The effect of rumen digesta inoculation on the time course of recovery from classical diet-induced milk fat depression in dairy cows

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ABSTRACT

Ten ruminally cannulated cows were used in a cross-over design that investigated the effect of rumen digesta inoculation from non-milk fat-depressed cows on recovery from classical diet-induced milk fat depression (MFD) characterized by reduced fat yield, reduced de novo milk fat synthesis, and increased alternate *trans* isomers. Two additional cows fed a high-fiber and low-polyunsaturated fatty acid (FA) diet (31.8% neutral detergent fiber, 4.2% FA, and 1.2% C18:2) 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-polyunsaturated FA diet (induction; 26.1% neutral detergent fiber, 5.8% FA, and 1.9% C18:2), resulting in a 30% decrease in milk fat yield. A recovery phase followed where all cows were switched to the high-forage, low-polyunsaturated FA 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 yield progressively decreased during recovery. Milk fat concentration increased progressively during the recovery phase and no effect of treatment existed at any time point. Also, no treatment effect of milk fat yield was detected. The concentration of milk de novo FA increased progressively during recovery for both treatments and was higher for inoculated compared with control cows on d 6. In agreement, milk fat concentration of *trans*-10,*cis*-12 conjugated linoleic acid decreased progressively in both treatments and was lower in inoculated cows on d 3 and 6. Ruminal inoculation from non-milk fat-depressed cows did not change milk fat yield, but slightly accelerated the rate of recovery of de novo FA synthesis and normal ruminal FA biohydrogenation, demonstrating a possible oppor-

tunity for other interventions that improve the ruminal environment to accelerate recovery from this condition.

Key words: milk fat depression, ruminal inoculation, dairy cow

INTRODUCTION

Low fat syndrome in dairy cows, referred to as classical diet-induced milk fat depression (MFD), is characterized by a dramatic reduction in milk fat yield in response to highly fermentable diets and diets high in unsaturated FA (Bauman and Griinari, 2003). Although both de novo and preformed FA are decreased, a larger decrease in de novo synthesized FA occurs. In ruminants, the dietary factors that cause diet-induced MFD are associated with altered rumen fermentation and the production of unique bioactive FA from biohydrogenation (BH) of dietary PUFA. Dietary factors such as PUFA and diet fermentability affect both the extent of BH and the specific intermediates formed (Jenkins et al., 2008; Fuentes et al., 2009), presumably through modification of the microbial population of the rumen (Tajima et al., 2001; Weimer et al., 2010b). *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 uptake, transport, synthesis, and esterification in the mammary gland (Harvatine et al., 2009).

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 highly fermentable and high-PUFA diets is much slower. For example, feeding a low-fiber and high-oil diet (29.5% NDF and 6.9% FA) to lactating dairy cows caused MFD in 9 d, and milk fat yield was recovered in 15 d by feeding a higher fiber and lower fat ration (36.9% NDF and 2.6% FA; Rico and Harvatine, 2013). Interestingly, a 2-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, and during recovery, *trans*-10,*cis*-12 CLA progressively decreased and *cis*-

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9,*trans*-11 CLA initially increased before returning to baseline. The delayed onset of diet-induced MFD, the lag to peak concentrations of *trans*-FA associated with MFD, and the secondary rise of the normal BH isomers during recovery suggests that ruminal adaptation has a greater effect on the time course compared with substrate for BH.

Satter and Bringe (1969) switched lactating cows between a high- and a low-forage diet, with or without a simultaneous near-total rumen content exchange from cows fed the same diet, and observed that both induction of and recovery from MFD were accelerated by rumen exchange relative to diet switch alone. This suggests that adaptation of the rumen environment or the microbial population is a key limiting factor. Furthermore, Weimer et al. (2010b) reported extensive shifts in ruminal bacteria communities during diet-induced MFD. Lastly, fecal microbiota transplants are increasingly used for treatment of several disorders in humans, with astounding results (Borody and Khoruts, 2012). Our objective was to test the effect of ruminal inoculation from a non-milk fat-depressed cow on the rate of recovery from classical diet-induced MFD after a diet switch. We hypothesized that the recovery of normal BH intermediates and milk fat synthesis would be accelerated by inoculation with ruminal microflora from a non-milk fat-depressed cow.

