Evaluation of propylene glycol and glycerol infusions as treatments for ketosis in dairy cows

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ABSTRACT

To evaluate propylene glycol (PG) and glycerol (G) as potential treatments for ketosis, we conducted 2 experiments lasting 4 d each in which cows received one bolus infusion per day. Blood was collected before infusion, over 240 min postinfusion, as well as 24 h postinfusion. Experiment 1 used 6 ruminally cannulated cows (26 ± 7 d in milk) randomly assigned to 300-mL infusions of PG or G (both $\geq 99.5\%$ pure) in a crossover design experiment with 2 periods. Within each period, cows were assigned randomly to infusion site sequence: abomasum (A)-cranial reticulorumen (R) or the reverse, R-A. Glucose precursors were infused into the R to simulate drenching and the A to prevent metabolism by ruminal microbes. Glycerol infused in the A increased plasma glucose concentration the most (15.8 mg/dL), followed by PG infused in the R (12.6 mg/dL), PG infused in the A (9.11 mg/dL), and G infused in the R (7.3 mg/dL). Infusion of PG into the R increased plasma insulin and insulin area under the curve (AUC) the most compared with all other treatments (7.88 vs. 2.13 $\mu\text{IU/mL}$ and 321 vs. 31.9 $\text{min} \times \mu\text{IU/mL}$, respectively). Overall, PG decreased plasma BHBA concentration after infusion (-6.46 vs. -4.55 mg/dL) and increased BHBA AUC ($-1,055$ vs. -558 $\text{min} \times \text{mg/dL}$) compared with G. Plasma NEFA responses were not different among treatments. Experiment 2 used 8 ruminally cannulated cows (22 ± 5 d in milk) randomly assigned to treatment sequence in a Latin square design experiment balanced for carryover effects. Treatments were 300 mL of PG, 300 mL of G, 600 mL of G (2G), and 300 mL of PG + 300 mL of G (GPG), all infused into the R. Treatment contrasts compared PG with each treatment containing glycerol (G, 2G, and GPG). Propylene glycol increased plasma glucose (14.0 vs. 5.35 mg/dL) and insulin (7.59 vs. 1.11 $\mu\text{IU/mL}$) concentrations compared with G, but only tended to increase glucose and insulin concentrations

compared with 2G. Propylene glycol increased AUC for glucose (1,444 vs. 94.3 mg/dL) and insulin (326 vs. 6.58 $\text{min} \times \mu\text{IU/mL}$) compared with G, and tended to increase insulin AUC compared with 2G. Propylene glycol was not different from GPG for glucose, insulin, or BHBA responses. Propylene glycol decreased plasma BHBA concentration (-10.3 vs. -4.21 mg/dL) and increased BHBA AUC ($-1,578$ vs. -1.42 $\text{min} \times \text{mg/dL}$) compared with G, but not compared with 2G. In general, and compared with G, GPG decreased plasma NEFA concentrations after infusions and PG decreased plasma NEFA concentrations early but not late after infusions. We conclude that a 300-mL dose of PG is more effective at increasing plasma glucose concentration than G and at least as effective as 600 mL of G or a combination of G and PG when administered in the cranial reticulorumen.

Key words: fresh cows, glucose precursors, ketosis, postpartum

INTRODUCTION

Insulin resistance and low plasma insulin concentration in the peripartum period stimulates fat mobilization, elevating plasma NEFA concentration. Greater NEFA supply stimulates hepatic oxidation of FA, increases hepatic export of ketone bodies, and increases the risk of hepatic lipidosis. Increased hepatic oxidation of NEFA likely induces satiety, decreasing feed intake and increasing fat mobilization even more during this period of high metabolic demands (Allen et al., 2009). Elevated concentration of ketone bodies in body fluids, referred to as ketosis, is indicative of excessive fat mobilization, and is often associated with inappetence and decreased milk yield and alertness.

