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Induction of and recovery from milk fat depression occurs progressively in dairy cows switched between diets that differ in fiber and oil concentration

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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 MFD. A high-fiber, low-oil diet was fed during the control and recovery periods, and a low-fiber, high-oil (LFHO) diet was fed during the induction period. Milk yield was not affected by treatment. Milk fat percentage and yield decreased progressively during induction and were lower by d 3 and 5, respectively. Milk fat concentration and yield increased progressively when cows were fed the recovery diet and were not different from control on d 19 and 15, respectively. Yield of de novo synthesized fatty acids (FA) decreased progressively during the induction period and was lower than that of controls by d 5. A biphasic response was seen for milk fat *trans* isomers, where *trans*-11 C18:1 and *cis*-9,*trans*-11 conjugated linoleic acid (CLA) were elevated initially and *trans*-10 C18:1 and *trans*-10,*cis*-12 CLA increased progressively during the induction period. A similar 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 increasing slightly above control levels during the second phase. Recovery from diet-induced MFD occurs gradually with a short lag when dietary fiber and oil concentrations are corrected. This time course provides a framework to identify factors causing MFD and set expectations during recovery from MFD.
Key words: biohydrogenation, conjugated linoleic acid, milk fat depression

INTRODUCTION

Low fat syndrome, also referred to as milk fat depression (**MFD**), was first documented more than 150 yr ago (Van Soest, 1994) and represents a challenge for the efficiency and profitability of modern dairies. Milk fat depression is characterized by up to a 50% 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 FA from metabolism of dietary 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 conjugated linoleic acid (**CLA**) is one of multiple biohydrogenation intermediates known to inhibit milk fat synthesis in the mammary gland, and its mechanism of action involves the downregulation 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 1 wk 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) because 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 MFD and recover milk fat fol-

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lowing dietary modification is crucial in determining causes of MFD on 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.

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 2009. Animals were housed individually in tiestalls with sawdust and rubber mattresses, and they had continuous access to water. Treatments were (1) a high-fiber, low-oil diet (control; 36.9% NDF and 1.1% PUFA); (2) induction of MFD by feeding a low-fiber, high-oil diet (**LFHO**; 29.5% NDF and 3.7% PUFA; Table 1; Supplemental Table S1: http://dx.doi.org/10.3168/jds.2013-6820); and (3) recovery from MFD by feeding the control diet after induction (recovery). Cows were randomly assigned to a treatment sequence in a repeated design that allowed analysis of recovery from a MFD diet (Table 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 long. Cows were treated with bST (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 intake was recorded daily. Each 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

Table 1. Ingredient and chemical composition of experimental diets

Item	LFHO[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	4.7	5.3
Grass hay/straw[4]	4.3	3.3
Soybean oil	2.9	—
Aminoplus[5]	—	4.2
Cottonseed hulls	3.0	2.9
Minerals and vitamins mix[6]	3.3	3.1
Chemical composition, g/100 g of DM unless otherwise stated; n = 3		
CP[7]	15.5	16.6
NDF[7]	29.5	36.9
ADF[7]	18.3	24.6
FA	6.9	2.6
Starch	27.0	18.0
Ash	5.7	7.5
Ca[8]	0.74	0.97
P[8]	0.38	0.41

[1]LFHO = low NDF high soy oil diet.

[2]Contained 34.2% DM.

[3]Contained 37.7% DM.

[4]Contained 88.3% DM.

[5]Aminoplus (Archer Daniels Midland Co., Decatur, IL) is a soybean meal-based protein source (51% CP, 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 of Cu; 1,085 mg/kg of Zn; 181 mg/kg of Fe; 6.67 mg/kg of Se; 125,875 IU/kg of vitamin A (retinyl acetate); 31,418 IU/kg of vitamin D (activated 7-dehydrocholesterol); and 946 IU/kg of vitamin E (DL-α tocopheryl acetate).

[7]CP, NDF, ADF, and ash analyzed by Cumberland Valley Analytical Services (Maugansville, MD; n = 3 per diet).

[8]Formulated values for Ca and P.

