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Effects of feeding crude glycerin on performance and ruminal kinetics of lactating Holstein cows fed corn silage- or cottonseed hull-based, low-fiber diets

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ABSTRACT

The objective was to determine whether crude glycerin could partially replace concentrate ingredients in corn silage- or cottonseed hull-based diets formulated to support minimal milk fat production without reducing milk production. Multiparous, lactating Holstein cows (n = 24; 116 ± 13 d in milk) were assigned to dietary treatments arranged in a 2 × 3 factorial design; namely, 2 dietary roughage sources (cottonseed hulls or corn silage) and 3 dietary concentrations of glycerin [0, 5, or 10% on a dry matter (DM) basis]. Four different cows received each dietary treatment in each of 3 periods such that each diet was evaluated using 12 cows. Crude glycerin, produced using soybean oil, contained 12% water, 5% oil, 6.8% sodium chloride, and 0.4% methanol. Glycerin partially replaced ground corn, corn gluten feed, and citrus pulp. Diets of minimum fiber concentrations were fed to lactating dairy cows and resulted in low concentrations of milk fat (averaging 3.12% for cows fed diets without glycerin). The effects of glycerin on cow performance and ruminal measurements were the same for both dietary roughage sources with the exception of feed efficiency. Replacing concentrate with crude glycerin at 5% of dietary DM increased DM intake without increasing milk yield. Concentration and yield of milk fat were reduced when glycerin was fed at 10% of dietary DM. This was accompanied by a 30% reduction in apparent total-tract digestion of dietary neutral detergent fiber. Crude glycerin affected the microbial population in the rumen as evidenced by increased molar proportions of propionic, butyric, and valeric acids and decreased molar proportions of acetic acid. Efficiency of N utilization was improved as evidenced by lower concentrations of blood urea nitrogen and ruminal ammonia-N. Cows fed cottonseed hull-based diets consumed 5.3 kg/d more DM but produced only 1.7 kg/d more milk, resulting in reduced efficiency. Increased production of ruminal microbial protein, molar proportion of propionic acid, and passage of ruminal fluid resulted from feeding the cottonseed hull- versus corn silage-based diets, although apparent digestibilities of DM and neutral detergent fiber were reduced. Replacing 5 and 10% of concentrate ingredients with crude glycerin improved efficiency of 4% fat-corrected milk production when corn silage-based diets were fed but decreased it when cottonseed hull-based diets were fed.

Key words: glycerin, milk fat, rumen kinetics

INTRODUCTION

Glycerin is a co-product with biodiesel from the reaction of vegetable or animal fat with methanol. Approximately 10 L of glycerin is produced with every 100 L of biodiesel (Wen, 2012). As glycerin production increases, glycerin supply may exceed the demand for food and personal care products, such that it may become an increasingly important substitute for concentrate feedstuffs for livestock. Crude (DeFrain et al., 2004) and pure glycerin (Donkin et al., 2009; Osborne et al., 2009; Carvalho et al., 2011) have been consumed successfully at 5 to 15% of dietary DM by lactating dairy cows. Diets in these studies contained more than sufficient NDF from traditional forages such as corn silage (**CS**) and alfalfa (NRC, 2001), and milk fat yield was maintained when glycerin was included in the diet. Replacing dietary starch with glycerin when diets are of marginal fiber content may affect measures of milk fat. The effect of glycerin (10% of dietary DM) on dietary energy depended upon the starch concentration of the diet fed to wethers (Sudekum, 2007). All glycerin disappeared from the rumen within 4 h of dosing of Friesian cows (240 g dosed; Rémond et al., 1993) or bulls (200 g dosed; Kijora et al., 1998) via fermentation and absorption from the rumen. Therefore, glycerin may have a faster fermentation rate than starch. In a review article, Offner et al. (2003) reported that in situ digestion rate of starch in ground corn averaged 0.055 h^{-1}, and approximately 32% of corn starch escaped ru-

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minal fermentation when ruminal passage rate of solids was 0.06 h^{-1}.

Cottonseed hulls (**CSH**) are a co-product of the extraction of oil from cottonseeds and consist of lint and seed coat. They are a viable alternative as a dietary roughage source for lactating dairy cows, replacing traditional forages such as CS, despite their high fiber content (85% NDF; NRC, 2001) and low ADF digestibility (Brown et al., 1977). Lactating dairy cows usually consumed more DM and produced more milk when CSH were the major source of roughage in the diet compared with CS, alfalfa, or bermudagrass (Morales et al., 1989; Adams et al., 1995). Diets containing CSH as the major roughage source usually contain more ground corn and other concentrate ingredients than CS-based diets, thus increasing the dietary ratio of concentrate to roughage. High grain diets are associated with depressed concentration of milk fat (Jenkins and McGuire, 2006). The effect of including glycerin in CSH-based diets has not been examined previously.

The objective of the current study was to evaluate the effects of using glycerin to partially replace concentrates in diets of marginal fiber content on performance, nutrient digestibility, and ruminal kinetics of lactating dairy cows.

MATERIALS AND METHODS

Animals and Treatments

Approval by the Animal Research Committee of the University of Florida was obtained before initiation of the studies.