Incidence of clinical ketosis in dairy farms can range from 2 to 15% during the first month after parturition (Duffield, 2000). Costs associated with ketosis include treatment costs, loss in milk production, decreased reproductive efficiency, increased culling risk, and increased risk of other diseases (Walsh et al., 2007; Duffield et al., 2009; McArt et al., 2012). In an attempt to decrease lipomobilization in cows and the incidence

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and severity of ketosis on farms, evaluation of gluconeogenic precursor supplementation in transition cow diets has received a great deal of attention (Fisher et al., 1971, 1973; DeFrain et al., 2004; Chung et al., 2007). However, experiments directly comparing potential treatments for ketosis in lactating dairy cows are more limited (Gordon et al., 2013).

Goals of ketosis treatment are to stimulate gluconeogenesis, increase plasma glucose, and decrease lipolysis (Herd and Emery, 1992). A recent recommended base treatment for ketosis is 300 mL of propylene glycol (PG) administered orally once daily for 5 d (Gordon et al., 2013), and was based on a single study (McArt et al., 2011, 2012). However, the first reports evaluating PG as a gluconeogenic precursor to treat ketosis were presented decades ago (Johnson, 1954). Unfortunately, dosage of PG is restricted for its potential toxic effects (Johnson, 1954), which might be at least partially related to the production of sulfur-containing gases, emitted during fermentation of PG in the rumen (Trabue et al., 2007). Glycerol (G) is another gluconeogenic precursor that might be effective in the treatment of ketosis, and is safe to administer in larger amounts as a drench (Johnson, 1954; Osman et al., 2008). Until recently, the price of G was not competitive with PG. However, G is a byproduct of the processing of fats from the biodiesel or chemical industries, and its availability has increased from the expansion of the biodiesel industry, reducing its cost.

Christensen et al. (1997) determined that PG administered as a drench or top-dressed was more effective at increasing plasma insulin and decreasing plasma NEFA compared with PG mixed in a TMR. Because DMI of ketotic cows is often depressed, drenches rather than top-dressing is the preferred form of administration of gluconeogenic precursors when treating ketosis. The objective of these experiments was to evaluate the short-term effects of PG and G infusions on plasma glucose, insulin, NEFA, and BHBA responses to help assess their use as treatments for ketosis. We hypothesized that G would be more effective than PG (300 mL each) in increasing plasma glucose concentration when infused in the abomasum (A) but not when infused in the cranial reticulorumen (R) and that a combination of G and PG (300 mL of each) would be more effective than 300 mL of PG alone in increasing plasma glucose concentration when infused in the R.

MATERIALS AND METHODS

Animal Housing and Care

All experimental procedures were approved by the Institutional Animal Care and Use Committee at Mich-

igan State University (East Lansing). Each cow was housed in the same tie-stall throughout the experiment. Cows were fed once daily (1000 h) at 115% of expected intake and milked twice daily (0500 and 1630 h). The amounts of feed offered and refused were weighed for each cow daily.

Design and Treatment Diets

Experiment 1. Six ruminally cannulated multiparous Holstein cows at the Michigan State University Dairy Field Laboratory (26 ± 7 DIM; 638 ± 63 kg of BW; 2.02 ± 0.3 BCS; mean $\pm$ SD) were used in a cross-over design experiment. Cows were randomly assigned to stalls and to glucose precursor (GP) and infusion site (IS) sequences. Glucose precursor treatments were 300 mL of PG or 300 mL of G (both $\geq 99.5\%$ pure) and IS treatments were A or R. Glucose precursors were infused in the A to prevent metabolism by ruminal microbes and in the R, through the ruminal cannula, to simulate drenching. Each cow received one infusion per day. Treatment sequences for GP were randomly assigned on d 1; cows received the same GP on d 1 and 2 and the other GP on d 3 and 4. Treatment sequences for IS were randomly assigned on d 1 and 3; on d 2 and 4, cows were dosed at the other IS rather than the one assigned on d 1 and 3. Each cow received all possible combinations of treatments. The ingredient and nutrient composition of the diet, fed as TMR, is reported in Table 1. The diet was formulated to meet requirements according to NRC (2001).

