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Assessment of Ruminal Bacterial Populations and Protozoal Generation Time in Cows Fed Different Methionine Sources[1]

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ABSTRACT

Methionine supplemented as 2-hydroxy-4-(methylthio)-butanoic acid (HMB) has been suggested to alter bacterial or protozoal populations in the rumen. Our objective was to determine if source of Met would change microbial populations in the rumen and to compare those results to samples from the omasum. The ruminal and omasal samples were collected from cows fed control (no Met), DL-Met, HMB, or the isopropyl ester of HMB (HMBi; estimated 50% rumen protection) in a replicated 4×4 Latin square design. In one square, changes in protozoal populations were determined using microscopic counts and denaturing gradient gel electrophoresis (DGGE), whereas changes in bacterial populations were determined using DGGE and ribosomal intergenic spacer length polymorphism (RIS-LP). Neither the protozoal counts nor the DGGE banding patterns derived from protozoa were different among the dietary treatments or for ruminal vs. omasal samples. As revealed by both DGGE and RIS-LP, bacterial populations clustered by treatments in ruminal and especially in omasal samples. Using cows from both Latin squares, the flow of protozoal cells from the rumen was quantified by multiplying protozoal cell count in omasal fluid by the omasal fluid flow (using CoEDTA as a liquid flow marker) or was estimated by rumen pool size of cells multiplied by either the ruminal dilution rate of CoEDTA (after termination of CoEDTA dosing) or the passage rate of Yb-marked particles. Compared with the omasal fluid flow measurement (16.4 h), protozoal generation time was approximated much more closely using the particulate than the fluid passage rate from the rumen (generation times of 15.7 and 7.5 h, respectively). There seems to be minimal selective retention of protozoal genera in the rumen in dairy cattle fed every 2 h. Data support the validity of the omasal sampling technique under our conditions.
Key words: omasal sampling, denaturing gradient gel electrophoresis, ribosomal intergenic spacer analysis, methionine

INTRODUCTION

Overfeeding CP leads to excessive excretion of urinary N into the environment. Balancing the diet for metabolizable amino acids can maintain or even improve milk yield and milk component production while decreasing dietary concentration of CP (Noftsger and St-Pierre, 2003). When 2-hydroxy-4-(methylthio)-butanoic acid (**HMB**) plus rumen-protected Met was fed to cows in that study, the improvements in milk protein yield were higher than what could be expected by an additional supply of metabolizable Met, suggesting a potential benefit from stimulation of microbial protein synthesis by HMB. The isopropyl ester form of HMB (**HMBi**) was estimated to have about 50% ruminal escape (Noftsger et al., 2005) and can therefore be expected to have both ruminal and postruminal effects. The latter authors hypothesized that a ruminal response from degradable HMB would be mediated through protozoa. In a continuous culture experiment in which protozoa were washed out, bacterial N flow was not affected by source of HMB (Noftsger et al., 2003). In the same study, ribosomal intergenic spacer length polymorphism (**RIS-LP**), a culture-independent method, was used for determining changes in bacterial populations. The banding patterns tended to group by source of Met, but inoculation source for each period seemed to hide potential banding patterns. From a comprehensive interpretation of these results, we projected that HMB source might alter both bacterial and protozoal populations in vivo.

Recently, Firkins et al. (2006) highlighted how measurements of microbial N flow might be potentially biased by harvesting a microbial standard from the rumen to represent microbial biomass flowing out of the rumen. Some of the methodological issues include difficulties in obtaining a truly representative single microbial sample from the reticulorumen but also in de-

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termining how samples representing different microbial fractions are quantitatively partitioned to accurately quantify microbial N flowing from the rumen. Duodenal contents have been the predominant source of digesta to quantify ruminal microbial N outflow, but in these samples, all protozoa are lysed (Sylvester et al., 2005) and selective lysis of bacteria is also possible (Kennedy et al., 1984). Bacterial marker:N ratios differ for liquid- and solids-associated bacteria, so a weighted average should be used to obtain a standard that represents the total bacteria flowing out of the rumen. However, because of faster passage rate of liquid than particulate matter, the relative contribution of bacterial N arising from the liquid-associated bacteria should be higher in sites distal from the reticulorumen compared with the rumen (Ahvenjärvi et al., 2000). With the recent improvement of omasal sampling techniques (Noftsger et al., 2005; Firkins et al., 2006), we hypothesized that omasal sampling would allow collection of microbial samples that more accurately represent microbes passing from the rumen.