Trial 1. Multiparous, lactating Holstein cows (n = 24; 116 ± 13 DIM; 671 ± 58 kg of BW) at the University of Florida were assigned to dietary treatments arranged in a 2 × 3 factorial design; namely, 2 dietary roughage sources and 3 dietary concentrations of glycerin (West Central, Ralston, IA). The 2 dietary roughage sources were (1) CS and alfalfa hay (36 and 11% of DM, respectively) or (2) CSH and bermudagrass hay (25 and 10% of DM, respectively, Table 1). Crude glycerin, produced using soybean oil (West Central), was fed at 0, 5, or 10% of dietary DM. The crude glycerin contained 12% water, 6.8% sodium chloride, and 0.4% methanol (Table 2). Glycerin partially replaced ground corn, corn gluten feed, and citrus pulp. The dietary proportions of soybean meal and SoyPlus (heat-treated soybean meal, West Central) were increased to formulate isonitrogenous diets. The experiment was a partially balanced, incomplete block design consisting of three 27-d periods. Four cows received each dietary treatment in each of 3 periods such that each diet was evaluated using 12 cows. The first 14 d of each period was used to adapt cows to a new diet and the last 13 d was used for data collection. At each of 2 daily feedings (0830 and 1230 h), the glycerin was mixed with other dietary ingredients using a Data Ranger (American Calan Inc., Northwood, NH). Diets based on CS were formulated to support 41 kg/d of milk (24 kg/d of DMI) and those based on CSH to support 39 kg/d of milk (24 kg/d of DMI; National Research Council, 2001). However, it was expected that cows offered the CSH-based diet would eat more DM than cows offered the CS-based diet and therefore would match or exceed the milk yield predicted by the National Research Council (2001). Diets were offered as a TMR for ad libitum consumption, allowing for 10% orts. Water was available continuously in ad libitum amounts. Cows were housed in a freestall, open-sided barn fitted with Calan gates (American Calan Inc.) to allow measurement of feed intake by individual cows. Freestalls were bedded with sand and cleaned daily. Sufficient freestalls were available to provide at least 1 freestall per cow. Fans and misters were operated continuously in the barn for cooling purposes during the May to September study.

Weights of TMR offered and orts were recorded daily for each cow. Representative samples of concentrate mixes, CS, alfalfa hay, and bermudagrass hay were collected weekly and composited for each experimental period. Cottonseed hulls were mixed with concentrate ingredients as a batch mix. Corn silage was dried at 55°C for 48 h in a forced-air oven, and all feedstuffs were ground through the 1-mm screen of a Wiley mill (A. H. Thomas, Philadelphia, PA) before compositing within each period. Feedstuff samples were analyzed for DM (105°C for 8 h), OM (512°C for 8 h), NDF (Van Soest et al., 1991) using heat-stable α-amylase, and N (vario MAX CN, model ID 25.00-5003; Elementar, Hanau, Germany). Protein was calculated by multiplying N × 6.25.

Body weight was monitored by weighing cows on 3 consecutive days at the beginning and end of each period. Cows were milked daily at 0700 and 1900 h, and milk weights recorded electronically. Milk samples were collected at 2 consecutive milkings on d 18 and 19 of each period for determination of fat, protein, and SCC. Somatic cell scores were generated as described by Norman et al. (2000) for statistical analysis of SCC. Samples were analyzed by Southeast Dairy Laboratory (Belleview, FL) as per the International Dairy Federation (method 141C:2000; ISO, 2000) using mid-infrared technologies (Bentley 2000, Bentley Instruments, Chaska, MN).

Blood samples (~10 mL) were collected from coccygeal vessels into Vacutainer tubes containing sodium heparin (Becton Dickinson and Co., Franklin Lakes, NJ) on d 15 of each period just before the a.m. feeding.

Table 1. Ingredient and chemical composition of corn silage and experimental diets based on 1 of 2 roughage sources (corn silage and cottonseed hulls) with 0, 5, or 10% dietary glycerin

		Corn silage			Cottonseed hulls		
Item	Corn silage	0%	5%	10%	0%	5%	10%
Ingredient							
Glycerin		0	5	10	0	5	10
Corn silage		35.6	35.6	35.6	—	—	—
Alfalfa hay		11.3	11.3	11.3	—	—	—
Cottonseed hulls		2.5	2.5	2.5	25.1	25.1	25.1
Bermudagrass hay		—	—	—	10.3	10.3	10.3
Ground corn		17.6	12.6	7.6	32.3	27.3	22.3
Corn gluten feed		8.0	7.7	7.4	5.6	4.8	4.1
Soybean meal		6.9	7.2	7.4	9.1	9.8	10.5
Soy Plus[1]		6.0	6.3	6.6	8.0	8.7	9.4
Citrus pulp		8.0	7.7	7.4	5.5	4.9	4.2
Mineral and vitamin mix[2]		4.1	4.1	4.1	4.1	4.1	4.1
Total		100	100	100	100	100	100
Chemical							
Dry matter, %	40.0	57.0	57.1	57.2	87.7	87.9	88.1
Ash, % of DM	4.2	7.1	7.2	7.2	6.1	6.5	6.8
CP, % of DM	8.6	17.5	17.1	16.5	17.2	17.5	17.7
NDF, % of DM	32.7	25.5	24.7	23.8	33.6	32.8	32.0
ADF, % of DM	15.5	18.9	18.6	18.3	25.1	24.7	24.4
Starch,[3] % of DM	39.9	29.0	25.8	23.0	27.3	23.0	19.1
NE_L,[3] Mcal/kg of DM	1.50	1.57	1.57	1.58	1.50	1.50	1.50
Ca, % of DM	0.16	0.94	0.95	0.94	0.77	0.76	0.75
P, % of DM	0.29	0.43	0.43	0.42	0.39	0.39	0.39
K, % of DM	1.58	1.52	1.53	1.52	1.33	1.34	1.34
Mg, % of DM	0.18	0.38	0.39	0.40	0.37	0.38	0.38
Na, % of DM	0.02	0.39	0.58	0.76	0.41	0.57	0.73
Cl, % of DM	0.38	0.25	0.60	0.80	0.24	0.44	0.63
Fe, mg/kg of DM	1,022	529	523	516	189	191	192
Cu, mg/kg of DM	5	25	27	29	31	34	37
Mn, mg/kg of DM	17	75	71	66	76	88	98
Zn, mg/kg of DM	31	65	67	68	59	62	65

[1]West Central (Ralston, IA).