Experiment 2. Eight ruminally cannulated multiparous Holstein cows at the Michigan State University

Table 1. Ingredient and nutrient composition of diets fed during experiments 1 and 2

Item	Diet	
	Experiment 1	Experiment 2
Ingredient, % of DM		
Corn silage	29.1	34.5
Alfalfa silage	19.8	19.8
Alfalfa hay	9.90	9.90
Dry ground corn	21.9	16.8
Soybean meal	15.6	15.3
Vitamin-mineral mix ¹	3.67	3.67
Nutrient composition		
DM, %	50.1	46.9
Starch, % of DM	26.0	25.0
NDF, % of DM	28.8	31.0
Forage NDF, % of DM	25.6	28.4
CP, % of DM	17.0	16.5

¹Vitamin-mineral mix contained (DM basis): 34.5% sodium chloride, 29.4% calcium carbonate, 13.4% magnesium oxide, 12.5% monocalcium phosphate, 5.40% soybean oil, and 4.85% trace minerals and vitamins.

Dairy Field Laboratory (22 ± 5 DIM; 671 ± 59 kg of BW; 2.46 ± 0.6 BCS; mean $\pm$ SD) were used in a replicated ($n = 2$) 4×4 Latin square design experiment, balanced for carryover effects. Cows were blocked by parturition date and randomly assigned to stalls and treatment sequence. Infusion site was always R. Glucose precursor treatments were 300 mL of PG (**PG**), 300 mL of G (**G**), 600 mL of G (**2G**), or 300 mL G + 300 mL of PG (**GPG**). Each cow received one infusion per day, and all cows received each treatment. The ingredient and nutrient composition of the diet, fed as TMR, is reported in Table 1. The diet was formulated to meet requirements according to NRC (2001).

Data and Sample Collection

The same data and samples were collected during both experiments. Feed offered and refused was measured daily. Milk yield was recorded and milk samples were collected daily at each milking and stored with preservative (Bronopol tablet; D&F Control Systems, San Ramon, CA) at 4°C for component analysis (Universal Lab Services, East Lansing, MI). Samples of all diet ingredients (0.5 kg) and orts from each cow (~12.5%) were collected daily during both experiments and stored in plastic bags at -20°C until processed. Body weight and BCS were recorded once for each cow for characterization. Body condition was scored by 2 trained investigators on a 5-point scale, where 1 = thin and 5 = fat, as described by Wildman et al. (1982).

To minimize stress, all cows were fitted with a single jugular catheter (left or right jugular vein). Catheterization was performed 2 d before the beginning of the experiment, according to Bradford et al. (2006). Catheter patency was checked daily until removed at the end of the experiment. Cows were blocked from feed at 0700 h and were not allowed access until all blood samples were collected. Infusion treatments were administered in a bolus either in the R or the A through a tube passed through the ruminal cannula. Blood samples were collected from the jugular vein 20 and 10 min before infusion, immediately before infusion, and at 10, 20, 30, 40, 50, 60, 80, 100, 120, 150, 180, and 240 min postinfusion as well as 24 h postinfusion. Blood samples were collected into 2 evacuated tubes, one containing potassium EDTA and the other containing potassium oxalate with sodium fluoride as a glycolytic inhibitor, and placed in ice until centrifugation. Both tubes were centrifuged at $2,000 \times g$ for 15 min within 1 h of sample collection, and plasma was harvested and stored at -20°C until analysis. Catheters were flushed with 5 to 10 mL of sterile 4.2% Na citrate after each blood collection.

Sample Analysis

Feed and orts samples were dried in a 55°C forced-air oven for 72 h and analyzed for DM concentration for calculation of DMI. Feed ingredients were composited by experiment and by block within experiment 2 and analyzed for DM, NDF, CP, and starch contents as described in Piantoni et al. (2013). Milk samples were analyzed for fat, true protein, lactose, MUN, and SCC by infrared spectroscopy (AOAC International, 1997), by the Michigan Herd Improvement Association (Universal Lab Services). Commercial kits were used to determine plasma concentrations of insulin (Coat-A-Count RIA kit, Siemens Healthcare Diagnostics, Deerfield, IL), NEFA [NEFA-HR (2) kit; Wako Chemicals USA Inc., Richmond, VA], and BHBA (kit no. 2240, Stanbio Laboratory, Boerne, TX). Plasma glucose concentration was analyzed using a glucose oxidase method (PGO Enzyme Product No. P7119, Sigma Chemical Co., St. Louis, MO). Insulin concentrations were detected in only 35% of the samples; nondetectable values were assigned an average concentration of one-half the limit of detection, according to Hornung and Reed (1990).

