Effects of feeding different amounts of supplemental glycerol on ruminal environment and digestibility of lactating dairy cows

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ABSTRACT

A replicated 3×3 Latin square study was conducted to evaluate the effects of replacing a portion of ground corn in the diet with dietary glycerol on rumen environment, blood metabolites, and nutrient digestibility. Six rumen-cannulated Holstein cows, averaging 56 ± 18 DIM and 38.0 ± 8.2 kg of milk/d, were used in the study. Experimental periods included 3 wk for treatment adjustment period followed by 1 wk for data collection. Diets were corn silage based and balanced to be isocaloric and isonitrogenous. Treatments were 0 g of glycerol/d (control, CON), 200 g of glycerol/d (G2), and 400 g of glycerol/d (G4). Dry matter intake (DMI) decreased as the amount of glycerol fed increased. Milk yield was higher for CON and G2 cows in comparison with G4 cows. Milk fat percentage and yield were reduced when glycerol was fed compared with CON cows but increased the milk protein percentage at the highest concentration of dietary glycerol. These changes resulted in decreased energy-corrected milk yield and efficiency (milk/DMI) in diets supplemented with G4 compared with CON. No differences were observed in ruminal pH and ammonia concentrations. Molar proportions of acetate, valerate, and acetate:propionate ratio decreased, whereas propionate increased as the amount glycerol fed increased. Molar proportions of butyrate were greatest when glycerol was included in the diet compared with CON. Nutrient intake and digestion were not different among treatments. Results of this trial suggest that feeding increasing amounts of glycerol may decrease DMI and alters ruminal fermentation, resulting in reduced yield of milk, fat, and energy-corrected milk.

Key words: dietary glycerol, volatile fatty acid, efficiency

INTRODUCTION

The growth of the ethanol industry has increased the need for alternatives to corn in lactating cow diets. Biodiesel production has increased in the United States from 690 million gallons in 2009 to 802 million gallons in 2011 (Biofuels Digest, 2011). Industry growth is expected to increase availability and promote favorable pricing of glycerol, which is a byproduct of base-catalyzed transesterification of oil in the formation of methyl and ethyl FA esters in the production of biodiesel (Thompson and He, 2006) and the main byproduct of ethanol fermentation processing (Michnick et al., 1997). Purified glycerol is used in the food, pharmaceutical, and cosmetic industries, but the cost of refining crude glycerol to a high purity is cost prohibitive for many small refining operations (Pachauri and He, 2006). This leads to potentially a large source of crude glycerol available to livestock producers. According to the Food and Drug Administration (FDA, 2006), glycerol is recognized as a safe feed ingredient in animal diets. Glycerol is an odorless, colorless, hygroscopic, sweet-tasting liquid that has the potential to replace corn in the diet and has been predicted to have a feed value of 100 to 120% of corn, making it a viable alternative to corn (Hippen et al., 2008). Linke et al. (2004) calculated the NE_L value of glycerol to be approximately 1.05 Mcal/kg, which is 20% greater than corn. Schröder and Südekum (1999) calculated the energy density of glycerol to range between 0.90 to 1.03 Mcal of NE_L /kg. Also, several studies have reported that glycerol enhances rumen fermentation and improves feed efficiency (Garton et al., 1961; Dirksen et al., 1985; Hippen et al., 2008). Dietary glycerol seems to be extensively fermented to propionate by ruminal bacteria (Czerkawski and Breckenridge, 1972; Remond et al., 1993; Bergner et al., 1995), although level of feeding and method of delivery may affect the amount of glycerol that escapes fermentation (Kijora et al., 1998; DeFraen et al., 2004).

The increased availability may create an opportunity to use glycerol as an energy source in dairy rations and has led to a corresponding decline in price projections (Yazdani and Gonzalez, 2007). These changes

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may favor the use of glycerol in rations fed to livestock, especially with the rising corn costs and more crops being diverted to biofuel production.

