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Effects of differing levels of glycerol on rumen fermentation and bacteria

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ABSTRACT

The effects of substituting corn with glycerol as a feed alternative were investigated using continuous fermenters. Four fermenters were used in a 4 × 4 Latin square design with four 10 days consecutive periods. Treatments diets contained 0 (T1), 36 (T2), 72 (T3) and 108 (T4) g glycerol/kg dry matter (DM). Diets consisted of 600 g/kg alfalfa hay and 400 g/kg concentrate (DM basis) and glycerol replaced the corn in the concentrate. Effluents were collected from each fermenter during the last 3 days of each period and analyzed for nutrients composition. On day 10 of each period, additional samples were collected from each fermenter at 3 h after the morning feeding and analyzed for volatile fatty acids (VFA), ammonia-nitrogen (NH_3-N), and microbial DNA concentration. Results showed that neutral detergent fiber (aNDFom) digestibility decreased (P<0.05) with the T3 and T4 diets compared with the T1 diet. Glycerol substitution had no effects on fermenters pH, NH_3-N concentration, and digestibility coefficients of DM and acid detergent fiber (ADFom). The molar proportion for acetate decreased (P<0.05) while the molar proportions for butyrate, valerate and isovalerate increased (P<0.05) with the glycerol diets compared with the T1 diet. The DNA concentrations for *Butyrivibrio fibrisolvens* and *Selenomonas ruminantium* decreased (P<0.05) with the T3 and T4 diets compared with the T1 diet. The DNA concentration for *Clostridium proteoclasticum* also decreased (P<0.05) with glycerol substitution. No differences in the DNA concentrations for *Ruminococcus albus* and *Succinivibrio dextrinosolvens* among diets were observed. Results from this study suggest that substituting corn with glycerol at low level had no adverse affects on fermentation, digestion or ruminal bacteria. Higher substitution levels, however, may adversely affect rumen fermentation through reducing fiber digestion, acetate production and bacterial populations.

1. Introduction

Governmental encouragement of a biofuels economy has strongly impacted not only crop production agriculture, but also animal production agriculture. The increase in biofuel production in the United States has resulted in higher market prices for corn and other feedstuffs and increased feed costs for livestock producers. A result of this is that both the increase in corn prices and the reduction of availability of corn for animal feed have resulted in the search for alternate feed sources. Glycerol

Abbreviations: ADFom, acid detergent fiber; CP, crude protein; DM, dry matter; FA, fatty acid; aNDFom, neutral detergent fiber; NH_3-N, ammonia-nitrogen; OM, organic matter; PCR, polymerase chain reaction; VFA, volatile fatty acid.

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Table 1
Ingredients, chemical, and fatty acids composition of treatment diets.

Ingredient (g/kg DM)	Treatments[a]				
	T1	T2	T3	T4	MSE
Alfalfa hay	600	600	600	600	
Corn, ground	240	204	168	132	
Glycerol[b]	0	36	72	108	
Soybean meal, 48% CP	94	100	108	118	
Soy hulls	60	54	46	36	
Limestone	3	3	3	3	
Mineral mix[c]	3	3	3	3	
Chemical composition (g/kg DM)					
DM (g/kg diet)	875	880	874	867	
CP	162	158	161	161	
ADFom	297	298	286	274	
aNDFom	423	416	387	379	
Ether extract	31.4	29.4	27.3	25.2	
Ca	10.3	10.3	10.3	10.2	
P	3.0	3.0	2.9	2.8	
Mg	2.4	2.3	2.3	2.3	
Fatty acids composition (mg/g DM)					
C16:0	2.29	2.56	2.48	2.45	
C18:0	0.46	0.46	0.51	0.45	
C18:1 *c9*	2.17	1.76	1.43	1.37	
C18:2 *c9c12*	5.16	4.47	4.11	3.64	
C18:3 *c9c12c15*	0.59	0.68	0.75	0.79	

[a] T1 = no glycerol (control); T2 = 36 g glycerol/kg DM; T3 = 72 g glycerol/kg DM; T4 = 108 g glycerol/kg DM.
[b] Grade glycerol (995 ml/L).
[c] Contained (g/kg): NaCl (955 to 9.8), Zn (10.0), Mn (7.5), Fe (6.0), Mg (0.5), Cu (0.32), I (0.28), and Co (0.11).

is a by-product of base-catalyzed transesterification of oil in the formation of methyl and ethyl fatty acid (FA) esters in the production of biodiesel. Glycerol is a carbohydrate molecule ($C_3H_8O_3$) with a net energy concentration of 1.98–2.29 Mcal/kg which is approximately equal to the energy contained in corn starch (Schröder and Südekum, 1999).

