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Feeding value of glycerol as a replacement for corn grain in rations fed to lactating dairy cows

S. S. Donkin,*[1] S. L. Koser,* H. M. White,* P. H. Doane,† and M. J. Cecava†
*Department of Animal Sciences, Purdue University, West Lafayette, IN 47907
†Archer Daniels Midland Company, Decatur, IL 62521

ABSTRACT

Growth of the corn ethanol industry has created a need for alternatives to corn for lactating dairy cows. Concurrent expansion in soydiesel production is expected to increase availability and promote favorable pricing for glycerol, a primary co-product material. The objective of this study was to determine the feeding value of glycerol as a replacement for corn in diets fed to lactating dairy cattle. Sixty lactating Holstein cows housed in individual tie stalls were fed a base diet consisting of corn silage, legume forages, corn grain, soyhulls, roasted soybeans, and protein supplements. After a 2-wk acclimation period, cows were fed diets containing 0, 5, 10, or 15% refined glycerol for 56 d. Cows were milked twice daily and weekly milk samples were collected. Milk production was 36.3, 37.2, 37.9, and 36.2 ± 1.6 kg/d and feed intake was 23.8, 24.6, 24.8, and 24.0 ± 0.7 kg/d for 0, 5, 10, and 15% glycerol treatments, respectively, and did not differ except for a modest reduction in feed intake during the first 7 d of the trial for 15% glycerol (treatment × time effect). Milk composition was not altered by glycerol feeding except that milk urea nitrogen was decreased from 12.5 ± 0.4 to 10.2 ± 0.4 mg/dL with glycerol addition. Cows fed diets containing 10 and 15% glycerol gained more weight than those fed rations containing 0 or 5% glycerol but body condition scores did not differ with glycerol feeding. The data indicate that glycerol is a suitable replacement for corn grain in diets for lactating dairy cattle and that it may be included in rations to a level of at least 15% of dry matter without adverse effects on milk production or milk composition.
Key words: glycerol, energy, biofuel

INTRODUCTION

Glycerol is a byproduct of base-catalyzed transesterification of oil in the formation of methyl and ethyl fatty acid esters in the production of biodiesel (Thompson and He, 2006) and is a main by-product of ethanol fermentation processing (Michnick et al., 1997). Approximately 0.92 kg of crude glycerol is produced for every 10 L of biodiesel produced. Recent growth of the biofuels industry, including biodiesel production, has prompted forecasting of glycerol surpluses (Crandell, 2004). Opportunities may exist to use glycerol as an energy source for livestock. Although glycerin is generally recognized as safe for use in animal feed (FDA, 2006) there is little information available on feeding rates and production responses in lactating dairy cattle fed moderate to high amounts of glycerol.

The use of glycerol in the treatment of ketosis was reported as early as 1954 (Johnson et al., 1955) and evaluation of glycerol as a ketosis treatment was further explored in the 1970s (Fisher et al., 1971, 1973). Glycerol inclusion levels in those studies were between 150 and 472 g/d (Fisher et al., 1971, 1973; Khalili et al., 1997). More recently, glycerol has been reexamined as a preventative aid for metabolic problems associated with transition cows. Goff and Horst (2001) used up to 3 L in treatment and prevention of ketosis, and DeFrain et al. (2004) fed 0.86 kg/d to transition dairy cattle. Feeding 162.5 g/d of glycerol in a dry product containing 65% food-grade glycerol did not alter feed intake, milk yield and components, blood metabolites, or serum insulin concentrations during the first 3 wk of lactation but tended to increase milk production 3 wk after cessation of feeding (Chung et al., 2007). Although these studies demonstrate the potential value of glycerol as an energy source in ketosis management, they do not address the potential for using glycerol as a primary feed ingredient in rations fed to lactating dairy cows.

There are only a few studies for which glycerol feeding rates approached 5% or more of the ration on a DM basis. Schröder and Südekum (1999) fed diets containing 10% glycerol to dairy cattle, effectively replacing over one-half of the starch in the diet, without negatively affecting intake, ruminal digestibility, rumen microbial synthesis, or total-tract nutrient digestibility in steers. Feeding diets containing 3.6% glycerol to mid-lactation

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[1]Corresponding author: sdonkin@purdue.edu

dairy cows had no effect on intake, milk production, or gross milk composition but slightly altered the profile of fatty acids in milk; it increased rumen propionate and butyrate at the expense of acetate (Khalili et al., 1997). Feeding 0.86 kg of glycerol per day, or 5.4% of ration DM, beginning 14 d before calving through 21 d in milk did not have any effects on milk production or feed intake (DeFrain et al., 2004). Feeding 500 mL of glycerol per day, or approximately 3.1% of DM, from 3 wk before calving to 70 d in milk caused an increase milk yield and milk protein content (Bodarski et al., 2005). Collectively, these experiments indicate that glycerol may be added to diets for lactating cows without deleterious effects. However, the upper limit of glycerol inclusion has not been elucidated. The objective of the present experiment was to determine the effects of feeding refined glycerol as a replacement for corn grain on feed intake, milk production, milk composition, and total-tract nutrient digestion in lactating cows. Food-grade refined glycerol was used to avoid possible confounding effects of impurities often associated with biodiesel-source crude glycerol.

