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# Alterations in ruminal bacterial populations at induction and recovery from diet-induced milk fat depression in dairy cows

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## ABSTRACT

Ten ruminally cannulated Holstein cows were used in a crossover design that investigated changes in ruminal bacterial populations in response to induction and recovery from diet-induced milk fat depression (MFD). Further, the effect on the ruminal microbiota of the cows with diet-induced milk fat depression inoculated with rumen contents from non-milk fat-depressed donor cows was evaluated. Milk fat depression was induced during the first 10 d of each period by feeding a low-fiber, high-starch, and high-polyunsaturated fatty acid diet (26.1% neutral detergent fiber, 28.1% starch, 5.8% total fatty acids, and 1.9% C18:2), resulting in a 30% decrease in milk fat yield. Induction was followed by a recovery phase, where all cows were switched to a high-fiber, low-starch, and low-polyunsaturated fatty acid diet (31.8% neutral detergent fiber, 23% starch, 4.2% total fatty acids, and 1.2% C18:2) and were allocated to (1) control (no inoculation) or (2) ruminal inoculation with donor cow digesta (8 kg/d for 6 d). Ruminal samples were collected at the end of induction (d 10) and during recovery (d 13, 16, and 28), separated to solid and liquid fractions, extracted for DNA, PCR- amplified for the V1-V2 region of the 16S rRNA gene, and analyzed for bacterial diversity. Results indicated that bacterial communities were different between fractions. In each fraction, differences were significant between the induction (d 10) and recovery (d 13, 16, and 28) periods; however, differences were less apparent with time during the recovery period. The MFD (d 10) was typified by a reduction in the relative sequence abundance of *Bacteroidetes* and an increase in the relative sequence abundance of *Firmicutes* and *Actinobacteria* across both fractions. At the genus level, relative sequence abundance of unclassified *Lachnospiraceae*, *Butyrivibrio*, *Bulleidia*, and *Coriobacteriaceae* were higher on d 10 and were positively correlated with *trans*-10,*cis*-12 CLA and the *trans*-10 isomer, suggesting their potential role in altered biohydrogenation reactions. A switch to the recovery diet resulted in a sharp increase in the *Bacteroidetes* lineages and a decrease in *Firmicutes* members on d 13; however, this shift appears to stabilize by d 28, indicating the restoration process for ruminal bacteria from an altered state is gradual and complex. Inoculation of 10% of rumen contents from non-MFD donor cows to MFD cows revealed this procedure had transient effects on only a few bacterial populations, and such effects disappeared after d 16 following cessation of inoculation. It can be concluded that alterations in milk FA profiles at induction are preceded by microbial alterations in the rumen driven by dietary changes.
**Key words:** biohydrogenation, milk fat depression, ruminal microbiome, fatty acid isomers

## INTRODUCTION

Diet-induced milk fat depression (**MFD**) in dairy cows is characterized by a specific reduction in milk fat yield in response to bioactive fatty acids produced in the rumen when feeding rapidly fermentable diets that are high in PUFA (Bauman and Griinari, 2003). Milk fat depression is a classic example of the interactions between dietary nutrients, the gastrointestinal microbiota and tissue physiology (Rico et al., 2015a). In ruminants, dietary PUFA are converted to saturated fats by microbes through biohydrogenation (**BH**) in the rumen. It has been reported during the course of diet-induced MFD that rumen fermentation is altered, causing a shift in BH pathways, resulting in specific *trans* FA that inhibit milk fat synthesis in the mammary gland, leading to MFD (Harvatine et al., 2009). The severity of MFD is dependent upon the rate and extent of BH and the specific intermediates formed in the rumen, which are influenced by the concentrations of PUFA and fermentable carbohydrates in the diet

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(Jenkins et al., 2008; Fuentes et al., 2009). Therefore, MFD in dairy cows is the direct consequence of rumen microbial activity that is responsive to diet. However, the interaction between dietary factors and the rumen microbiota and their gene repertoire leading to MFD is not well understood.