Although selective retention of isotrichid protozoa in the reticulum or lysis of entodiniomorphid protozoa could bias omasal sampling for protozoa, Firkins and Yu (2006) concluded that these potential biases probably are less problematic in the lactating dairy cow with very high ruminal passage rates than in prior studies with cattle or sheep fed at much lower DMI as percentages of BW. They also explained why many studies with protozoal count data in the rumen and omasum should not be used to assess protozoal lysis rates or generation times. For example, bypass of drinking water and protozoal chemotaxis for particulate matter should cause protozoal counts in the omasum to be less than corresponding counts in the rumen fluid irrespective of protozoal sequestration in the reticulum or potential autolysis in the omasal fluid. Comparison of ruminal pool size of protozoal cells with omasal flow of protozoal cells, the mathematically correct way to represent protozoal generation time in vivo, has seldom been measured and, to our knowledge, not at all with high-producing dairy cattle (Firkins and Yu, 2006).

The use of cultivation-based techniques for enumeration of ruminal bacteria has its own limitations regarding the ability to characterize microbial communities, but these techniques still avoid the important pitfall that an unknown majority of bacteria are currently uncultivable (Firkins and Yu, 2006). Culture-independent techniques targeting the DNA encoding the synthesis of small subunit ribosomal RNA allow direct analysis and quantification of microorganisms. Analysis of RIS-LP has been used for profiling bacterial populations in continuous culture (Noftsger et al., 2003) and to fractionate ruminal bacterial subpopulations (Larue et al., 2005). Recently, denaturing gradient gel electrophoresis (**DGGE**) has been used to profile predominant bacterial (Larue et al., 2005) and protozoal (Regensbogenova et al., 2004) populations in the rumen and to compare ruminal and duodenal protozoal profiles (Sylvester et al., 2005).

The hypotheses of this experiment were that 1) HMB and HMBi promote changes in ruminal bacterial and protozoal populations, 2) RIS-LP analysis and DGGE allow the detection of changes in ruminal microbial populations resulting from supplementation of a source of Met, and 3) collection of samples from the omasum represents bacteria and protozoa leaving the rumen. The objectives of this study were 1) to compare the effects of Met supplementation on microbial populations in ruminal and omasal samples, 2) to compare RIS-LP and DGGE for the potential to detect changes in the microbial community profiles, and 3) to compare microbial community profiles based on molecular procedures or protozoal counting between the rumen and omasum. Ruminal and omasal samples that were used in this study were used for derivation of data from a companion study (Noftsger et al., 2005).

MATERIALS AND METHODS

Animals and Experimental Design

Eight ruminally cannulated Holstein cows were assigned to a replicated 4 × 4 Latin square design. For characterization of microbial populations, only one of the squares was analyzed. For outflow and generation time data using total protozoal counts, data from both Latin squares were combined for regression analyses. The dietary treatments were 1) no supplemental methionine (control), 2) HMB at 0.10% of DM, 3) HMBi at 0.13% of DM, and 4) DL-Met at 0.088% of DM. Methionine was supplemented on an equimolar basis across treatments, and the amount of additional Met or Met precursor on a Met basis supplied by each treatment diet was calculated to be 22 g/d at 25 kg/d of DMI. Treatments were added to a basal diet consisting of 57% concentrate and 43% forage, with approximately 70% of the forage DM from corn silage and 30% from alfalfa hay (Noftsger et al., 2005). Experimental periods consisted of 28 d, with d 1 through 14 serving to adjust cows to new diets, d 15 through d 20 to adjust cows to a restricted feeding regimen, and d 21 through d 28 for collection of data. Starting on d 15, cows were restricted to 95% of their respective average ad libitum DMI determined during the prior 2 wk of adjustment. Using automatic feeders (Ankom Technology, Macedon, NY), cows received approximately one-twelfth of their daily feed allowance every 2 h. Care and handling of the animals was conducted as outlined in the guidelines of The Ohio