Statistical Analysis

Data were analyzed using the fit model procedure of JMP (version 11.0, SAS Institute Inc., Cary, NC) according to the following models:

$$\begin{aligned} \text{Experiment 1: } Y_{ijklmn} = & \mu + P_i + T_j + P_iT_j + S_k \\ & + T_jS_k + D(P_i)_l + U_m + C_n + e_{ijklmn}, \end{aligned}$$

where Y_{ijklmn} = dependent variable, μ = overall mean, P_i = fixed effect of period ($i = 1$ to 2), T_j = fixed effect of GP treatment ($j = 1$ to 2), P_iT_j = interaction between period and GP treatment, S_k = fixed effect of IS treatment ($k = 1$ to 2), T_jS_k = interaction between GP and IS treatments, $D(P_i)_l$ = fixed effect of day ($l = 1$ to 2) within period, U_m = fixed effect of sequence for site ($m = 1$ to 2), C_n = random effect of cow ($n = 1$ to 6), and e_{ijklmn} = residual.

$$\begin{aligned} \text{Experiment 2: } Y_{ijkl} = & \mu + S_i + T_j + C(S_i)_k \\ & + P_l + K_jP_l + e_{ijkl}, \end{aligned}$$

where Y_{ijkl} = dependent variable, μ = overall mean, S_i = fixed effect of square ($i = 1$ to 2), T_j = fixed effect of GP treatment ($j = 1$ to 4), $C(S_i)_k$ = random effect of cow within square ($k = 1$ to 4), P_l = fixed effect of period ($l = 1$ to 4), T_jP_l = interaction between treatment

and period, and e_{ijkl} = residual. Contrasts evaluated were PG versus G, PG versus 2G, and PG versus GPG.

Plasma glucose, insulin, BHBA, and NEFA responses to infusions over time were analyzed with the GLIMMIX procedure of SAS v.9.2 (SAS Institute Inc.) as repeated measures using the models described above for each experiment. We used first-order autoregressive as the covariate structure, unless otherwise specified. Denominator degrees of freedom were estimated by the Kenward-Rogers method. Treatment differences within each time point were analyzed using the SLICE option. The Bonferroni adjustment was applied to decrease the probability of type I error when multiple treatment means were compared.

Normality of the residuals was checked with normal probability and box plots and homogeneity of variances with plots of residuals versus predicted values. Significance was determined at $P \leq 0.05$ for main effects and $P \leq 0.10$ for interactions, and tendencies were declared at $P \leq 0.10$ for main effects and $P \leq 0.15$ for interactions. All data were expressed as least squares means and standard error of the means, unless otherwise specified. All cows were in apparent good health throughout the experiment, and all data were included in the analysis.

RESULTS

Experiment 1

Glucose precursor and IS treatments interacted to affect plasma glucose concentration ($P < 0.01$; Table 2). Glycerol increased plasma glucose concentration more than PG when infused in the A (15.8 vs. 9.11 mg/dL) but less than PG when infused in the R (7.30 vs. 12.6 mg/dL). A similar trend for an interaction of treatments ($P = 0.11$) was observed for glucose area under the curve (AUC); G tended to increase glucose AUC more than PG when infused in the A (1,545 vs. 1,103 min $\times$ mg/dL) but less than PG when infused in the R (237 vs. 1,073 min $\times$ mg/dL; Table 2). Glycerol infused into the A increased plasma glucose concentration and AUC more than the other treatments. An interaction between GP, IS, and time was detected for glucose response to infusions ($P = 0.009$); G infused in the A increased plasma glucose concentration the most and G infused in the R the least compared with the other treatments, and treatment differences were not detectable at 180 min after infusions (Figure 1A).

