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Shifts in bacterial community composition in the rumen of lactating dairy cows under milk fat-depressing conditions[1]

P. J. Weimer,*†[2] D. M. Stevenson,* and D. R. Mertens*
*USDA-ARS, US Dairy Forage Research Center, Madison, WI 53706
†Department of Bacteriology, University of Wisconsin–Madison, 53706

ABSTRACT

Eighteen ruminally cannulated dairy cattle were fed a series of diets (in 28-d periods) designed to elicit different degrees of milk fat depression (MFD) for the purpose of relating MFD to ruminal bacterial populations. Cows were fed a TMR containing 25% starch (DM basis) supplied as corn silage, a slowly fermented starch (SFS treatment, period 1), then switched to a TMR containing 27% starch, much of it supplied as ground high-moisture corn, a rapidly fermented starch (RFS treatment, period 2). In period 3, the RFS diet was amended with 13.6 mg of monensin/kg of DM (RFS/Mon treatment), and in period 4, the cows were returned to the RFS diet without monensin (RFS/Post treatment). Effect of both starch source and monensin on milk fat percentage varied by cow, and cluster analysis identified 4 pairs of cows having distinct milk fat patterns. Archived ruminal liquors and solids from the 4 pairs were processed to isolate bacterial DNA, which was subjected to automated ribosomal intergenic spacer analysis followed by correspondence analysis to visualize bacterial community composition (BCC). One pair of cows (S-responsive) showed MFD on RFS feeding, but displayed no additional MFD upon monensin feeding and a fat rebound upon monensin withdrawal. The second pair of cows (M-responsive) showed no MFD upon switch from the SFS diet to the RFS diet, but displayed strong MFD upon monensin feeding and no recovery after monensin withdrawal. Both groups displayed major shifts in BCC upon dietary shifts, including dietary shifts that both did and did not change milk fat production. The third pair of cows (SM-responsive) displayed reduction of milk fat on both RFS and RFS/Mon diets, and fat returned to the levels on the RFS diet upon monensin withdrawal; these cows showed a more gradual shift in BCC in response to both starch source and monensin. The fourth pair of cows (nonresponsive) did not display changes in milk fat percentage with dietary treatment and showed only minor shifts in BCC with dietary treatment. Regardless of milk fat response, BCC did not reassemble its original state upon monensin withdrawal, though the difference was strongest in M-responsive cows. One amplicon length (representing a single bacterial species) was elevated in most, but not all, MFD-susceptible (S-, M-, or SM-responsive) cows relative to milk fat-nonresponsive cows, whereas 2 amplicon lengths displayed reduced abundance under MFD conditions. Overall, this study demonstrates an association between MFD and wholesale shifts of microbial communities in the rumen.

Key words: bacterial community composition, milk fat depression, monensin, rumen

INTRODUCTION

Because of the strong influence of milk fat on milk pricing, milk fat depression (**MFD**, defined as the reduction in the percentage or yield of milk fat) is a serious economic problem for dairy producers. Modern dairy diets containing high concentrations of highly digestible cereal grains are widely used to maximize milk production, but such diets often induce MFD (Emery, 1988; Peterson et al., 2003; Nielsen et al., 2006). The problem can be exacerbated further in some cows by certain feed additives, such as monensin, that are known to promote changes in ruminal microbial populations (Van Beukelen et al., 1984; Ramanzin et al., 1997; Oelker et al., 2009). Elucidating the mechanism underlying MFD thus has been of longstanding interest to dairy nutritionists. Evidence accumulated over the past decade has focused attention on certain long-chain unsaturated fatty acids as proximal regulators of mammary lipogenesis (Bauman and Griinari, 2003). These long-chain unsaturated fatty acids can be produced or modified by some members of the ruminal microbiota, for example, through biohydrogenation reactions (Harfoot and Hazlewood, 1988; Wallace, 2008). Consequently, induction of MFD may result from alteration of the populations or activities of particular