MATERIALS AND METHODS

Cows and Dietary Treatments

Sixty lactating Holstein cows with an average DIM of 173 ± 47 were housed in individual tie stalls at the Purdue Dairy Research and Education Center and fed a base ration balanced to meet NRC (2001) guidelines for 600-kg Holstein dairy cows producing 40 kg of milk/d with 3.8% of milk fat and 3.0% true protein. The base ration contained corn silage, alfalfa haylage, hay, dry-rolled corn, vitamins, and minerals (Table 1). After 2 wk of adjustment to the facilities, cows were stratified by milk production and parity and randomly assigned to the base diet containing no added glycerol (0% glycerol) or to diets containing 5, 10, or 15% added refined glycerol (99.5% USP-grade glycerine; Pt Sumi Ashi Oleochemicals Industry, Jakarta, Indonesia) as a percentage of ration DM (Table 1). Glycerol and corn gluten meal in an 86:14 ratio (by weight) replaced an equal weight of corn grain in the diets. The addition of corn gluten meal was intended to adjust for the protein that was removed with corn grain. Diets were offered once daily for ad libitum intake (10 to 15% weighbacks) for 56 d. Feed refusals were measured daily and feed intake was determined by difference.

Cows were milked twice daily and milk samples were obtained weekly at 2 consecutive milkings and analyzed for fat, protein, lactose, total solids, MUN, and somatic cells by DairyOne (Ithaca, NY). Body weights and condition scores were obtained before initiation of treatments and at the end of the experiment. Body condition was scored by 2 trained individuals based on a 5-point scale (Wildman et al., 1982). Blood samples were collected on d 56 of the experiment into vacutainers containing oxalate and sodium fluoride by venipuncture of a coccygeal vessel. Plasma was analyzed for glucose concentrations (Glucose Test, procedure no. 1075, Stanbio Laboratory Inc., Boerne, TX) using the procedure of Trinder (1969).

Feed Analysis and Total-Tract Digestibility

Samples of TMR were collected weekly and ground to pass a 4-mm screen. Composite samples were formed for wk 1 through 4 and wk 5 through 8 and analyzed by a commercial laboratory (Dairy One, Ithaca, NY) for DM, CP, ADF, and minerals by wet chemistry following AOAC (2000) procedures and for NDF following the method of Goering and Van Soest (1970). Target composition of the diets was 17.0% CP, 28.3% NDF, 19.2% ADF, 1.56 Mcal of NE_L/kg, 1% Ca, and 0.4% P with an anticipated intake of 26.1 kg/d. Analyzed composition met or exceeded the ration target due, in part, to variations in forage (hay and haylage) quality during the experiment.

Fecal samples were collected once per day during the last 3 d of the feeding period (d 53 through 56) and sampling was shifted 8 h in time during the 3-d collection schedule. Samples of diets were collected for 3 d before initiation of fecal collection. Fecal and TMR samples were dried in a forced-air oven at 60°C and ground to pass through a 1-mm screen. Composite samples of diets and fecal samples from each cow were analyzed for acid detergent insoluble ash (Van Keulen and Young, 1977), N, ADF, and NDF as described above. Gross energy of samples was determined with an adiabatic bomb calorimeter (Parr Instrument Co. Inc., Moline, IL). Apparent digestibility coefficients were determined as described previously (Van Keulen and Young, 1977). Cumulative efficiency of feed utilization for milk, maintenance, and gain were calculated individually for each animal during the 56-d feeding period. Maintenance requirements were determined based on BW (NRC, 2001). Energy associated with a change in BW for the 56-d period was calculated based on BCS at the beginning of the trial and change in BW during the subsequent period (NRC, 2001; Table 2–5). Milk energy was determined from average milk composition for each cow over the 56-d period and NE_L calculations described by the NRC (2001) using the coefficient 0.0563 for milk true protein. Overall energetic efficiency was calculated as the sum of milk, maintenance, and BW gain (or loss) for the 56-d experiment divided by cumulative DMI for the corresponding interval.

Table 1. Ingredient and nutrient composition of diets

Item	Glycerol, % of diet DM			
	0	5	10	15
Ingredient, % of DM				
Corn silage	31.9	31.9	31.9	31.9
Alfalfa haylage	10.0	10.0	10.0	10.0
Alfalfa hay	12.1	12.2	12.2	12.1
Soyhulls	7.7	7.7	7.7	7.6
Soybean meal	6.6	6.6	6.6	6.6
Roasted soybeans	5.4	5.4	5.4	5.4
Fish meal	0.7	0.7	0.7	0.7
Urea	0.3	0.3	0.3	0.3
Megalac R[1]	1.0	1.0	1.0	1.0
Corn, ground	20.0	14.2	8.4	2.8
Glycerol	—	5.0	10.0	15.0
Corn gluten meal	—	0.8	1.6	2.4
Mineral/vitamin[2]	4.3	4.3	4.3	4.3
Chemical composition[3]				
CP	18.1 (0.57)	17.5 (1.13)	18.2 (0.49)	18.1 (0.99)
ADF	19.1 (0.64)	19.2 (0.57)	19.4 (1.06)	19.3 (1.13)
NDF	30.9 (1.27)	32.4 (1.70)	29.7 (1.41)	31.0 (1.40)
NE_L, Mcal/kg	1.69 (0.00)	1.67 (0.02)	1.69 (0.00)	1.69 (0.02)
Ca	1.03 (0.04)	1.01 (0.08)	1.04 (0.03)	1.05 (0.06)
P	0.41 (0.01)	0.39 (0.01)	0.41 (0.01)	0.41 (0.02)
Mg	0.34 (0.01)	0.31 (0.01)	0.32 (0.01)	0.33 (0.01)
K	1.88 (0.35)	1.85 (0.33)	1.88 (0.35)	1.88 (0.31)
Na	0.25 (0.02)	0.24 (0.02)	0.28 (0.00)	0.27 (0.05)

[1]Church & Dwight Co., Princeton, NJ.