The use of glycerol in livestock diets, ruminants in particular, is not novel; however, this interest has been renewed due to the increased availability and favorable pricing of glycerol. Although the effects of feeding glycerol to ruminant animals either as an energy supplement (Fisher et al., 1973; DeFrain et al., 2004; Bodarski et al., 2005) or as a substitute for corn (Donkin et al., 2007; Culp et al., 2009; Parsons et al., 2009) on performance of animals and feed intake are well documented, little is known about the effects of glycerol on fermentation pattern and microbes in the rumen. Wang et al. (2009) reported linear increases in rumen propionate and butyrate molar proportions and a linear reduction in acetate to propionate ratio with increasing glycerol doses (100, 200 and 300 g/head/day) in steers' diet. In contrast, rumen molar proportions for acetate, propionate and butyrate were unaffected by feeding glycerol to beef cattle at levels up to 120/kg of concentrate DM (Mach et al., 2009). Roger et al. (1992) reported that the addition of glycerol at 0.05 (v/v) to the *in vitro* media greatly inhibited the growth and cellulolytic activity of rumen bacteria and fungi. Additionally, Paggi et al. (2004) reported that the cellulolytic activity of ruminal extract was reduced as glycerol concentration in rumen cultures increased from 50 to 300 mM. The main objectives of this study were to measure the effects of substituting corn with glycerol at different levels on fermentation and DNA concentration of selected rumen bacteria using continuous fermenters.

2. Materials and methods

2.1. Experimental protocol

Four dual-flow continuous culture systems, as described by Teather and Sauer (1988), were used in 4 × 4 Latin square design with 4 periods of 10 days each (first 7 days for adaptation and last 3 days for samples collection). Glycerol was included in the treatment diets at 0 (T1), 36 (T2), 72 (T3) and 108 (T4) g/kg (DM basis) substituting 0.0, 0.15, 0.30 and 0.45 of the corn, respectively. Treatment diets (60:40 forage:concentrate ratio; DM basis) were fed at 45 g/day (DM basis) in three equal portions at 0800, 1500 and 2400 h. The forage consisted of alfalfa pellets while the concentrate mix contained corn, soybean meal, soy hulls, minerals and vitamins (Table 1). Grade glycerol (995 ml/L; Sigma–Aldrich Chemical Company St. Louis, MO) was used in this study to avoid any impact of potential contaminates found in crude glycerol.

2.2. Continuous culture

Two ruminally fistulated Holstein cows fed a total mixed ration (60:40 forage:concentrate ratio; DM basis) were used for collection of ruminal contents. Whole ruminal contents were collected 2–4 h after the morning feeding, transferred to the laboratory in sealed bags, mixed, and then strained using a double-layered cheese cloth. Approximately 600 mL of the

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ruminal fluid was added to each of the four fermenters, containing 100 mL of prewarmed buffer. Anaerobic conditions in fermenters were maintained by infusing CO_2 at 40 mL/min. Cultures were stirred (BCD Caframo Stirrer, Fisher, St. Louis, MO, USA) continuously at 45 rpm and fermenter pH was measured daily before addition of feed using a portable pH meter at 0800, 1500 and 2400 h. Fermenter temperature was maintained at 39 °C using a circulating water bath. Buffer was delivered continuously at a flow rate of 1.16 mL/min (0.10 h^{-1} liquid dilution rate), using a precision pump. Flow rate of each fermenter was recorded every day at 08:00.

