). However, the concentration of de novo synthesized FA was increased 9.8% by inoculation on d 6 (Figure 6-2; $P = 0.03$). By the random regression analysis, the rate of recovery of 16 C FA concentration was increased ($P < 0.001$) by inoculation, whereas the rate of decrease in preformed FA concentration was negligibly higher in control. The random regression analysis did not fit recovery of milk de novo FA concentration, perhaps due to insufficient resolution of the data set, therefore, regression parameters for this variable are not reported.

There was a treatment by time interaction on milk fat concentration of *cis*-9, *trans*-11 CLA and a tendency for a treatment by time interaction for *trans*-11 C18:1 (Table 6-3). Both *cis*-9, *trans*-11 CLA and *trans*-11 C18:1 were decreased by inoculation on d 12 (29 and 25% reduction, respectively; Figure 6-3). In addition, by the regression analysis the rate of decrease in *cis*-9, *trans*-11 CLA and *trans*-11 C18:1 was increased ($P < 0.001$) by inoculation. There was no effect of treatment or treatment by time interaction, but an effect of time on milk fat concentration of *trans*-10 C18:1 and *trans*-10, *cis*-12 CLA (Table 6-3). However, on d 6, inoculation decreased *trans*-10 C18:1 and *trans*-10, *cis*-12 CLA concentrations in milk fat by 39 and 28%, respectively by ($P < 0.05$; Figure 6-3). Accordingly, the rate of decrease in the concentration of *trans*-10

C18:1 was greater in inoculated cows ($P < 0.001$). In contrast, the rate of recovery of *trans*-10, *cis*-12 CLA was increased by inoculation, although the difference was negligible ($P < 0.001$; Table 6-4).

There was no effect of treatment ($P > 0.7$) or treatment by time interaction ($P > 0.5$) for C14 and C16 desaturase indexes, although there was an effect of time ($P < 0.01$; Table 6-3). Both indexes decreased progressively and reached a nadir on d 12 of recovery from MFD. Treatments did not differ at any time point (data not shown).

Discussion

The production of bioactive FA by rumen microbes during MFD is dependent on the rumen environment, amount of PUFA substrate available for BH, and the rumen microbial population (Jenkins et al., 2008). Importantly, these factors have multiple complex interactions. The ruminal inoculation treatment used in this experiment was intended to test that hypothesis that inoculation of ruminal microorganisms from a non-MFD cow would accelerate reestablishment of a normal rumen microflora, normal BH pathways and capacity, and recovery from diet-induced MFD. The amount of inoculum transferred (8 kg/d) is approximately 10% of rumen digesta weight. An effective dose is difficult to predict, but the level was selected to provide a feasible, but substantial transfer, while maintaining the endogenous rumen environment and minimizing disruption of rumen donors. The effects of direct fed microbials (Francisco et al., 2002; Chiquette et al., 2008) or of near complete switch of ruminal digesta (Satter and Bringe, 1969; Weimer et al., 2010a) on performance of dairy cows have previously been

studied. In contrast, the intent of the present experiment was to specifically study the effects of adaptation of the rumen flora in response to inoculation with a microbial community associated with normal ruminal fermentation.

The time course of diet-induced MFD induction and recovery were previously described in Chapter 3 using similar diets, and provided the basis for the length of the induction and recovery phases of the current experiment. Specifically, near complete MFD induction occurred by approximately 10 d using a similar high corn silage, low fiber, and high PUFA diet. Importantly, the experimental design does not require reaching a maximal reduction. However, milk fat must be decreased to allow examination of recovery. In the current experiment, greater than a 15% reduction in milk fat concentration was successfully achieved in 8 cows in period 1 and 6 cows in period 2. The recovery diet was designed to allow recovery from MFD, as it was higher in NDF and lower in PUFA than the induction diet.

Previously, a high-resolution time course experiment observed that milk fat concentration and yield progressively increased were completely recovered in 19 d when cows were fed a moderate NDF and low PUFA diet following MFD induction (Chapter 3). In the current experiment, milk fat percent progressively increased and plateaued near d 15 and was not affected by inoculation treatments at any time point (Figure 6-1). Milk fat yield was not changed during recovery. The recovery diet resulted in a substantial milk yield loss presumably due to the lower fermentability of the recovery diet. However, milk FA profile clearly shows classical recovery from MFD. Specifically, MFD is characterized by a larger reduction in de novo synthesized FA that results in a decrease in the proportion of FA less than 16 C. The progressive

changes in the proportion of de novo and preformed FA indicates recovery from MFD even though milk fat yield was not increased over time. Additionally, the inoculation increased the proportion of de novo FA on d 6 indicating an acceleration of recovery. A further increase in milk fat or recovery of normal milk FA profile may have been achievable beyond d 18 in the current experiment; however, the hypothesis is based on the rate of recovery after diet modification and the impact of short-term inoculation.

Others have reported differing effects of probiotic treatments on milk fat in dairy cows. Milk fat concentration tended to increase when transition dairy cows where ruminally infused with a daily dose of 2×10^{11} cells of *Prevotella bryantii* (25A), while no effects where observed on DMI or milk yield (Chiquette et al., 2008). In contrast, administration of a propionibacteria culture topdress (17 g/d) decreased DMI, but had no effect on milk yield or milk fat concentration in early lactation cows (Francisco et al., 2002).

A two-phase response has been previously reported for milk fat *trans* FA during recovery (Chapter 3). During the first phase, from approximately d 1 to 10, milk fat *trans*-10 C18:1 and *trans*-10, *cis*-12 CLA decrease, and during the second phase *trans*-11 C18:1 and *cis*-9, *trans*-11 CLA rise slightly before returning to baseline. In the current experiment *trans*-10 C18:1 and *trans*-10, *cis*-12 CLA were decreased on d 6 by inoculation representing a modest acceleration of the decrease of the alternative biohydrogenation pathway. Additionally, *trans*-11 C18:1 and *cis*-9, *trans*-11 CLA were decreased on d 12 by inoculation representing a possible dampening of the second phase. The increased de novo synthesized FA taken together with changes in *trans*

isomers during the first and second phases of recovery provide support for a modest acceleration of recovery by the digesta inoculation.

Milk fat desaturase indexes are commonly used as a proxy of the activity of stearoyl-CoA desaturase enzyme (Perfield et al., 2006; Harvatine et al., 2009a). In the present experiment, the C14 and C16 desaturase indexes progressively decreased during recovery from MFD in agreement with the progressive decrease in both C14 and C16 desaturase indexes during recovery from diet-induced MFD reported in Chapter 3.

Conclusions

The rates of recovery of mammary de novo FA synthesis and normal ruminal BH pathways were modestly accelerated by ruminal inoculation with digesta from non-MFD cows. The mechanism likely involves the restoration of normal microbial populations that have a higher capacity for BH of PUFA and utilize BH pathway that do not produced milk fat depressing intermediates. Other rumen modifiers may have similar effects on acceleration of recovery from diet-induced MFD through probiotic or prebiotic mechanisms.

Table 6-1. Ingredient and chemical composition of diets.

Item	Induction[1]	Recovery
Ingredients, g/100 of DM		
Corn silage[2]	36.8	37.0
Alfalfa haylage[3]	6.6	17.3
Ground corn	17.4	9.2
Roasted soybeans	9.7	5.2
Canola meal	9.5	9.4
Cookie meal	5.3	5.8
Grass hay/straw[4]	3.7	5.4
Sugar cane molasses	2.6	2.3
Soybean oil	1.6	-
Optigen[5]	0.5	0.5
Cottonseed hulls	3.8	5.4
Minerals and vitamins mix[6]	2.7	2.5
Chemical composition[7] (g/100 g DM)		
CP	16.9	16.9
NDF	26.1	31.8
ADF	15.5	20.8
Starch	28.1	23.0

[1]Induction is a lower forage and higher oil diet fed during the induction phase of each period and Recovery is a higher forage diet fed during the recovery phase of each period.

[2]Contained 37% DM, 8.4% CP, and 37.2% NDF.

[3]Contained 36.3% DM, 21.45% CP, and 39.9% NDF.

[4]Contained 89% DM, 7.6% CP, and 71.2% NDF.

[5]Optigen is a non-protein N source (256% CP, DM basis), Alltech Inc., (Lexington, KY).

[6]Contained (%, as fed basis): 45.8 dried corn distillers grains with solubles; 35.8 limestone (38% Ca); 8.3 magnesium oxide (54% Mg); 6.4 salt; 1.73 vitamin ADE premix; 1.09 selenium premix (0.06% selenium); and 0.88 trace mineral mix. Composition (DM basis): 11% CP; 18% NDF; 5.2% fat; 14.9% Ca; 0.35% P; 4.58% Mg; 0.41% K; 0.31% S; 357 mg/kg Cu; 1,085 mg/kg Zn; 181 mg/kg Fe; 6.67 mg/kg Se; 125,875 IU/kg vitamin A; 31,418 IU/kg vitamin D; and 946 IU/kg vitamin E (Cargill Animal Nutrition, Roaring Spring, PA)

[7]Analyzed by Cumberland Valley Analytical Services (Maugansville, MD; n = 2 per diet).

Table 6-2. Effect of ruminal inoculation on DMI and milk yield and composition during recovery from diet-induced milk fat depression.

	Treatment[1]			*P*-value[2]		
Item	Control	INOC	SEM	Trt	Time	Trt x Time
DMI, kg/d	26.7	26.9	1.3	0.93	0.02	0.89
Milk Yield, kg/d						
Milk	35.7	31.9	1.9	0.16	<0.001	0.91
Fat	1.29	1.09	0.10	0.18	0.70	0.68
Protein	1.14	1.04	0.05	0.21	<0.001	0.87
Milk Composition, %						
Fat, %	3.52	3.56	0.15	0.85	<0.01	0.76
Protein, %	3.20	3.28	0.04	0.21	0.41	0.87
Milk FA by source, g/d[3]						
< 16 C	254	264	35	0.75	<0.01	0.63
> 16 C	458	444	50	0.78	0.17	0.45

[1]Control = no inoculation; INOC = ruminal inoculation with 8 kg of donor cow digesta/d from d 1 to 6 during recovery from diet-induced milk fat depression.

[2] Trt = Treatment effect; Time = effect of day in recovery from milk fat depression.

[3]FA = fatty acids. FA < 16 C originate from de novo synthesis in the mammary gland and FA > 16 C originate from extraction from plasma.

Table 6-3. Effect of ruminal inoculation on milk fatty acid composition during recovery from diet-induced milk fat depression.