[2]Contained 43.52% dried molasses, 13.06% calcium carbonate, 10.88% sodium bicarbonate, 8.70% dicalcium phosphate, 4.35% salt, 3.92% trace mineral-vitamin premix (16.11% Ca, 2.11% S, 31,505 mg/kg Zn, 8,036 mg/kg Cu, 26,020 mg/kg Mn, 140 mg/kg Se, 473 mg/kg Co, 284 mg/kg I, 1,440 kIU/kg vitamin A, 416 kIU/kg vitamin D, 6,647 IU/kg vitamin E), 3.48% magnesium oxide (58%), 3.48% DCAD plus, 3.48% XP yeast culture (Diamond V Mills, Cedar Rapids, IA), 3.48% Omnigen-AF (Prince-Agri Products, Quincy, IL), 0.87% Dynamate, 0.42% Niacin, and 0.35% vitamin E 20000.

[3]Mean analysis for composite samples (n = 2) and associated standard deviations (in parentheses).

Urine Sampling and Purine Analysis

Spot urine samples were obtained from all animals during the final week of the experiment. Urine samples were acidified to pH <4 with 4 *M* HCl and frozen for later analysis. Analysis of purine derivatives and creatinine was performed at the University of Nebraska using an HPLC instrument (Waters Corp., Milford, MA) with urine volume assumed to be 28 mg/kg BW of creatinine output (Janicek et al., 2008). The ratio of purine derivative output (allantoin plus uric acid) to creatinine was used to compare relative differences in microbial CP production and supply (Chen and Gomes, 1992).

Data Analysis

The data were analyzed using the MIXED procedure (SAS Inst. Inc., Cary, NC). The model accounted for the main effects of diet (0, 5, 10, or 15% glycerol) and, when appropriate, the day (or week) of experiment effect and the interaction effect of diet by day (or week) of experiment. Compound symmetry covariance structure was used to evaluate variables measured. Milk production, feed intake, and milk:DMI were analyzed with repeated measures by day. Average weekly milk production, DMI, and milk composition were analyzed using repeated measures by week. Means were different if $P < 0.05$ and tended to differ if $0.05 \leq P \leq 0.10$. Orthogonal contrasts tested linear, quadratic, and cubic responses to glycerol. Single degree of freedom contrasts between 0% glycerol and 5, 10, or 15% glycerol were used to characterize time × treatment responses when present. Values reported are least squares means and associated standard errors.

RESULTS

Cows fed glycerol consumed similar amounts of feed and produced a similar amount of milk during the 8-wk feeding period as cows fed a diet containing no added glycerol (Table 2). There was a time × treatment response ($P < 0.05$) for feed intake, milk production, and efficiency. Feed intake decreased ($P < 0.05$) with inclusion of 15% glycerol during the first 7 d of the trial (Figure 1) but this effect was transient and no intake differences were detected during the subsequent 7 wk of the experiment. Average daily milk production was not

Table 2. Effect of glycerol on feed intake, milk production, BW change, BCS, and plasma glucose

	Glycerol, % of diet DM					Treatment and contrast P-values[1]			
Item	0	5	10	15	SEM	Overall	Linear	Quadratic	Cubic
Milk production,[2] kg/d	36.3	37.2	37.9	36.2	1.6	0.89	0.74	0.52	0.79
DMI,[2] kg/d	23.8	24.6	24.8	24.0	0.7	0.76	0.54	0.38	0.95
Milk: DMI, kg/kg	1.55	1.52	1.52	1.53	0.05	0.99	0.87	0.87	0.81
3.5% FCM,[2] kg/d	34.3	34.3	35.4	33.1	1.6	0.80	0.75	0.48	0.53
Milk fat, kg/d	1.33	1.28	1.33	1.27	0.06	0.68	0.47	0.55	0.45
Milk protein, kg/d	1.00	1.04	1.06	1.04	0.04	0.65	0.56	0.28	0.76
Milk lactose, kg/d	1.66	1.68	1.76	1.67	0.08	0.69	0.83	0.33	0.51
Milk solids, kg/d	4.31	4.33	4.48	4.30	0.19	0.75	0.99	0.37	0.53
SCC, × 1,000 cells/mL	275	490	137	144	111	0.18	0.27	0.52	0.07
MUN, mg/dL	12.5	10.9	10.7	10.2	0.4	0.01	0.01	0.23	0.17
Milk fat, %	3.70	3.52	3.58	3.58	0.11	0.45	0.16	0.92	0.44
Milk protein, %	2.79	2.84	2.86	2.89	0.06	0.78	0.42	0.52	0.92
Milk lactose, %	4.64	4.62	4.70	4.66	0.07	0.68	0.81	0.43	0.37
Milk solids, %	12.05	11.89	12.03	12.04	0.19	0.74	0.47	0.70	0.45
BCS change	0.07	0.08	0.12	0.12	0.05	0.91	0.52	0.95	0.79
BW change, kg	31.5	40.7	49.7	51.6	4.6	0.01	0.01	0.43	0.74
Plasma glucose, mg/dL	51.9	54.9	55.9	58.2	2.03	0.21	0.04	0.87	0.73

[1]Probability that treatment means and contrasts are not different.
[2]Treatment × day interaction effect ($P < 0.05$).

altered in response to glycerol feeding although there was a time × treatment response ($P < 0.05$). Mean weekly milk production did not differ among the diets. Fat-corrected milk production followed a similar pattern as milk production. Milk composition for the 8-wk feeding period was not altered in response to glycerol feeding except for MUN, which linearly decreased ($P < 0.05$) with increasing glycerol in the diet. Blood glucose concentrations increased in a linear manner with the addition of glycerol to the diet (Table 2).