Table 2. Treatment assignment of a repeated design to study the recovery from diet-induced milk fat depression

Assignment	Pre-experiment	Period 1	Period 2	Period 3
1	Control	Control	Induction	Recovery
2	Induction	Recovery	Control	Induction
3	Control	Induction	Recovery	Control

screen (A. H. Thomas, Philadelphia, PA). Samples of TMR were composited by period and analyzed for DM, CP, and select minerals by wet chemistry procedures according to AOAC International (2000) and for NDF and ADF according to Van Soest et al. (1991; Cumberland Valley Analytical Services Inc., Maugansville, MD; Table 1).

Cows were milked twice daily at 0500 and 1700 h and milk yield determined by an integrated milk meter (AfiMilk, SAE Afikim, Afikim, Israel). Milk was sampled every other day at both milkings. One subsample was stored at 4°C with preservative (Bronolab-WII, Advanced Instruments Inc., Norwood, MA) until analyzed for fat (filter B) and protein by infrared spectroscopy [Fossomatic 4000 Milko-Scan and 400 Fossomatic, Foss Electric, Hillerød, Denmark; AOAC International (2000; method 972.160), Dairy One Laboratory, 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 day 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-catalyzed transmethylation according to Chouinard et al. (1999). Fatty acid 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 × 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, which was increased by 2°C/min to 190°C and held for 15 min. 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 Prep Inc.) 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 calculate the proportion of FA in milk triglycerides (**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 indices were calculated for each sample as the ratio of product to substrate plus 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), $C_k(S_i)$ 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 versus induction and control versus recovery at each time point. 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 analysis.

RESULTS

Treatment by time interactions were significant ($P < 0.01$) for all reported variables, except for milk yield ($P = 0.34$).