Glucose precursor and IS treatments also interacted to affect plasma insulin concentration and AUC (both $P < 0.05$; Table 2). Even though the greatest glucose response was observed when G was infused in the A, the greatest insulin response was observed when PG was infused into the R. Glycerol increased plasma insu-

lin concentration more than PG when infused in the A (2.36 vs. 1.88 μ IU/mL) but less than PG when infused in the R (2.15 vs. 7.88 μ IU/mL). Insulin AUC response to treatments followed the same pattern; G increased insulin AUC more than PG when infused in the A (83.9 vs. 49.0 min $\times$ μ IU/mL) but less than PG when infused in the R (-37.3 vs. 321 min $\times$ μ IU/mL). From 20 to 60 min postinfusion, PG in the R increased plasma insulin concentration by over 100% compared with the other 3 treatments, but treatment differences disappeared over time (interaction $P < 0.0001$; Figure 1B).

In general, no interactions were detected between GP and IS treatments for plasma BHBA or NEFA responses except for plasma BHBA concentration 24 h postinfusion; PG infused in the R increased plasma BHBA concentration the most and PG infused in the A the least compared with the other treatments (interaction $P = 0.01$; Table 2). Overall, PG decreased BHBA concentration and AUC compared with G (-6.46 vs. -4.55 mg/dL and $-1,055$ vs. -558 min $\times$ mg/dL, respectively; both $P \leq 0.05$; Figure 1D). Abomasal infusions tended to decrease BHBA (-6.42 vs. -4.59 mg/dL; $P = 0.06$) and increased BHBA AUC ($-1,014$ vs. -599 mg/dL; $P = 0.05$) compared with R infusions. When infused in the R, PG decreased plasma BHBA concentration compared with G from 60 to 120 min postinfusion ($P \leq 0.05$; Figure 1D). Treatments did not affect plasma NEFA concentration over time or AUC differently (Figure 1C). Even though treatments affected plasma metabolites and insulin responses, they did not affect production responses measured.

Experiment 2

Infusion of PG in the R increased plasma glucose (14.0 vs. 5.35 mg/dL; $P < 0.001$) and insulin (7.59 vs. 1.11 μ IU/mL; $P < 0.01$) concentrations compared with G, but only tended to increase plasma glucose and insulin concentrations compared with 2G (both $P < 0.10$; Table 3). Propylene glycol increased AUC for glucose (1,444 vs. 94.3 mg/dL; $P < 0.001$) and insulin (326 vs. 6.58 min $\times$ μ IU/mL; $P < 0.01$) compared with G, and tended to increase insulin AUC compared with 2G ($P < 0.10$). Responses to PG and GPG infusions did not differ significantly for glucose, insulin, or BHBA. Propylene glycol tended to decrease plasma NEFA concentration 24 h postinfusion compared with 2G only (484 vs. 599 mg/dL; $P = 0.06$). Infusion of PG decreased BHBA (-10.3 vs. -4.21 mg/dL) and increased BHBA AUC ($-1,578$ vs. -1.42 min $\times$ mg/dL; both $P < 0.01$) compared with G, but not compared with 2G or GPG.

Interactions between GP treatments and time were detected for plasma glucose ($P = 0.03$; Figure 2A), insulin ($P = 0.003$; Figure 2B), NEFA ($P = 0.03$; Fig-

Table 2. Dry matter intake, yields of milk and milk components, milk composition, and plasma glucose, insulin, NEFA, and BHBA responses to 300 mL of glycerol (G) or propylene glycol (PG) infused either in the abomasum (A) or cranial reticulorumen (R) in experiment 1¹