Initial BW was 605, 603, 578, and 595 ± 17 kg for cows fed glycerol at 0, 5, 10, or 15% of diet DM, respectively, and did not differ ($P > 0.05$) among treatment groups at trial initiation. All cows gained weight during the 8 wk of the trial but cows fed the highest amount of glycerol gained the greatest amount of BW (Table 2). Cows fed 10 and 15% glycerol gained more weight than cows fed the control diet, whereas weight change was similar for cows fed 0 and 5% glycerol diets. Body condition scores were 2.66, 2.57, 2.52, and 2.58 ± 0.07 at the beginning of the experiment for 0, 5, 10, or 15% glycerol, respectively, and did not differ among treatments. Changes in BCS were positive and although they mirrored the effects of glycerol on BW, these changes were small and did not differ among treatments.

Apparent digestibility of DM and OM increased with glycerol addition to the diet (Table 3). There was no difference in DM or OM intake. Dry matter digestibility responded quadratically to the replacement of corn with glycerol. Intake of NDF decreased linearly ($P < 0.05$) with glycerol addition to the diet and although there was an overall tendency for glycerol to reduce NDF digestibility, this response was not linear. The digestibility of NDF was reduced with 5% glycerol compared with no addition but was similar between 0, 10, and 15% glycerol. Nitrogen digestibility showed a quadratic response ($P < 0.05$) to increasing glycerol inclusion in the diet. Although gross energy intake was unaffected, there was greater apparent digestibility of energy for the glycerol diets and this occurred in a quadratic ($P < 0.05$) manner. Microbial CP synthesis was estimated from urinary purines (Table 4) and did not differ with glycerol feeding despite linear, cubic, and quadratic response for purine derivatives.

Overall efficiency of feed utilization during the 56-d experiment did not differ among the treatment groups (Table 3). There was no difference in the efficiency of use of feed DM for milk production; however, the calculated partial efficiency of feed for BW gain was increased in a linear manner with increasing glycerol inclusion in the diet.

DISCUSSION

Increased production of biofuels has increased the coproduction of glycerol and has led to a corresponding decline in price projections (Yazdania and Gonzalez, 2007). These dynamics may favor the use of glycerol in rations fed to livestock, especially in light of diversion of corn and other grains toward ethanol production. Although there is evidence supporting the use of glycerol in transition dairy cow diets, there is little information on the potential use of glycerol as a primary ingredient in rations for lactating dairy cows. The present data

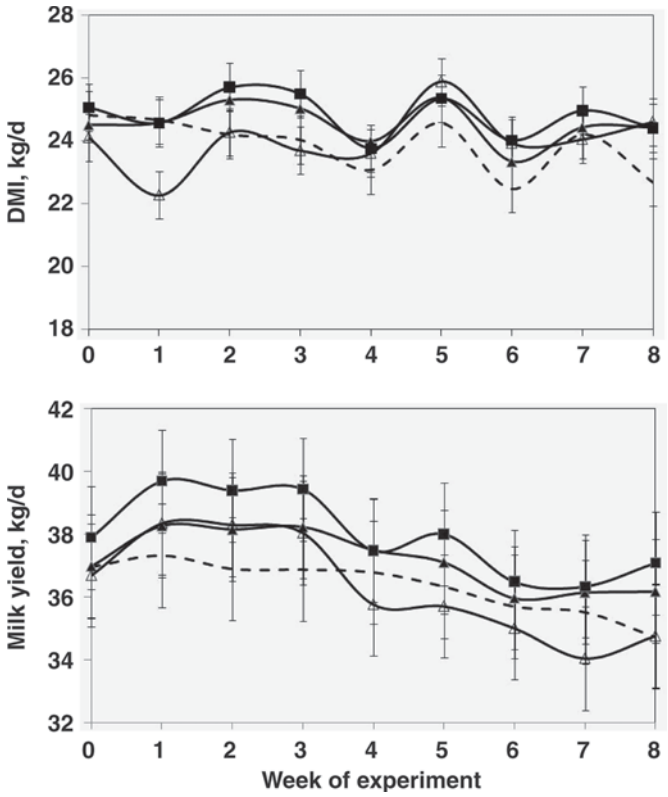

Figure 1. Effect of feeding glycerol for 56 d on DMI and milk production. Cows were fed rations containing 0 (– – –), 5 (–■–), 10 (–▲–), or 15% (–Δ–) glycerol during a 56-d feeding period. Feed intake and milk production were measured daily. Data are expressed as weekly means, and data for wk 0 represent the 7 d immediately before initiation of treatments. There was a treatment × week of experiment effect ($P < 0.05$) on milk production and feed intake. Single degree of freedom tests revealed that feed intake was reduced ($P < 0.05$) for cows fed 15% glycerol compared with 0% glycerol during the first week of the experiment. Corresponding standard errors are 1.6 and 0.7 kg for milk and DMI, respectively.

indicate that glycerol can be used to partially replace corn grain without negative consequences on milk production or composition.

Recent research indicates no effect of glycerol on feed intake. Glycerol administered as a topdress from −14 to +21 d relative to calving decreased prepartum intake but was without effect during the postpartum interval (DeFrain et al., 2004). A recent experiment in which a dry glycerol product was fed to transition cows during the prepartum period showed no effect on intake (Chung et al., 2007). In contrast, feeding 300 or 500 mL of glycerol per day beginning at calving had a transient effect of increasing DMI after 5 wk of feeding (Bodarski et al., 2005). In other experiments, feeding glycerol to mid-lactation dairy cows had no effect on feed intake (Khalili et al., 1997). The purity of glycerol has been suggested as a determining factor for adverse effects of glycerol, particularly in the case of reduced feed intake (Chung et al., 2007). Impurities in biodiesel-derived glycerol may adversely affect intake (Schröder and Südekum, 1999); however, the use of food-grade glycerol in the present experiment eliminated this possibility. The time × treatment effect on intake observed in the present experiment suggested an effect of glycerol on intake that was transient and it was observed only when glycerol exceeded 10% of the diet DM.