	Treatment[1]			*P*-value[2]		
FA, % of total FA[3]	Control	INOC	SEM	Trt	Time	Trt x Time
trans-10 C18:1	1.06	0.92	0.10	0.35	<0.001	0.35
trans-11 C18:1	1.10	1.03	0.08	0.47	0.03	0.10
cis-9, *trans*-11 CLA[4]	0.66	0.64	0.04	0.71	<0.001	0.06
trans-10, cis-12 CLA	0.010	0.008	0.001	0.27	<0.001	0.18
FA < 16 C[5]	23.9	24.64	0.39	0.17	<0.001	0.39
FA > 16 C	42.67	41.56	1.03	0.34	<0.001	0.79
16 C	25.77	26.47	0.69	0.41	<0.001	0.68
Sum of *trans* C18:1	4.12	3.76	0.22	0.27	<0.001	0.44
C14 desaturase index[6]	0.08	0.09	0.004	0.78	<0.001	0.58
C16 desaturase index	0.052	0.053	0.003	0.83	<0.001	0.94

[1]Control = no inoculation; INOC = ruminal inoculation with 8 kg of donor cow digesta/d from d 1 to 6 during recovery from diet-induced milk fat depression.

[2]Trt = Treatment effect; Time = effect of day in recovery from milk fat depression.

[3]FA = fatty acids.

[4]CLA = conjugated linoleic acid.

[5]Fatty acids < 16 C originate from de novo synthesis in the mammary gland, fatty acids > 16 C originate from extraction from plasma and 16 C fatty acids originate from both sources.

[6]C14 desaturase index = C14:1/(C14:1 + C14:0); C16 desaturase index = C16:1/(C16:1 + C16:0).

Table 6-4. Effect of ruminal inoculation on milk composition regression parameters during recovery from diet-induced milk fat depression

		Estimates[2]			*P*-value[3]		
Item	Trt[1]	Int	L	Q	Int	L	Q
Milk Fat							
%	Control	2.87	0.10	-0.0030	<0.001	<0.001	<0.001
	INOC	2.60	0.16	-0.0048			
kg/d	Control	1.44	0.01	-	<0.001	<0.001	-
	INOC	1.25	0.02	-			
Milk FA by Source, % of Total FA[4]							
< 16 C	Control	22.07	0.2031	-	<0.001	<0.001	-
	INOC	23.65	0.1476	-			
16 C	Control	24.15	0.219	-	<0.001	<0.01	-
	INOC	24.19	0.297	-			
> 16 C	Control	46.08	-0.338	-	<0.001	<0.001	-
	INOC	44.12	-0.326	-			
Milk *trans* FA, % of Total FA							
trans-10 C18:1	Control	4.50	-0.577	0.0196	<0.001	<0.001	<0.01
	INOC	5.31	-0.811	0.0305			
trans-11 C18:1	Control	1.21	-0.009	-	<0.0001	<0.01	-
	INOC	1.66	-0.049	-			
cis-9, *trans*-11 CLA[5]	Control	0.89	-0.024	-	<0.001	<0.001	-
	INOC	0.12	-0.04	-			
trans-10, cis-12 CLA	Control	0.04	-0.006	0.0002	<0.001	<0.001	<0.001
	INOC	0.03	-0.005	0.0002			

[1]Control = no inoculation; INOC = ruminal inoculation with 8 kg of donor cow digesta/d from d 1 to 6 during recovery from diet-induced milk fat depression.
[2]Intercept (Int) and slopes of the regression terms; Trt = Treatment effect; L = linear and Q = quadratic effects of day in recovery from milk fat depression.
[3]Probability for effect of regression term. L = interaction of treatment and the linear effect of of day in recovery from milk fat depression; and Q = interaction of treatment and the quadratic effect of day in recovery from milk fat depression.
[4]FA < 16 C originate from de novo synthesis in the mammary gland, FA > 16 C originate from extraction from plasma and 16 C FA originate from both sources.
CLA = conjugated linoleic acid.
[5]C14 desaturase index = C14:1/(C14:1 + C14:0); C16 desaturase index = C16:1/(C16:1 + C16:0).

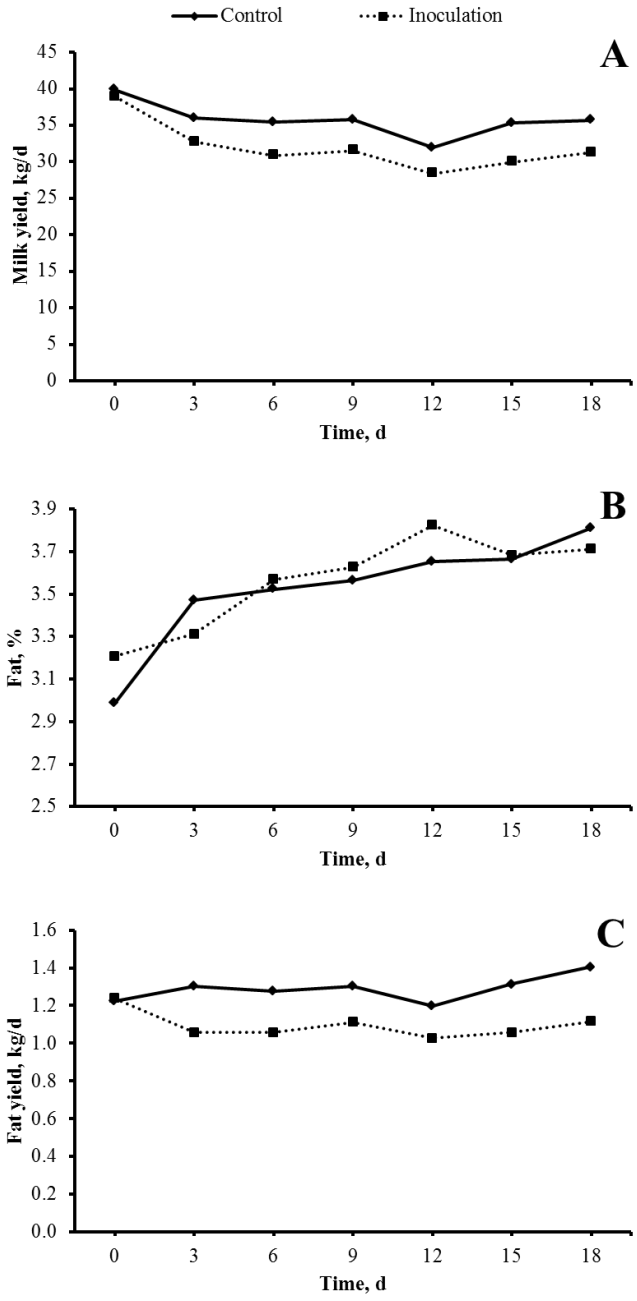


Figure 6-1. Effect of ruminal inoculation with digesta from a non-milk fat depressed cow for 6 days on milk yield and milk fat concentration and yield during recovery from diet-induced milk fat depression compared to control.

Panel A: Milk fat yield by time, Panel B: Milk fat Concentration by time, and Panel C: Milk fat yield by time. Preplanned contrasts tested the difference between Control and Inoculation at each time point (* = $P < 0.05$; SEM = 1.9, 0.15, and 0.10, for milk yield, milk fat concentration, and milk fat yield, respectively).

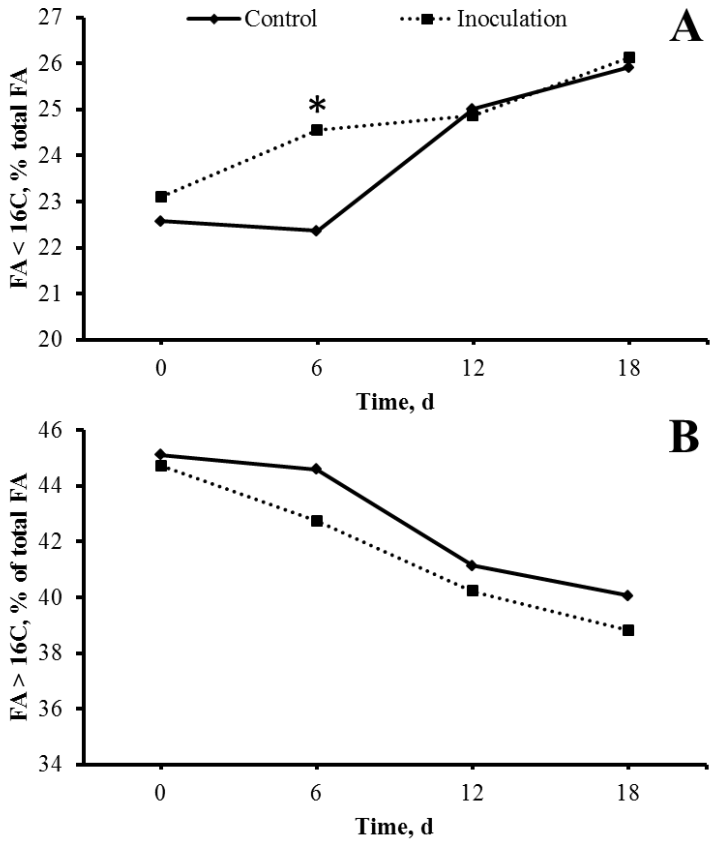


Figure 6-2. Effect of ruminal inoculation with digesta from a non-milk fat depressed cow for 6 days on de novo and preformed fatty acid concentration in milk fat during recovery from diet-induced milk fat depression compared to control.

Panel A: de novo fatty acid concentration by time and Panel B: preformed fatty acid by time. Preplanned contrasts tested the difference between Control and Inoculation at each time point (* = $P < 0.05$; SEM = 0.39 and 1.03, for de novo and preformed FA, respectively).