MATERIALS AND METHODS

Experimental Design and Treatments

All experimental procedures were approved by the Pennsylvania State University Institutional Animal Care and Use Committee (University Park). Twelve ruminally cannulated Holstein cows were housed in tie-stalls at the Pennsylvania State University Dairy Production Research and Teaching Center. Ten cows (197 ± 94 DIM; 41 ± 9.8 kg milk/d; mean $\pm$ 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; 39 ± 9.8 kg milk/d; mean $\pm$ SD) fed a high-fiber, low-PUFA diet (31.8% NDF, 4.2% total FA, and 1.2% C18:2; Table 1) were used as rumen digesta donors. A total of 20 kg/d (as-is basis) were removed from each donor cow during the inoculation period. This is equivalent to approximately 25% of the expected rumen digesta pool (Oba and Allen, 2003; Harvatine and Allen, 2006). Each experimental 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 recombinant bST every 14 d (Posilac; Elanco Animal Health, Greenfield, IN).

Table 1. Ingredients and chemical composition of diets

Item	Diet ¹	
	Induction	Recovery
Ingredient, 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
Mineral and vitamin mix ⁶	2.7	2.5
Chemical composition, ⁷ g/100 g of DM		
CP	16.9	16.9
NDF	26.1	31.8
ADF	15.5	20.8
Starch	28.1	23.0
Total FA	5.8	4.2
C18:1n-9	1.4	0.9
C18:2n-6	1.9	1.2
C18:3n-3	0.3	0.3
Σ Unsaturated FA	3.8	2.6

¹Induction is a low-fiber and high-PUFA diet fed during the induction phase of each period, and recovery is a high-forage and low-PUFA diet fed during the recovery phase of each period.

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

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

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

⁵Optigen is an NPN source (256% CP, DM basis; Alltech Inc., Lexington, KY).

⁶Composition (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 of Cu/kg, 1,085 mg of Zn/kg, 181 mg of Fe/kg, 6.67 mg of Se/kg, 262,105 IU of vitamin A/kg, 65,421 IU of vitamin D/kg, and 1,970 IU/kg of vitamin E (Cargill Animal Nutrition, Roaring Spring, PA).

⁷Crude protein, NDF, ADF, and starch were analyzed by Cumberland Valley Analytical Services (Maugansville, MD; n = 2 per diet).

During the MFD induction phase (10 d) all cows were fed a low-fiber, high-PUFA diet (Table 1; Supplemental Table S1; <http://dx.doi.org/10.3168/jds.2013-7342>). During the subsequent MFD recovery phase, cows were fed a high-fiber, low-PUFA diet (recovery), with or 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-milk fat-depressed donor cows. Similarly, for control cows, 8 kg of rumen contents were evacuated and returned on the same days. The high-forage, low-PUFA 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 contents were determined weekly for diet adjustment and DMI determination (forced-air oven at 55°C for 72 h). All individual feed ingredients were sampled weekly and stored at

–20°C, thawed at room temperature, and DM content determined similarly. Individual feeds were ground in a Wiley mill through a 1-mm screen (Arthur 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 International (2000); NDF and ADF according to Van Soest et al. (1991), using heat-stable amylase and sodium sulfite; and starch according to Hall (2009). Total FA concentration and FA profile of the TMR was determined by gas chromatography after direct methylation (Sukhija and Palmquist, 1988).