[2]Formulated to provide (per kilogram of premix) 24% CP, 9% Ca, 1% P, 4% K, 3% Mg, 10% Na, 2% Cl, 1.1% S, 158 mg of Fe, 500 mg of Cu, 1,200 mg of Mn, 1,500 mg of Zn, 8.25 mg of Se, 20 mg of I, 147,756 IU of vitamin A, 43,750 IU of vitamin D, and 787 IU of vitamin E (DM basis). Trace minerals were supplied in inorganic form.

[3]Calculated using NRC (2001).

Blood was stored on ice for transport and centrifuged at 3,000 × *g* for 15 min to separate plasma. Plasma was stored at –20°C until analyzed. A Technicon Autoanalyzer (Technicon Instruments Corp., Chauncey, NY) was used to measure plasma glucose (Bran and Luebbe Industrial Method 339-19; Gochman and Schmitz, 1972) and BUN (Bran and Luebbe Industrial Method 339-01; Marsh et al., 1965).

Table 2. Chemical composition of crude glycerin

Item	Value
Glycerin	80.3[1]
Water, %	12.4
Cl, % of DM	4.07
Na, % of DM	2.72
Ca, % of DM	0.03
P, % of DM	0.01
Mg, % of DM	0.01
K, % of DM	0.01
S, % of DM	0.01
Cu, mg/kg	1
Fe, mg/kg	11
Mn, mg/kg	1
Zn, mg/kg	3
Methanol, % of DM	0.4

[1]Calculated as 100 minus sum of all other components.

At 1030 h on d 14 and 15 of each period, spot samples of urine were collected and frozen at –20°C until analyzed. To estimate microbial protein synthesis in the rumen, creatinine and allantoin analyses were performed as described by Vagnoni et al. (1997). Gelatin capsules containing 10 g of Cr_2O_3 were administered orally via a balling gun twice daily at 0430 and 1630 h on d 16 to 25 of each period. Fecal grab samples were collected before administration of the capsules on d 21 to 25 of each period. Fecal samples from daily collections were dried for 96 h at 55°C in a forced air oven and ground to pass the 1-mm screen of a Wiley mill (A. H. Thomas). Samples of dried feces were composited across the 10 sampling times to obtain one fecal

sample per cow per period. Feces were analyzed for Cr by atomic absorption spectrophotometry (Williams et al., 1962; AAnalyst 800, Perkin Elmer, Waltham, MA), and for DM, OM, N, and NDF using the previously described methods. Apparent digestibility of DM, OM, CP, and NDF were calculated by the marker ratio technique (Schneider and Flatt, 1975).

Trial 2. Four ruminally fistulated, multiparous, lactating Holstein cows (590 ± 51 kg of BW) were assigned to 4 diets set in a 2 × 2 factorial design arranged within a 4 × 4 Latin square. The 4 dietary treatments were CS and alfalfa hay-based or CSH and bermudagrass hay-based diets containing 0 or 10% glycerin (DM basis; West Central Soy). Each period lasted 20 d, of which the first 13 d served as an adjustment period and the last 7 d was used for data collection. Feeding, housing, and milking management were as described in trial 1.

On d 14, ruminal fluid (~50 mL) was collected hourly for 8 h starting at feeding. Using a suction strainer system consisting of a 1-L side-armed flask equipped with a rubber bulb and a perforated hard plastic tube inserted into the ruminal fluid, samples were collected from at least 3 locations within the rumen. The pH was measured immediately upon collection (Accumet model 15 pH meter, Fisher Scientific, Pittsburgh, PA). A subsample of 5 mL was acidified with 50% sulfuric acid to a pH <2 and centrifuged at 3,000 × *g* for 20 min. The supernatant was collected and frozen immediately at –20°C. Upon thawing, samples were centrifuged at 13,500 × *g* for 20 min. The VFA were analyzed using a UV detector (Spectroflow 757, ABI Analytical Kratos Division, Ramsey, NJ) in an HPLC system (L7485, Hitachi, Tokyo, Japan) set to a flow press rate of 0.700 mL/min and a column temperature of 45°C.

Chromium-mordanted fiber was used as an inert marker to determine thfe passage rate of solids from the rumen. Bermudagrass hay fed in the study was Cr-mordanted according to the method of Udén et al. (1980) and weighed (3 ± 0.02 g) into gelatin capsules. At 0930 h on d 15, 9 gelatin capsules were administered orally via a balling gun. Fecal grab samples were collected at 0, 8, 20, 24, 28, 32, 44, 50, 56, 68, 74, and 80 h after the gelatin capsules were administered. Fecal samples were stored at –20°C until analyzed. Upon thawing, fecal samples were dried at 55°C in a forced-air oven and ground to pass the 1-mm screen of a Wiley mill. Feces were analyzed for Cr as described previously. Passage of solids from the rumen was determined as the slope of descending portion of the curve of a graph depicting changes in chromium concentration over time.

Cobalt-EDTA, prepared according to Binnerts et al. (1968), was used as a marker to measure passage rate of liquid from the rumen. At 0830 h on d 19 after the morning milking and before feeding, 1 L of 50% Co-EDTA was delivered to at least 5 locations within the rumen of each cow using a plastic tube. After the contents in the rumen were mixed thoroughly by hand, ruminal fluid was collected as described previously, once before the addition of Co-EDTA and 12 times after the addition in 1-h intervals. Samples were stored at –20°C until analyzed. Concentration of Co in ruminal fluid samples was analyzed by atomic absorption spectrophotometry (AAnalyst 800, Perkin Elmer). The slope of the natural log of the Co concentration plotted against hour of sampling was considered the liquid passage rate coefficient.

On d 20, all solid and liquid contents of the rumen were removed through the cannula at 0900 h before the morning feeding and weighed to determine rumen volume. Contents were sampled throughout the removal process and composited, and DM was determined (105°C for 48 h). The removed rumen contents were returned to the rumen.

