J. Dairy Sci. 93:5902–5912
doi:10.3168/jds.2010-3500

Host specificity of the ruminal bacterial community in the dairy cow following near-total exchange of ruminal contents[1]

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ABSTRACT

The purpose of this study was to examine the stability and host specificity of a cow's ruminal bacterial community following massive challenge with ruminal microflora from another cow. In each of 2 experiments, 1 pair of cows was selected on the basis of differences in ruminal bacterial community composition (BCC), determined by automated ribosomal intergenic spacer analysis (ARISA), a culture-independent "community fingerprinting" technique. Each pair of cows was then subjected to a 1-time exchange of >95% of ruminal contents without changing the composition of a corn silage/alfalfa haylage-based TMR. In experiment 1, the 2 cows differed ($P < 0.01$) in prefeed ruminal pH (mean = 6.88 vs. 6.14) and prefeed total VFA concentration (mean = 57 vs. 77 m*M*), averaged over 3 d. Following exchange of ruminal contents, ruminal pH and total VFA concentration in both cows returned to their preexchange values within 24 h. Ruminal BCC also returned to near its original profile, but this change required 14 d for 1 cow and 61 d for the other cow. In experiment 2, the 2 other cows differed in prefeed ruminal pH (mean = 6.69 vs. 6.20) and total VFA concentration (mean = 101 vs. 136 m*M*). Following exchange of ruminal contents, the first cow returned to its preexchange pH and VFA values within 24 h; the second cow's rumen rapidly stabilized to a higher prefeed pH (mean = 6.47) and lower prefeed VFA concentration (mean = 120 m*M*) that was retained over the 62-d test period. Both cows reached somewhat different BCC than before the exchange. However, the BCC of both cows remained distinct and were ultimately more similar to that of the preexchange BCC than of the donor animal BCC. The data indicate that the host animal can quickly reestablish its characteristic ruminal pH and VFA concentration despite dramatic perturbation of its ruminal microbial community. The data also suggest that ruminal BCC displays substantial host specificity that can reestablish itself with varying success when challenged with a microbial community optimally adapted to ruminal conditions of a different host animal.

Key words: bacterial community, rumen, rumen pH, volatile fatty acid

INTRODUCTION

The microbial community of the rumen is responsible for converting ingested feed into the VFA that serve as the major energy source for the ruminant host, and for providing a substantial portion of the host's protein requirements (Hungate, 1966). Ruminal bacteria are the most numerous of the microbial inhabitants (Russell, 2002), and until recently, the number of different bacterial species in the rumen was vastly underestimated (Krause and Russell, 1996; Stevenson and Weimer, 2007), largely because most bacterial species have resisted the laboratory cultivation that ordinarily precedes species identification and characterization.

Culture-independent community fingerprinting techniques such as automated ribosomal intergenic spacer analysis (**ARISA**) have greatly expanded our understanding of the diversity of the ruminal bacterial population and have indicated that the bacterial community composition (**BCC**) of dairy cattle can differ substantially, even among animals fed the same diet and with similar milk yields and compositions (Weimer et al., 2010; Welkie et al., 2010). This raises the possibility that ruminal BCC may be optimally adapted to its particular ruminant host, and can display substantial resistance to colonization by bacterial strains not adapted to the recipient host. Host specificity would explain in part the inconsistent effects of targeted additions of pure bacterial strains to the rumen. Such additions have produced mixed results (compare Jones and Megarrity, 1986 and Gregg et al., 1998 with Varel et al., 1995 and Krause et al., 1999) that appear to depend largely on whether the added strain can fill an empty niche in the microbial community (Weimer, 1998). On

Received June 2, 2010.
Accepted August 26, 2010.
[1]Mention of specific products is for informational purposes only and does not imply a warranty or endorsement over other products not mentioned.
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the basis of these reports, we hypothesized that the ruminal BCC displays sufficient host specificity to resist establishment of allochthonous (non-native) bacterial strains, even when the latter are inoculated in very large dosages.