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[2]Corresponding author: Paul.Weimer@ars.usda.gov

microbial species involved in these reactions; however, it has been difficult to assign the production or conversion of these acids to specific ruminal microbes. Maia et al. (2007) reported that *trans*-10,*cis*-12 conjugated linoleic acid (**t10c12-CLA**), the long-chain unsaturated fatty acid most closely associated with MFD (Bauman and Griinari, 2003), was not produced by any of 23 ruminal bacterial species tested in pure culture. Ruminal metabolism of long-chain unsaturated fatty acids is also poorly understood. *Butyrivibrio fibrisolvens* (Rosenfeld and Tove, 1971) is perhaps the best-characterized of the biohydrogenating bacteria, and Boeckaert et al. (2008) demonstrated that accumulation of *trans*-$C_{18:1}$ fatty acids in the rumen is associated with changes in the relative population sizes of certain strains of *Butyrivibrio*, although they did not examine milk fat response in their study. However, the importance of *B. fibrisolvens* in biohydrogenation reactions in the rumen is unclear because this species typically displays very low ruminal abundance even when quantified by sensitive molecular methods (Forster et al., 1997; Stevenson and Weimer, 2007; Weimer et al., 2008).

Anecdotal evidence suggests that diets containing both highly fermentable starch [e.g., high-moisture corn (**HMC**)] and monensin increase the incidence of MFD. Recent evidence using quantitative real-time PCR (**qPCR**) with relative quantification indicated that monensin decreased the relative abundance of several bacterial species, including *B. fibrisolvens* and *Megasphaera elsdenii*, although their abundance, even in the absence of monensin, was very small (<0.01% of total bacterial 16S rRNA gene copies, a measure of population abundance; Weimer et al., 2008). Moreover, that study revealed that the contributions by most of the 16 taxa examined to total 16S rRNA gene copies were unaffected by monensin. Evaluation of clone libraries constructed from 16S rRNA genes obtained from bulk ruminal DNA have revealed that most (perhaps 90% or more) ruminal bacteria show <97% sequence identity to cultivated species (Whitford et al., 1998; Tajima et al., 1999, 2000), suggesting that these unidentified strains represent novel species that have to date resisted cultivation (Wallace, 2008). Moreover, qPCR studies have shown that the 13 most commonly studied ruminal bacterial species contribute <7% of the total 16S rRNA gene copies in ruminal contents (Stevenson and Weimer, 2007). It is thus highly likely that a substantial number of currently uncultured species may respond to the presence of feed additives like monensin, and that at least some of these species may be directly involved in the production or consumption of the long-chain unsaturated fatty acids that regulate milk fat synthesis or may affect populations of other species that may be more directly involved. During the past decade, several methods based on amplification and quantitation of taxon-specific segments of genomic DNA have been developed for profiling entire bacterial communities (Fisher and Triplett, 1999; Giraffa and Neviani, 2001; Danovaro et al., 2006; Loisel et al., 2006). We used one such community fingerprinting technique, automated ribosomal intergenic spacer analysis (**ARISA**), to examine the effect of starch fermentability and the feeding and withdrawal of monensin on ruminal bacterial community composition (**BCC**) in lactating dairy cows that displayed different patterns of MFD.

MATERIALS AND METHODS

Experimental Design

The experiment was designed to determine differential effect of monensin feeding and withdrawal on milk fat production under conditions of high starch fermentability and to relate any differential milk fat response among cows to the population structure of the ruminal bacterial community. Specifically, we sought to identify internally coherent groups of cows that differed, or did not differ, in milk fat production in response to starch fermentability, or monensin, or both, for post-hoc examination of bacterial populations in archived ruminal contents. This strategy permitted reducing the total number of samples that required the labor-intensive process of purifying bulk ruminal DNA from a very large sample set.

Experiments were performed in accordance with a research protocol approved by the University of Wisconsin–Madison's Research Animal Resource Center. Eighteen lactating, ruminally cannulated Holstein cows (7 primiparous, 11 multiparous; mean BW = 599 kg, DIM = 50–134, milk yield = 30–52 kg/d) that had never received monensin were maintained in adjacent tie-stalls at the US Dairy Forage Research Center farm near Prairie du Sac, Wisconsin. Cows were fed the TMR once daily at approximately 0830 h, had continuous access to water, and were milked twice daily. Refusals were collected and weighed before each feeding, and a handful of refusals from individual cows were collected just before feeding on each of the last 7 d of each period (as defined later); these samples were composited by cow and stored frozen before compositional analysis.

The 2 primary sources of starch for the diets were corn silage and HMC, known to differ greatly in ruminal fermentability (Owens et al., 1986). Both were ensiled in tower silos for >200 d before the start of the experiment. The corn silage was ensiled with minimal kernel processing (cracking of the grain). The HMC was finely ground (Clay Hammer Mill, Cedar Falls, IA) each day during removal from the silo to decrease