Previously, glycerol has been a cost-prohibitive addition to the diet because of its value in other fields, but with growing biodiesel production and corn costs, the addition of glycerol to the ration is a possible choice for many producers. The objective of this study was to determine the effects of feeding diets with different levels of glycerol on rumen function, blood metabolites, and nutrient digestibility in lactating Holstein cows.

MATERIALS AND METHODS

The study was conducted May to July of 2008 at the University of Georgia, Tifton Campus Dairy Research Center. The protocol for the trial was approved by the University of Georgia Institutional Animal Care and Use Committee.

Cows and Management

Six early-lactation rumen-cannulated Holstein cows (4 multiparous and 2 primiparous) were used in the replicated 3×3 Latin square design study. Cows averaged 56 ± 18 DIM, 38.0 ± 8.2 kg/d of milk, $3.77 \pm$

0.97% milk fat, and $2.72 \pm 0.2\%$ milk protein at the beginning of the study. Cows were housed in a freestall barn with access to individual freestalls, free access to water, and fed behind Calan doors (American Calan Inc., Northwood, NH). Training for Calan doors was initiated in early April, and the study began in early May and continued until the end of July. Cows were cooled using fans and high-pressure misters. All diets contained 15 g/907 kg of DM of Rumensin (Elanco Animal Health, Greenfield, IN).

Feed was mixed and delivered once daily. The amount of feed offered was adjusted each day to achieve 7 to 10% refusals. Diets were balanced to be isocaloric and isonitrogenous (Table 1). Cows were milked twice daily at 0400 and 1500 h during the study.

Cows were ranked by average daily milk yield, stage of lactation, and ECM during the standardization period, blocked into pairs by rank, and each pair was assigned randomly to 1 of the 3 experimental diets within block and progressed through the three 4-wk periods until exposed to all treatments.

Experimental Treatments and Design

The experimental design for this study was a replicated 3×3 Latin square. Each period consisted of 3

Table 1. Ingredient and chemical composition of experimental diets containing supplemental glycerol¹

Item	Diet ²		
	CON	G2	G4
Ingredient, % of DM			
Corn silage	38.5	38.5	38.5
Alfalfa hay	10.6	10.6	10.6
Whole cottonseed	1.82	1.82	1.82
Ground corn	23.9	22.9	22.1
Wet brewers grain	12.1	12.1	12.1
Concentrate mix ³	13.2	13.2	13.2
Glycerol	0	0.78	1.57
DM, ⁴ %	50.0 $\pm$ 0.5	49.3 $\pm$ 0.8	49.2 $\pm$ 0.6
Chemical composition, ⁴ % of DM			
NDF	39.6 $\pm$ 1.8	40.9 $\pm$ 1.9	39.7 $\pm$ 1.5
ADF	26.1 $\pm$ 1.1	27.1 $\pm$ 1.6	26.3 $\pm$ 1.1
CP	17.2 $\pm$ 0.5	16.6 $\pm$ 0.3	16.7 $\pm$ 0.6
RUP ⁵	7.14	6.86	6.89
RDP ⁵	10.05	9.74	9.81
Ether extract	4.4 $\pm$ 0.3	4.7 $\pm$ 0.3	4.7 $\pm$ 0.6

¹Samples were collected 4 times during the collection week and compiled for analysis (n = 3).

²CON = control: no glycerol added; G2 = control + 200 g of supplemental glycerol/d; G4 = control + 400 g of supplemental glycerol/d.