Milk Sampling and Analysis

The time course of recovery from MFD 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 MilkoScan and 400 Fossomatic (Foss Electric A/S, Hillerød, Denmark); AOAC International, 2000; method 972.160; Dairy One Coop. Inc.]. A second subsample was stored at –20°C without preservative until analyzed for FA composition.

Milk FA analysis was performed as described by Rico and Harvatine (2013). Briefly, milk fat was composited by day based on milk fat yield at each milking, and lipids were extracted with hexane:isopropanol, and transmethylated in the presence of sodium methoxide. Quantification of FAME was performed by gas chromatography using a fused-silica capillary column (SP-2560; 100 m × 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 are reported as NEFA as described by Rico and Harvatine (2013).

Statistical Analysis

Data were statistically analyzed as a crossover design using PROC MIXED of SAS with repeated measures (version 9.3; SAS Institute Inc., Cary, NC). The model was $Y_{ijklmn} = \mu + S_i + P_j + C_k(S_i) + X_1 + T_m + D_n + T_m \times D_n + e_{ijklmn}$, 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 10), X_1 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 used, based on the Akaike and Bayesian information criteria. The Kenward-Rogers denominator degrees of freedom adjustment was used. The preplanned contrast tested the effect of inoculation at each time point.

Data for some variables were logarithmically 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, which typically included less than 3 data points per variable. Cow periods that did not result in MFD induction (less than a 15% decrease in milk fat concentration) were excluded from the analysis. This reduction in milk fat concentration was successfully achieved in 8 cows in period 1 and 6 cows in period 2.

RESULTS

Induction of MFD

The induction diet successfully reduced milk fat concentration and yield in both periods (Table 2). The induction diet reduced milk fat yield by 29% and milk fat concentration by 30%, whereas milk yield and milk protein yield and concentration were not changed.

DMI and Milk Production and Composition

No effect of treatment or treatment by time interaction was detected for DMI or milk yield [26.8 ± 1.27 and 33.8 ± 1.86 kg/d (mean $\pm$ SEM), respectively; Table 3]. An effect of time on milk yield was detected, with both treatments progressively decreasing during the recovery phase; however, the biggest reduction occurred between d 0 and 3 (5.0 kg/d average reduction; Figure 1). Milk protein concentration and yield were not affected by rumen inoculation, but milk protein yield decreased over time (Table 3; Supplemental Figure S1; <http://dx.doi.org/10.3168/jds.2013-7342>).

Milk fat concentration increased progressively after switching to the recovery diet; however, no difference existed between treatments at any time point (Figure 1). In contrast, milk fat yield remained constant during recovery and no effect of treatment existed at any time point (Figure 1). No treatment or treatment by time interaction was detected for the yield of milk de novo or preformed FA (<16 C and >16 C, respectively; Supplemental Figure S2; <http://dx.doi.org/10.3168/jds.2013-7342>). An effect of time on the yield of de novo FA ($P < 0.001$) was detected, where yield of de

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

Item	Day in induction ¹			SE	<i>P</i> -value ²
	d 0	d 7	d 10		
Milk yield, kg/d					
Milk	39.72	40.68	40.42	2.62	0.92
Fat	1.62	1.24	1.15	0.17	<0.001
Protein	1.25	1.27	1.27	0.1	0.95
Milk composition, %					
Fat	4.03	3.02	2.83	0.41	<0.001
Protein	3.08	3.07	3.11	0.10	0.63

¹Milk fat depression was induced by feeding a low-fiber and high-unsaturated-fat diet. Samples were taken the day before the diet and after 7 and 10 d.

²Main effect of day of the induction period.

novo FA increased progressively, but was not different between treatments at any time point during recovery from MFD (Supplemental Figure S2).

Milk FA Profile and Δ^9 -Desaturase Indices

An effect of time was detected, but no effect of treatment or treatment by time interaction on milk fat concentration of de novo (<16 C), 16-C, and preformed FA (>16 C; Table 4). In both treatments, the proportion of de novo and 16-C FA increased progressively, whereas the proportion of preformed FA decreased progressively during recovery from MFD (Figure 2). However, the concentration of de novo synthesized FA was increased 11% by inoculation on the last day of digesta switching (d 6; Figure 2A; $P < 0.01$).

