Inhibition of Ruminal Methanogenesis Modulates Microbial Energy Metabolism via H_2 Rerouting

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Beef and dairy cattle are the nexus between plants and humans. They provide highly nutritious and bioavailable milk and meat from cellulose, which is inedible to us. However, methane (CH_4) emissions from beef and dairy cattle contribute to roughly 10% of the global anthropogenic greenhouse gas emissions. Enteric methanogenesis represents up to 12% of dietary energy loss, resulting in reduced feed efficiency. Mitigation of ruminal methanogenesis provides an opportunity for beef and dairy producers to increase feed efficiency, reduce feed costs, and minimize environmental impact.

Feed additives and CH_4 abatement strategies typically target one of two processes, i.e. modulate microbial thermodynamics or inhibit enzyme kinetics. Thermodynamics is related to availability of free energy ($\Delta G°$). Conversion of NO_3^- to NH_3 in the rumen has a $\Delta G°$ of -599.6 kJ/mol, compared to a $\Delta G°$ of -131.0 kJ/mol for hydrogenotrophic methanogenesis. Enzyme kinetics are affected by the affinity of enzymes (K_m) to bind to substrate, thereby entailing the possibility of direct inhibition. Bromochloromethane (BCM) competitively inhibits the cobamide-dependent methyl transfer of methanogenesis pathways. The objective of the present study was to evaluate the *in vitro* effects of $NaNO_3$ and BCM as thermodynamic and enzyme inhibitors, respectively, on CH_4 by mixed cultures of rumen microbes. We hypothesized that: 1) $NaNO_3$ will inhibit CH_4 but allow microbial fermentation by directing H_2 toward alternative sinks; and 2) BCM will inhibit CH_4 and reduce microbial fermentation by limiting the use of H_2 in alternative sinks. We tested three levels of $NaNO_3$ and BCM in batch cultures consisting of 100-mL glass bottles fitted with airtight rubber screw caps. Each culture bottle received 30 mL of inoculum prepared by mixing filtered rumen contents with artificial saliva in a 1:2 ratio. Experimental diets were prepared using ground alfalfa hay and a concentrate mix (ground corn and soybean meal) to provide three forage-to-concentrate ratios: 70:30, 50:50, and 30:70. Diets were weighed in duplicate and incubated at 39℃ for 24 hours. After 24 h of incubation, headspace CH_4, culture pH, NH_3-N, and short chain fatty acids (SCFAs) were measured. Microbial DNAs from sample aliquots were extracted, amplified using universal and archaea-specific 16S rRNA and fungal ITS2 primers, and sequenced using Illumina MiSeq 2x300. The sequence data were sorted using Cutadapt and analyzed using the DADA2 pipeline.

Both $NaNO_3$ and BCM decreased ($p < 0.01$) CH_4 by 95% and 98%, respectively. $NaNO_3$ increased ($p < 0.01$) NH_3-N, pH, acetate and propionate but decreased ($p < 0.01$) butyrate without affecting ($p > 0.10$) total SCFA concentration. In contrast, BCM increased ($p < 0.01$) propionate and decreased ($p < 0.01$) acetate and total SCFA. Cultures receiving BCM had appreciably greater ($p < 0.01$) concentrations of gaseous H_2; no increase in gaseous H_2 was detected in cultures receiving $NaNO_3$ compared to control. $NaNO_3$ enriched microbes capable of nitrate/nitrite reduction and succinate/propionate production such as *Prevotella* spp., whereas BCM enriched *Megasphaera elsdenii* and *Desulfovibrio*. *Methanobrevibacter* were reduced in the BCM-treated culture compared to cultures receiving $NaNO_3$. Both $NaNO_3$ and BCM drastically reduced CH_4 and increased propionate; the abundance of *M. elsdenii* suggests acrylate pathway predominated with BCM induced inhibition of CH_4. The results offer key insights into microbial strategies to alter energy dispersion in the absence of methanogenesis as a primary H_2 sink.