³Composition of concentrate mix (% of DM): 2.04% Pro-Lak (H. J. Baker and Brother Inc., Westport, CT), 6.06% soybean meal 48, 0.151% Ca 17%:P 21%, 0.182% potassium magnesium sulfate, 0.454% potassium bicarbonate, 0.151% limestone, 0.151% urea 45%N, 0.027% Availa-4 (Zinpro Corp., Eden Prairie, MN), 0.154% MgO, 0.227% salt; 0.530% Na bicarbonate, 0.188% yeast, 0.121% trace mineral premix (2.91% Ca; 0.38% Mg; 1.76% S; 144 mg/kg of Co; 9,523 mg/kg of Cu; 1,465 mg/kg of Fe; 842 mg/kg of I; 28,617 mg/kg of Mn; 220 mg/kg of Se; 25,343 mg/kg Zn), 0.01% vitamin premix (4,210,830 IU/kg of vitamin A; 1,684,330 IU/kg of vitamin D; 21,045 IU/kg of vitamin E), and 0.229% Rumensin 3 (Elanco Animal Health, Greenfield, IN).

⁴Results are presented as means $\pm$ SD.

⁵Calculated using the Spartan ration program (Michigan State University, East Lansing).

wk for treatment adjustment followed by 1 wk for data collection. Dietary treatments consisted of a control without supplemental glycerol (**CON**), control plus 200 g of supplemental glycerol/d (**G2**), and control plus 400 g of supplemental glycerol/d (**G4**; Table 1). Glycerol was mixed into the TMR using a top-loading Super Data Ranger (American Calan Inc.) with whole cottonseed as a carrier. The glycerol was added to the whole cottonseed and mixed and the coated cottonseed was then added to the TMR in place of ground corn. Glycerol contained 80 to 85% glycerol with no more than 14% moisture, 7% sodium chloride, and methanol content of 18 ppm (analysis provided by ADM Nutrition Alliance Inc., Quincy, IL).

Data Collection

The amount of feed offered and refused was recorded daily during the 7-d collection week. Milk yield was recorded at each milking (2×) using electronic meters (Alpro; DeLaval, Kansas City, MO) and summed daily during the collection weeks. Milk samples were collected from 4 consecutive milkings each collection period and analyzed for concentrations of fat, protein, and SCC (Southeast Milk Inc., Belleview, FL). Energy-corrected milk yield was calculated as $ECM = (0.327 \times \text{kg of milk}) + (12.95 \times \text{kg of fat}) + (7.65 \times \text{kg of protein})$ (Tyrrell and Reid, 1965).

Blood samples from the coccygeal vessel were collected once during the each collection week for analysis of serum glucose and urea N using a Boehringer Mannheim/Hitachi 912 automated chemistry analyzer (Roche Laboratory Systems, Indianapolis, IN). Rumen samples were collected on d 3 of each collection week at 0, 2, 4, 6, 8, and 10 h after feeding. Approximately 50 mL of ruminal fluid was collected and strained through 3 layers of cheesecloth and immediately analyzed for pH and ammonia levels. A 10-mL subsample was immediately mixed with 2 mL of metaphosphoric acid (25% wt/vol), centrifuged at $10,000 \times g$ for 10 min, and the supernatant was collected and frozen for later analyses of VFA (Erwin et al., 1961) using a Thermo Scientific Trace GC Ultra (Thermo Fisher Scientific Inc., Bellefonte, PA).

Samples of TMR, refusals, and ingredients were collected 4 times during the collection week. The DM content was determined by drying in a forced-air oven at 55°C for 48 h. Samples were composited by period and ground to pass through a 1-mm screen using a Wiley mill (Thomas Scientific, Swedesboro, NJ) for analysis of DM, CP, ether extract (AOAC, 1990), NDF (Van Soest et al., 1991), and ADF (AOAC, 1990).

Digestibility Study

Cows were fed 23 g/d (DM basis) of Cr_2O_3 mixed into the experimental diet beginning 10 d before the 4-d collection period each collection week. Powdered Cr_2O_3 was mixed with ground corn as a carrier at a rate of 3.82 g of corn/kg of DM in an upright mixer. The Cr_2O_3 corn blend was added to the diet and mixed daily. Daily feed and ort samples were collected during the 4-d collection period. Fecal grab samples were collected during the collection period at 12-h intervals, with the collection time advancing by 3 h each day to represent 0300, 0600, 0900, 1200, 1500, 1800, 2100, and 2400 h. Fecal samples were frozen at -5°C until composited by cow. Samples of feed, orts, and feces were compiled by cow and dried at 55°C and ground to pass through a 1-mm screen using a Wiley mill and stored for analysis of CP, NDF, and ADF as described previously. Chromium content of feed, orts, and feces was determined using atomic absorption spectrophotometry (AAnalyst 100/300; Perkin Elmer, Bellefonte, PA) following wet ashing (Ferret et al., 1999).

