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Impact of Crude Glycerin Supplementation on Rumen and Duodenal Microbial Populations in Forage Diets

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Summary

Seven ruminally and duodenally fistulated beef steers were fed 0%, 4%, 8%, and 12% crude glycerin in forage-based diets. Rumen and duodenal samples were used to determine the effects of crude glycerin on the prevalence of five selected species of ruminal bacteria. In the rumen, Anaerovibrio lipolytica and Selenomonas ruminantium increased while Butyrivibrio fibrosolvens, Fibrobacter succinogenes, and Megasphaera elsdenii were unaffected. In the duodenum, A. lipolytica, S. ruminantium, and B. fibrosolvens increased while F. succinogenes and M. elsdenii were unaffected. Changes in the relative abundance of microbial species may help explain animal performance responses to crude glycerin.

Introduction

In an effort to keep feed costs low, beef cattle producers often incorporate byproducts as feed ingredients into the beef cattle diet. The primary byproduct of biodiesel production is crude glycerin which has become more abundant with industry growth, at times making it a cost effective feed additive. Crude glycerin has been shown to increase performance when fed in moderate amounts. Decreases in the acetate to propionate ratio commonly reported with the addition of crude glycerin may have positive impacts on F:G. However, the impacts of crude glycerin on fiber digestion are unclear and changes in ruminal microbial populations may help understand this relationship. However changes in microbial community composition during glycerin supplementation are undocumented.

The objective of this study was to determine the effects of crude glycerin on the abundance of five species of rumen microbes that are known to influence energy metabolism and fiber digestion within the rumen. The five species investigated in this study were *Selenomonas ruminantium*, *Megasphaera elsdenii*, *Butyrivibrio fibrosolvens*, *Anaerovirbrio lipolytica*, and *Fibrobacter succinogenes*. Species were selected based on metabolic pathways and substrates utilized by these microbes. The lipid digester *A. lipolytica* was the major species of interest because of its ability to metabolize glycerol. It was hypothesized that this species would increase in abundance with increasing glycerin concentrations. Fiber digesters were included to investigate the influence of glycerin on fiber digestion.

Procedure

Seven ruminally and duodenally fistulated beef steers were used in a four diet, four period, row by column transformation with dietary treatments of 0%, 4%, 8%, and 12% glycerin. The basal diet consisted of wheat straw, soybean hulls, and soybean meal. Crude glycerin replaced soybean hulls and soybean meal increased with increasing concentration of crude glycerin to ensure sufficient nitrogen in the diet. Diets and digestion parameters from this study are reported elsewhere (*2016 Beef Report*, pp. 40–43).

Samples were collected from ruminal and duodenal cannulas 8 hours post-feeding on the last day of a 21-day period. Total DNA was extracted from rumen and duodenal samples and the microbial species abundance was quantified using quantitative realtime PCR with species-specific primers. Real-time assays were performed using the SYBR Green reporter assay and the relative fold change in the rumen and duodenum were calculated using the ΔΔ CT method relative to the control for each species. The 16S rRNA gene was used to normalize the data before fold change was calculated. Data were analyzed using

Table 1. Fold Change in Selected Ruminal Microbial Species in Response to Crude Glycerin

Species	Dietary crude glycerin inclusion				SE	*P*-value	
	0%	4%	8%	12%		linear	quadratic
A. lipolytica	1.00	3.73	15.67	13.69	0.81	< 0.01	0.18
B. fibrosolvens	1.00	0.54	1.41	0.86	0.77	0.83	0.91
M. elsdenii	1.00	1.59	5.35	2.02	1.18	0.27	0.52
F. succinogenes	1.00	1.04	3.77	1.52	0.49	0.31	0.30
S. ruminantium	1.00	1.91	18.47	21.44	1.12	< 0.01	0.74

Table 2. Fold Change in Selected Duodenal Microbial Species in Response to Crude Glycerin

Species	Dietary crude glycerin inclusion				SE	*P*-value	
	0%	4%	8%	12%		linear	quadratic
A. lipolytica	1.00	1.29	8.07	8.85	0.70	< 0.01	0.87
B. fibrosolvens	1.00	13.97	17.09	21.35	0.39	< 0.01	< 0.01
M. elsdenii	1.00	1.47	1.35	1.35	0.51	0.61	0.59
F. succinogenes	1.00	0.82	0.99	1.13	0.33	0.87	0.90
S. ruminantium	1.00	1.11	4.02	4.33	0.42	< 0.01	0.97

the mixed procedures of SAS. The model included glycerin concentration, animal, and period. Contrasts were developed to test linear and quadratic change in species abundance as glycerin inclusion increased.

Results

In the rumen, *S. ruminantium* linearly increased up to 21-fold at 12% glycerin supplementation (Table 1; $P = 0.02$) and *A. lipolytica* linearly increased up to 16-fold at 8% glycerin supplementation ($P < 0.01$). *F. succinogenes*, *B. fibrosolvens*, and *M. elsdenii* abundance did not change within the rumen ($P > 0.27$). In the duodenum, *S. ruminantium* and *A. lipolytica* linearly increased up to 4-fold (Table 2; $P < 0.001$) and up to 9-fold ($P < 0.001$) at 12% glycerin supplementation, respectively. *F. succinogenes* and *M. elsdenii* populations showed no significant change in the duodenum ($P > 0.86$). *B. fibrosolvens* increased quadratically up to 21-fold at 12% glycerin supplementation in the duodenum ($P < 0.001$).

An increase in *A. lipolytica* is indicative of an increase in propionate which could positively impact animal performance. An increase in *S. ruminantium* indicates an increase in lactate utilization within the rumen. Since there was no change in the abundance of *M. elsdeni* (a lactate utilizer), the data suggest that the primary metabolic pathway being utilized for lactate utilization is the succinate pathway. An insignificant effect on *F. succinogenes* suggests that fiber digestion is not affected or may not be negatively affected by an increase in dietary glycerin. Overall, the data suggest that crude glycerin inclusion at moderate levels in the beef cattle diet may have positive effects on feed efficiency because of the increase in propionate producing microbial species.

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