Statistical Analyses

Trial 1. Measurements of DMI, milk yield and composition, feed efficiency, apparent digestibility, BW change, plasma concentrations of glucose and urea nitrogen, and microbial protein synthesis were analyzed by using the Mixed procedure of SAS (version 9.2; SAS Institute, 1999). The statistical model used was as follows:

$$Y_{ijkl} = \mu + F_i + G_j + FG_{ij} + C_k + P_l + \varepsilon_{ijkl},$$

where Y_{ijkl} is the observed measurement, μ is the overall population mean, F_i is the fixed effect of roughage source i, G_j is the fixed effect of glycerin feeding rate j, FG_{ij} is the interaction of roughage source i and glycerin feeding rate j, C_k is the random effect of cow k, P_l is the fixed effect of period l, and ε_{ijkl} is the residual. Single degree of freedom orthogonal polynomial contrasts (linear and quadratic effects) for an increasing concentration of glycerin in the diet were tested, as well as interactions of roughage source and orthogonal contrasts of glycerin. Results are reported as least squares means. Significance was declared at $P < 0.05$ and a statistical trend at $P \geq 0.05$ but ≤ 0.10.

Trial 2. All variables measured, except for those repeatedly measured over time (e.g., ruminal pH, VFA, and ammonia), were analyzed by the same SAS procedure and model as that for trial 1. For the analysis of repeatedly measured variables, hour and hour × treatment interaction terms and a repeated-measures statement with the autoregressive 1 covariance struc-

Table 3. Effect of roughage (R) source and glycerin (GLY) feeding on DMI, milk production and composition, and BW change of Holstein cows fed diets based on 1 of 2 roughage sources (corn silage or cottonseed hulls) with 0, 5, or 10% dietary glycerin[1]

	Corn silage			Cottonseed hulls				Orthogonal contrasts (*P*-value)		
Item	0%	5%	10%	0%	5%	10%	SEM	R	GLY	R × GLY
DMI, kg/d	24.7	25.1	23.4	28.2	31.6	29.2	0.7	<0.01	<0.01[2]	NS[3]
DMI, % of BW	3.65	3.67	3.42	4.05	4.54	4.19	0.11	<0.01	<0.01[2]	NS
Milk, kg/d	34.4	35.6	35.6	37.7	36.6	36.4	0.7	<0.01	NS	NS
Milk fat, %	3.10	3.20	3.01	3.15	3.32	3.05	0.08	NS	0.02[2]	NS
Milk fat, kg/d	1.05	1.14	1.06	1.18	1.21	1.11	0.03	<0.01	0.02[2]	NS
Milk true protein, %	2.88	2.86	2.86	2.95	3.01	3.00	0.03	<0.01	NS	NS
Milk true protein, kg/d	0.99	1.01	1.01	1.11	1.09	1.09	0.02	<0.01	NS	NS
Milk SCC, × 10^3 cells/mL	3.2	2.5	3.0	3.1	3.5	3.3	0.4	NS	NS	NS
4% FCM, kg/d	29.6	31.3	30.1	32.8	32.7	31.3	0.6	<0.01	NS	NS
4% FCM per kg of DMI	1.19	1.25	1.28	1.19	1.04	1.07	0.04	<0.01	NS	0.02[4]
BW change, kg/d	0.16	0.15	−0.13	0.74	0.75	0.55	0.23	<0.01	NS	NS

[1]Diets based on corn silage were formulated to support 41 kg/d of milk (24 kg/d of DMI) and those based on cottonseed hulls to support 46 kg/d of milk (28 kg/d of DMI; NRC, 2001).

[2]Quadratic effect.

[3]$P > 0.05$.

[4]Linear effect.

ture were added to the model. Autoregressive 1 was the best covariance structure based upon the smallest Akaike's information criterion values. Other covariance structures tested included compound symmetry, unstructured, and heterogeneous compound symmetry and autoregressive 1. Results are reported as least squares means. Significance was declared at $P < 0.05$ and a statistical trend at $P \geq 0.05$ but ≤ 0.10.

RESULTS AND DISCUSSION

Trial 1

As planned, the CS-based diets had a minimum fiber concentration, averaging 24.7% NDF and 18.6% ADF (DM basis; Table 1). The CSH-based diets had greater fiber concentration due to the high fiber content of CSH but much of the fiber was of smaller particle length. Dietary concentrations of CP ranged from 16.5 to 17.7% (DM basis). However, intake of the 16.5% CP diet exceeded the MP requirement of milk production by cows in the current study (National Research Council, 2001). Cows demonstrated no sorting of the CSH-based diet, and sorting of the CS-based diet was minimal. As expected, DMI of the CSH-based diet was 21.6% greater ($P < 0.01$) than that of the CS-based diet (Table 3). In previous studies, feeding a nonpelleted CSH-based diet to lactating dairy cows stimulated intake by 24.3% (replacing CS; Harris et al., 1983), 15.5% (replacing alfalfa hay; Villavicencio et al., 1968), and 22.8% (replacing ground corrugated boxes; Peavy et al., 1980). This increase may be due to the smaller particle size and greater specific gravity of CSH compared with the other roughage sources fed. Even at lower dietary inclusion rates, CSH acted as an intake stimulant. When CSH replaced CS at 8% of dietary DM, DMI by lactating dairy cows increased by 6.3% (Kononoff and Heinrichs, 2003).

Across roughage sources, DMI (kg/d and % of BW) increased and then returned to the baseline level as the proportion of glycerin in the diet increased from 0 to 5 to 10% (quadratic effect, $P < 0.01$; Table 3). This increase differs from that in most other published studies when glycerin was consumed at 4 to 6% of dietary DM. Pregnant, nonlactating dairy cows ate less DM when the TMR was top-dressed with an 80% glycerin product (DeFrain et al., 2004) or when glycerin (99.7% glycerin) was added to the drinking water of periparturient dairy cows (Osborne et al., 2009). However, DMI by lactating dairy cows was unchanged when glycerin was mixed in the TMR (Donkin et al., 2009). Feeding glycerin at 10 to 11% of dietary DM did not affect the DMI of lactating cows (Donkin et al., 2009; Carvalho et al., 2011). The increased DMI by cows consuming diets of 5% glycerin in the current study is similar to that reported for cows fed diets in which liquid or dried molasses replaced high-moisture shelled corn at 4.9 to 5.6% of dietary DM in 2 studies (Broderick and Radloff, 2004). Both glycerin and liquid molasses are sweet, viscous, and rapidly and extensively fermented by ruminal microorganisms.