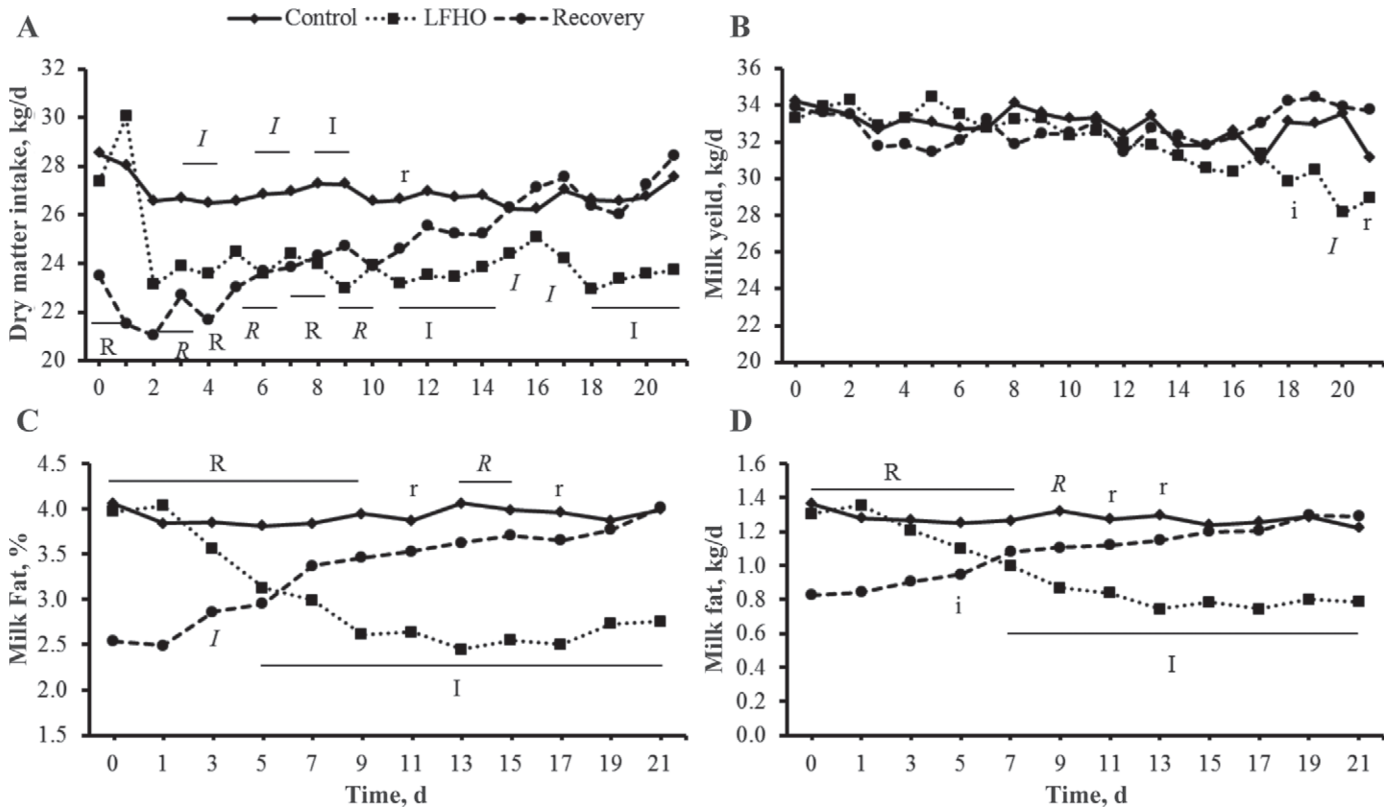

Figure 1. Time course of DMI (A; kg/d), milk yield (B; kg/d), milk fat percentage (C), and milk fat yield (D; kg/d) of cows fed a low-fiber, high-oil diet (LFHO), a high-fiber, low-oil diet (control), or a high-fiber, low-oil diet after LFHO (recovery). Preplanned contrasts tested the difference between 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 = 1.28, 2.48, 0.18, and 0.09 for DMI, milk yield, fat yield, and fat percentages, respectively.

Diet Description

The LFHO diet used to induce milk fat depression had a lower NDF and a higher PUFA concentration compared with the control diet (29.5 vs. 36.9% NDF and 6.9 vs. 2.6% FA, respectively; Table 1). Dietary MUFA and PUFA concentrations were higher in LFHO compared with the control diet (17.5 vs. 7.0 and 37.3 vs. 11.6 g/kg of diet DM, respectively; Supplemental Table S1; http://dx.doi.org/10.3168/jds.2013-6820).

DMI and Milk Production and Composition

Dry matter intake decreased progressively from d 1 of induction and was, on average, 3.1 kg/d lower than control from d 6 to 21 (Figure 1A). During recovery, DMI increased progressively and was not different from control by d 11.

We observed no main effect of treatment on milk yield. However, on d 20 of induction, milk yield in the LFHO cows was lower than that of controls ($P < 0.05$; Figure 1B). Milk fat content and yield in the treatment group decreased progressively from d 1 of induction and were lower than those of control group by d 3 and 5 ($P < 0.05$), respectively. Milk fat content and yield reached a nadir around d 9 (Figures 1C and 1D). During induction, milk fat concentration was reduced 34 ± 3.6% (mean ± SD) compared with that of control from d 9 to 21. During recovery, milk fat concentration progressively increased from d 1, tended to be different from control on d 11 and 17 ($P = 0.09$), and was not different from control on d 19 to 21 ($P > 0.40$). Similarly, milk fat yield recovered progressively, tended to be different from control on d 11 and 13 ($P = 0.07$), and was not different from control from d 15 to 21 ($P > 0.30$).

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 (Supplemental Figure S1A; http://dx.doi.org/10.3168/jds.2013-6820). Milk protein percentage was 6.1 ± 0.6% (mean ± SD) higher during MFD induction compared with control from d 11 and 21. Milk protein yield was not affected by treatment, except on d 1, where

it was higher in recovery than in control ($P < 0.05$; Supplemental Figure S1B; http://dx.doi.org/10.3168/jds.2013-6820).

Milk De Novo and Preformed FA

Concentration of milk FA <16 carbons (de novo synthesized) decreased progressively during induction, tended to be lower than control on d 3 ($P = 0.09$), and reached a nadir on d 5 ($P < 0.001$; Figure 2A). During recovery, the concentration of milk FA <16 carbons increased progressively. In contrast, the concentration of milk FA >16 carbons (preformed FA) increased during induction, reached a plateau on d 5, and decreased during recovery (Figure 2B). Changes in individual de novo and preformed FA paralleled these patterns (Supplemental Table S2; http://dx.doi.org/10.3168/jds.2013-6820). Similar to milk fat concentration, yield of FA <16 carbons decreased progressively during induction and was lower than control by d 5 ($P < 0.001$; Figure 2C). In contrast, yield of FA >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 2D). During recovery, yield of FA >16 carbons was lower than control only on d 0 and 3 ($P < 0.05$) and was higher than control on d 21 ($P < 0.05$).