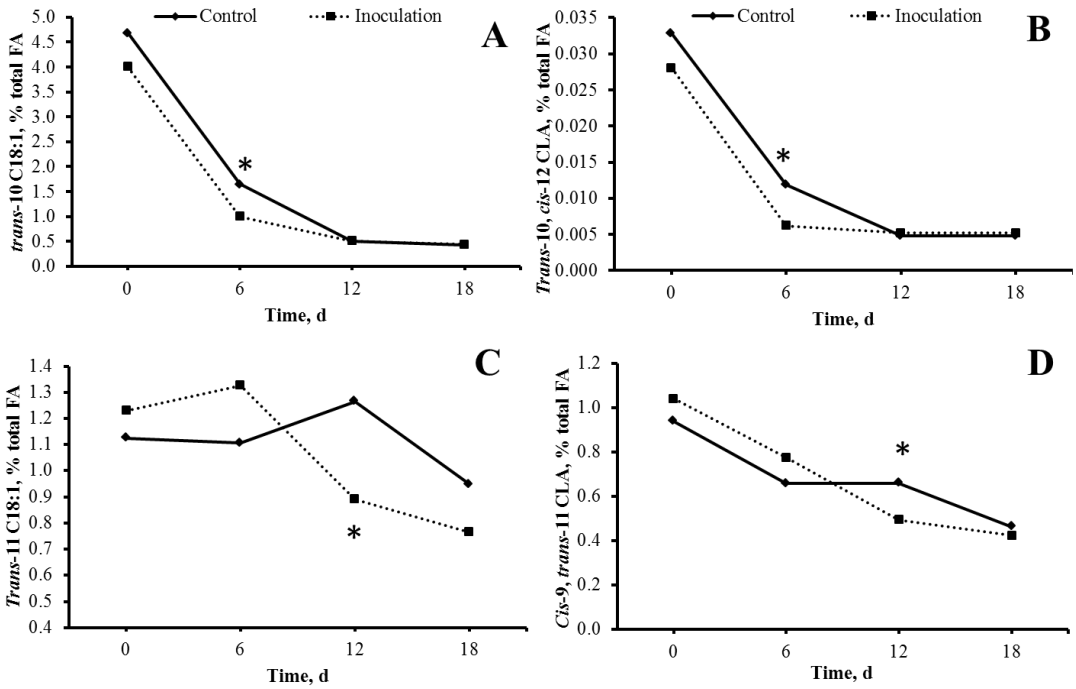


Figure 6-3. Effect of ruminal inoculation with digesta from a non-milk fat depressed cow for 6 days on milk trans fatty acid concentration during recovery from diet-induced milk fat depression compared to control.

Panel A: *trans*-10 C18:1, Panel B: *trans*-10, *cis*-12 CLA, Panel C: *trans*-11 C18:1, and Panel D: *cis*-9, *trans*-11 CLA. Preplanned contrasts tested the difference between Control and Inoculation at each time point (* = $P < 0.05$; SEM = 0.10, 0.001, 0.08, and 0.04, for *trans*-10 C18:1, *trans*-10, *cis*-12 CLA, *trans*-11 C18:1, and *cis*-9, *trans*-11 CLA, respectively).

Chapter 7

Integrative Discussion

Milk fat concentration and fatty acid profile are affected by several factors such as genetics and stage of lactation, but are especially responsive dietary factors. High concentrate, low forage and high oil diets are commonly associated to milk fat depression and the mechanism involves the alteration of ruminal fermentation and flora that results in increased production of biohydrogenation (BH) intermediates that inhibit mammary lipid synthesis (Bauman and Griinari, 2001; Weimer et al., 2010b). Induction of milk fat depression and the associated changes in milk FA profile are known to occur progressively (Roy et al., 2006; Shingfield et al., 2006). However, prior to the studies discussed in this dissertation, the time course of recovery from diet-induced milk fat depression had not been specifically investigated. In addition to restoration of milk fat concentration and yield, recovery from milk fat depression was studied in terms of return to normal ruminal BH pathways and recovery of mammary lipid synthesis, considering these two factors are key to the phenotype of classic diet induced MFD (Bauman and Griinari, 2001).

Knowledge of the time course of induction of and recovery from MFD is of applied importance for the management of this condition on dairy farms, as it allows to identify the diet changes that caused MFD, as well as to set expectations regarding how long to wait for a diet correction to have an effect before making additional changes. Our initial characterization showed that similarly to induction of MFD, recovery from MFD also occurs progressively, and that ruminal adaptation is a key rate-limiting step. The mammary gland responds very rapidly to absorption of *trans*-10, *cis*-12 CLA, as

evidenced by the reductions in milk fat synthesis observed within 12 h of abomasal CLA infusion. (Harvatine and Bauman, 2011). A key observation from our initial characterization of recovery is the lag between changes in dietary nutrients and changes in milk fat yield, which highlights the importance of gradual ruminal adaptation to the new diet before observing recovery of milk fat synthesis.

Based on this study, increasing diet fermentability and PUFA concentration can cause MFD in 7 to 13 days, whereas after correcting these risk factors it may take 11 to 15 days to rescue milk fat. The observed reduction in milk fat was mainly explained by progressive increases in FA intermediates in the altered BH pathway (i.e. *trans*-10 C18:1 and *trans*-10, *cis*-12 CLA), and a concomitant reduction in de novo FA secretion in milk. Similarly, feeding a mixture of fish and sunflower oil at 4.5 % of DM in high fiber, corn silage-based diets, Shingfield et al. (2006) reported a progressive decrease in milk fat yield associated with a gradual increase in *trans*-10 C18:1 and *trans*-10, *cis*-12 CLA, and drastic reductions in de novo synthesized FA.

In addition, our study of corrections of key dietary factors to accelerate recovery from MFD showed that reduction of dietary PUFA concentration is the most important factor for rapid recovery of milk fat synthesis and restoration of normal BH pathways. Regardless of the lower initial milk fat concentration in this experiment, similarly to our first characterization of recovery from MFD, changes in de novo FA synthesis and restoration of normal BH occurred progressively and to a similar extent when diet fermentability and PUFA, or PUFA alone were corrected. However, the high oil diet slowed down the decrease in the altered BH pathway and failed to recover de novo FA synthesis. This is likely due to the toxic effect that PUFA have on bacteria, which results in turn in alterations of ruminal BH pathways (Maia et al., 2007; Lourenço et al., 2010; Maia et al., 2010). Importantly the low forage diet allowed for higher DMI and milk

yield. This is also of practical importance, as the goal of dairy nutritionists is to correct MFD without reducing milk yield due to limitation of energy intake in higher fiber diets. Minimizing dietary PUFA concentration should be the first correction to recover from MFD. Secondly, excess diet fermentability should be evaluated, as it may lead to subacute ruminal acidosis, which is associated with impaired ruminal fermentation and MFD (Plaizier et al., 2008).

We observed that monensin had little impact on recovery of milk fat synthesis and restoration of normal ruminal BH, whereas correction of dietary PUFA and diet fermentability were more important. Previous research has shown that monensin supplementation can exacerbate MFD in higher PUFA diets by further increasing the accumulation of intermediates in the altered BH pathway (Alzahal et al., 2008; He et al., 2012). Interestingly, relative to the induction diet, the recovery diets contained little PUFA and the previously reported slowing down of BH and the accumulation of intermediates reported by others (Fellner et al., 1997) was not expected to be an issue considering the lower content of PUFA in the recovery relative to the induction diet.

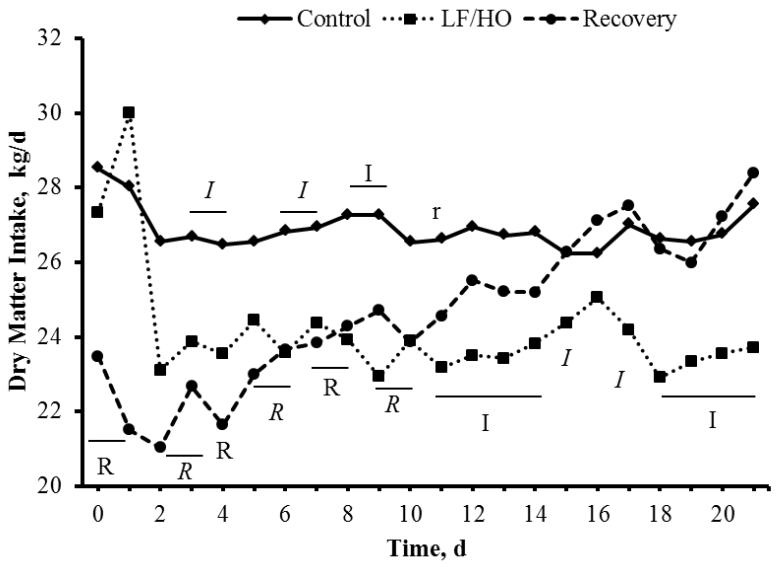
Interestingly, ruminal inoculation with normal ruminal flora showed the potential of direct-fed microbials to accelerate recovery from MFD, and although the observed effect on de novo FA and restoration of normal BH was modest, it suggested that similar approaches that help promote faster adaptation of ruminal flora could lead to benefits in recovery of milk fat synthesis. Other feed additives that help normalizing the ruminal environment like yeast may have benefit when MFD occurs (Longuski et al., 2009). Stabilization of the ruminal environment by this type additive could be of importance in situations where diet fermentability is increased to support higher milk yield.

Lastly, future research efforts could focus on the effect of different dietary interventions known have an impact on ruminal fermentation, BH and milk fat yield,

such as supplementation of yeast culture, vitamin E, sugar, and dietary buffers on the restoration of normal ruminal BH pathways and recovery of milk fat synthesis.

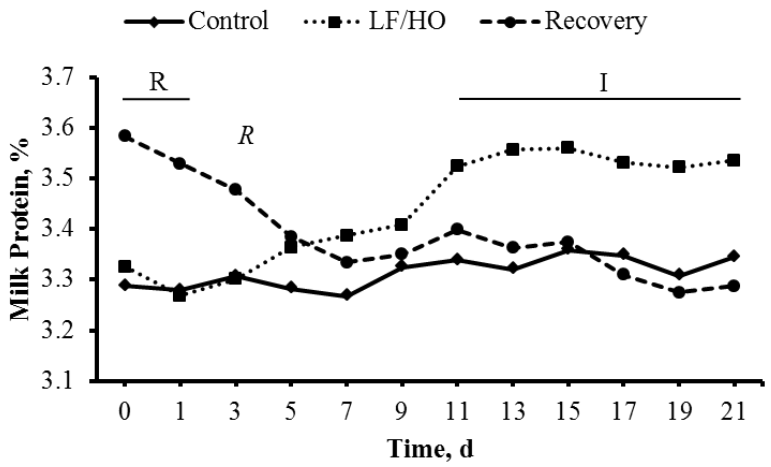
Appendix

Time course of dry matter intake, milk protein and milk FA profile during induction of and recovery from diet-induced milk fat depression



Appendix figure 7-1. Time course of dry matter intake during induction and recovery from diet-induced milk fat depression.

Shown is the dry matter intake of cows fed a low fiber, high oil diet (Induction; LF/HO), a high fiber diet, low oil (Control), or a high fiber, low oil diet after LF/HO (Recovery). Preplanned contrasts tested the difference between Control and Induction (*I* = $P < 0.01$, I = $P < 0.05$, and i = $P < 0.1$) and between Control and Recovery [R = $P < 0.01$, *R* = $P < 0.05$, and r = $P < 0.1$; SEM = 1.94].



Appendix figure 7-2. Time course of milk protein concentration during induction and recovery from diet-induced milk fat depression.