2.3. Sample collection and analysis

Starting on days 7, 8 and 9 of each period, the overflow (effluent) was collected into 2 L plastic flasks, approximately 3/4th immersed into ice. Collected effluents were homogenized by stirring and approximately 0.25 (v/v) subsamples were pooled into one sample and stored at −80 °C until further analysis. Effluent samples were thawed in a 50 °C water bath and then centrifuged (Beckman J2-21, GMI, Inc. Ramsey, MN, USA) in 250 mL plastic bottles at 500 × *g* for 5 min. The supernatant was discarded, and to the sediment, more samples was added and centrifuged again. Finally the remaining sediments were freeze dried for at least 48 h and ground through 1 mm screen Wiley mill. Dry matter of TMR was determined by drying at 105 °C for 48 h (AOAC, 1990; method 930.15). Samples were analyzed for CP (method 976.05), ether extract (method 920.39), and ash (method 942.05) according to AOAC methods (2000). The neutral detergent fiber (aNDFom) was determined using the (Van Soest et al., 1991) procedure. The heat stable amylase and sodium sulphite were used to determine aNDFom. The acid detergent fiber (ADFom) content was determined according to AOAC (1990; method 973.18). The neutral detergent fiber and ADFom values were measured on organic matter (OM) basis. Treatment diets and effluent samples were methylated using the sodium methoxide ($NaOCH_3$) and HCl two step procedures as outlined by Kramer et al. (1997) and analyzed in duplicate for fatty acids (FA) as described by AbuGhazaleh and Jacobson (2007).

On day 10 of each period, samples were collected from each fermenter at 3 h post morning feeding for VFA, ammonia-nitrogen (NH_3-N) and bacterial analysis. Before collection of samples, the fermenter speed was increased to 190–200 rpm to ensure thorough mixing. Samples for VFA analyses were prepared and analyzed as described by Jenkins (1987), using 2-ethylbutyric acid as an internal standard. Ammonia–N samples were centrifuged at 2000 × *g* at 4 °C for 10 min and the supernatant was acidified with 0.5 ml of 0.1 N HCl then analyzed for NH_3-N by a TECO DIAGNOSTICS KIT (Anaheim, CA, USA) using a spectrophotometer (Thermo Spectronic Genesys 5 Spectrophotometer, Artisan Scientific, Champaign, IL, USA).

Bacterial samples (10 mL) collected from fermenters were frozen immediately in liquid nitrogen and stored at −80 °C to await bacterial analysis. Samples were thawed and centrifuged at 500 × *g* for 15 min to remove liquid associated bacteria, and the supernatant was discarded. To remove any trapped liquid associated bacteria, approximately 4 mL of phosphate buffered saline was then added to sediment, centrifuged again at 500 × *g* for 15 min, and the supernatant was discarded. The DNA from pellets, containing the solid-associated bacteria, was then extracted using the MO BIO Ultraclean™ Microbial DNA isolation kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA). Concentrations of DNA were measured by BioPhotometer (Eppendorf Scientific, Inc., NY, USA).

The five rumen bacteria targeted in this study were purchased from DSMZ (German Resource Center for biological material, Braunschweig, Germany) or ATCC (The Global Bioresource Center, Manassas, VA, USA). *Butyrivibrio fibrisolvens* (DSM 3071), *Ruminococcus albus* (DSM 20455), *Selenomonas ruminantium* (DSM 2150), and *Succinivibrio dextrinosolvens* (DSM 3072) were obtained from DSMZ. *Clostridium proteoclasticum* was obtained from ATCC (ATCC 51982). Primers used in this study are shown in Table 2. Primer pair for total bacteria was described by Denman and McSweeney (2006). Primer pairs for *S. ruminantium,* and *S. dextrinosolvens* were as described by Tajima et al. (2001). Primer pair for *C. proteoclasticum* was found online (using IDT, Integrated DNA Technologies). Primer pairs for *B. fibrisolvens* and *R. albus* were designed online through BLAST program. Sequence region specific for a given species (with 0.97 similarity) were found online using the BLAST family programs in GenBank to ensure the specificity of primers. These primers were also tested for the requirements imposed by real-time quantitative PCR.

Table 2
PCR primers for detection of selected ruminal bacteria.