Milk trans FA

Induction caused a rapid increase in concentrations of milk fat *trans*-11 C18:1 and *cis*-9,*trans*-11 CLA, with maximum concentrations of 2.5 and 1.7% of FA reached on d 3 and 5, respectively (Figure 3A and 3C). After this peak, both FA progressively decreased, with *trans*-11 C18:1 decreasing below control levels on d 15, 19, and 21 ($P < 0.05$). Contents of milk fat *trans*-10 C18:1 and *trans*-10,*cis*-12 CLA increased progressively during induction and were higher than control on d 1 and 3 of the induction period, respectively ($P < 0.05$; Figure 3B and 3D). A near-peak concentration of *trans*-10 C18:1 (4.85% of FA) was achieved on d 9 of the induction period, whereas concentration of *trans*-10,*cis*-12 CLA

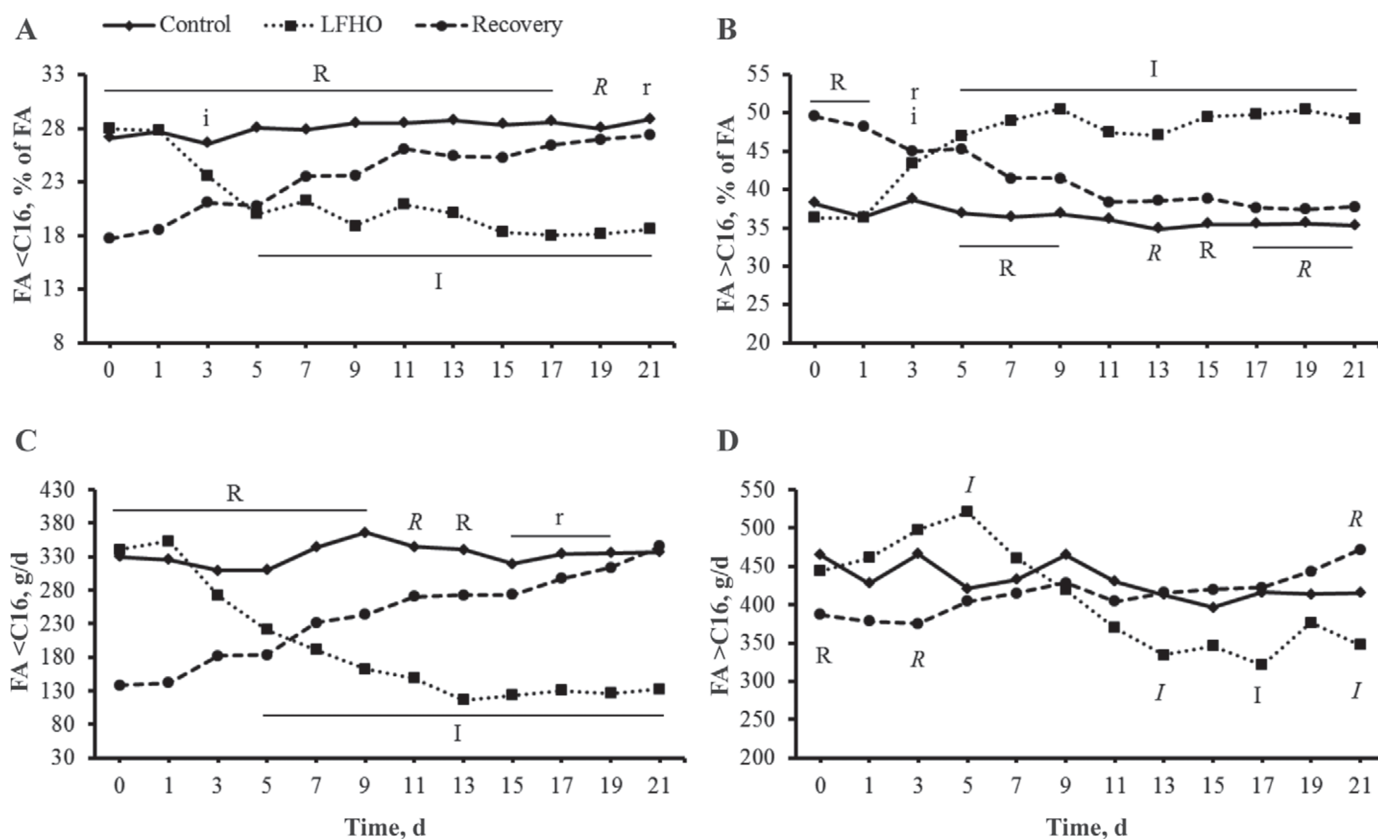

Figure 2. Time course of milk fat concentration and yield of de novo (panels A and C, respectively) and preformed (panels B and D, respectively) fatty acids of cows fed a low-fiber, high-oil diet (LFHO), a high-fiber, low-oil diet (control), or a high-fiber, low-oil diet after LFHO (recovery). Preplanned 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.

