Prior Adaptation Improves Crude Glycerin Utilization by Cattle

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Introduction

Crude glycerin has increased in availability as a feedstock for cattle as a result of expansion of the biodiesel industry in the United States. This byproduct, when ingested by cattle, is fermented by ruminal bacteria to yield volatile fatty acids that are used as sources of energy by cattle. The primary component of crude glycerin is glycerol, and the fermentation of glycerol is carried out by specific populations of microorganisms. Anecdotal observations from our previous research with crude glycerin in feedlot cattle have suggested that a period of adaptation may be necessary to achieve optimal utilization of the byproduct. Our objective in this study was to evaluate this adaptive response by measuring *in vitro* digestion by ruminal microbes that were obtained from cattle fed diets with or without added glycerin.

Experimental Procedures

Four ruminally cannulated Holstein steers were placed into individual pens in an indoor barn at the Kansas State University Beef Cattle Research Center. Animals were assigned to two finishing diets, which contained either 0 or 15% crude glycerin (100% dry matter basis). Diets were composed of 10% corn silage, corn gluten feed, soybean hulls, dry-rolled corn, and a vitamin mineral supplement (Table 1). After 14 days of adaptation, ruminal contents were collected from each animal, strained through 8 layers of cheese cloth, placed into a preheated thermos, and transported to the Pre-harvest Food Safety Laboratory. The strained ruminal fluid was placed into a large separatory funnel, sparged with carbon dioxide gas, and placed into a 98°F room for 40 minutes and allowed to stratify into layers. The clarified liquid layer was mixed 1:2 with McDougall's buffer and used as the initial microbial inoculums for *in vitro* cultures.

To measure total fermentative activity and composition of fermentative gasses, total mixed rations and buffered ruminal fluid were placed into fermentation flasks equipped with pressure sensitive membranes and radio frequency transmitters that recorded volume of fermentative gasses at 5-minute intervals. Two flasks containing the buffered microbial inoculum without substrate were included as negative controls. The energy substrates used in the remaining flasks consisted of the total mixed rations shown in Table 1, which contained 0 or 15% crude glycerin. The different diet substrates were added to flasks containing buffered inoculums from adapted steers, as well as from steers that had not been previously adapted to glycerin. Samples of fermentative gasses were removed from the head space of each sealed flask after 0 and 24 hours of incubation to determine concentrations of carbon dioxide and methane using a gas chromatograph. At the end of the 24-hour fermentation, contents of the flasks were chilled in an ice bath to terminate microbial activity. The contents were centrifuged, and a 4-mL sample of supernatant was combined with 1 mL of metaphosphoric acid and used to characterize concentrations of major volatile fatty acids.

To measure *in vitro* dry matter disappearance, 30 mL of buffered ruminal contents from cattle fed diets without added glycerin were added to fermentation tubes. An aliquot of the control diet (no glycerin) was added to a portion of the tubes, and the diet containing 15% glycerin was added to others. The process was repeated using ruminal contents from animals previously adapted to diets containing glycerin. Tubes were gassed with carbon dioxide and placed into a 98°F incubator. The tubes were gently swirled every 3 hours during the 24-hour incubation period. At the end of the incubation period, the tubes were chilled and then centrifuged at 30,000 × g for 20 minutes. The clear fluid supernatant was discarded from each tube, and the remaining pellet was dried overnight 221°F. Weight of the resulting residue was measured and subsequently used to determine *in vitro* dry matter disappearance.

The study was arranged as a 2 × 2 factorial, with factor 1 as the diet to which steers were adapted, and factor 2 as type of substrate added to cultures. Data were analyzed using the MIXED procedure of SAS (SAS Institute, Cary, NC).

Results and Discussion

Figure 1 illustrates the effects of donor animal diet and *in vitro* substrate on the disappearance of dry matter (an estimate of digestion) from our cultures. There was a tendency for an interaction ($P = 0.06$) between diet of the donor animals and substrate used during the fermentation. When glycerin was added to *in vitro* cultures containing ruminal inoculum from unadapted animals, digestion was mildly depressed, but digestibility increased slightly when glycerin was added to cultures containing ruminal inoculum from adapted animals. This result supports our hypothesis that prior adaptation is needed to optimize glycerin utilization and is consistent with anecdotal observations of the same from previous feedlot cattle experiments. Total production of volatile fatty acids (Table 2) was not influenced by diets of the donor animals or by substrate utilized in the *in vitro* fermentations. Substrate utilized in the *in vitro* fermentations, but not diet of donor animals, increased production of both propionate and butyrate ($P < 0.01$). Increases in propionate production with glycerol supplementation are consistent with observations from previous experiments, and generally are viewed as energetically favorable. Increases in butyrate also may be viewed as beneficial, because butyrate is the principal energy substrate used by epithelial cells lining the rumen. Production of fermentative gasses (Table 3), including methane and CO_2, was largely unaffected by diets of donor animals or substrate used in the *in vitro* fermentations ($P > 0.10$).

Implications

This study suggests that prior adaptation may result in modest improvements in digestion of diets containing glycerin.