Target bacterium	Primer sequences (5′–3′)		Annealing temp. (°C)	Product size (bp)
Total bacteria	Forward	CGGCAACGAGCGCAACCC	60	130
	Reverse	CCATTGTAGCACGTGTGTAGCC		
Butyrivibrio fibrisolvens	Forward	TAACATGAGTTTGATCCTGGCTC	62	136
	Reverse	CGTTACTCACCCGTCCGC		
Ruminococcus albus	Forward	GTTTTAGGATTGTAAACCTCTGTCTT	60	270
	Reverse	CCTAATATCTACGCATTTCACCGC		
Selenomonas ruminantium	Forward	TGCTAATACCGAATGTTG	54	513
	Reverse	TCCTGCACTCAAGAAAGA		
Succinivibrio dextrinosolvens	Forward	TGGGAAGCTACCTGATAGAG	57	854
	Reverse	CCTTCAGAGAGGTTCTCACT		
Clostridium proteoclasticum	Forward	TCCTAGTGTAGCGGTGAAATG	62	188
	Reverse	TTAGCGACGGCACTGAATGCCTAT		

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Table 3
Effect of substituting corn with glycerol on nutrient digestibility.

	Treatments[a]				
	T1	T2	T3	T4	MSE
DM	0.536	0.543	0.540	0.519	1.54
aNDFom	0.386[a]	0.389[a]	0.334[b]	0.319[b]	2.79
ADFom	0.295	0.298	0.246	0.251	3.38

Values within rows with different superscript letters (a and b) are significantly different (P<0.05).
[a] T1 = no glycerol (control); T2 = 36 g glycerol/kg DM; T3 = 72 g glycerol/kg DM; T4 = 108 g glycerol/kg DM.

The five purchased pure bacteria were grown on specific media in our laboratory in Hungate tubes as specified by DSMZ and ATCC. Tubes were incubated for 3–6 days at 37 °C to allow bacterial growth. Cultures were transferred to fresh medium from incubated tubes for 2 or 3 times to avoid dead cells. Approximately 1 mL of the culture was used to extract DNA using the MO BIO DNA isolation kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA). Dilutions of purified genomic DNA from the pure bacteria were used to construct specific calibration curves.

Individual species-specific real time quantitative PCR (qPCR) was performed using Bio-Rad iCycler MyiQ single color real-time PCR detection system (Bio-Rad Laboratories, Inc., Hercules, CA, USA), using fluorescence detection of SYBR green mix. Briefly 12.5 μL SYBR green mix, 2 μL of each primer, sample DNA (starting concentration; Table 2), and RNAse free water were added to make a total volume of 25 μL. Amplification involved one cycle at 95 °C for 10 min for initial denaturation and then 40 cycles of 95 °C for 30 s followed by annealing at the temperatures shown in Table 2 for 30 s and then at 72 °C for 1 min. Detection of the fluorescent product was set at the last step of each cycle. Standard curves, DNA sample quantification and melting curve analyses were obtained using iQ5 Optical System Software (version 2.1, Bio-Rad Laboratories, Inc., Hercules, CA, USA). Melting curve analysis was performed after each amplification step to determine the specificity of PCR product. The melting curve was obtained by slow heating with a 0.1 °C/s increment from 65 °C to 95 °C, with fluorescence collection at 0.1 °C intervals. Samples were amplified in triplicate along with dilution standards of known bacterial DNA concentrations. Samples and standards were assayed on the same plate to allow for the relative quantification of bacterial DNA present in each sample.

2.4. Statistical analysis

Data was analyzed as a 4 × 4 Latin square design using the PROC MIXED of SAS (SAS Institute, Inc., Cary, NC). The statistical model included: fermenters, diet, and period. Fixed effects were diet and period. Random effect was fermenter. Results are expressed as least square means with standard error of the means. The significance threshold was set at P<0.05.

3. Results

The ingredients, chemical and FA composition of the treatment diets are presented in Table 1. Dietary ADFom, aNDFom, ether extract, oleic acid (C18:1*c*9) and linoleic acid (C18:2*c*9*c*12) content decreased as proportion of glycerol in diets increased due to feeding less soy hulls and corn. Dietary CP, Ca, P and Mg content were similar among treatment diets. The effects of the treatment diets on nutrient digestibility are presented in Table 3. Substituting glycerol for corn had no effect on DM digestibility, however, feeding glycerol at 72 (T3) and 108 (T4) g/kg DM reduced (P<0.05) aNDFom digestibility and tended (P<0.12) to reduce ADFom digestibility when compared with the T1 diet.