Rico and Harvatine (2013) and Rico et al. (2014) have described models for induction and recovery from classic diet-induced MFD. By feeding a low-fiber, high-starch, high-PUFA diet (26.1% NDF, 28.1% starch, 5.8% total fatty acids, and 1.9% C18:2) to lactating dairy cows, those authors induced MFD and noted a reduction by 30% in milk fat yield by 10 d. After establishment of MFD, cows were switched to a high-fiber, low-starch, low-PUFA diet (31.8% NDF, 23% starch, 4.2% total fatty acids, and 1.2% C18:2) and recovery from MFD was achieved in 15 d. In response to these dietary changes, a 2-phase adaptation of milk FA profiles was reported during both induction and recovery periods. During the induction phase, *cis*-9,*trans*-11 CLA peaked on d 3 and progressively reduced, whereas *trans*-10,*cis*-12 CLA progressively increased during the induction period. However, in the recovery period, *cis*-9,*trans*-11 CLA initially increased and returned to baseline and *trans*-10,*cis*-12 CLA gradually decreased. Rico et al. (2014) concluded that the time course for induction and recovery from MFD was dependent on ruminal adaptation more than the dietary substrate available for BH. Nonetheless, how microbes respond to these dietary changes and which microbes are associated with different CLA isomers are not known.

The rumen microbiota is composed of bacteria, protozoa, fungi, and archaea that live in a symbiotic relationship and are collectively responsible for microbial fermentation in the rumen (Morgavi et al., 2013). Evidence indicates that changes in dietary components such as NDF, CP, and starch can significantly alter the composition of the rumen microbial communities (Pitta et al., 2010, 2014a,c,d). Whereas dietary structural carbohydrates and NFC and proteins are needed for microbial growth in the rumen, supplementation of fatty acids (**FA**) is not necessary, as microbes can synthesize their own FA (Garton, 1977). Elevated concentrations of PUFA are considered toxic to rumen microbes because PUFA form complexes with the cell walls of rumen microbes and interfere with rumen fermentation (Kim et al., 2002). Higher concentrations of PUFA and starch in the diet can alter the BH pathways leading to MFD (Rico and Harvatine, 2013). However, a paucity of information exists on the role of microbes in BH and the mechanism of action leading to MFD. Utilizing the induction-recovery model of diet-induced MFD (Rico et al., 2014), the current study investigated the changes in bacterial populations based on their relative sequence abundance at induction and their change as dairy cows recovered from MFD, with dietary intervention alone and dietary intervention combined with inoculation from non-MFD cows.

## MATERIALS AND METHODS

The recovery ruminal samples used for bacterial community analysis in our study were collected and archived from a recently published report (Rico et al., 2014) that investigated production responses and included FA profiles during induction and recovery from MFD. Experimental details were previously described by Rico et al., (2014) and were approved by the Pennsylvania State University Institutional Animal Care and Use Committee. Briefly, 10 ruminally cannulated Holstein cows were randomly assigned to treatment sequences in a crossover design with 28-d periods. Two additional cows fed a high-fiber, low-starch, low-PUFA diet (31.8% NDF, 23% starch, 4.2% total FA, and 1.2% C18:2; Supplemental Table S1; https://doi.org/10.3168/jds.20176-12514) were used as rumen digesta donors. Each treatment period included a 10-d MFD induction and an 18-d MFD recovery phase. During the MFD induction phase, the 10 treatment cows were fed a low-fiber, high-starch, high-PUFA diet (26.1% NDF, 28.1% starch, 5.8% total FA, and 1.9% C18:2; Supplemental Table S1). During the subsequent MFD recovery phase, the cows were fed a high-fiber, low-starch, low-PUFA diet (31.8% NDF, 23% starch, 4.2% total FA, and 1.2% C18:2; Supplemental Table S1) with or without ruminal digesta inoculation. Rumen digesta inoculation of the 5 treated cows included removal of 8 kg of whole-rumen digesta at 1300 h on d 11 to 16 (recovery period) and replacement with digesta from the non-MFD donor cows. On each day of inoculation, rumen digesta was collected from 5 different locations of the rumen (cranial dorsal, cranial ventral, central, caudal dorsal, and caudal ventral) in both donor animals, mixed, and about 8 kg was transferred to each recipient. Rumen samples (approximately 300 g) were collected from the 10 experimental cows on d 13, 16, and 28, strained through 0.5-mm mesh, and solid and liquid fractions were frozen and stored at −80°C. The same procedure was carried out to archive subsamples of the mixed donor digesta samples.

### *DNA Extraction and PCR*

The rumen samples were processed for genomic DNA using a modified version of the PSP Spin Stool DNA Plus Kit (Invitek, Berlin, Germany) as per the method