Shown is the milk protein concentration of cows fed a low fiber, high oil diet (Induction; LF/HO), a high fiber diet, low oil (Control), or a high fiber, low oil diet after LF/HO (Recovery). Preplanned contrasts tested the difference between Control and Induction ($I = P < 0.01$, I = $P < 0.05$, and i = $P < 0.1$) and between Control and Recovery [R = $P < 0.01$, $R = P <$ 0.05, and r = $P < 0.1$; SEM = 0.10].

Appendix table 7-1. Major fatty acid (FA) composition of diets fed.

	Treatment	
Fatty acid, g/kg of diet DM	LF/HO[1]	Control
C14:0	0.13	0.14
C14:1	1.06	0.69
C16:0	8.52	4.31
C16:1, n-7	0.12	0.10
C18:0	2.24	0.76
C18:1, n-9	16.27	6.24
C18:2, n-6	33.54	9.77
C18:3, n-3	3.60	1.64
Σ MUFA[2]	17.45	7.03
Σ PUFA[3]	37.14	11.41
Σ Unsaturated FA	54.70	18.59
Unknown	3.24	2.56

[1]LF/HO = low fiber high soy oil diet.

[2]Sum of total monounsaturated fatty acids.

[3]Sum of total polyunsaturated fatty acids.

Appendix table 7-2.Time course of individual milk fatty acids of cows fed a low fiber high soy oil diet (Induction), a high fiber diet (Control), or a high fiber diet after Induction (Recovery).

					P-values				
					-----Contrasts-----				
Day	Control	Induction[1]	Recovery[2]	SEM	Control vs LFHO[1]	Control vs Recovery	Treatment	Day	Treatment x day
Fatty Acid									
C4:0									
0	4.22	4.28	1.92	0.19	0.81	<0.001	<0.001	0.01	<0.001
1	3.78	3.83	2.01	0.12	0.70	<0.001			
3	3.65	3.34	2.72	0.23	0.31	<0.01			
5	4.35	2.89	3.02	0.19	<0.001	<0.001			
7	3.75	2.88	3.37	0.17	<0.01	0.09			
9	4.36	2.49	3.55	0.17	<0.001	<0.01			
11	3.88	2.45	3.49	0.19	<0.001	0.11			
13	3.83	2.31	3.52	0.18	<0.001	0.14			
15	4.19	2.27	3.71	0.18	<0.001	0.04			
17	3.98	2.22	3.57	0.16	<0.001	0.05			
19	3.66	2.07	3.73	0.12	<0.001	0.51			
21	4.07	2.17	4.17	0.26	<0.001	0.79			
C6:0									
0	2.46	2.59	0.98	0.09	0.25	<0.001	<0.001	0.31	<0.001
1	2.40	2.39	1.08	0.07	0.91	<0.001			
3	2.23	1.93	1.51	0.18	0.23	<0.01			
5	2.59	1.65	1.50	0.10	<0.001	<0.001			

7	2.45	1.59	1.77	0.08	<0.001	<0.001			
9	2.54	1.33	1.91	0.10	<0.001	<0.001			
11	2.39	1.26	2.09	0.11	<0.001	0.03			
13	2.39	1.32	2.06	0.11	<0.001	0.01			
15	2.51	1.22	2.11	0.10	<0.001	<0.01			
17	2.47	1.20	2.15	0.09	<0.001	<0.01			
19	2.32	1.10	2.27	0.07	<0.001	0.46			
21	2.62	1.17	2.41	0.12	<0.001	0.15			
C8:0									
0	1.30	1.38	0.51	0.05	0.15	<0.001	<0.001	<0.01	<0.001
1	1.37	1.38	0.58	0.04	0.65	<0.001			
3	1.27	1.09	0.81	0.11	0.24	<0.01			
5	1.38	0.89	0.75	0.05	<0.001	<0.001			
7	1.40	0.88	0.94	0.05	<0.001	<0.001			
9	1.35	0.71	0.97	0.05	<0.001	<0.001			
11	1.36	0.69	1.15	0.07	<0.001	0.02			
13	1.38	0.74	1.12	0.06	<0.001	<0.01			
15	1.36	0.65	1.11	0.05	<0.001	<0.01			
17	1.41	0.66	1.20	0.05	<0.001	<0.01			
19	1.34	0.60	1.28	0.04	<0.001	0.10			
21	1.42	0.62	1.28	0.06	<0.001	0.05			
C10:0									
0	2.98	3.13	1.33	0.13	0.27	<0.001	<0.001	<0.001	<0.001
1	3.23	3.30	1.52	0.12	0.47	<0.001			
3	3.03	2.59	1.96	0.25	0.19	<0.01			
5	3.13	2.04	1.79	0.14	<0.001	<0.001			
7	3.32	2.10	2.25	0.14	<0.001	<0.001			
9	3.06	1.69	2.25	0.14	<0.001	<0.001			

11	3.28	1.73	2.76	0.18	<0.001	0.02			
13	3.39	1.88	2.67	0.16	<0.001	<0.01			
15	3.17	1.46	2.56	0.13	<0.001	<0.001			
17	3.37	1.64	2.88	0.12	<0.001	<0.001			
19	3.27	1.51	3.04	0.13	<0.001	0.03			
21	3.27	1.55	2.99	0.15	<0.001	0.09			
C12:0									
0	3.48	3.55	2.07	0.17	0.68	<0.001	<0.001	<0.001	<0.001
1	3.76	3.85	2.30	0.15	0.53	<0.001			
3	3.58	3.12	2.58	0.26	0.17	<0.01			
5	3.62	2.56	2.36	0.18	<0.001	<0.001			
7	3.82	2.79	2.94	0.22	<0.01	<0.01			
9	3.57	2.34	2.74	0.17	<0.001	<0.001			
11	3.86	2.67	3.31	0.25	<0.01	0.08			
13	4.04	2.61	3.21	0.19	<0.001	<0.01			
15	3.75	2.15	3.10	0.16	<0.001	<0.001			
17	3.96	2.25	3.46	0.14	<0.001	<0.001			
19	3.93	2.25	3.58	0.14	<0.001	<0.01			
21	3.75	2.23	3.53	0.17	<0.001	0.22			
C14:0									
0	10.76	10.85	8.04	0.35	0.80	<0.001	<0.001	<0.001	<0.001
1	11.18	11.19	8.56	0.31	0.97	<0.001			
3	10.76	9.57	9.25	0.49	0.05	0.02			
5	10.92	8.27	9.21	0.47	<0.001	0.01			
7	11.19	8.86	10.28	0.44	<0.001	0.08			
9	11.19	8.23	10.17	0.34	<0.001	<0.01			
11	11.37	8.57	11.12	0.36	<0.001	0.45			
13	11.66	8.49	10.84	0.32	<0.001	<0.01			

15	11.14	8.17	10.55	0.31	<0.001	0.02			
17	11.33	8.22	11.02	0.34	<0.001	0.31			
19	11.30	8.08	11.09	0.32	<0.001	0.42			
21	11.15	8.22	11.02	0.39	<0.001	0.75			
C14:1 *cis*-9									
0	0.94	1.01	1.44	0.09	0.42	<0.001	<0.01	0.09	<0.001
1	1.05	0.96	1.42	0.08	0.90	<0.001			
3	1.00	1.03	1.14	0.13	0.01	0.04			
5	1.00	0.94	1.12	0.10	<0.001	<0.01			
7	1.06	1.00	1.02	0.10	<0.001	0.09			
9	0.99	1.24	1.01	0.10	<0.001	0.05			
11	1.09	1.28	1.06	0.11	<0.001	0.51			
13	0.98	1.51	1.10	0.09	<0.001	0.53			
15	0.99	1.32	1.11	0.09	<0.001	0.17			
17	1.01	1.37	1.16	0.11	<0.001	0.43			
19	1.10	1.25	1.04	0.10	<0.001	0.27			
21	1.04	1.30	0.98	0.10	<0.001	0.85			
C15:0									
0	0.95	1.03	0.96	0.04	0.14	0.87	<0.001	0.04	0.02
1	1.02	0.91	0.99	0.05	0.06	0.68			
3	1.02	0.87	1.09	0.06	0.04	0.35			
5	1.00	0.77	1.03	0.05	<0.01	0.72			
7	1.01	0.82	1.05	0.04	<0.01	0.43			
9	1.01	0.91	1.01	0.04	<0.01	0.96			
11	1.04	0.96	1.05	0.04	0.12	0.85			
13	1.10	0.95	1.06	0.04	<0.01	0.35			
15	1.07	0.87	1.03	0.04	<0.01	0.38			
17	1.04	0.87	1.01	0.05	0.01	0.60			

19	1.05	0.85	0.99	0.04	<0.01	0.14			
21	1.06	0.91	1.00	0.05	0.02	0.32			
C16:0									
0	26.55	27.19	22.41	0.73	0.42	<0.001	<0.001	0.01	<0.001
1	27.00	26.92	23.07	0.66	0.90	<0.001			
3	26.32	23.36	23.87	0.92	0.01	0.04			
5	27.12	21.01	24.58	0.72	<0.001	<0.01			
7	27.04	20.92	25.80	0.69	<0.001	0.09			
9	27.21	20.85	25.97	0.64	<0.001	0.05			
11	27.07	21.41	26.55	0.75	<0.001	0.51			
13	27.59	22.20	27.12	0.73	<0.001	0.53			
15	27.85	22.26	27.07	0.62	<0.001	0.17			
17	27.65	22.22	27.02	0.74	<0.001	0.43			
19	27.43	21.63	26.72	0.66	<0.001	0.27			
21	27.35	21.65	27.24	0.66	<0.001	0.85			
C16:1 *cis*-9									
0	1.56	1.76	2.84	0.16	0.20	<0.001	<0.01	0.01	<0.001
1	1.66	1.46	2.66	0.17	0.22	<0.001			
3	1.61	1.65	2.14	0.24	0.89	0.08			
5	1.57	1.54	2.09	0.19	0.91	0.03			
7	1.61	1.59	1.67	0.16	0.90	0.64			
9	1.56	2.19	1.66	0.18	<0.01	0.62			
11	1.58	2.18	1.65	0.20	0.01	0.74			
13	1.47	2.52	1.64	0.25	<0.01	0.57			
15	1.53	2.17	1.77	0.31	0.12	0.54			
17	1.57	2.54	1.65	0.19	<0.001	0.73			
19	1.73	2.34	1.64	0.17	<0.01	0.56			
21	1.63	2.53	1.57	0.17	<0.001	0.76			