The effect of the treatment diets on fermentation is presented in Table 4. No differences were found on fermenters' pH, NH_3-N concentration, or molar proportion of propionate. The molar proportion for acetate decreased (P<0.05) with feeding

Table 4
Effect of substituting corn with glycerol on fermenters pH, ammonia–N and volatile fatty acids (mol/100 mol).

	Treatments[a]				
	T1	T2	T3	T4	MSE
pH	6.53	6.54	6.57	6.53	0.026
NH_3-N, (mg/dl)	9.63	8.53	8.69	9.47	0.938
Acetate	43.44[a]	39.6[b]	37.83[b]	33.86[c]	2.087
Propionate	26.91	26.06	27.38	28.55	1.499
Acetate:propionate	1.67[a]	1.53[a,b]	1.40[b,c]	1.22[c]	0.152
Butyrate	21.11[b]	25.06[a]	25.91[a]	26.96[a]	1.221
Isobutyrate	1.15[a]	1.01[a]	1.15[a]	1.04[b]	0.061
Valerate	4.22[c]	4.77[b]	5.16[b]	6.13[a]	0.321
Isovalerate	3.18[b]	3.42[a]	3.57[a]	3.45[a]	0.113
Total VFA (mM)	75.05[b]	74.56[b]	77.79[a,b]	80.96[a]	2.581

Values within rows with different superscript letters (a, b and c) are significantly different (P<0.05).
[a] T1 = no glycerol (control); T2 = 36 g glycerol/kg DM; T3 = 72 g glycerol/kg DM; T4 = 108 g glycerol/kg DM.

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Table 5
Effect of substituting corn with glycerol on the DNA concentration (pg) of selected rumen bacteria.

	Treatments[a]				
	T1	T2	T3	T4	MSE
Total bacteria	28.9[a]	32.3[a]	33.4[a]	19.7[b]	4.32
Butyrivibrio fibrisolvens	110.0[a]	97.2[a,b]	78.1[b,c]	58.6[c]	10.21
Selenomones ruminantium	2.5[a]	2.1[a]	0.6[b]	0.5[b]	0.32
Ruminoccocus albus	27.2	21.1	23.9	17.5	6.59
Clostridium proteoclasticum	1.8[a]	1.1[b]	1.0[b]	0.9[b]	0.23
Selenomonas dextrinosolvens	9.0E−3	6.9E−3	10.0E−3	7.8E−3	1.79E−03

Values within rows with different superscript letters (a, b and c) are significantly different (P<0.05).
[a] T1 = no glycerol (control); T2 = 36 g glycerol/kg DM; T3 = 72 g glycerol/kg DM; T4 = 108 g glycerol/kg DM.

glycerol and was the lowest with the T4 diet. The acetate to propionate ratio decreased (P<0.05) with the T3 and T4 diets relative to the T1 diet. The molar proportion for isobutyrate decreased (P<0.05) only with the T4 diet. Compared with the T1 diet, the molar proportions for butyrate and isovalerate increased (P<0.05) with feeding glycerol but were similar among the glycerol diets. The molar proportion for valerate also increased (P<0.05) with feeding glycerol, relative to the T1 diet, and was highest with the T4 diet. Total VFA concentration was highest with the T4, relative to the T1 diet, but similar among other diets.

The effects of substituting glycerol for corn on the DNA concentration of selected ruminal bacteria are presented in Table 5. The glycerol supplemented did not impact the DNA concentrations of *R. albus* and *S. dextrinosolvens*. Relative to the T1 diet, the DNA concentrations for *S. ruminantium* and *B. fibrisolvens* decreased (P<0.05) with the T3 and T4 diets. The DNA concentrations for *C. proteoclasticum* decreased (P<0.05) with feeding glycerol but were similar among the glycerol diets.

The effects of the treatment diets on effluent FA composition are presented in Table 6. With the exception for C18:1 *trans,* C18:1*c*9 and C18:2 *c*9*c*12, substituting glycerol for corn had no effects on effluent FA composition. Compared with the T1 diet, the proportions (g/100 g FA) of C18:1 *trans* decreased (P<0.05) with the T3 and T4 diets. The proportions of C18:1*c*9 and C18:2 *c*9*c*12 in effluents decreased (P<0.05) with glycerol feeding but were similar (P>0.05) among the glycerol diets.