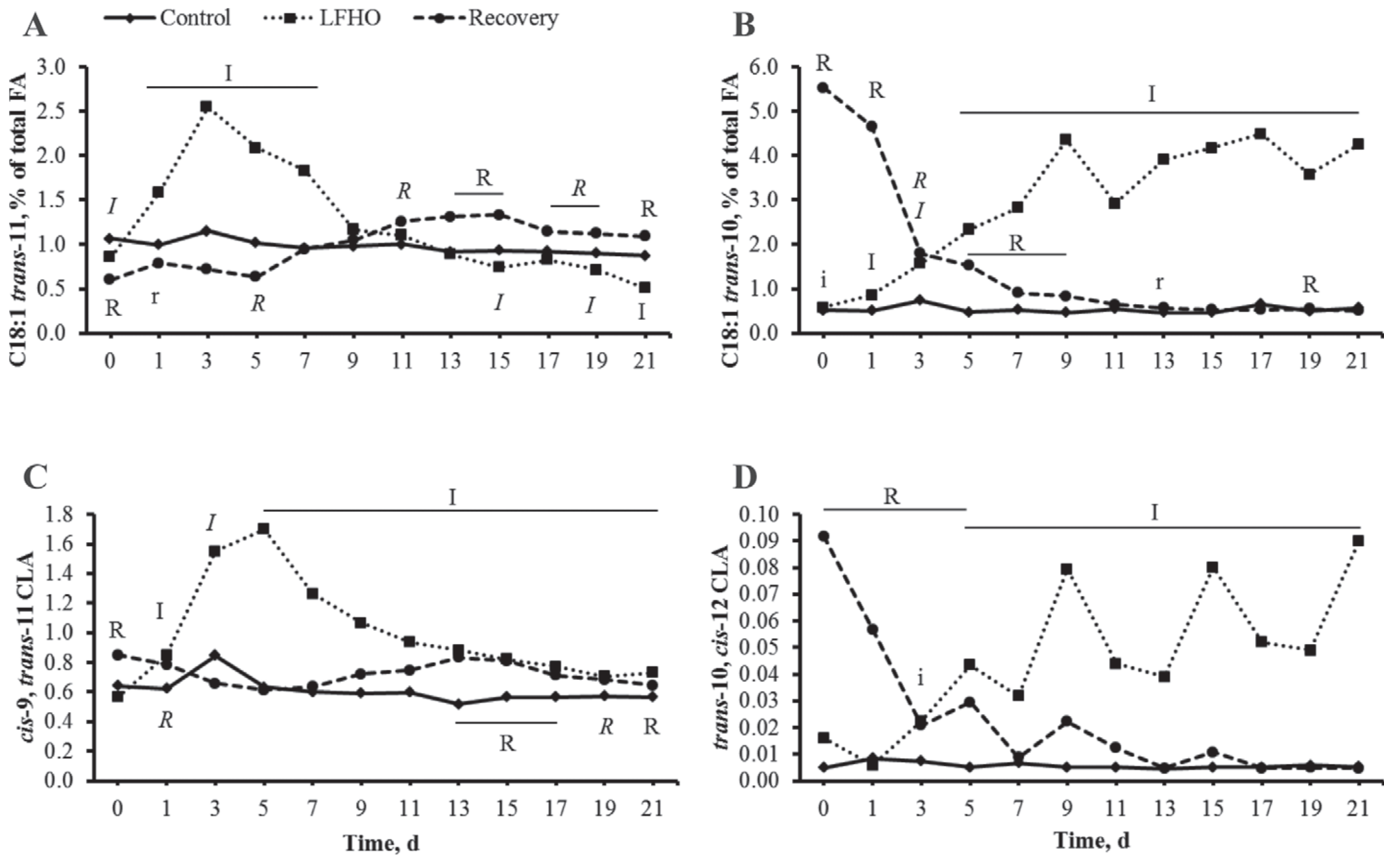

Figure 3. Time course of C18:1 *trans*-11 (A), *trans*-10 (B), *cis*-9,*trans*-11 conjugated linoleic acid (CLA; C) and *trans*-10,*cis*-12 CLA (D) in milk fat (% of total FA) of cows fed a low-fiber, high-oil diet (LFHO), a high-fiber, low-oil diet (control), or a high-fiber, low-oil diet after LFHO (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, and *trans*-10, and *cis*-9,*trans*-11 and *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 contents 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 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 Δ^9-Desaturase Indices

During induction, milk C14 and C16 desaturase indices increased progressively and reached a plateau around d 9 (Figures 4A and 4B). During recovery, both indices decreased gradually, reaching a nadir on d 7.

DISCUSSION