Milk fat concentrations of *trans*-10 C18:1 and *trans*-10,*cis*-12 CLA, intermediates of the alternative pathway

of ruminal BH, decreased progressively during recovery (Figure 3A and 3B) and a treatment by time interaction was detected for both FA ($P < 0.05$; Table 4). Inoculation decreased *trans*-10 C18:1 by 44% compared with the control on the last day of digesta switching (d 6; $P < 0.01$), whereas it reduced *trans*-10,*cis*-12 CLA on d 3 and 6 by over 50% ($P < 0.05$).

A treatment by time interaction was detected for milk fat concentration of *trans*-11 C18:1 ($P = 0.03$) and a tendency for a treatment by time interaction for *cis*-9,*trans*-11 CLA ($P = 0.07$; Table 4), which are intermediates of the normal pathway of ruminal BH. In agreement with the decreased utilization of the alternate BH pathway, *trans*-11 C18:1 tended to be increased ($P = 0.09$) and *cis*-9,*trans*-11 CLA was increased by inoculation on d 6 ($P = 0.02$). Additionally, *trans*-11 C18:1 tended to be decreased on d 9 ($P = 0.05$) and was decreased on d 12 by 31% ($P = 0.04$; Figure 3C). The

Table 3. Effect of ruminal inoculation on DMI and milk yield and composition during recovery from diet-induced milk fat depression

Item	Treatment ¹			<i>P</i> -value ²		
	Control	INOC	SEM	Trt	Time	Trt × 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	1.90	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, ³ g/d						
<16 C	270	271	39	0.99	<0.001	0.71
>16 C	458	465	50	0.61	0.17	0.68

¹Control = 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.

²Trt = treatment effect; time = effect of day in recovery from milk fat depression.

³Fatty acids <16 C originate from de novo synthesis in the mammary gland and FA >16 C originate from plasma.

Figure 1. Effect of daily ruminal inoculation (from d 1 to 6) with digesta from non-milk fat-depressed cows (inoculation) on milk yield and milk fat concentration and yield during recovery from diet-induced milk fat depression compared with the control. (A) Milk yield; (B) milk fat concentration; (C) milk fat yield. Treatments did not differ at any time point ($P > 0.10$). Standard error of the mean = 1.9, 0.15, and 0.10, for milk yield, milk fat concentration, and milk fat yield, respectively.

cis-9,trans-11 CLA concentration decreased progressively in both treatments, although it was increased by inoculation on d 6 (37%; $P = 0.02$; Figure 3D).

No effect of treatment ($P > 0.7$) or treatment by time interaction ($P > 0.5$) was detected for C14 and C16 desaturase indices, although an effect of time ($P < 0.01$; Table 4) was detected. Both indices decreased progressively and reached a nadir on d 12 of recovery from MFD. Treatments had no effect on the desaturase indices 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 substrate entering the rumen was immediately changed during the recovery period, but the composition of rumen digesta and the rumen environment had a slightly longer lag. However, we have observed increased rumen pH by d 3 of recovery in other similar experiments (unpublished data). The ruminal inoculation treatment used in the current experiment was intended to reintroduce a normal rumen microbiota and determine if reestablishment of normal BH pathways and capacity and recovery from MFD could be accelerated.