Glycerin did not affect milk yield (mean of 36.0 kg/d) when included in either the CS-based or CSH-based diets. This agrees with previous research in which glycerin was consumed with CS and alfalfa silage/hay as the primary forage sources (DeFrain et al., 2004; Donkin et al., 2009; Osborne et al., 2009), which suggests that glycerin provided similar amounts of NE_L as the feed-

stuffs it replaced. Milk yield was 1.7 kg/d greater ($P < 0.01$) in cows fed the CSH-based diets, likely due to their greater DMI (Table 3). Likewise, lower producing cows (24 kg/d) ate 4.5 kg/d more DM and produced 1.4 kg/d more milk when CS was replaced by CSH at 40% of dietary DM (Harris et al., 1983).

Concentration of milk fat was low for the control groups (0% glycerin) in this study, averaging 3.12% across roughage sources (Table 3). The dietary concentrations of NDF and ADF in the CS-based diets were at the lower end of the recommended values (NRC, 2001) so lower milk fat concentrations were expected. Dietary concentrations of NDF and ADF in the CSH-based diets were above recommendations (NRC, 2001) but inclusion of CSH in the diet will reduce daily chewing activity (Kononoff and Heinrichs, 2003) of lactating dairy cows, which might influence milk fat concentration. Including glycerin at 10% of dietary DM across CS- and CSH-based diets decreased milk fat concentration (quadratic effect, $P = 0.02$; Table 3). The quadratic decrease in milk fat concentration for cows fed diets of 10% glycerol was confirmed by performing a follow-up statistical test of the 0% plus 5% glycerol diets versus the 10% glycerol diet, for which $P = 0.03$. Others reported no effect of glycerin at intakes of about 10% of dietary DM on milk fat concentration (Donkin et al., 2009; Osborne et al., 2009; Carvalho et al., 2011), although the base concentration of milk fat was much greater (3.70, 5.51, and 4.01%, respectively) than that observed in the current study. Feeding glycerin (10% of dietary DM) in diets with borderline dietary or effective fiber may result in lowered milk fat concentration. This milk fat reduction brought about by glycerin feeding was accompanied by a 30% decrease (linear effect, $P = 0.02$) in NDF digestibility of diets containing 10% versus 0% glycerin across roughage sources (Table 4). Similar to milk fat concentration, milk fat yield followed a quadratic pattern ($P = 0.02$), as cows fed the most glycerin produced the least amount of milk fat. Production of 4% FCM was unchanged by feeding increasing amounts of glycerin, as variation in milk yield and milk fat concentration within and across treatments prevented the significant effect of glycerin on milk fat concentration and yield from carrying over to yield of 4% FCM. Production of ECM tended ($P = 0.09$) to be reduced by 3.5 kg/d in dairy cows fed crude glycerin (430 to 860 g/d) due to a tendency ($P = 0.13$) for reduced production of milk fat (DeFrain et al. 2004). Concentration and yield of milk true protein were unaffected by glycerin feeding, averaging 2.92% and 1.05 kg/d, respectively (Table 3). As glycerin intake increased, efficiency of 4% FCM production increased linearly when CS and alfalfa hay were the main roughage sources, but efficiency decreased linearly when CSH and bermudagrass hay replaced them (roughage source by glycerin interaction, $P = 0.02$). Feeding glycerin, an extensive and quickly digestible energy source, in high (65%) concentrate diets (CSH) had a negative effect on efficiency of conversion of feed to milk compared with adding it to moderate (51%) concentrate diets. Efficiency of milk production was not changed by feeding glycerin in diets of 46% (Donkin et al., 2009) or 41% concentrate (Osborne et al., 2009; Carvalho et al., 2011) but tended to be lower when dietary concentrate contributed 52% of dietary DM (DeFrain et al., 2004). Cows consuming diets of greater dietary concentrate appear to be susceptible to lower milk efficiency when fed glycerin. Change in BW over each 27-d period was not affected by glycerin feeding.

Source of dietary roughage did not influence milk fat concentration in the current study. Likewise, replacing all of the CS (Harris et al., 1983) or long alfalfa hay (Villavicencio et al., 1968) with CSH (30% of dietary DM) did not influence milk fat concentration. Due to increased milk yield, yield of milk fat was increased 7.7% ($P < 0.01$) by feeding CSH. Concentration of true

Table 4. Effect of roughage (R) source and glycerin (GLY) feeding on apparent digestibility coefficients, ruminal microbial protein synthesis, and plasma concentrations of BUN and glucose of lactating Holstein cows fed diets based on 1 of 2 roughage sources (corn silage or cottonseed hulls) with 0, 5, or 10% dietary glycerin

	Corn silage			Cottonseed hulls				Orthogonal contrasts (P-value)		
Item	0%	5%	10%	0%	5%	10%	SEM	R	GLY	R × GLY
Apparent digestibility										
DM	63.8	64.2	62.4	55.8	58.2	55.5	2.0	<0.01	NS[1]	NS
OM	67.8	67.6	65.7	57.4	60.1	57.5	1.9	<0.01	NS	NS
CP	63.3	60.6	58.4	61.9	64.5	61.2	2.5	NS	NS	NS
NDF	40.1	39.3	26.9	26.5	27.0	20.2	4.3	<0.01	0.02[2]	NS
Ruminal microbial protein, g/d	1,664	1,488	1,629	1,718	1,760	1,858	84	0.01	NS	NS
BUN, mg/dL	14.6	14.3	12.5	17.7	16.7	15.0	0.73	<0.01	<0.01[2]	NS
Glucose, mg/dL	69.9	69.6	71.8	70.2	70.7	70.6	1.8	NS	NS	NS

[1] $P > 0.05$.