4. Discussion

The reduction in aNDFom digestibility seen in this study with substituting glycerol for corn at high levels (T3 and T4) is consistent with the findings of Paggi et al. (2004) who found a 0.07 and 0.17 reduction in carboxymethylcellulose digestibility when glycerol concentrations in ruminal cultures were increased from 50 to 200 and 300 mM, respectively. The reduction in aNDFom digestibility in this study may have resulted from glycerol effect on rumen microbes (discussed below). Roger et al. (1992) showed that the growth, adhesion and cellulolytic activity of two ruminal cellulolytic species were inhibited when glycerol was included in cultures at high concentration (0.05; v/v) but not at low concentration (<0.01; v/v). Paggi et al. (2004) also showed that the cellulolytic activity of ruminal extract was reduced as glycerol concentration in culture increased.

Substituting glycerol for corn in the diet altered rumen fermentation pattern toward more butyrate, valerate and isovalerate and less acetate. The reduction in the molar proportion of acetate and acetate to propionate ratio was consistent with the reduction in aNDFom digestibility with the T3 and T4 diets. Studies that have reported reduction in aNDFom digestibility have also reported reductions in acetate concentration and acetate to propionate ratio (Ribeiro et al., 2005; Castillejos et al., 2006). Surprisingly, despite the reduction in starch availability, as a result of substituting glycerol for corn, the molar proportion for propionate was not affected. Previous studies have showed that glycerol is mostly fermented into

Table 6
Effect of substituting corn with glycerol on fermenters fatty acids concentration (g/100 g FA).

Fatty acid	Treatments[a]				
	T1	T2	T3	T4	MSE
C16:0	17.21	16.92	16.66	16.52	0.581
C16:1*t*	0.19	0.19	0.24	0.23	0.038
C16:1*c*	0.27	0.26	0.33	0.34	0.058
C17:0	0.69	0.69	0.67	0.78	0.057
C17:1	0.23	0.21	0.25	0.20	0.046
C18:0	28.61	27.64	27.42	28.83	1.580
C18:1*trans*	11.03[a]	10.46[a,b]	9.95[b,c]	9.20[c]	0.527
C18:1 *c*9	6.29[a]	5.27[b]	4.91[b]	4.62[b]	0.396
C18:2 *c*9*c*12	6.37[a]	4.96[b]	4.94[b]	4.59[b]	0.611
C18:3 *c*9*c*12*c*15	1.32	1.22	1.23	0.98	0.218

Values within rows with different superscript letters (a, b and c) are significantly different (P<0.05).
[a] T1 = no glycerol (control); T2 = 36 g glycerol/kg DM; T3 = 72 g glycerol/kg DM; T4 = 108 g glycerol/kg DM.

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propionate (Garton et al., 1961; Bergner et al., 1995). Additionally, drenching cows with 1 kg of glycerol (Linke et al., 2004) or supplementing steers with glycerol (200 or 300 g/d; Wang et al., 2009) have been shown to increase rumen propionate relative to control (no glycerol). The increase in propionate formation from the fermented glycerol used in this study may have offset any reduction in propionate. The increase in the molar proportion of butyrate with glycerol substitution level is consistent with the findings of others (Remond et al., 1993; Wang et al., 2009) who reported that the molar proportion of butyrate in the VFA mixture increased at the expense of acetate when glycerol was supplemented at increasing levels. Additionally, Czerkawski and Breckenridge (1972) showed that propionate and butyrate were the primary short-chain FA of glycerol fermentation. The increase in molar proportions of valerate and isovalerate with increased glycerol substitution suggests their formation during glycerol fermentation.