Rumen probiotic treatments can be achieved through feeding specific exogenously cultivated microbial species (Francisco et al., 2002; Chiquette et al., 2008; Aikman et al., 2011) or transfer of rumen digesta or fluid. Rumen digesta transfer was selected for the current experiment because it is expected that many species are important for the establishment of normal BH, and the most important species for this process in vivo are not well established. Cole (1991) investigated the effect of transferring approximately half of the rumen contents between fed and fasted sheep and observed little effect of digesta on DMI, feeding behavior, and rumen function, thus concluding that postabsorptive mechanisms predominated. However, rumen digesta inoculation (300 g/d) from sheep fed plants containing toxic tannins (*Acacia angustissima*) provided protection to nonadapted sheep (Saarisalo et al., 1999). Additionally, near complete exchange of ruminal digesta was investigated in dairy cows (Satter and Bringe, 1969; Weimer et al., 2010a), but did not provide an assessment of the modification of the microbial community or a dissection of the effect of digesta composition from the microbial community. In contrast, the intent of the present experiment was to specifically study the response to inoculation with a microbial community as-

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

FA, % of total FA	Treatment ¹		SE	P-value ²		
	Control	INOC		Trt	Time	Trt × time
<i>trans</i> -10 C18:1	1.08	0.92	0.10	0.03	<0.001	0.04
<i>trans</i> -11 C18:1	1.10	0.99	0.08	0.33	<0.001	0.03
<i>cis</i> -9, <i>trans</i> -11 CLA	0.66	0.67	0.043	0.85	<0.001	0.07
<i>trans</i> -10, <i>cis</i> -12 CLA	0.010	0.008	0.001	0.07	<0.001	0.05
FA <16 C ³	23.1	23.6	0.35	0.17	<0.001	0.35
FA >16 C	42.8	41.9	1.40	0.07	<0.001	0.86
16-C FA	26.1	27.2	0.76	0.27	0.01	0.82
C14 desaturase index ⁴	0.10	0.11	0.01	0.39	<0.001	0.38
C16 desaturase index	0.05	0.06	0.01	0.59	<0.001	0.91

¹Control = 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.

²Trt = treatment effect; time = effect of day in recovery from milk fat depression.

³Fatty acids <16 C originate from de novo synthesis in the mammary gland, >16-C FA originate from plasma, and 16-C FA originate from both sources.

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

sociated with normal ruminal BH. The amount of inoculum transferred (8 kg/d) in the current experiment is approximately 10% of whole-rumen digesta (as-is basis; Oba and Allen, 2003; Harvatine and Allen, 2006). 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 digesta donors.

The time course of diet-induced MFD induction and recovery were previously described by Rico and Harvatine (2013) 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 did not require reaching a maximal reduction. However, milk fat must be decreased to allow examination of recovery. Cow periods that failed to decrease milk fat concentration more than 15% were removed. The recovery diet was designed to allow recovery from diet-induced MFD, as it was higher in NDF and lower in PUFA content than the induction diet.

A previous time-course experiment observed that milk fat concentration and yield progressively increased and were completely recovered in 15 d when cows were fed a moderate-NDF and low-PUFA diet following MFD induction (Rico and Harvatine, 2013). In the current experiment, milk fat percentage progressively increased and plateaued near d 15 and was not affected by inoculation treatments at any time point (Figure 1). Milk fat yield was not changed during recovery. The recovery diet resulted in a substantial loss of milk yield, presumably due to the lower fermentability of the recovery diet. Decreasing diet fermentability will

commonly decrease milk production and it appears that this occurred in the recovery diet. This general decrease in milk production makes interpretation of recovery of milk fat yield difficult. However, the milk FA profile shows recovery from classical diet-induced MFD. Specifically, MFD is characterized by a larger reduction in de novo synthesized FA, which results in a decrease in the proportion of FA less than 16 C and an increase in the concentration of the intermediates of the alternative BH pathway (e.g., *trans*-10,*cis*-12 CLA). The progressive changes in the proportion of de novo and preformed FA and the rapid decrease in alternative BH intermediates indicates recovery from classical diet-induced MFD, even though milk fat yield was not increased over time. A further increase in milk fat or recovery of the 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 effect of short-term inoculation. Importantly, the inoculation treatment accelerated the recovery of the proportion of de novo synthesized FA on d 6 and appeared to shift the recovery of de novo synthesized FA by approximately 3 d. The inoculation treatment was terminated on d 6 and did not appear to have any carryover effect on de novo milk fat synthesis; however, there was little room for additional recovery.