Milk production was similar for cows fed glycerol compared with the control diet. There was a time × treatment response on milk production. The nature of this interaction is not readily apparent as there were no differences between milk production for cows fed the control diet compared with cows fed 5, 10, or 15% glycerol in each week. The data indicate that cows fed glycerol experienced a slight numerical increase in milk production during the first 3 wk of the experiment. Cows fed 0, 5, or 10% glycerol then followed a similar pattern of production but cows fed 15% glycerol experienced a numerical reduction in milk production. Taken together, these data contribute to a time × treatment response but a lack of overall treatment effect. The lack of glycerol effects on milk production agrees with previous studies in which transition cows (DeFrain et al., 2004; Chung et al., 2007), early postpartum cows (Fisher et al., 1973), and mid-lactation cows (Khalili et al., 1997) were fed glycerol. In contrast, the addition of 300 and 500 mL of glycerol as a topdress once daily to rations fed to dairy cattle from 2 wk before calving through 10 wk of lactation resulted in greater milk production compared with cows fed a control diet (Bodarski et al., 2005). The available data suggest that glycerol is not detrimental to milk production if fed during the transition period or during lactation. Data from the present study indicate that 15% glycerol can be fed in a TMR without negatively affecting milk production.

Power calculations were performed using the POWER procedure of SAS for 2 sample means and applying the variance for milk production and DMI obtained from the PROC MIXED analysis from the present experiment. When $\alpha = 0.05$, the power of the test for DMI was 0.68. Therefore, the probability of making the correct decision to reject the null hypothesis (i.e., that the treatment means are equal) is 68%. The corresponding power of the hypothesis test for milk production is 34%. A power of the hypothesis test of 80% when utilizing 15 cows per treatment group and the standard deviation associated with the present experiment would require differences between treatment means of at least 6.6 kg/d for milk production and 2.7 kg/d for DMI. The low power associated with the hypothesis test limits the inference of the current experiment and should be considered when contrasting the current data with other determinations of the relative feeding value for glycerol compared with corn grain.

Table 3. Effect of feeding glycerol on nutrient intake and total tract digestibility in lactating dairy cows

	Glycerol, % of diet DM					Treatment and contrast *P*-values[1]			
Item	0	5	10	15	SEM	Overall	Linear	Quadratic	Cubic
Intake									
DMI, kg	24.2	24.4	24.9	24.0	0.7	0.81	0.98	0.42	0.58
OM, kg	22.6	22.8	23.3	22.4	0.7	0.81	0.97	0.45	0.55
NDF, kg	7.90	6.62	6.90	7.12	0.21	0.01	0.03	0.01	0.09
Nitrogen, g	580.1	605.1	595.9	540.3	17.1	0.05	0.10	0.02	0.87
Gross energy, Mcal	98.9	101.0	105.7	100.6	3.0	0.41	0.46	0.23	0.36
Digestibility, apparent total-tract									
DMI, %	76.8	79.1	80.7	79.7	0.8	0.01	0.01	0.05	0.63
OM, %	77.8	79.9	81.6	80.7	0.8	0.02	0.01	0.07	0.59
NDF, %	66.1	61.1	63.8	63.3	1.3	0.07	0.34	0.09	0.07
Nitrogen, %	74.5	77.1	77.0	74.0	0.9	0.03	0.67	0.01	0.96
Gross energy, %	77.2	79.6	81.6	80.3	0.9	0.01	0.01	0.05	0.49
Energetic efficiency,[2] Mcal/kg of DMI									
Overall	1.55	1.55	1.57	1.58	0.04	0.90	0.50	0.87	0.80
Milk	1.04	1.02	1.03	1.00	0.03	0.85	0.49	0.87	0.60
BW change	0.51	0.53	0.54	0.58	0.02	0.10	0.01	0.63	0.71

[1]Probability that treatment means and contrasts are not different.

[2]Calculated thus: overall = (milk energy + maintenance + BW change)/DMI; milk = milk energy/DMI; BW change/DMI.

Milk composition was unaffected by glycerol feeding with the exception that MUN decreased. The lack of effects of glycerol on milk composition are consistent with previous reports when glycerol was fed to transition cows (DeFrain et al., 2004; Chung et al., 2007) or mid-lactation cows (Khalili et al., 1997) but in contrast to observed effects of prolonged feeding from precalving through 10 wk of lactation (Bodarski et al., 2005). A tendency for decreased MUN was observed with glycerol feeding to transition cows (DeFrain et al., 2004) and BUN decreased in mid-lactation cows when glycerol was fed in combination with fatty acids (Khalili et al., 1997). Decreased MUN and BUN suggest greater N efficiency; however, bacterial N content of rumen fluid (Kijora et al., 1998) and ^{15}N incorporation into bacterial N fractions in vitro are both reportedly decreased by glycerol (Bergner et al., 1995). Estimates of rumen microbial CP production in the present study indicate that glycerol did not alter rumen protein synthesis. Any changes in N efficiency with glycerol feeding, therefore, may be attributed to changes in postruminal or postabsorptive N metabolism.

Apparent total-tract digestibility increased for DM, OM, N, and gross energy with increasing glycerol in the diet. These data contrast with previous observations demonstrating a lack of effect of glycerol on total-tract digestibility of DM, OM, N, and NDF (Khalili et al., 1997). Glycerol, when added in combination with plant-derived fatty acids, increased lipid digestibility but had no effect when added alone (Khalili et al., 1997). It is unclear if the interaction of glycerol and fatty acids that results in an increase in FCM production (Khalili et al., 1997) is a consequence of increased lipid digestibility. The apparent digestibility of lipid was not measured in the current experiment.