C17:0									
0	0.50	0.49	0.35	0.012	0.20	<0.001	<0.001	<0.001	<0.001
1	0.51	0.46	0.37	0.018	0.02	<0.001			
3	0.50	0.42	0.50	0.021	0.01	0.98			
5	0.47	0.36	0.51	0.014	<0.001	0.01			
7	0.50	0.38	0.54	0.016	<0.001	0.05			
9	0.51	0.35	0.53	0.016	<0.001	0.22			
11	0.51	0.36	0.52	0.018	<0.001	0.53			
13	0.54	0.36	0.55	0.014	<0.001	0.45			
15	0.51	0.36	0.50	0.013	<0.001	0.58			
17	0.50	0.35	0.50	0.016	<0.001	0.87			
19	0.52	0.36	0.53	0.015	<0.001	0.60			
21	0.48	0.35	0.50	0.012	<0.001	0.09			
C18:0									
0	9.81	9.23	9.29	0.41	0.16	0.22	0.02	<0.01	<0.001
1	9.07	8.38	9.49	0.43	0.13	0.36			
3	9.17	9.31	9.68	0.66	0.86	0.54			
5	9.38	10.97	9.90	0.50	0.01	0.37			
7	9.09	11.26	9.57	0.43	<0.001	0.28			
9	9.40	10.33	9.84	0.51	0.12	0.46			
11	9.01	10.09	9.73	0.38	<0.01	0.04			
13	9.61	9.75	9.08	0.52	0.97	0.36			
15	9.37	10.12	9.06	0.40	0.08	0.41			
17	9.01	10.30	9.10	0.46	0.02	0.86			
19	8.56	10.62	9.24	0.46	<0.01	0.18			
21	9.18	10.14	9.80	0.43	0.04	0.17			
C18:1, *trans*-4									
0	0.028	0.026	0.037	0.002	0.55	<0.01	<0.001	0.11	<0.01

1	0.027	0.038	0.034	0.004	0.04	0.14			
3	0.035	0.044	0.028	0.004	0.13	0.26			
5	0.027	0.033	0.031	0.002	0.04	0.18			
7	0.026	0.033	0.028	0.003	0.08	0.61			
9	0.024	0.037	0.028	0.003	<0.01	0.15			
11	0.026	0.032	0.025	0.003	0.09	0.60			
13	0.033	0.027	0.024	0.004	0.28	0.12			
15	0.025	0.034	0.026	0.002	<0.001	0.47			
17	0.027	0.031	0.024	0.003	0.33	0.45			
19	0.028	0.029	0.024	0.003	0.83	0.27			
21	0.026	0.035	0.027	0.002	<0.01	0.73			
C18:1, *trans*-5									
0	0.020	0.022	0.040	0.003	0.59	<0.001	<0.001	0.02	<0.001
1	0.021	0.038	0.038	0.004	<0.01	<0.01			
3	0.023	0.040	0.031	0.006	0.02	0.25			
5	0.020	0.031	0.024	0.003	0.01	0.31			
7	0.021	0.034	0.025	0.003	<0.01	0.19			
9	0.019	0.040	0.021	0.004	<0.001	0.58			
11	0.011	0.033	0.029	0.009	0.10	0.14			
13	0.024	0.029	0.021	0.002	<0.01	0.14			
15	0.018	0.039	0.019	0.002	<0.001	0.50			
17	0.016	0.034	0.016	0.003	<0.001	0.82			
19	0.022	0.033	0.019	0.003	0.02	0.51			
21	0.020	0.035	0.019	0.002	<0.001	0.74			
C18:1, *trans*-6-8									
0	0.36	0.35	1.20	0.077	0.48	<0.001	<0.001	<0.01	<0.001
1	0.35	0.51	1.06	0.079	<0.01	<0.001			
3	0.42	0.72	0.55	0.106	0.01	0.19			

5	0.38	0.76	0.48	0.060	<0.001	0.02			
7	0.37	0.88	0.42	0.069	<0.001	0.16			
9	0.34	1.10	0.39	0.075	<0.001	0.05			
11	0.33	1.21	0.38	0.076	<0.001	0.04			
13	0.33	0.97	0.35	0.070	<0.001	0.47			
15	0.34	0.96	0.36	0.076	<0.001	0.46			
17	0.34	0.90	0.35	0.051	<0.001	0.51			
19	0.33	0.87	0.35	0.052	<0.001	0.27			
21	0.35	0.94	0.35	0.063	<0.001	0.72			
C18:1, *trans*-9									
0	0.29	0.32	0.96	0.078	0.74	<0.001	<0.001	0.63	<0.001
1	0.27	0.37	0.73	0.048	0.08	<0.001			
3	0.35	0.53	0.41	0.055	0.01	0.43			
5	0.30	0.59	0.36	0.052	<0.001	0.31			
7	0.27	0.63	0.32	0.050	<0.001	0.33			
9	0.27	0.63	0.30	0.077	<0.01	0.79			
11	0.27	0.75	0.29	0.080	<0.001	0.86			
13	0.26	0.72	0.28	0.068	<0.001	0.86			
15	0.26	0.67	0.28	0.074	0.00	0.80			
17	0.25	0.75	0.28	0.063	<0.001	0.73			
19	0.29	0.65	0.28	0.047	<0.001	0.80			
21	0.30	0.76	0.28	0.090	<0.01	0.87			
C18:1, *trans*-10									
0	0.52	0.47	5.63	0.53	0.06	<0.001	<0.001	<0.001	<0.001
1	0.51	0.86	4.62	0.47	<0.001	<0.001			
3	0.74	1.55	1.85	0.55	0.05	0.02			
5	0.47	2.34	1.52	0.40	<0.001	<0.001			
7	0.53	2.82	0.93	0.46	<0.001	0.01			

9	0.47	4.85	0.83	0.56	<0.001	<0.001			
11	0.55	4.34	0.65	0.65	<0.001	0.29			
13	0.48	3.90	0.57	0.42	<0.001	0.07			
15	0.46	5.05	0.53	0.53	<0.001	0.10			
17	0.66	4.49	0.53	0.82	<0.001	0.29			
19	0.46	3.58	0.56	0.34	<0.001	<0.01			
21	0.46	4.59	0.51	0.45	<0.001	0.09			
C18:1, *trans*-11									
0	1.07	0.86	0.60	0.09	0.03	<0.001	0.02	<0.001	<0.001
1	1.00	1.58	0.79	0.16	<0.01	0.08			
3	1.15	2.55	0.72	0.62	0.02	0.14			
5	1.02	2.08	0.64	0.31	<0.01	0.02			
7	0.96	1.83	0.88	0.24	<0.01	0.63			
9	0.98	1.17	1.05	0.14	0.26	0.66			
11	1.00	1.11	1.28	0.09	0.25	0.01			
13	0.92	0.90	1.35	0.07	0.59	<0.001			
15	0.93	0.78	1.33	0.12	0.11	<0.01			
17	0.92	0.82	1.15	0.08	0.19	0.02			
19	0.90	0.72	1.12	0.10	0.04	0.04			
21	0.88	0.49	1.09	0.07	<0.001	<0.01			
C18:1, *trans*-12									
0	0.52	0.54	0.79	0.04	0.60	<0.001	<0.001	<0.01	<0.001
1	0.47	0.73	0.72	0.05	<0.01	<0.01			
3	0.63	0.81	0.46	0.09	0.14	0.17			
5	0.54	0.80	0.51	0.03	<0.001	0.32			
7	0.46	0.71	0.47	0.04	<0.001	0.97			
9	0.52	0.81	0.49	0.04	<0.001	0.44			
11	0.47	0.72	0.46	0.06	<0.01	0.88			

13	0.50	0.68	0.46	0.03	<0.001	0.25			
15	0.53	0.73	0.49	0.03	<0.001	0.25			
17	0.44	0.69	0.44	0.05	<0.01	0.94			
19	0.52	0.65	0.51	0.03	<0.01	0.79			
21	0.55	0.71	0.53	0.03	<0.001	0.29			
C18:1, *cis*-9									
0	21.70	21.15	26.18	0.96	0.59	<0.01	<0.001	<0.01	<0.001
1	20.81	19.32	25.16	0.87	0.09	<0.001			
3	21.37	21.01	26.31	1.14	0.79	0.00			
5	21.03	23.22	27.24	1.15	0.12	<0.01			
7	20.89	23.99	24.21	1.02	0.01	0.01			
9	21.00	25.31	24.19	0.97	<0.01	<0.01			
11	20.42	24.82	21.56	1.02	<0.01	0.30			
13	19.22	24.69	22.14	0.90	<0.001	0.00			
15	19.90	25.78	22.50	0.91	<0.001	0.01			
17	20.38	25.63	21.89	0.90	<0.001	0.10			
19	19.95	27.17	21.37	0.80	<0.001	0.05			
21	20.12	25.96	21.29	0.90	<0.001	0.21			
C18:2, *cis*-9, *cis*-12									
0	2.29	2.35	4.01	0.23	0.83	<0.001	<0.001	<0.01	<0.001
1	2.19	2.61	3.48	0.15	0.01	<0.001			
3	2.40	3.29	2.94	0.25	0.01	0.09			
5	2.24	3.91	2.90	0.16	<0.001	<0.01			
7	2.13	3.73	2.47	0.20	<0.001	0.13			
9	2.12	3.95	2.52	0.14	<0.001	<0.01			
11	2.18	3.70	2.32	0.17	<0.001	0.41			
13	1.91	3.44	2.24	0.19	<0.001	0.10			
15	2.07	3.59	2.32	0.13	<0.001	<0.01			

17	2.07	3.82	2.14	0.16	<0.001	0.61			
19	2.22	4.05	2.16	0.23	<0.001	0.82			
21	2.33	3.89	2.23	0.22	<0.001	0.72			
C20:0									
0	0.14	0.14	0.11	0.013	0.93	0.04	<0.001	0.01	0.14
1	0.11	0.11	0.08	0.020	0.95	0.22			
3	0.14	0.10	0.12	0.014	0.03	0.31			
5	0.15	0.13	0.12	0.015	0.22	0.10			
7	0.14	0.11	0.13	0.020	0.23	0.66			
9	0.15	0.12	0.16	0.009	<0.01	0.70			
11	0.15	0.12	0.15	0.011	0.06	0.67			
13	0.16	0.11	0.15	0.011	<0.01	0.71			
15	0.15	0.11	0.13	0.014	0.03	0.29			
17	0.14	0.12	0.13	0.019	0.52	0.81			
19	0.13	0.11	0.14	0.010	0.02	0.43			
21	0.15	0.13	0.15	0.011	0.08	0.69			
C18:3, *cis*-9, *cis*-12, *cis*-15									
0	0.36	0.37	0.43	0.03	0.69	0.06	<0.001	<0.001	0.08
1	0.41	0.50	0.42	0.03	0.02	0.78			
3	0.42	0.49	0.43	0.03	0.11	0.74			
5	0.38	0.47	0.41	0.02	<0.01	0.29			
7	0.43	0.51	0.43	0.02	<0.01	0.95			
9	0.38	0.48	0.44	0.02	<0.001	0.00			
11	0.44	0.47	0.47	0.03	0.56	0.52			
13	0.38	0.43	0.47	0.03	0.12	0.02			
15	0.39	0.42	0.42	0.02	0.16	0.07			
17	0.43	0.53	0.45	0.02	<0.01	0.45			
19	0.45	0.53	0.42	0.03	0.06	0.38			