Statistical Analysis

Data were analyzed as a 3×3 Latin square design using PROC MIXED of SAS version 9.1 (SAS Institute, 2004). Significance was defined as $P < 0.05$ and trends as $0.05 \leq P \leq 0.15$. Values are reported are least squares means and associated standard errors. Model 1 was used for analysis of intake, production, blood, and apparent digestibility. Model 2 was used for analysis of VFA, pH, and ammonia and included effects of time, period, and treatment. Interactions tested were hour by treatment for ruminal data. Sample time was included as a repeated measure for model 2. When significance was determined, pairwise *t*-tests with a Tukey adjustment were performed between treatment means using the PDIFF statement in SAS. The models were as follows:

$$Y_{ijk} = \mu + \beta_i + P_j + C_k + \varepsilon_{ijk};$$

$$Y_{ijln} = \mu + \beta_i + P_j + C_k + H_l + \beta H_{il} + \varepsilon_{ijkln},$$

where Y_{ijk} and Y_{ijln} = variable responses, μ = general mean, β_i = fixed effect of treatment ($i = 1, \dots, 3$), P_j = fixed effect of period ($j = 1, \dots, 3$), C_k = fixed effect of cow ($k = 1, \dots, 6$), H_l = fixed effect of hour ($l = 0, \dots, 10$), βH_{il} = interaction of treatment and hour, and ε_{ijk} and ε_{ijkln} = residual errors.

Table 2. Performance and blood metabolites of lactating Holstein cows fed diets supplemented with different amounts of supplemental glycerol (n = 72 milk samples; n = 18 serum samples)

Item	Diet ¹			SE	P-value
	CON	G2	G4		
Milk, kg/d	37.9 ^a	37.3 ^a	35.5 ^b	0.52	0.004
Fat, %	3.46 ^a	3.31 ^b	3.35 ^b	0.03	0.004
Fat, kg/d	1.33 ^a	1.24 ^b	1.19 ^b	0.03	0.0009
Protein, %	2.76 ^a	2.75 ^a	2.81 ^b	0.005	0.0001
Protein, kg/d	1.04	1.02	1.00	0.02	0.06
ECM, kg/d	37.6 ^a	36.1 ^{ab}	34.6 ^b	0.61	0.003
Efficiency					
ECM/DMI	1.54	1.55	1.48	0.02	0.10
Milk/DMI	1.55 ^{ac}	1.60 ^{ab}	1.51 ^c	0.03	0.05
Serum metabolite					
Glucose, mg/dL	62.7	62.5	63.3	1.3	0.89
Urea N, mg/dL	21.7	21.8	20.5	0.4	0.09

^{a-c}Means within a row with unlike superscripts differ ($P < 0.05$).

¹CON = control: no glycerol added; G2 = control + 200 g of supplemental glycerol/d; G4 = control + 400 g of supplemental glycerol/d.

RESULTS AND DISCUSSION

The chemical composition of the experimental diets is reported in Table 1. Nutrient concentrations were similar for all diets except that concentrations of ether extract were slightly higher when glycerol was included in the diet.