The repeated design allowed investigation of the time course of induction and recovery of MFD while accounting for cow and period variation. The low NDF and high PUFA diet was selected to mimic conditions that commonly lead to reduced milk fat on farms. These 2 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). Even though rations are not formulated to be deficient in NDF 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 because of unexpected changes in diet composition due to changes in forage 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

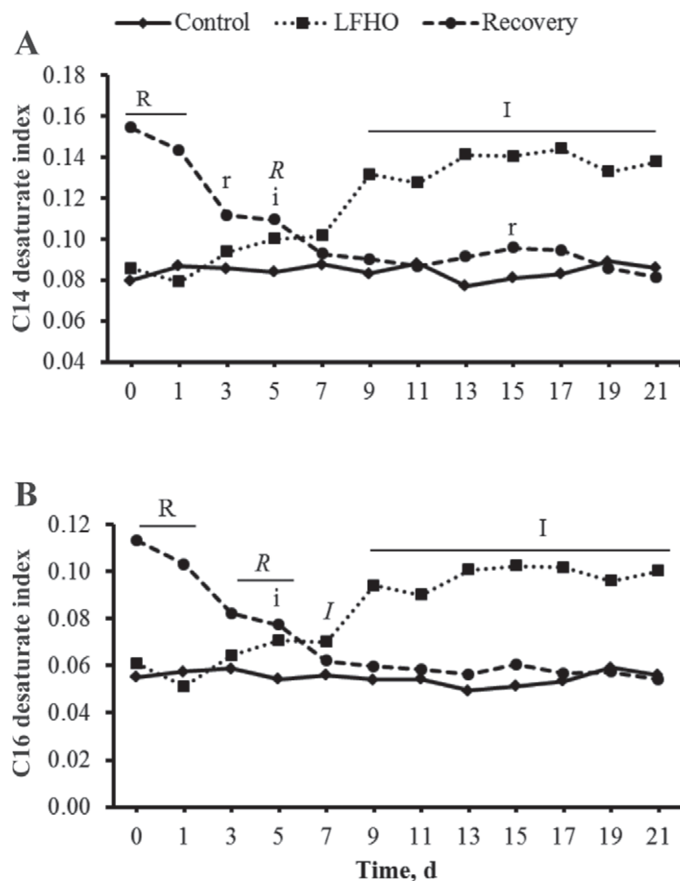

Figure 4. Time course of milk C14 (A) and C16 (B) desaturase indices of cows fed a low-fiber, high-oil diet (LFHO), a high-fiber, low-oil diet (control), or a high-fiber, low-oil diet after LFHO (recovery). Preplanned contrasts tested the difference between control and induction (I = $P < 0.01$, $I = P < 0.05$, 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 indices, respectively.

and recovery represents a drastic change in nutrient composition where NDF is adequate and PUFA are minimized.