21	0.40	0.46	0.39	0.03	0.16	0.69			
CLA[3], *cis*-9, *trans*-11									
0	0.64	0.57	0.85	0.05	0.11	<0.001	<0.001	<0.001	<0.001
1	0.63	0.85	0.79	0.06	0.01	0.04			
3	0.62	1.54	0.66	0.17	<0.01	0.84			
5	0.63	1.70	0.61	0.08	<0.001	0.85			
7	0.60	1.26	0.64	0.11	<0.001	0.81			
9	0.59	1.07	0.72	0.07	<0.001	0.15			
11	0.60	0.94	0.75	0.08	<0.01	0.15			
13	0.52	0.88	0.83	0.06	<0.001	<0.001			
15	0.57	0.82	0.81	0.05	<0.001	<0.001			
17	0.57	0.77	0.71	0.05	<0.001	<0.01			
19	0.57	0.71	0.68	0.05	<0.01	0.01			
21	0.57	0.73	0.65	0.04	<0.001	0.01			
CLA[2], *trans*-10, *cis*-12									
0	0.005	0.008	0.092	0.009	0.79	<0.001	<0.001	<0.001	<0.001
1	0.009	0.006	0.057	0.007	0.76	<0.001			
3	0.008	0.023	0.022	0.007	0.09	0.11			
5	0.005	0.044	0.030	0.006	<0.001	<0.01			
7	0.007	0.032	0.011	0.005	<0.001	0.34			
9	0.005	0.065	0.022	0.007	<0.001	0.05			
11	0.005	0.048	0.013	0.007	<0.001	0.36			
13	0.008	0.039	0.005	0.005	<0.001	0.62			
15	0.005	0.066	0.011	0.007	<0.001	0.52			
17	0.006	0.053	0.005	0.005	<0.001	0.81			
19	0.008	0.049	0.005	0.004	<0.001	0.52			
21	0.005	0.090	0.005	0.006	<0.001	0.95			

Unknowns									
0	6.57	6.51	7.56	0.17	0.80	<0.001	<0.001	<0.001	<0.01
1	7.00	7.55	7.56	0.18	0.03	0.03			
3	7.69	8.18	7.91	0.21	0.10	0.44			
5	6.39	7.12	7.36	0.28	0.06	0.01			
7	7.07	7.22	7.49	0.20	0.56	0.13			
9	6.55	7.47	7.38	0.23	0.01	0.01			
11	7.14	7.13	7.30	0.27	0.98	0.65			
13	7.23	7.71	7.33	0.28	0.18	0.77			
15	6.50	7.22	7.06	0.22	0.02	0.04			
17	6.74	7.48	7.31	0.25	0.04	0.10			
19	7.23	7.20	7.14	0.17	0.91	0.71			
21	6.71	7.13	6.79	0.19	0.09	0.74			

[1] Milk fat depression induced by a low forage and high oil diet.

[2] A high forage and low fat diet following induction of milk fat depression.

[3] CLA = conjugated linoleic acid.

References

Ahnadi, C. E., N. Beswick, L. Delbecchi, J. J. Kennelly, and P. Lacasse. 2002. Addition of fish oil to diets for dairy cows. II. Effects on milk fat and gene expression of mammary lipogenic enzymes. J. Dairy Res. 69:521-531.

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

Alzahal, O., N. E. Odongo, T. Mutsvangwa, M. M. Or-Rashid, T. F. Duffield, R. Bagg, P. Dick, G. Vessie, and B. W. McBride. 2008. Effects of monensin and dietary soybean oil on milk fat percentage and milk fatty acid profile in lactating dairy cows. J. Dairy Sci. 91:1166-1174.

Alzahal, O., M. M. Or-Rashid, S. L. Greenwood, M. S. Douglas, and B. W. McBride. 2009. The effect of dietary fiber level on milk fat concentration and fatty acid profile of cows fed diets containing low levels of polyunsaturated fatty acids. J. Dairy Sci. 92:1108-1116.

Annison, E. F., R. Bickerstaffe, and J. L. Linzell. 1974. Glucose and fatty acid metabolism in cows producing milk of low fat content. J Agric Sci 82:87-95.

AOAC. 2000. Official Methods of Analysis. 17th ed. Association of Official Analytical Chemists, Arlington, VA.

Bailey, K. W., C. M. Jones, and A. J. Heinrichs. 2005. Economic returns to Holstein and Jersey herds under multiple component pricing. J. Dairy Sci. 88:2269-2280.

Banks, W., J. L. Clapperton, A. K. Girdler, and W. Steele. 1984. Effect of inclusion of different forms of dietary fatty acid on the yield and composition of cow's milk. J. Dairy Res. 51:387-395.

Bauman, D. E. 1992. Bovine Somatotropin: Review of an Emerging Animal Technology. J. Dairy Sci. 75:3432-3451.

Bauman, D. E., R. E. Brown, and C. L. Davis. 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 W. B. Currie. 1980. Partitioning of nutrients during pregnancy and lactation: a review of mechanisms involving homeostasis and homeorhesis. J. Dairy Sci. 63:1514-1529.

Bauman, D. E. and J. M. Griinari. 2001. Regulation and nutritional manipulation of milk fat: low-fat milk syndrome. Livest. Prod. Sci. 70:15-29.

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

Bauman, D. E., K. J. Harvatine, and A. L. Lock. 2011. Nutrigenomics, Rumen-Derived Bioactive Fatty Acids, and the Regulation of Milk Fat Synthesis. Annu. Rev. Nutr. 31:299-319.

Bauman, D. E., C. J. Peel, W. D. Steinhour, P. J. Reynolds, H. F. Tyrrell, A. C. Brown, and G. L. Haaland. 1988. Effect of bovine somatotropin on metabolism of lactating dairy cows: influence on rates of irreversible loss and oxidation of glucose and nonesterified fatty acids. J. Nutr. 118:1031-1040.

Baumgard, L. H., B. A. Corl, D. A. Dwyer, A. Saebo, and D. E. Bauman. 2000. Identification of the conjugated linoleic acid isomer that inhibits milk fat synthesis. Am. J. Physiol. Regul. Integr. Comp. Physiol. 278:R179-184.

Baumgard, L. H., E. Matitashvili, B. A. Corl, D. A. Dwyer, and D. E. Bauman. 2002. *Trans*-10, *cis*-12 conjugated linoleic acid decreases lipogenic rates and expression of genes involved in milk lipid synthesis in dairy cows. J. Dairy Sci. 85:2155-2163.

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

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.

Bell, J. A., J. M. Griinari, and J. J. Kennelly. 2006. Effect of safflower oil, flaxseed oil, monensin, and vitamin E on concentration of conjugated linoleic acid in bovine milk fat. J. Dairy Sci. 89:733-748.

Bell, J. A. and J. J. Kennelly. 2003. Short communication: Postruminal infusion of conjugated linoleic acids negatively impacts milk synthesis in Holstein cows. J. Dairy Sci. 86:1321-1324.

Broderick, G. A., N. D. Luchini, S. M. Reynal, G. A. Varga, and V. A. Ishler. 2008. Effect on Production of Replacing Dietary Starch with Sucrose in Lactating Dairy Cows. J. Dairy Sci. 91:4801-4810.

Broderick, G. A. and W. J. Radloff. 2004. Effect of molasses supplementation on the production of lactating dairy cows fed diets based on alfalfa and corn silage. J. Dairy Sci. 87:2997-3009.

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.

Chiquette, J., M. J. Allison, and M. A. Rasmussen. 2008. Prevotella bryantii 25A used as a probiotic in early-lactation dairy cows: effect on ruminal fermentation characteristics, milk production, and milk composition. J. Dairy Sci. 91:3536-3543.

Choi, N.-J., J. Y. Imm, S. Oh, B.-C. Kim, H.-J. Hwang, and Y. J. Kim. 2005. Effect of pH and oxygen on conjugated linoleic acid (CLA) production by mixed rumen bacteria from cows fed high concentrate and high forage diets. Anim. Feed Sci. Technol. 123–124, Part 2:643-653.

Chouinard, P. Y., L. Corneau, D. M. Barbano, L. E. Metzger, and D. E. Bauman. 1999. Conjugated linoleic acids alter milk fatty acid composition and inhibit milk fat secretion in dairy cows. J. Nutr. 129:1579-1584.

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

Cole, N. A. 1991. Effects of animal-to-animal exchange of ruminal contents on the feed intake and ruminal characteristics of fed and fasted lambs. J. Anim. Sci. 69:1795-1803.

Corl, B. A., S. T. Butler, W. R. Butler, and D. E. Bauman. 2006. Short communication: Regulation of milk fat yield and fatty acid composition by insulin. J. Dairy Sci. 89:4172-4175.

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

Davis, C. L. and R. E. Brown. 1970. Low-fat milk syndrome. Pages 545-565 in Physiology of Digestion and Metabolism in the Ruminant. A. T. Phillipson, ed. Oriel Press, Newcastle upon Tyne, UK.

de Veth, M. J., J. M. Griinari, A. M. Pfeiffer, and D. E. Bauman. 2004. Effect of CLA on milk fat synthesis in dairy cows: comparison of inhibition by methyl esters and free fatty acids, and relationships among studies. Lipids 39:365-372.

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

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

Duffield, T. F., K. E. Leslie, D. Sandals, K. Lissemore, B. W. McBride, J. H. Lumsden, P. Dick, and R. Bagg. 1999. Effect of prepartum administration of monensin in a controlled-

release capsule on milk production and milk components in early lactation. J. Dairy Sci. 82:272-279.

Duffield, T. F., A. R. Rabiee, and I. J. Lean. 2008a. A meta-analysis of the impact of monensin in lactating dairy cattle. Part 1. Metabolic effects. J. Dairy Sci. 91:1334-1346.

Duffield, T. F., A. R. Rabiee, and I. J. Lean. 2008b. A meta-analysis of the impact of monensin in lactating dairy cattle. Part 2. Production effects. J. Dairy Sci. 91:1347-1360.

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.