[2] Linear decrease due to increasing dietary concentration of glycerin.

Table 5. Effect of dietary roughage (R) source and glycerin (GLY) on pH, VFA, and ammonia-N of ruminal fluid from lactating Holstein cows fed diets based on 1 of 2 roughage sources (corn silage or cottonseed hulls) with 0 or 10% dietary glycerin

	Corn silage		Cottonseed hulls			Orthogonal contrasts (P-value)[1]		
Item	0%	10%	0%	10%	SEM	R	GLY	R × GLY
pH	6.15	5.99	6.25	6.18	0.12	NS[2]	NS	NS
Acetic acid, molar %	57.0	48.0	52.5	46.8	0.7	<0.01	<0.001	0.04
Propionic acid, molar %	20.6	26.9	25.2	28.4	0.7	<0.001	<0.001	0.04
Isobutyric acid, molar %	3.3	3.2	3.0	3.0	0.1	0.03	NS	NS
Butyric acid, molar %	12.0	13.9	12.6	14.0	0.3	NS	<0.001	NS
Isovaleric acid, molar %	3.8	3.5	3.1	3.5	0.2	NS	NS	NS
Valeric acid, molar %	3.3	4.5	3.6	4.3	0.1	NS	<0.001	0.04
Total, mol/L	107.4	107.2	103.9	93.4	3.4	0.03	NS	NS
Acetate:propionate ratio	2.80	1.82	2.13	1.72	0.09	0.001	0.001	0.005
Ammonia-N, mg/dL	12.3	10.1	15.1	11.6	0.8	0.02	<0.01	NS

[1]Treatment by hour interaction was not significant for any dependent variable.
[2]$P > 0.05$.

protein of milk increased from 2.87 to 2.99% by the CSH diet, likely because it had a greater proportion of concentrate (65 vs. 51%, DM basis) than the CS diet. This increased supply of readily fermentable energy increased ($P = 0.01$) the production of microbial protein in the rumen (Table 4), which likely contributed to the increased concentration of protein in the milk. As a consequence of increased concentration of milk true protein and milk yield, production of milk true protein increased ($P < 0.01$) 9.3%, from 1.00 to 1.08 kg/d. Similar to the increase of uncorrected milk yield of 1.7 kg/d, yield of 4% FCM was increased ($P < 0.01$) by 1.9 kg/d due to feeding of the CSH-based diet. Efficiency was reduced ($P < 0.01$) from 1.24 to 1.10 kg of 4% FCM per kilogram of DMI when CSH-based diets replaced CS-based diets. This reduction was due to the dramatic increase in DMI of CSH-based diets with a less efficient conversion to milk energy but a greater increase in BW gain. Gain of BW increased ($P < 0.01$) from 0.1 to 0.7 kg/d for cows fed the CSH-based diets. Because of the large diversion of dietary energy to BW, efficiency of milk production was reduced for cows fed the CSH-based diets compared with the CS-based diets.

Feeding glycerin had no effect on apparent digestibility of DM, OM, and CP of CS- or CSH-based diets across roughage sources (Table 4). However, as stated previously, digestibility of NDF decreased linearly ($P = 0.02$) as glycerin increased in the diet from 0 to 10% across both roughage sources. In agreement, total-tract digestibility of NDF tended to be less ($P = 0.07$) in lactating cows fed diets containing 5, 10, or 15% compared with 0% glycerin (DM basis, Donkin et al., 2009). This decrease in NDF digestibility was not accompanied by a more acidic ruminal fluid pH (Table 5). In vitro growth of the fibrolytic organism *Ruminococcus flavefaciens* was partially inhibited when glycerin made up >1% of the growth medium (Roger et al., 1992). However, growth of the fungus *Neocallimastis frontalis* was depressed by as little as 0.1% glycerin in the medium. Based upon liquid volume and turnover in the rumen (Table 6), concentration of glycerin in the rumen of cows fed diets of 10% glycerin averaged 1%. Production of microbial protein, as calculated from creatinine and allantoin secretion in urine, was not affected by glycerin feeding (Table 4), which agrees with results of Donkin et al. (2009).

Table 6. Effect of dietary roughage (R) source and glycerin (GLY) on ruminal contents and passage rates of lactating Holstein cows fed diets based on 1 of 2 roughage sources (corn silage or cottonseed hulls) with 0 or 10% dietary glycerin

	Corn silage		Cottonseed hulls			Orthogonal contrasts (P-value)[1]		
Item	0%	10%	0%	10%	SEM	R	GLY	R × GLY
% of DM	13.7	12.3	19.1	18.0	0.9	0.001	NS[2]	NS
Solids, wet kg	69.3	68.9	66.2	67.7	2.0	NS	NS	NS
Solids, DM kg	9.3	8.5	12.9	12.3	0.7	0.003	NS	NS
Solids passage rate, h^{-1}	0.060	0.076	0.064	0.061	0.010	NS	NS	NS
Liquid volume, L	60.1	60.6	53.4	55.6	1.7	0.02	NS	NS
Liquid dilution rate, h^{-1}	0.144	0.132	0.162	0.168	0.005	0.002	NS	NS

[1]Treatment by hour interaction was not significant for any dependent variable.
[2]$P > 0.05$.