The tendency for a reduction in NDF digestibility was not matched by reductions in OM or DM digestibility or by a corresponding reduction in intake. Glycerol in

Table 4. Effect of feeding glycerol on daily excretion of urinary creatinine, allantoin, uric acid, and estimated rumen microbial CP production

	Glycerol, % of diet DM					Treatment and contrast *P*-values[1]			
Item	0	5	10	15	SEM	Overall	Linear	Quadratic	Cubic
Creatinine, m*M*	1.25	1.30	1.14	1.40	0.06	0.61	0.26	0.10	0.03
Allantoin, m*M*	3.85	3.95	3.72	4.37	0.14	0.31	0.04	0.05	0.06
Uric acid, m*M*	0.51	0.51	0.46	0.56	0.03	0.93	0.42	0.10	0.11
Purine derivatives,[2] m*M*	4.36	4.46	4.18	4.93	0.16	0.36	0.04	0.05	0.05
Purine:creatinine ratio	3.55	3.50	3.70	3.54	0.13	0.84	0.76	0.69	0.32
Microbial CP,[3] g/d	2,318	2,232	2,290	2,277	61	0.69	0.89	0.75	0.67

[1]Probability that treatment means and contrasts are not different.

[2]Total purine derivatives (allantoin + uric acid).

[3]Microbial CP, g/d = ({[purine derivative production − (0.385 × $BW^{0.75}$)]/0.85} × 70 × 6.25)/(0.13 × 0.83 × 1,000), where purine derivative production = [(28 × BW)/113.1] × (purine:creatinine ratio).

excess of 1% in bacterial culture media inhibited growth and cellulolytic activity of *Ruminococcus flavefaciens* and *Fibrobacter succinogenes* in vitro, although bacterial adherence to cellulose was not affected (Roger et al., 1992). There appeared to be an adaptation to glycerol feeding in vivo that was reflected in a reduction in cellulose fermentation in vitro but no effect on starch fermentation was observed (Rémond et al., 1993). The current data suggest that glycerol may decrease total-tract NDF digestibility; however, additional data are necessary to confirm this response and to determine the site of its occurrence and causation.

Body weight change increased with increasing level of glycerol. This effect was not reflected in a corresponding change in BCS. One unit of BCS represents a change of 82 kg of live weight for Holstein dairy cows (NRC, 2001); therefore, a change in BCS of 0.6 would be anticipated for cows fed 15% glycerol based on the observed changes in BW. A portion of the lack of agreement for effect of glycerol on BW and BCS may be due to differences in water intake and hydration state of cows fed glycerol. There is considerable interest in the potential for ergogenic properties of glycerol in endurance athletes and potential for increased time to exhaustion and peak power output (Goulet et al., 2008). The benefits of glycerol for athletic activity may be due to altered hydration state, changes in nutrient metabolism, or both (Nelson and Robergs, 2007). Glycerol increases hydration state in horses by stimulating renal water conservation and enhancing voluntary water intake (Schott et al., 2001). In humans and rats, a single loading dose of glycerol may increase total body water up to 5% (Koenigsberg et al., 1995). The hyperhydrated state may be achieved through a decrease in urinary water loss or an increase in water absorption. The difference in BW gain for cows fed 15% glycerol compared with no added glycerol is approximately 3.3%. Measures of hydration status were not performed in the present experiment; however, if glycerol acts to enhance hydration state in dairy cows, as it does in other species, these hydration effects may be beneficial during heat stress or other physiological and environmental challenges.

Dietary glycerol appears to be extensively fermented to propionate by ruminal bacteria (Czerkawski and Breckenridge, 1972; Rémond et al., 1993; Bergner et al., 1995) although level of feeding and method of delivery may influence the amount of glycerol that escapes fermentation (Kijora et al., 1998; DeFrain et al., 2004). The density of glycerol (1.26 g/mL) matches closely the optimal density for passage from the rumen through the rumen-reticulo orifice (King and Moore, 1957; desBordes and Welch, 1984; Neel et al., 1995). Therefore, the amount that escapes the rumen when glycerol is delivered as a drench may be greater than when fed as a TMR. A rapid increase in blood glucose when glycerol is delivered as a drench, compared with feeding, supports this hypothesis (Linke et al., 2004). Data from the present experiment also demonstrate an increase in circulating glucose with increasing glycerol although the case of this increase cannot be determined from a single glucose sample.

Outflow to the abomasum represented 7 to 18% of a 1,200-g pulse-dose of glycerol infused through a rumen cannula in nonlactating dairy cows (Rémond et al., 1993). Other experiments support a rapid disappearance of glycerol from the rumen. When glycerol is pulse-dosed through the rumen cannula at a level matching 10% of daily DMI, only 5% of the dose is detectable after 4 h (Kijora et al., 1998). It has been suggested that only one-half of the disappearance of glycerol from the rumen can be accounted for by bacterial fermentation and therefore suggests direct absorption of glycerol from the rumen (Rémond et al., 1993). Glycerol transport across epithelia in other mammals is mediated by homologous water channel proteins, aquaporins (**AQP**), some of which also function as glycerol transporters known as aquaglyceroporins (Rojek et al., 2008). The AQP3 transporter has been identified as a prime glycerol transporter in the intestines of rats and humans but has also been localized in eye, kidney, stomach, spleen, and erythrocyte. Alternatively, AQP7 in mouse has been localized to adipose tissue where it serves, along with glycerol kinase, to direct adiposity and the fate of glycerol released through lipolysis (MacDougald and Burant, 2005). The presence of aquaglyceroporins in bovine rumen tissues has not been reported but direct absorption of glycerol across the rumen or intestinal epithelium could be a determinant of the intermediary metabolism of glycerol and its role as a glucose precursor.