Erasmus, L. J., P. M. Botha, and A. Kistner. 1992. Effect of yeast culture supplement on production, rumen fermentation, and duodenal nitrogen flow in dairy cows. J. Dairy Sci. 75:3056-3065.

Erdman, R. A. 1988. Dietary Buffering Requirements of the Lactating Dairy Cow: A Review1. J. Dairy Sci. 71:3246-3266.

Fellner, V., F. D. Sauer, and J. K. Kramer. 1997. Effect of nigericin, monensin, and tetronasin on biohydrogenation in continuous flow-through ruminal fermenters. J. Dairy Sci. 80:921-928.

Foote, M. R., S. L. Giesy, G. Bernal-Santos, D. E. Bauman, and Y. R. Boisclair. 2010. t10,c12-CLA decreases adiposity in peripubertal mice without dose-related detrimental effects on mammary development, inflammation status and metabolism. Am. J. Physiol. Regul. Integr. Comp. Physiol. 299:R1521-1528.

Francisco, C. C., C. S. Chamberlain, D. N. Waldner, R. P. Wettemann, and L. J. Spicer. 2002. Propionibacteria fed to dairy cows: effects on energy balance, plasma metabolites and hormones, and reproduction. J. Dairy Sci. 85:1738-1751.

Fuentes, M. C., S. Calsamiglia, P. W. Cardozo, and B. Vlaeminck. 2009. Effect of pH and level of concentrate in the diet on the production of biohydrogenation intermediates in a dual-flow continuous culture. J. Dairy Sci. 92:4456-4466.

Gama, M. A. S., P. C. Garnsworthy, J. M. Griinari, P. R. Leme, P. H. M. Rodrigues, L. W. O. Souza, and D. P. D. Lanna. 2008. Diet-induced milk fat depression: Association with changes in milk fatty acid composition and fluidity of milk fat. Livest. Sci. 115:319-331.

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. The Journal of Agricultural Science 105:27-30.

Gervais, R. and P. Y. Chouinard. 2008. Effects of intravenous infusion of conjugated diene 18:3 isomers on milk fat synthesis in lactating dairy cows. J. Dairy Sci. 91:3568-3578.

Gervais, R., J. W. McFadden, A. J. Lengi, B. A. Corl, and P. Y. Chouinard. 2009. Effects of intravenous infusion of trans-10, cis-12 18:2 on mammary lipid metabolism in lactating dairy cows. J. Dairy Sci. 92:5167-5177.

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.

Gregg, K., B. Hamdorf, K. Henderson, J. Kopecny, and C. Wong. 1998. Genetically modified ruminal bacteria protect sheep from fluoroacetate poisoning. Appl. Environ. Microbiol. 64:3496-3498.

Griinari, J. M., B. A. Corl, S. H. Lacy, P. Y. Chouinard, K. V. Nurmela, and D. E. Bauman. 2000. Conjugated linoleic acid is synthesized endogenously in lactating dairy cows by Delta(9)-desaturase. J. Nutr. 130:2285-2291.

Griinari, J. M., D. A. Dwyer, M. A. McGuire, D. E. Bauman, D. L. Palmquist, and K. V. Nurmela. 1998. Trans-octadecenoic acids and milk fat depression in lactating dairy cows. J. Dairy Sci. 81:1251-1261.

Griinari, J. M., M. A. McGuire, D. A. Dwyer, D. E. Bauman, and D. L. Palmquist. 1997. Role of insulin in the regulation of milk fat synthesis in dairy cows. J. Dairy Sci. 80:1076-1084.

Hall, M. B. 2009. Determination of starch, including maltooligosaccharides, in animal feeds: comparison of methods and a method recommended for AOAC collaborative study. Journal of AOAC International 92:42-49.

Hara, A. and N. S. Radin. 1978. Lipid extraction of tissues with a low-toxicity solvent. Anal. Biochem. 90:420-426.

Harfoot, C. G. and G. P. Hazlewood. 1997. Lipid Metabolism in the rumen. Pages xxi, 719 p. in The Rumen microbial ecosystem. 2nd ed. P. N. Hobson and C. S. Stewart, ed. Blackie Academic & Professional, London ; New York.

Harvatine, K. J. and M. S. Allen. 2006. Fat supplements affect fractional rates of ruminal fatty acid biohydrogenation and passage in dairy cows. J. Nutr. 136:677-685.

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 D. E. Bauman. 2011. Characterization of the acute lactational response to trans-10, cis-12 conjugated linoleic acid. J. Dairy Sci. 94:6047-6056.

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

Harvatine, K. J., J. W. Perfield, II, and D. E. Bauman. 2009b. Expression of enzymes and key regulators of lipid synthesis is upregulated in adipose tissue during CLA-induced milk fat depression in dairy cows. J. Nutr. 139:849-854.

He, M., K. L. Perfield, H. B. Green, and L. E. Armentano. 2012. Effect of dietary fat blend enriched in oleic or linoleic acid and monensin supplementation on dairy cattle performance, milk fatty acid profiles, and milk fat depression. J. Dairy Sci. 95:1447-1461.

Heid, H. W. and T. W. Keenan. 2005. Intracellular origin and secretion of milk fat globules. Eur J Cell Biol 84:245-258.

Hristov, A. N., G. Varga, T. Cassidy, M. Long, K. Heyler, S. K. Karnati, B. Corl, C. J. Hovde, and I. Yoon. 2010. Effect of Saccharomyces cerevisiae fermentation product on ruminal fermentation and nutrient utilization in dairy cows. J. Dairy Sci. 93:682-692.

Ipharraguerre, I. R. and J. H. Clark. 2003. Usefullness of ionophores for lactating dairy cows: a review. Anim. Feed Sci. Technol. 106:39-57.

Jacobs, A. A., J. van Baal, M. A. Smits, H. Z. Taweel, W. H. Hendriks, A. M. van Vuuren, and J. Dijkstra. 2011. Effects of feeding rapeseed oil, soybean oil, or linseed oil on stearoyl-CoA desaturase expression in the mammary gland of dairy cows. J. Dairy Sci. 94:874-887.

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

Jenkins, T. C., V. Fellner, and R. K. McGuffey. 2003. Monensin by fat interactions on trans fatty acids in cultures of mixed ruminal microorganisms grown in continuous fermentors fed corn or barley. J. Dairy Sci. 86:324-330.

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

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

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

Kalscheur, K. F., B. B. Teter, L. S. Piperova, and R. A. Erdman. 1997. Effect of dietary forage concentration and buffer addition on duodenal flow of trans-C18:1 fatty acids and milk fat production in dairy cows. J. Dairy Sci. 80:2104-2114.

Kennedy, A., K. Martinez, S. Schmidt, S. Mandrup, K. LaPoint, and M. McIntosh. 2010. Antiobesity mechanisms of action of conjugated linoleic acid. The Journal of nutritional biochemistry 21:171-179.

Khalili, H. and P. Huhtanen. 1991. Sucrose supplements in cattle given grass silage-based diet. 2. Digestion of cell wall carbohydrates. Anim. Feed Sci. Technol. 33:263-273.

Khorasani, G. R. and J. J. Kennelly. 2001. Influence of carbohydrate source and buffer on rumen fermentation characteristics, milk yield, and milk composition in late-lactation Holstein cows. J. Dairy Sci. 84:1707-1716.

Kritchevsky, D. 2000. Antimutagenic and some other effects of conjugated linoleic acid. Br. J. Nutr. 83:459-465.

Lean, I. J., M. Curtis, R. Dyson, and B. Lowe. 1994. Effects of sodium monensin on reproductive performance of dairy cattle. I. Effects on conception rates, calving-to-conception intervals, calving-to-heat and milk production in dairy cows. Australian veterinary journal 71:273-277.

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. Tyburczy, D. A. Dwyer, K. J. Harvatine, F. Destaillats, Z. Mouloungui, L. Candy, and D. E. Bauman. 2007. *Trans*-10 octadecenoic acid does not reduce milk fat synthesis in dairy cows. J. Nutr. 137:71-76.

Longuski, R. A., Y. Ying, and M. S. Allen. 2009. Yeast culture supplementation prevented milk fat depression by a short-term dietary challenge with fermentable starch. J. Dairy Sci. 92:160-167.

Loor, J. J. and J. H. Herbein. 1998. Exogenous conjugated linoleic acid isomers reduce bovine milk fat concentration and yield by inhibiting de novo fatty acid synthesis. J. Nutr. 128:2411-2419.

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.

Mackle, T. R., D. A. Dwyer, K. L. Ingvartsen, P. Y. Chouinard, J. M. Lynch, D. M. Barbano, and D. E. Bauman. 1999. Effects of insulin and amino acids on milk protein concentration and yield from dairy cows. J. Dairy Sci. 82:1512-1524.

Maia, M. R., 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 Microbiol 10:52.

Maia, M. R., 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.

Martel, C. A., E. C. Titgemeyer, L. K. Mamedova, and B. J. Bradford. 2011. Dietary molasses increases ruminal pH and enhances ruminal biohydrogenation during milk fat depression. J. Dairy Sci. 94:3995-4004.

Maxin, G., H. Rulquin, and F. Glasser. 2011. Response of milk fat concentration and yield to nutrient supply in dairy cows. Animal 5:1299-1310.

McClymont, G. L. and S. Vallance. 1962. Depression of blood glycerides and milk-fat synthesis by glucose infusion. The Proceedings of the Nutrition Society 21:41-42.

McGuffey, R. K., L. F. Richardson, and J. I. D. Wilkinson. 2001. Ionophores for dairy cattle: current status and future outlook. J. Dairy Sci. 84:E194-E203.

Medeiros, S. R., D. E. Oliveira, L. J. Aroeira, M. A. McGuire, D. E. Bauman, and D. P. Lanna. 2010. Effects of dietary supplementation of rumen-protected conjugated linoleic acid to grazing cows in early lactation. J. Dairy Sci. 93:1126-1137.

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

O'Donnell-Megaro, A. M., J. L. Capper, W. P. Weiss, and D. E. Bauman. 2012. Effect of linoleic acid and dietary vitamin E supplementation on sustained conjugated linoleic acid production in milk fat from dairy cows. J. Dairy Sci. 95:7299-7307.

Oba, M. and M. S. Allen. 2000. Effects of brown midrib 3 mutation in corn silage on productivity of dairy cows fed two concentrations of dietary neutral detergent fiber: 1. Feeding behavior and nutrient utilization. J. Dairy Sci. 83:1333-1341.

Odongo, N. E., R. Bagg, G. Vessie, P. Dick, M. M. Or-Rashid, S. E. Hook, J. T. Gray, E. Kebreab, J. France, and B. W. McBride. 2007. Long-term effects of feeding monensin on methane production in lactating dairy cows. J. Dairy Sci. 90:1781-1788.