The five bacteria tested in this study were chosen because on their role in the degradation of fiber (*B. fibrisolvens*, *R. albus*) starch and sugars (*S. ruminantium*, *S. dextrinosolvens*) and protein (*C. proteoclasticum*) in the rumen (Tajima et al., 2001; Russell, 2002). Among the five bacteria examined, only three appear to be affected by glycerol substitution, particularly at higher substitution levels. The DNA concentration of *B. fibrisolvens* was reduced with theT3 and T4 diets indicating that high levels of glycerol affected their growth. The reduction in the DNA concentration for *B. fibrisolvens* with the T3 and T4 diets corresponded with the reduction in aNDFom digestibility seen with these diets. Although the mechanism is not unknown, glycerol may have interfered with *B. fibrisolvens* adhesion to feed particles making nutrients less accessible to the bacterial cells. Adhesion to feed particles is the preliminary stage in rumen microbial degradation (Akin, 1979; Cheng et al., 1984). Roger et al. (1992) reported that adhesion, growth and cellulolytic activity for two rumen cellulolytic bacteria (*Ruminococcus flavefaciens* and *Fibrobacter succinogenes*) were reduced when glycerol was included in growth medium at 0.05 (v/v). Interestingly, the DNA concentrations of *R. albus* were not affected by glycerol substitution indicating that *R. albus* is less sensitive than *B. fibrisolvens* to the inhibitory effect of glycerol at the tested levels. Sensitivity of rumen microbes to glycerol may also depend on the level of inclusion. Roger et al. (1992), for example reported that including glycerol in the growth medium at 0.02 (v/v) slowed the growth (increased lag phase) of *F. succinogenes* but not *R. flavefaciens*. Additionally, they found that although the growth of *R. flavefaciens* and *F. succinogenes* were not affected when glycerol was included in medium at 0.005 (v/v), the growth of the *Neocallimastix frontalis*, fungi was strongly inhibited. Although the DNA concentration for *R. albus* was not affected by glycerol substitution at high levels, it's still also possible that glycerol decreased the capacity of *R. albus* to degrade fiber rather than the bacteria as such by modifying bacteria cell membrane permeability and therefore affecting bacterial cellulolytic enzymatic activities.

S. ruminantium and *S. dextrinosolvens* are non-fibrolytic bacteria that ferment soluble carbohydrates in the rumen (Tajima et al., 2001). *S. ruminantium* was reported to be the predominant bacterium present in continuous cultures when incubated along with other cellulolytic bacteria (Chen and Weimer, 2001). The DNA concentration for *S. ruminantium* was lower with the T3 and T4 diets relative to the T1 and T2 diets. The reduction in the DNA concentration for *S. ruminantium* with the high substitution levels may have resulted from either the reduction in starch and sugars availability with these diets as a result of corn replacement and/or adhesion of *S. ruminantium* to feed particles. Although the DNA concentration for *C. proteoclasticum* was lower with glycerol diets relative to control, *C. proteoclasticum* appears to be less sensitive to glycerol level of inclusion.

The reduction in *trans* C18:1 concentration found with substituting glycerol for corn at high levels (T3 and T4) is consistent with the low oleic acid and linolenic acid content with these diets. *Trans* C18:1 are produced in the rumen as intermediates during the biohydrogenation of dietary C18 unsaturated FA by rumen microorganisms (Harfoot and Hazlewood, 1988; AbuGhazaleh et al., 2005). Relative to the T1 diet, the intake of oleic acid was reduced by 0.34 and 0.37 whereas the intake of linoleic acid was reduced by 0.20 and 0.29 with the T3 and T4 diets, respectively. The reduction in *trans* C18:1 concentration with glycerol substitution may be also explained in part by the changes in rumen bacterial population, *B. fibrisolvens* and *C. proteoclasticum* in particular, seen with glycerol substitution. Recent studies have demonstrated that these two bacteria play a fundamental and central role in the rumen biohydrogenation process (Wallace et al., 2006; Maia et al., 2007; Paillard et al., 2007). Maia et al. (2007) identified *B. fibrisolvens* as the most important cellulolytic bacteria involved in the process of biohydrogenation and *trans* FA formation in the rumen.

5. Conclusion

Substituting glycerol for corn resulted in no changes ($P>0.05$) in fermenters pH, NH_3-N concentration, propionate molar proportion and DM digestibility. The molar proportion for acetate decreased while the molar proportions for butyrate, valerate and isovalerate increased with glycerol substitution. Including glycerol in the diet at 72 and 108 g/kg DM reduced aNDFom digestibility, acetate to propionate ratio, the DNA concentration for *B. fibrisolvens* and *S. ruminantium*. These results suggest that substituting corn with glycerol at low level had no adverse affects on fermentation, digestion or ruminal bacteria. Higher substation levels may adversely affect fiber digestion and bacterial population and negatively impact acetate production.

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