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

The LFHO diet reduced milk fat by 34%, which is in the upper range of reductions observed in diet-induced MFD (Harvatine et al., 2009a). The extent of MFD in the current experiment is of a higher degree than normally observed on farms (Bailey et al., 2005). However, the model used serves to test the time course of induction and recovery of MFD. Considering that dietary induction of MFD requires changes in rumen microbial populations and ruminal metabolism of FA, we 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 oil [4.5% of DM; fish and sunflower oil (1:2 wt/wt)] 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, when feeding a high-concentrate, low-forage diet, Peterson et al. (2003) reported a 25% decrease in milk fat concentration by d 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 MFD 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 lower resolution experiment (i.e., less frequent time points) showed a progressive recovery of milk fat concentration that was almost complete 2 wk after the correction of dietary NDF (Weiss, 2012). In the current experiment, the time course of recovery was similar to that of induction. Recovery of milk fat was achieved around d 15 after restoration of adequate dietary NDF 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 longer than the response of the mammary gland to CLA, which occurs between 14 and 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 the 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 biohydrogenation (**BH**) of linoleic acid (C18:2 *cis*-9,*cis*-12) have been described

previously (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, whereas, 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 the use of milk fat to characterize the time course of adaptation in rumen BH. In the present experiment, the LFHO 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 MFD. 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 was caused by a limited capacity of the final steps of the normal BH pathway. The observed lag between substrate provision (PUFA) and peak concentrations of *trans*-11 C18:1 suggests that modification of the BH capacity of the microbial population has a greater effect on the capacity 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, whereas *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 BH, presumably due to a major change in microbial metabolism or microbial populations (Lourenço et al., 2010; Weimer et al., 2010). Previous studies have reported a similar time course of changes in BH intermediates in milk (Shingfield et al., 2006) and rumen digesta (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 more 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 (PUFA) provision and peak concentrations of *trans*-10 C18:1 suggests that modification of the microbial population has a greater effect on the predominant pathway of BH 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 (by 3- and 4.2-fold, respectively) between d 0 and 3. Concurrently, *trans*-11 C18:1 progressively increased, whereas *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, whereas the alternate isomers remained low. Interestingly, the control and recovery diets were the same, and differences in milk FA may represent further adaptations in the microbial population occurring after the alternate isomers have subsided. The second phase of recovery may represent a period of decreased activity of the last steps of BH similar to that observed during the first phase of induction. This may be a period of increased susceptibility for relapse into MFD; however, more investigation of this period is required. 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 to a MFD diet (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 (<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 (e.g., 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, whereas 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 2-stage response. Interestingly, in contrast to the progressive decrease observed for de novo FA, a 5-d lag occurred in the depression of preformed FA output in milk.

The milk fat desaturase indices are commonly used as an indication of stearoyl-CoA desaturase enzyme activity. In the current experiment, the reduction in milk fat was inversely related to the desaturase indices, as these indices increased during induction and decreased during recovery. The desaturase indices 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. In agreement with our results, others have reported an increased desaturase index during MFD (Gama et al., 2008). Interestingly, abomasal infusion of near maximally effective *trans*-10,*cis*-12 CLA doses rapidly and robustly decreased the

desaturase index (Harvatine and Bauman, 2011), but infusions of low doses of *trans*-10,*cis*-12 CLA did not alter the desaturase index (Peterson et al., 2002). The discrepancy between MFD experiments may be due to differences in the dose of *trans*-10,*cis*-12 CLA or the production of other bioactive isomers, some of which may increase desaturase activity. Finally, this provides further evidence that inhibition of FA desaturation is not a functional mechanism of MFD (see review by 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 occurred 7 to 14 d before MFD, which provides a time window for further investigation. After identification of MFD, dietary adjustments that will recover milk fat are expected to do so in 2 to 3 wk, allowing a realistic expectation to be set to determine the success of an intervention. Mechanistically, the time course of induction and recovery from MFD demonstrates the importance of dietary factors on milk fat synthesis, suggesting that modification of rumen microbial metabolism or population is a key limiting step in both processes. Moreover, this study represents 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 and recovery of MFD occurred progressively after a short lag. During induction, ruminal BH intermediates in milk followed a 2-phase response, with an initial slowing of the normal pathway followed by a progressive increased utilization of the alternate pathway. Intermediates of FA BH in milk also appeared to follow a 2-phase response during recovery. Our data indicate that the modification of ruminal BH pathways is a rate-limiting step of induction and recovery from diet-induced MFD, as the time course was much slower than previously observed for abomasal CLA infusions. Additionally, it appears that modification of ruminal microbial metabolism or populations, rather than supply of PUFA, is the rate-limiting step for modification of BH intermediates during induction of and recovery from 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 a framework to further study ways to accelerate recovery from MFD.

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