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), whereas no effects were observed on DMI or milk yield (Chiquette et al., 2008). In contrast, administration of a propionibacteria culture top-dress (17 g/d) decreased DMI, but had no

Figure 2. Effect of daily ruminal inoculation (from d 1 to 6) with digesta from non-milk fat-depressed cows (inoculation) on the concentration of milk FA by source during recovery from diet-induced milk fat depression compared with the control. (A) De novo FA; (B) 16-C FA; (C) preformed FA. Preplanned contrasts tested the difference between the control and inoculation at each time point. ** $P < 0.01$; † $P < 0.1$; SEM = 0.35, 0.76, and 1.40, for de novo, 16-C, and preformed FA, respectively.

effect on milk yield or milk fat concentration in early-lactation cows (Francisco et al., 2002). The ability to develop probiotic treatments depends on the identification of specific microbial species with important

functional roles in BH that grow competitively under conditions of milk fat-challenging diets.

A 2-phase response has been previously reported for milk fat *trans* FA during recovery (Rico and Harvatine, 2013). During the first phase, from approximately d 1 to 10 of recovery, milk fat *trans*-10 C18:1 and *trans*-10,*cis*-12 CLA concentrations decrease and during the second phase, *trans*-11 C18:1 and *cis*-9,*trans*-11 CLA concentrations increase slightly before returning to a nadir. In the current experiment, inoculation decreased the utilization of the alternate pathway as evidenced by decreased milk fat *trans*-10 C18:1 and *trans*-10, *cis*-12 CLA concentrations during the first phase (d 6). In agreement, a greater use of the normal BH pathway was observed during this phase as milk fat concentration of *trans*-11 C18:1 and *cis*-9,*trans*-11 CLA concentrations increased. Additionally, inoculation appears to have increased the capacity of the normal BH pathway during the second phase of recovery as the normal BH pathway isomers are decreased on d 9 and 12. The increased concentration of de novo synthesized FA, taken together with the changes in *trans* FA isomers during the first and second phases of recovery, demonstrate a slight acceleration of recovery from classical diet-induced MFD by the ruminal digesta inoculation. Recently, fecal microbiota transplants from healthy donors that provide complete and stable microbial communities have resulted in improved recovery from *Clostridium difficile* infections and restored normal bowel function in human subjects (Borody and Khoruts, 2012), demonstrating the effectiveness of a similar approach.

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., 2009). 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 by Rico and Harvatine (2013).

CONCLUSIONS

Milk fat yield was not changed, but the recovery of the concentration of de novo synthesized FA and normal ruminal BH pathways were slightly accelerated by ruminal inoculation with digesta from non-milk-fat-depressed cows. The mechanism likely involves the restoration of normal microbial populations that have a higher capacity for BH of PUFA and utilize BH pathways that do not produce milk fat-depressing intermediates.

Figure 3. Effect of daily ruminal inoculation (from d 1 to 6) with digesta from non-milk fat-depressed cows (inoculation) on the concentration of milk *trans* FA during recovery from diet-induced milk fat depression compared with the control. (A) *trans*-10 C18:1; (B) *trans*-10, *cis*-12 CLA; (C) *trans*-11 C18:1; (D) *cis*-9, *trans*-11 CLA. Preplanned contrasts tested the difference between the control and inoculation at each time point. ** $P < 0.01$; * $P < 0.05$; † $P < 0.1$; SEM = 0.10, 0.08, 0.001, and 0.04, for *trans*-10 C18:1, *trans*-10, *cis*-12 CLA, *trans*-11 C18:1, and *cis*-9, *trans*-11 CLA, respectively.

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