Milk yield was reduced ($P = 0.004$) by 1.8 and 2.4 kg/d for G4 compared with CON and G2. Inclusion of glycerol in the diet resulted in a reduction in milk fat percentage ($P = 0.004$) and yield ($P = 0.0009$) compared with CON. In contrast, milk protein percentage increased ($P = 0.0001$) at the higher level of glycerol supplementation compared with CON and G2. These results are in contrast to earlier work reported by Donkin and Doane (2007) in which no differences were observed in milk yield or composition when glycerol replaced corn in the diet. Chung et al. (2007) included dried glycerol (food grade, 65% glycerol) in the diet of transition cows and also reported no effect on milk yield or composition. Linke et al. (2004) fed glycerol at the rate of 498 or 998 g/d in the diet of mid-lactation Holstein and Brown Swiss cows in a 3×3 Latin square, with no effect on DMI, milk yield, or FCM, but observed an improvement in feed efficiency with the inclusion of glycerol with milk to feed ratios of 1.46, 1.59, and 1.60 for the control, 498 and 998 g/d, respectively. Feed efficiency (milk/DMI) was reduced ($P = 0.05$) for G4 compared with G2 and CON. Also, ECM was reduced ($P = 0.003$) for G4 compared with CON and a tendency ($P = 0.10$) for lower efficiency (ECM/DMI) was found in the current study. These results are in agreement with DeFrain et al. (2004) who reported reduced ECM when glycerol was fed. No differences

were observed among treatments in concentrations of serum glucose or urea N, although a trend ($P = 0.09$) for reduced serum urea N was observed with increasing amounts of glycerol in the diet (Table 2).

No differences were observed for the interaction of treatment and sampling time; thus, only the main effects are reported. No differences were observed among treatments for ruminal pH (average 6.1), but a weak trend ($P = 0.14$) existed for decreased ruminal ammonia concentrations with the addition of glycerol to the diet. Ruminal ammonia concentrations were 12.1, 11.7, and 9.8 for CON, G2, and G4, respectively (Table 3). No differences were observed in total VFA concentration among treatments. Molar proportions of acetate, valerate, and acetate:propionate ratio decreased ($P = 0.0001$), whereas molar proportions of propionate ($P = 0.0002$) increased as the amount of glycerol fed increased. Molar proportions of butyrate were highest ($P = 0.0001$) for diets supplemented with glycerol compared with CON. No differences were observed in molar proportions of isobutyrate or isovalerate.

The effects of supplemental glycerol on ruminal VFA concentrations are in agreement with previous reports. Schröder and Südekum (1999) examined the effects of feeding glycerol on ruminal fermentation using ruminally cannulated steers and reported that although the addition of glycerol did not affect diet digestibility, it did decrease the acetate:propionate ratio, linearly increased ruminal butyrate concentrations, and stimulated increased water intake. Schröder and Südekum (1999) concluded that the inclusion of dietary glycerol would be beneficial to the dairy cow because it would increase ruminal propionate, increasing the supply of gluconeogenic substrate to the liver. Also, the increased

Table 3. Ruminal pH and VFA concentrations of lactating Holstein cows fed diets supplemented with different amounts of supplemental glycerol (n = 108 rumen fluid samples)¹

Item	Diet ²			SE	P-value
	CON	G2	G4		
pH	6.1	6.0	6.1	0.04	0.64
Ammonia, %	12.1	11.7	9.8	0.86	0.14
Total VFA, mmol	46.7	52.2	49.0	2.3	0.24
Composition, %					
Acetate	61.1 ^a	59.1 ^b	57.6 ^c	0.39	0.0001
Propionate	23.3 ^a	24.1 ^b	25.4 ^c	0.35	0.0002
Butyrate	11.4 ^a	12.3 ^b	12.4 ^b	0.15	0.0001
Isobutyrate	1.3	1.2	1.2	0.06	0.91
Isovalerate	1.5	1.5	1.5	0.04	0.53
Valerate	1.5 ^a	1.7 ^b	1.8 ^b	0.05	0.0001
Acetate:propionate	2.7 ^a	2.5 ^b	2.3 ^c	0.05	0.0001

^{a-c}Means within a row with unlike superscripts differ ($P < 0.05$).

¹No treatment by sampling time interactions ($P > 0.10$).