An increase in the partial efficiency of DMI for BW gain with an increasing level of glycerol in the diet suggests actions to alter postabsorptive metabolism in favor of body tissue gain. These effects are observed in the absence of offsetting losses output of milk energy and suggest improved energetic efficiency with glycerol feeding. Changes in energetic efficiency may be due to effect of glycerol on rumen fermentation, postabsorptive effects, or a combination. Recent data from transition cows given glycerol as a topdress (Chung et al., 2007) or supplied in water (Osborne et al., 2009) indicate postpartum reductions in plasma BHBA with glycerol. These data suggest a shift to reduce fatty acid oxidation to ketones or to increase utilization of ketones by extrahepatic tissues when glycerol is fed. Glycerol has been shown to increase palmitate uptake while simultaneously decreasing β-oxidation in myocytes (Gambert

et al., 2005). Elevated glycerol concentrations have been observed in adipocytes from AQP-7–deficient mice, which leads to elevated glycerol concentrations and triglyceride accumulation in adipose tissue (Rodríguez et al., 2006); therefore, circulating glycerol concentrations may serve to modulate adipose triglyceride turnover. These data point to as-yet unexplored possibilities for postruminal glycerol metabolism in the dairy cow to modulate postabsorptive lipid metabolism.

Results from this study clearly indicate that glycerol is a valuable feed ingredient for lactating dairy cows. Glycerol may be included at 15% of the ration for lactating dairy cows without deleterious effects. Feeding glycerol in place of corn is an alternative strategy for formulating diets for lactating cows. Additional information is needed to determine the effects of extending the duration of glycerol feeding on intake, production, and health. Likewise, the nature of glycerol effects on total-tract digestibility needs to be further explored, in particular for diets where animal fiber digestibility may limit animal performance. Because the present study was conducted with 99.9% pure food-grade glycerol, caution is warranted for extrapolating these data to the feed value of crude biodiesel glycerol. The value of the latter will be reduced if substantial contaminants are present or if diluents including water, fat, methanol, and minerals are present (Thompson and He, 2006). If methanol is a contaminant, levels must be consistent with federal and state regulations for biodiesel glycerol to be considered acceptable as animal feed.

CONCLUSIONS

Glycerol is a suitable feed ingredient to replace corn grain in rations fed to lactating dairy cows. There were no deleterious effects on feed intake, milk production, or milk composition when glycerol substituted for up to 15% of ration DM.

REFERENCES

AOAC. 2000. Official Methods of Analysis. 17th ed. AOAC Int., Gaithersburg, MD.

Bergner, H., C. Kijora, Z. Ceresnakova, and J. Szakacs. 1995. In vitro studies on glycerol transformation by rumen microorganisms. Arch. Tierernahr. 48:245–256.

Bodarski, R., T. Wertelecki, F. Bommer, and S. Gosiewski. 2005. The changes of metabolic status and lactation performance in dairy cows under feeding TMR with glycerin (glycerol) supplement at periparturient period. Electronic Journal of Polish Agricultural Universities Animal Husbandry 8:1–9. http://www.ejpau.media.pl/volume8/issue4/art-22.html

Chen, X. B., and M. J. Gomes. 1992. Estimation of microbial protein supply to sheep and cattle based on urinary excretion of purine derivatives—An overview of the technical details. Int. Feed. Res. Unit, Occas. Publ. Rowett Res. Inst., Bucksburn, Aberdeen, UK.

Chung, Y. H., D. E. Rico, C. M. Martinez, T. W. Cassidy, N. Noirot, A. Ames, and G. A. Varga. 2007. Effects of feeding dry glycerin to early postpartum Holstein dairy cows on lactational performance and metabolic profiles. J. Dairy Sci. 90:5682–5691.

Crandall, L. 2004. Glycerol abundance cause for concern. Inform 15:146–147.

Czerkawski, J. W., and G. Breckenridge. 1972. Fermentation of various glycolytic intermediates and other compounds by rumen microorganisms, with particular reference to methane production. Br. J. Nutr. 27:131–146.

DeFrain, J. M., A. R. Hippen, K. F. Kalscheur, and P. W. Jardon. 2004. Feeding glycerol to transition dairy cows: Effects on blood metabolites and lactation performance. J. Dairy Sci. 87:4195–4206.

desBordes, C., and J. G. Welch. 1984. Influence of specific gravity on rumination and passage of indigestible particles. J. Anim. Sci. 59:470.

Fisher, L. J., J. D. Erfle, G. A. Lodge, and F. D. Sauer. 1973. Effects of propylene glycol or glycerol supplementation of the diet of dairy cows on feed intake, milk yield and composition, and incidence of ketosis. Can. J. Anim. Sci. 53:289–296.

Fisher, L. J., J. D. Erfle, and F. D. Sauer. 1971. Preliminary evaluation of the addition of glucogenic materials to the rations of lactating cows. Can. J. Anim. Sci. 51:721–727.

FDA. 2006. Code of Federal Regulations, 21 CFR 582.1320, Title 21, Vol. 6, 21CFR582.1320. http://edocket.access.gpo.gov/cfr_2002/aprqtr/21cfr582.1320.htm

Gambert, S., C. Héliès-Toussaint, and A. Grynberg. 2005. Regulation of intermediary metabolism in rat cardiac myocyte by extracellular glycerol. Biochim. Biophys. Acta 1736:152–162.

Goering, H. K., and P. J. Van Soest. 1970. Forage Fiber Analysis (Apparatus, Reagents, Procedures and Some Applications). Agric. Handbook No. 379. ARS-USDA, Washington, DC.