One approach to examine the specificity between the host animal and its ruminal microflora is to cross-inoculate ruminal contents between cows. Several such cross-inoculations have been reported in the literature (Satter and Bringe, 1969; Cole, 1991), but these studies have focused on animal responses (particularly feed intake and the profiles of pH and VFA), without examination of the effects on population distributions of the many members of the microbial community. We report here that near-complete exchange of ruminal contents between pairs of cows generally results in a reassembly of the bacterial communities over a period of approximately 2 to 9 wk, to resemble, to varying degrees, those before the exchange. In addition, the exchange is accompanied by a surprisingly rapid return to the pre-exchange pH and VFA profiles, suggestive of a major influence of the cow on its own ruminal chemistry.

MATERIALS AND METHODS

Two ruminal exchange experiments were conducted with lactating, ruminally fistulated Holstein cows according to a protocol approved by the University of Wisconsin–Madison Research Animal Resource Center. Cows were maintained in tiestalls, fed once daily, milked twice daily, and had continuous access to water. The first experiment comprised 2 cows whose ruminal BCC (determined by ARISA and correspondence analysis; see below) was shown to be the most different among 5 cows tested. This experiment was conducted November 17, 2008, to January 23, 2009, at the University of Wisconsin Dairy Cattle Instructional Center in Madison. The cows were fed a TMR that contained (DM basis) 35.1% corn silage, 23.1% alfalfa haylage, 13.4% coarse-ground corn, 10% distillers dried grains, 6.4% soybean meal, 3.2% corn gluten feed, 0.69% $CaCO_3$, 0.63% soy hulls, 0.4% $NaHCO_3$, 0.23% iodized NaCl, 0.12% $CaHPO_4$, 0.11% DynaMate, 0.09% MgO, and supplemental vitamins and trace minerals. The second experiment comprised 2 cows whose BCC was shown to be the most different among 18 cows tested. This test was conducted May 18 to July 21, 2009, at the US Dairy Forage Research Center farm near Prairie du Sac, WI. These cows were fed once daily with a TMR that contained (DM basis) 29.6% corn silage, 27.9% alfalfa haylage, 25.5% corn grain, 7.4% roasted soybeans, 2.6% vitamin and mineral mix, 1.9% whole cottonseed, 1.8% dried distillers grains, 1.8% soybean meal, and 1.5% blood meal, along with Rumensin 80 (Elanco Animal Health, Greenfield, IN) to provide monensin at 13 mg/kg of diet DM.

Ruminal sampling was conducted just before feeding on 2 successive days (−2 and −1) before the exchange of ruminal contents. On the day of the exchange, ruminal contents were sampled just before (**PRE**) and just after (**POST**) exchanging ruminal contents, and subsequently just before feeding on various days as indicated in the Results section. Samples were placed on ice and transported back to the laboratory, and then archived at −80°C for analysis at the conclusion of each experiment.

The exchanges themselves were accomplished by hand-emptying the rumens, first by withdrawing hand samples of wet solids, and eventually by removal of residual ruminal liquor using a 0.25-L plastic cup. During emptying, the experimenters wore shoulder-length polyethylene gloves (Nasco, Ft. Atkinson, WI), and care was taken to open folds in the rumen wall to access additional contents. Although direct measurements were not employed, we estimate that we removed approximately 95% of the ruminal contents from each cow. The removed solids and liquids were placed in precleaned large plastic garbage cans. Upon conclusion of the ruminal emptying, the wet solid ruminal contents from the donor cows were hand-stuffed into the rumen of the recipient cow, and the residual liquor was then poured into the rumen. For both experiments, the exchanges were completed within approximately 30 min.

Ruminal pH and temperature were measured at each ruminal sampling using a model 340i datalogging pH meter fitted with a SenTix 41 electrode (WTW, Weinheim, Germany). The pH measurement system was calibrated at pH 7.00 and 4.00 immediately before use. Fermentation acids (VFA and lactate) were determined by HPLC, as described previously (Weimer et al., 1991). Comparisons of BCC were performed using ARISA, a culture-independent community fingerprinting technique that examines the relative abundance of the internally transcribed sequence between the 16S and 23S rRNA genes present in all bacteria. The methods for DNA isolation, PCR amplification of the internal transcribed spacer sequence, separation of amplicons by ARISA, and correspondence analysis of the amplicon profiles obtained by capillary electrophoresis, have been described (Weimer et al., 2010). Amplicon profiles were compared using an analysis of similarity (**ANOSIM**). The ANOSIM R-value was calculated from a Bray-Curtis distance table (Ludwig and Reynolds, 1988) according to the method of Clarke (1993). Calculations were performed using custom software written in the C programming language.