²CON = control: no glycerol added; G2 = control + 200 g of supplemental glycerol/d; G4 = control + 400 g of supplemental glycerol/d.

ruminal butyrate would support the growth of ruminal epithelial tissue and possibly increase nutrient absorption from the rumen as proposed by Dirksen et al. (1985).

DeFrain et al. (2004) conducted a transition cow study with the inclusion of glycerol. Those researchers reported that rumen fluid collected postpartum from cows offered glycerol had a greater total VFA concentration, increased molar proportions of propionate, and decreased acetate:propionate ratio compared with the control. Butyrate concentrations also showed a tendency to increase linearly in cows fed glycerol treatment compared with CON.

Linke et al. (2004) reported an increase in molar proportions of propionate and butyrate with increasing glycerol without any difference in DMI, milk yield, or

FCM. They also reported molar proportions of acetate decreased when cows were provided supplemental glycerol through drenching or mixing in their diet. Molar proportions of propionate and butyrate increased with either route of administration with peak concentration observed 4 h after consumption. Concentrations of glycerol in blood plasma increased when cows were drenched or tubed but not when they were fed glycerol. Plasma insulin and BHBA concentrations increased when cows were drenched or tubed compared with those fed glycerol in their diets. These researchers concluded that for glycerol to be gluconeogenic it must be delivered in water to associate with the liquid fraction of the rumen contents or be able to bypass the rumen and be absorbed as glycerol and converted to glucose by the liver. Glycerol that is available to the rumen microbes

Table 4. Nutrient intake and apparent digestibility of lactating Holstein cows fed diets supplemented with different amounts of supplemental glycerol (n = 108 intake observations; n = 144 fecal samples)

Item	Diet ¹			SE	P-value
	CON	G2	G4		
Intake, kg/d					
DM	24.3 ^a	23.1 ^b	23.4 ^b	0.24	0.003
CP	3.5	3.1	3.1	0.1	0.21
NDF	7.8	7.4	7.5	0.4	0.70
ADF	5.2	4.9	4.8	0.3	0.60
Apparent digestibility, %					
DM	68.5	66.9	65.6	2.3	0.66
CP	71.8	69.1	68.2	1.8	0.36
NDF	47.5	46.3	48.6	3.5	0.90
ADF	44.9	43.6	40.9	4.1	0.78

^{a,b}Means within a row with unlike superscripts differ ($P < 0.05$).

¹CON = control: no glycerol added; G2 = control + 200 g of supplemental glycerol/d; G4 = control + 400 g of supplemental glycerol/d.

is highly fermentable and is converted to propionic and butyric acids. Garton et al. (1961) conducted in vitro incubations of glycerol and observed that almost 25% of glycerol had disappeared after 2 h and almost 90% of the glycerol was undetectable after an 8-h incubation.

The DMI was reduced ($P = 0.003$) with the inclusion of glycerol for both G2 and G4 compared with CON (Table 4). This is in contrast to a previous trial (Boyd et al., 2011) in which no differences in DMI were observed when lactating cows were fed diets supplemented with up to 400 g of glycerol/d for 10 wk. Other researchers (DeFrain et al., 2004; Linke et al., 2004) have not observed any effect on DMI with the inclusion of glycerol in the diet. However, no differences in nutrient intake or apparent digestibility (Table 4) were observed among treatments. These results are in agreement with previous research reported by Schröder and Südekum (1999) and Donkin and Doane (2007) who reported no effects on nutrient digestibility with the inclusion of glycerol in the diet.

CONCLUSIONS

Inclusion of dietary glycerol in the ration of dairy cows affected milk yield and components, ECM, and ruminal fermentation patterns. The effects on ruminal fermentation are in agreement with several earlier studies. Addition of rumen-fermentable glycerol decreased molar proportions of acetate and acetate:propionate ratio and increased molar proportions of propionate and butyrate, which should increase the supply of gluconeogenic precursors and support the growth of ruminal epithelial tissue and potentially increase nutrient absorption from the rumen.

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