Goff, J. P., and R. L. Horst. 2001. Oral glycerol as an aid in the treatment of ketosis/fatty liver complex. J. Dairy Sci. 84(Suppl. 1):153. (Abstr.)

Goulet, E. D., S. F. Rousseau, C. R. Lamboley, G. E. Plante, and I. J. Dionne. 2008. Pre-exercise hyperhydration delays dehydration and improves endurance capacity during 2 h of cycling in a temperate climate. J. Physiol. Anthropol. 27:263–271.

Janicek, B. N., P. J. Kononoff, A. M. Gehman, and P. H. Doane. 2008. The effect of feeding dried distillers grains plus solubles on milk production and excretion of urinary purine derivatives. J. Dairy Sci. 91:3544–3553.

Johnson, R. B. 1955. The treatment of ketosis with glycerol and propylene glycol. Cornell Vet. 44:6–21.

Khalili, H., T. Varvikko, V. Toivonen, K. Hissa, and M. Suvitie. 1997. The effects of added glycerol or unprotected free fatty acids or a combination of the two on silage intake, milk production, rumen fermentation and diet digestibility in cows given grass silage based diets. Agric. Food Sci. Finl. 6:349–362.

Kijora, C., H. Bergner, K. P. Gotz, J. Bartelt, J. Szakacs, and A. Sommer. 1998. Research note: investigation on the metabolism of glycerol in the rumen of bulls. Arch. Tierernahr. 51:341–348.

King, K. W., and W. E. C. Moore. 1957. Density and size as factors affecting passage rate of ingesta in the bovine and human digestive tracts. J. Dairy Sci. 40:528.

Koenigsberg, P. S., K. K. Martin, H. R. Hlava, and M. L. Riedesel. 1995. Sustained hyperhydration with glycerol ingestion. Life Sci. 57:645–653.

Linke, P. L., J. M. DeFrain, A. R. Hippen, and P. W. Jordon. 2004. Ruminal and plasma responses in dairy cows to drenching or feeding glycerol. J. Dairy Sci. 87(Suppl. 1):343. (Abstr.)

MacDougald, O. A., and C. F. Burant. 2005. Obesity and metabolic perturbations after loss of aquaporin 7, the adipose glycerol transporter. Proc. Natl. Acad. Sci. USA 102:10759–10760.

Michnick, S., J. L. Roustan, F. Remize, P. Barre, and S. Dequin. 1997. Modulation of glycerol and ethanol yields during alcoholic fermentation in *Saccharomyces cerevisiae* strains overexpressed or disrupted for GPD1 encoding glycerol 3-phosphate dehydrogenase. Yeast 13:783–793.

Neel, J. P. S., E. C. Prigge, and E. C. Townsend. 1995. Influence of moisture content of forage on ruminal functional specific gravity and passage of digesta. J. Anim. Sci. 73:3094–3102.

NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Sci., Washington, DC.

Nelson, J. L., and R. A. Robergs. 2007. Exploring the potential ergogenic effects of glycerol hyperhydration. Sports Med. 37:981–1000.

Osborne, V. R., N. E. Odongo, J. P. Cant, K. C. Swanson, and B. W. McBride. 2009. Effects of supplementing glycerol and soybean oil in drinking water on feed and water intake, energy balance, and production performance of periparturient dairy cows. J. Dairy Sci. 92:698–707.

Rémond, B., E. Souday, and J. P. Jouany. 1993. In vitro and in vivo fermentation of glycerol by rumen microbes. Anim. Feed Sci. Technol. 41:121–132.

Rodríguez, A., V. Catalán, J. Gómez-Ambrosi, and G. Frühbeck. 2006. Role of aquaporin-7 in the pathophysiological control of fat accumulation in mice. FEBS Lett. 580:4771–4776.

Roger, V., G. Fonty, C. Andre, and P. Gouet. 1992. Effects of glycerol on the growth, adhesion, and cellulolytic activity of rumen cellulolytic bacteria and anaerobic fungi. Curr. Microbiol. 25:197–201.

Rojek, A., J. Praetorius, J. Frøkiaer, S. Nielsen, and R. A. Fenton. 2008. A current view of the mammalian aquaglyceroporins. Annu. Rev. Physiol. 70:301–327.

Schott, H. C., K. S. Patterson, and S. W. Eberhart. 2001. Glycerol hyperhydration in resting horses. Vet. J. 161:194–204.

Schröder, A., and K.-H. Südekum. 1999. Glycerol as a by-product of biodiesel production in diets for ruminants. In New Horizons for an Old Crop. Proc. 10th Int. Rapeseed Congr., Canberra, Australia, Paper No. 241. N. Wratten and P. A. Salisbury, ed. The Regional Institute Ltd., Gosford, New South Wales, Australia.

Thompson, J. C., and B. He. 2006. Characterization of crude glycerol from biodiesel production from multiple feedstocks. Appl. Eng. Agric. 22:261–265.

Trinder, P. 1969. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Ann. Clin. Biochem. 6:24–25.

Van Keulen, J., and B. A. Young. 1977. Evaluation of acid-insoluble ash as a natural marker in ruminant digestion studies. J. Anim. Sci. 44:282–287.

Wildman, E. E., G. M. Jones, P. E. Wagner, R. L. Boman, H. F. Troutt Jr., and T. N. Lesch. 1982. A dairy cow body condition scoring system and its relationship to selected production characteristics. J. Dairy Sci. 65:495–501.

Yazdania, S. S., and R. Gonzalez. 2007. Anaerobic fermentation of glycerol: A path to economic viability for the biofuels industry. Curr. Opin. Biotechnol. 18:213–219.