Ordway, R. S., V. A. Ishler, and G. A. Varga. 2002. Effects of Sucrose Supplementation on Dry Matter Intake, Milk Yield, and Blood Metabolites of Periparturient Holstein Dairy Cows. J. Dairy Sci. 85:879-888.

Palmquist, D. 2006. Milk fat: Origin of fatty acids and influence of nutritional factors thereon. Advanced Dairy Chemistry Volume 2 Lipids:43-92.

Pariza, M. W. 2004. Perspective on the safety and effectiveness of conjugated linoleic acid. Am. J. Clin. Nutr. 79:1132S-1136S.

Perfield, J. W., II, P. Delmonte, A. L. Lock, M. P. Yurawecz, and D. E. Bauman. 2006. *Trans*-10, *trans*-12 conjugated linoleic acid does not affect milk fat yield but reduces delta9-desaturase index in dairy cows. J. Dairy Sci. 89:2559-2566.

Perfield, J. W., II, A. L. Lock, J. M. Griinari, A. Saebo, P. Delmonte, D. A. Dwyer, and D. E. Bauman. 2007. *Trans*-9, *cis*-11 conjugated linoleic acid reduces milk fat synthesis in lactating dairy cows. J. Dairy Sci. 90:2211-2218.

Peterson, D. G., L. H. Baumgard, and D. E. Bauman. 2002. Short communication: milk fat response to low doses of *tran*-10, *cis*-12 conjugated linoleic acid (CLA). J. Dairy Sci. 85:1764-1766.

Peterson, D. G., E. A. Matitashvili, and D. E. Bauman. 2003. Diet-induced milk fat depression in dairy cows results in increased *trans*-10, *cis*-12 CLA in milk fat and coordinated suppression of mRNA abundance for mammary enzymes involved in milk fat synthesis. J. Nutr. 133:3098-3102.

Peterson, D. G., E. A. Matitashvili, and D. E. Bauman. 2004. The inhibitory effect of *trans*-10, *cis*-12 CLA on lipid synthesis in bovine mammary epithelial cells involves reduced proteolytic activation of the transcription factor SREBP-1. J. Nutr. 134:2523-2527.

Phipps, R. H., J. I. Wilkinson, L. J. Jonker, M. Tarrant, A. K. Jones, and A. Hodge. 2000. Effect of monensin on milk production of Holstein-Friesian dairy cows. J. Dairy Sci. 83:2789-2794.

Piperova, L. S., B. B. Teter, I. Bruckental, J. Sampugna, S. E. Mills, M. P. Yurawecz, J. Fritsche, K. Ku, and R. A. Erdman. 2000. Mammary lipogenic enzyme activity, *trans* fatty acids and conjugated linoleic acids are altered in lactating dairy cows fed a milk fat-depressing diet. J. Nutr. 130:2568-2574.

Plaizier, J. C., D. O. Krause, G. N. Gozho, and B. W. McBride. 2008. Subacute ruminal acidosis in dairy cows: The physiological causes, incidence and consequences. The Veterinary Journal 176:21-31.

Poppy, G. D., A. R. Rabiee, I. J. Lean, W. K. Sanchez, K. L. Dorton, and P. S. Morley. 2012. A meta-analysis of the effects of feeding yeast culture produced by anaerobic fermentation of Saccharomyces cerevisiae on milk production of lactating dairy cows. J. Dairy Sci. 95:6027-6041.

Pottier, J., M. Focant, C. Debier, G. De Buysser, C. Goffe, E. Mignolet, E. Froidmont, and Y. Larondelle. 2006. Effect of dietary vitamin E on rumen biohydrogenation pathways and milk fat depression in dairy cows fed high-fat diets. J. Dairy Sci. 89:685-692.

Qiu, X., M. L. Eastridge, K. E. Griswold, and J. L. Firkins. 2004. Effects of Substrate, Passage Rate, and pH in Continuous Culture on Flows of Conjugated Linoleic Acid and Trans C18:1. J. Dairy Sci. 87:3473-3479.

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

Roy, A., A. Ferlay, K. J. Shingfield, and Y. Chilliard. 2006. Examination of the persistency of milk fatty acid composition responses to plant oils in cows given different basal diets, with particular emphasis on trans-C18:1 fatty acids and isomers of conjugated linoleic acid. Animal Science 82:479-492.

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.

Russell, J. B. and A. J. Houlihan. 2003. Ionophore resistance of ruminal bacteria and its potential impact on human health. FEMS microbiology reviews 27:65-74.

Russell, J. B. and H. J. Strobel. 1989. Effect of ionophores on ruminal fermentation. Appl. Environ. Microbiol. 55:1-6.

Saebo, A., J. W. Perfield, II, P. Delmonte, M. P. Yurawecz, P. Lawrence, J. T. Brenna, and D. E. Bauman. 2005a. Milk fat synthesis is unaffected by abomasal infusion of the conjugated diene 18:3 isomers cis-6,trans-10, cis-12 and cis-6,trans-8,cis-12. Lipids 40:89-95.

Saebo, A., P. C. Saebo, J. M. Griinari, and K. J. Shingfield. 2005b. Effect of abomasal infusions of geometric isomers of 10,12 conjugated linoleic acid on milk fat synthesis in dairy cows. Lipids 40:823-832.

Satter, L. D. and A. N. Bringe. 1969. Effect of abrupt ration changes on milk and blood components. J. Dairy Sci. 52:1776-1780.

Seal, C. J. and C. K. Reynolds. 1993. Nutritional implications of gastrointestinal and liver metabolism in ruminants. Nutrition research reviews 6:185-208.

Shingfield, K. J. and J. M. Griinari. 2007. Role of biohydrogenation intermediates in milk fat depression. Eur J Lipid Sci Technol 109:799-816.

Shingfield, K. J., C. K. Reynolds, G. Hervas, J. M. Griinari, A. S. Grandison, and D. E. Beever. 2006. Examination of the persistency of milk fatty acid composition responses to fish oil and sunflower oil in the diet of dairy cows. J. Dairy Sci. 89:714-732.

Shingfield, K. J., A. Saebo, P. C. Saebo, V. Toivonen, and J. M. Griinari. 2009. Effect of abomasal infusions of a mixture of octadecenoic acids on milk fat synthesis in lactating cows. J. Dairy Sci. 92:4317-4329.

Siri-Tarino, P. W., Q. Sun, F. B. Hu, and R. M. Krauss. 2010. Meta-analysis of prospective cohort studies evaluating the association of saturated fat with cardiovascular disease. Am. J. Clin. Nutr. 91:535-546.

Strobel, H. J. and J. B. Russell. 1986. Effect of pH and Energy Spilling on Bacterial Protein Synthesis by Carbohydrate-Limited Cultures of Mixed Rumen Bacteria. J. Dairy Sci. 69:2941-2947.

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.

Tajima, K., R. I. Aminov, T. Nagamine, H. Matsui, M. Nakamura, and Y. Benno. 2001. Diet-dependent shifts in the bacterial population of the rumen revealed with real-time PCR. Appl. Environ. Microbiol. 67:2766-2774.

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.

Van Soest, P. J. 1994. Nutritional ecology of the ruminant. 2nd ed. Comstock Pub., Ithaca.

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

Wallace, R. J. 1994. Ruminal microbiology, biotechnology, and ruminant nutrition: progress and problems. J. Anim. Sci. 72:2992-3003.

Wallace, R. J., N. McKain, K. J. Shingfield, and E. Devillard. 2007. Isomers of conjugated linoleic acids are synthesized via different mechanisms in ruminal digesta and bacteria. J. Lipid Res. 48:2247-2254.

Weimer, P. J., D. M. Stevenson, H. C. Mantovani, and S. L. Man. 2010a. Host specificity of the ruminal bacterial community in the dairy cow following near-total exchange of ruminal contents. J. Dairy Sci. 93:5902-5912.

Weimer, P. J., D. M. Stevenson, and D. R. Mertens. 2010b. Shifts in bacterial community composition in the rumen of lactating dairy cows under milk fat-depressing conditions. J. Dairy Sci. 93:265-278.

Weimer, P. J., D. M. Stevenson, D. R. Mertens, and E. E. Thomas. 2008. Effect of monensin feeding and withdrawal on populations of individual bacterial species in the rumen of lactating dairy cows fed high-starch rations. Appl Microbiol Biotechnol 80:135-145.

Weiss, W. P. 2012. Use of a corn milling product in diets for dairy cows to alleviate milk fat depression. J. Dairy Sci. 95:2081-2090.

Williams, P. E., C. A. Tait, G. M. Innes, and C. J. Newbold. 1991. Effects of the inclusion of yeast culture (Saccharomyces cerevisiae plus growth medium) in the diet of dairy cows on milk yield and forage degradation and fermentation patterns in the rumen of steers. J. Anim. Sci. 69:3016-3026.

Zened, A., F. Enjalbert, M. C. Nicot, and A. Troegeler-Meynadier. 2013. Starch plus sunflower oil addition to the diet of dry dairy cows results in a trans-11 to trans-10 shift of biohydrogenation. J. Dairy Sci. 96:451-459.

Zened, A., A. Troegeler-Meynadier, T. Najar, and F. Enjalbert. 2012. Effects of oil and natural or synthetic vitamin E on ruminal and milk fatty acid profiles in cows receiving a high-starch diet. J. Dairy Sci. 95:5916-5926.

VITA

Daniel Rico

Daniel Rico grew up in central Colombia near Bogota. Although not raised in a farm, he always had a very strong interest in science, and particularly in animal biology. Daniel decided to enroll in the Animal Science program at the National University of Colombia, where he received his bachelor's degree in 2007. The focus for his undergraduate thesis was in the study of grass species and maturity on milk quality. During his internship on the last year of school, Daniel had the opportunity to work with Dr. Gabriella Varga at Penn State University on the study of the effects of a dry glycerol supplement from the biodiesel industry on the performance of early lactation dairy cows. Following completion of his bachelor's program, Daniel returned to Dr. Varga's laboratory at Penn State to pursue his master's degree, where the main focus of research was the study of the inclusion of glycerol in diets for dairy cows. Daniel received his Master's degree in the spring of 2009 and continued at Penn State working on his Ph.D. in Dr. Kevin Harvatine's lab, primarily studying the recovery from diet-induced milk fat depression in dairy cows. After completion of his Ph.D. in May 2009, Daniel will work with Dr. Yvan Chouinard and Dr. Rachel Gervais as a postdoctoral fellow in the Department of Animal science at Laval University in Quebec, Canada.