Table 7. Effect of roughage (R) source and glycerin (GLY) feeding on DMI and milk production and composition of rumen-cannulated Holstein cows fed diets based on 1 of 2 roughage sources (corn silage or cottonseed hulls) with 0 or 10% dietary glycerin[1]

	Corn silage		Cottonseed hulls			Orthogonal contrasts (*P*-value)		
Item	0%	10%	0%	10%	SEM	R	GLY	R × GLY
DMI, kg/d	22.1	22.1	28.4	26.9	0.7	0.001	NS[2]	NS
DMI, % of BW	3.58	3.63	4.65	4.40	0.12	0.001	NS	NS
Milk, kg/d	31.8	33.3	35.6	35.6	0.6	<0.01	NS	NS
Milk fat, %	2.78	2.82	3.03	2.90	0.07	NS	NS	NS
Milk fat, kg/d	0.90	0.94	1.08	1.03	0.05	0.05	NS	NS
Milk protein, %	2.99	2.98	2.98	3.07	0.05	NS	NS	NS
Milk protein, kg/d	0.95	0.99	1.06	1.09	0.03	0.03	NS	NS
SCS	4.3	4.0	2.5	2.9	0.8	NS	NS	NS
4% FCM, kg/d	26.2	27.5	30.4	29.7	1.0	0.04	NS	NS
Milk efficiency[3]	1.17	1.25	1.07	1.11	0.05	NS	NS	NS

[1]Diets based on corn silage were formulated to support 41 kg/d of milk (24 kg/d of DMI) and those based on cottonseed hulls to support 46 kg/d of milk (28 kg/d of DMI; NRC, 2001).

[2]$P > 0.05$.

[3]Kilograms of 4% FCM per kg of DMI.

Total-tract digestibility of DM, OM, and NDF were lower ($P < 0.01$) for CSH- compared with CS-based diets (Table 4). The NDF content of both CSH (85%) and bermudagrass (75%) is high and of lower digestibility compared with typical CS (Ruiz et al., 1995) and alfalfa hay feedstuffs (Moore et al., 1990; Torrent et al., 1994). Therefore, total-tract digestibility was expected to be lower for diets containing these feeds of greater NDF concentration. Despite a greater concentration of NDF and a lower NDF digestibility, DMI of the CSH-based diet was greater than that of the CS-based diets. The smaller particle size of the CSH probably allows them to leave the rumen with less extensive microbial digestion compared with CS.

Utilization of dietary N by ruminal microorganisms was improved with increased feeding of glycerin regardless of roughage source as evidenced by decreased mean concentration of BUN of 2.4 mg/100 mL between 0 and 10% glycerin diets (linear effect, $P < 0.01$; Table 4). Others reported decreased concentrations of MUN when glycerin replaced corn (DeFrain et al., 2004; Donkin et al., 2009). Valadares et al. (1999) reported a positive linear relationship between concentrations of plasma urea N and dietary NFC.

Plasma concentration of glucose averaged 70 mg/100 mL and was not changed by feeding glycerin. The effect of feeding glycerin on concentrations of plasma glucose of lactating cows has been inconsistent. Blood concentrations of glucose were unchanged (DeFrain et al., 2004; Osborne et al., 2009), increased (Donkin et al., 2009), and decreased (Carvalho et al., 2011) when lactating cows were consuming glycerin at 5 to 11% of dietary DM. These differences could be due to differences in sampling time of a single blood collection in relation to feeding time across studies, but not all studies provided this information. Glycerol is extensively fermented by ruminal bacteria to propionate (Table 6) so an increase in blood glucose due to glycerin consumption is certainly possible.

Trial 2

Responses of performance variables (DMI and milk yield, composition, and efficiency) of the 4 ruminally cannulated cows to treatments with 0 and 10% glycerin were very similar to those of the 24 intact cows (Tables 3 and 7). Exceptions included milk fat percentage and yield, which were not reduced by feeding glycerin, and milk protein percentage, which was not increased, and milk efficiency, which was not decreased when CSH-based diets were fed, although numerical trends were evident. A lack of statistical significance could be due to fewer experimental units used in trial 2 compared with trial 1.

Mean pH of ruminal fluid averaged 6.14 and was not affected by feeding glycerin (Table 5). Ruminal fluid pH was <6.0 for 4 of the 12 h, and treatment effects on pH were not different over the 12 samples collected (treatment × hour interaction, $P = 0.80$). Glycerin did not affect total VFA concentration, suggesting that the effect of glycerin was not different from that of starch on extent of fermentation. However, proportions of the individual VFA measured were affected by feeding glycerin. Feeding glycerin reduced the proportion of acetic acid and increased the proportions of propionic, butyric, and valeric acids ($P < 0.01$; Table 5). These results are in agreement with others supplying glycerin to cows at similar proportions of the dietary DM as used in the current study (Rémond et al., 1993; Carvalho et al., 2011). In vitro studies using ruminal fluid

have reported reduced proportions of acetic acid (Trabue et al., 2007) and increased proportions of propionic acid (Czerkawski and Breckenridge, 1972), butyric acid (Czerkawski and Breckenridge, 1972), and valeric acid (Trabue et al., 2007) with the addition of glycerin. Changes in patterns of VFA often are associated with shifts in the species of microbes in the rumen. Glycerin is metabolized by *Megasphaera elsdenii*, *Streptococcus bovis*, and *Selenomonas ruminantium* (Stewart et al., 1997). *Megasphaera elsdenii* has been associated with increased concentrations of butyric acid in ruminal fluid, which *M. elsdenii* produces from lactic acid (Klieve et al., 2003). Lactic acid was produced within 2 h of initiation of in vitro fermentation of glycerin and increased rapidly through 8 h of incubation (Trabue et al., 2007). Therefore, increased butyric acid in the current study may have resulted from an increased production of lactic acid by fermentation of glycerin, which, in turn, provided a substrate for *M. elsdenii* to produce butyric acid. *Selenomonas ruminantium*, capable of fermenting glycerin, is instrumental in the conversion of succinate produced by other microbes to propionate (Wolin et al., 1997). *Megasphaera elsdenii* also produces propionate as a fermentation end product. Feeding glycerin should increase the numbers of these organisms and result in shifts in VFA toward propionate and butyrate, as was detected in the current study. Butyrate concentrations in ruminal fluid are also in greater concentrations under greater numbers of ciliated protozoa (Whitelaw et al., 1972). It is unknown how glycerin feeding influences protozoal populations. Because of decreased acetic and increased propionic acids, the acetate to propionate ratio was reduced ($P = 0.001$) by feeding glycerin (Table 5). This decrease was most pronounced when cows were fed the CS-based diets (roughage source × glycerin interaction, $P = 0.005$), likely due to the relatively low acetate to propionate ratio for cows fed the CSH-based diet with 0% glycerin. This diet contained almost twice the proportion of ground corn compared with the CS-based diet (32.2 vs. 17.6% of DM; Table 1). The microbial fermentation of corn starch in the rumen usually results in less acetic and more propionic and valeric acids compared with that of structural carbohydrates. The addition of a highly fermentable feedstuff such as glycerin to a diet already rich in readily fermentable carbohydrates would not increase propionate as much as its addition to a diet containing less ground corn.