Table 1. Composition of experimental diets (100% dry matter basis)

	Treatments	
Ingredient, % of dry matter	Control	15% glycerin
Corn silage	10	10
Dry-rolled corn	31.0	13.3
Corn gluten feed	35	35
Soybean hulls	20	20
Supplement[1]	4.0	6.7
Crude glycerin	0	15
Nutrient analyses, %		
Dry matter	65.7	65.7
Crude protein	14.5	14.5
Neutral detergent fiber	37.2	35.9
Ether extract	3.03	2.30
Calcium	0.70	0.70
Phosphorus	0.53	0.50
Potassium	0.70	0.70

[1]Formulated to provide 0.3% salt, 0.1 ppm Co, 10 ppm copper, 0.6 ppm iodine, 60 ppm manganese, 0.25 ppm selenium, 60 ppm zinc, 1,000 IU/lb vitamin A, and 10 IU/lb vitamin E in the total diet on a 100% dry matter basis.

Table 2. Production of volatile fatty acids by *in vitro* cultures containing ruminal contents from adapted and unadapted steers when cultures were fed substrates consisting of diets containing 0 or 15% crude glycerin

	Unadapted donor		Adapted donor		*P*-value[a]		
Item, mM	Control	Glycerin	Control	Glycerin	Diet	Substrate	D × S
Total volatile fatty acids	24.2	25.2	12.4	27.9	0.47	0.20	0.26
Acetate	18.3	12.4	6.9	13.3	0.29	0.95	0.22
Propionate	4.2	7.7	3.5	9.8	0.54	<0.01	0.23
Butyrate	1.6	4.1	1.8	3.7	0.91	<0.01	0.70

[a] Diet refers to the finishing diet fed to donor animals; substrate refers to the diet that was included in the *in vitro* fermentations; D × S refers to the interaction between diet of the donor animal and substrate fed to the *in vitro* culture.

Table 3. Composition of fermentative gasses produced by *in vitro* cultures containing ruminal contents from adapted and unadapted steers when cultures were fed substrates consisting of diets with 0 or 15% crude glycerin

	Unadapted donor		Adapted donor		*P*-value[a]		
Item, mL	Control	Glycerin	Control	Glycerin	Diet	Substrate	D × S
Total gas	107.7	108.3	83.4	88.9	0.16	0.84	0.87
Methane	5.0	4.7	5.9	7.3	0.11	0.61	0.45
Carbon dioxide	29.3	25.7	18.0	21.2	0.24	0.98	0.61

[a] Diet refers to the finishing diet fed to donor animals; substrate refers to the diet that was included in the *in vitro* fermentations; D × S refers to the interaction between diet of the donor animal and substrate fed to the *in vitro* culture.

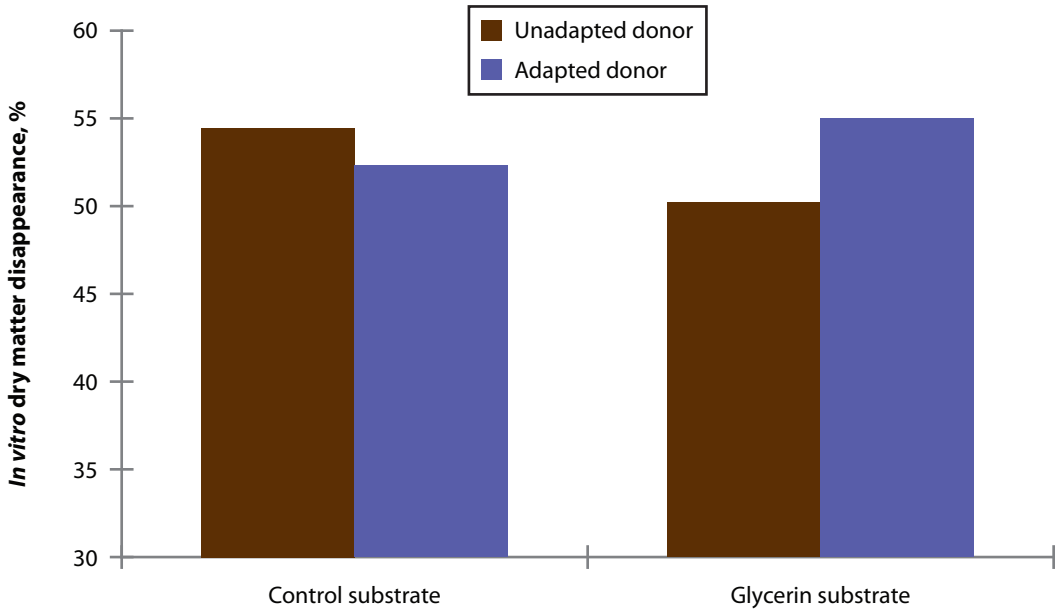

Figure 1. Percentage *in vitro* dry matter disappearance (24-hour incubation) from cultures containing ruminal microorganisms extracted from donor steers fed diets with or without added glycerin. Substrates fed to the *in vitro* cultures consisted of finishing diets containing 0 or 15% crude glycerin. SEM = 2.5. Interaction between donor diet and *in vitro* substrate, *P* = 0.06; effect of donor diet, *P* = 0.48; effect of *in vitro* substrate, *P* = 0.86.