The mean concentration of ammonia-N in ruminal fluid was reduced ($P < 0.01$) by 2 to 3 mg/100 mL across roughage sources by feeding glycerin (Table 5). This result is compatible with the lowered BUN values of cows fed glycerin in trial 1 (Table 4). These reductions in ammonia-N and urea are often interpreted as being indicative of improved efficiency of N utilization. If glycerin is more rapidly fermented than starch in the rumen, this interpretation may be correct. However, in a study in which 2 bulls were pulse-dosed with 200 g of glycerin, Kijora et al. (1998) reported numerically lower bacterial N in ruminal fluid over a 6-d period.

Ruminal pH of cows fed CS tended to be lower ($P < 0.09$) than that of cows fed CSH (6.07 vs. 6.21, Table 5). This more acidic condition was due to a greater ($P = 0.03$) concentration of total VFA (107.3 vs. 98.6 mol/L) in CS diets. Although DMI was greater in cows fed CSH (Table 7), a lower digestibility of CSH and bermudagrass hay compared with CS and alfalfa hay, as detected in trial 1 (Table 4), would result in a lower concentration of total VFA. These roughage source effects on pH and total VFA differ from those reported by Kononoff and Heinrichs (2003), in which a more acidic pH, but no change in VFA, was detected when CS was partially replaced with CSH at 7.8% of dietary DM for lactating dairy cows. However, Moore et al. (1990) reported no difference in mean ruminal fluid pH when CSH replaced alfalfa hay in flaked milo-based diets for steers (6.4 vs. 6.0, respectively). The concentration of ammonia-N was greater ($P = 0.02$) in ruminal fluid of cows fed CSH compared with CS (13.3 vs. 11.2 mg/dL; Table 5); similar results were reported by Kononoff and Heinrichs (2003). This likely reflects the greater intake of protein and the greater proportion of soybean meal (Table 1), a protein source of high RDP value (NRC, 2001), in the CSH- versus CS-based diets.

Feeding glycerin did not change the DM concentration of the ruminal contents, although, as expected, the contents were less dry (numerically) when liquid glycerin replaced the dry ingredients (15.1 vs. 16.4%; Table 6). The total weight of the ruminal contents was not affected by diet, but the dry weight tended to be less ($P = 0.10$) when glycerin was fed (10.4 vs. 11.1 kg), again likely due to the liquid glycerin replacing dry feeds. Passage rate of the solid particles and liquid phase from the rumen was not affected by feeding glycerin. Ruminal infusion of 500 g of salt increased water intake and liquid passage rate from the rumen of steers (Rogers et al., 1979). In the current study, an additional 150 to 180 g/d of NaCl was consumed from crude glycerin but was not enough to affect liquid passage rate. Source of dietary roughage influenced ruminal dynamics. Cows fed the CSH-based diets had drier ruminal contents (18.5 vs. 13.0%, $P = 0.001$) due to consumption of drier diets, more ruminal solids (12.6 vs. 8.9 kg, $P = 0.003$) due to greater DMI, and faster passage of liquids from the rumen (0.165 vs. 0.138 h^{-1}, $P = 0.002$), possibly because of the greater intake of free water when consuming the drier diet compared with cows fed the CS-based diets. Liquid passage rate was unchanged when CSH partially replaced CS at 8%

of dietary DM (Kononoff and Heinrichs, 2003), but the liquid passage rate increased linearly from 0.119 to 0.133 to 0.192 to 0.218 h^{-1} as the dietary concentration of CSH increased from 0 to 8 to 16 to 24% of dietary DM, partially replacing sorghum silage (Akinyode, 2002). This increased passage rate of liquids may carry with it the smaller feed particles (Woodford and Murphy, 1988) and aid in increasing DMI, but it may have contributed to the lower digestibility of the CSH-based diets. The passage rate of ruminal solids was unchanged by feeding CSH (Table 5) in spite of greater DMI. This lack of detected change may have been due to the Cr-mordanting of bermudagrass hay instead of CSH to estimate the solids passage rate. The flow of Cr-mordanted bermudagrass hay may not mimic that of CSH due to differences in specific gravity; therefore, the passage rate of the solids of the total diet may have been underestimated. However, passage rates of solids from the rumen of steers fed flaked milo-based diets with alfalfa hay, CSH, or wheat straw were not different (Moore et al., 1990).

CONCLUSIONS

Diets containing low amounts of effective fiber were fed to lactating dairy cows and resulted in low concentrations of milk fat. Partially replacing ground corn, corn gluten feed, and citrus pulp with crude glycerin at 5% of dietary DM increased DMI without increasing milk yield. Concentration and production of milk fat and apparent total-tract digestion of dietary NDF were reduced when crude glycerin was fed at 10% of dietary DM. Crude glycerin affected the microbial population in the rumen, as evidenced by shifts toward propionic and butyric acids and away from acetic acid while improving efficiency of N utilization. These effects were evident in both CS- and CSH-based diets.

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