Item	G		PG		SEM	Significance, <i>P</i> -value		
	A	R	A	R		Trt	IS	Trt × IS
Production response								
DMI, kg/d	20.4	20.1	21.0	20.6	0.67	0.33	0.53	0.95
Yield, kg/d								
Milk	41.1	41.2	41.8	40.6	1.63	0.94	0.44	0.40
Fat	1.55	1.66	1.67	1.59	0.14	0.85	0.87	0.32
Protein	1.18	1.18	1.21	1.16	0.07	0.76	0.44	0.37
Lactose	2.03	2.03	2.06	1.99	0.09	0.83	0.37	0.35
3.5% FCM	43.0	44.8	45.1	43.3	2.66	0.85	1.00	0.29
Milk composition								
Fat, %	3.76	4.05	3.97	3.89	0.30	0.89	0.56	0.37
Protein, %	2.86	2.86	2.87	2.86	0.12	0.88	0.95	0.84
Lactose, %	4.94	4.93	4.92	4.89	0.04	0.18	0.35	0.65
SCC, × 1,000/mL	22.0	16.8	26.8	23.1	10.0	0.13	0.22	0.83
MUN, mg/dL	14.9	14.1	14.9	14.4	0.87	0.65	0.06	0.68
Metabolic response								
Glucose								
Baseline, mg/dL	54.1	56.6	54.7	55.1	2.38	0.68	0.18	0.35
Delta, ² mg/dL	15.8	7.30	9.11	12.6	1.78	0.71	0.19	<0.01
AUC, min × mg/dL	1,545	237	1,103	1,073	289	0.56	0.08	0.11
24 h postinfusion, mg/dL	55.8	54.6	57.5	55.8	1.93	0.22	0.23	0.83
Insulin								
Baseline, µIU/mL	1.60	1.99	1.63	1.39	0.31	0.20	0.71	0.18
Delta, ² µIU/mL	2.36	2.15	1.88	7.88	1.07	<0.01	0.001	0.001
AUC, min × µIU/mL	83.9	−37.3	49.0	321	60.6	0.05	0.29	0.03
24 h postinfusion, µIU/mL	2.05	1.59	2.21	1.75	0.26	0.46	0.06	1.00
NEFA								
Baseline, µEq/L	404	358	427	411	87.6	0.39	0.47	0.75
Delta, ³ µEq/L	−165	−158	−166	−231	47.2	0.29	0.40	0.33
AUC, min × µEq/L	−1,814	6,373	8,033	1,464	8,575	0.73	0.91	0.35
24 h, postinfusion µEq/L	340	388	319	351	79.8	0.49	0.34	0.86
BHBA								
Baseline, mg/dL	9.53	9.39	12.5	9.20	0.96	0.09	0.04	0.07
Delta, ³ mg/dL	−5.39	−3.71	−7.45	−5.47	0.90	0.05	0.06	0.87
AUC, min × mg/dL	−756	−359	−1,271	−839	206	0.03	0.05	0.93
24 h postinfusion, mg/dL	10.5	9.91	7.61	11.9	0.73	0.56	0.03	0.01

¹Trt = treatments; IS = infusion site; AUC = area under the curve.²Delta = maximum value − baseline value.³Delta = minimum value − baseline value.

ure 2C), and BHBA ($P < 0.001$; Figure 2D) responses. In general, PG increased plasma glucose and insulin and decreased plasma BHBA concentrations compared with G throughout the sampling period. However, PG decreased plasma NEFA concentrations compared with G only early after infusions and not late (interaction $P = 0.03$). Compared with G, GPG decreased plasma NEFA concentrations after infusions throughout the sampling period. Both PG and GPG decreased plasma NEFA concentrations postinfusion similarly, but GPG maintained a lower plasma NEFA concentration later in the sampling period compared with PG. Even though infusions that contained G increased plasma glucose concentration faster after infusions compared with PG, PG maintained a higher plasma glucose concentration for a longer period of time after infusions (interaction $P = 0.03$). Infusion of G alone did not affect plasma glu-

cose, insulin, NEFA, or BHBA concentrations substantially compared with the other GP treatments (Figure 2). Compared with G only, PG decreased DMI (17.3 vs. 18.6 kg/d; $P = 0.02$) and milk protein concentration (2.86 vs. 2.97%; $P = 0.03$) and increased yields of 3.5% FCM (52.7 vs. 48.6 kg/d; $P = 0.03$) and milk fat (2.10 vs. 1.88 kg/d; $P = 0.04$).

DISCUSSION

Cows used in these experiments were in a lipolytic state but did not show signs of clinical ketosis. Because of variability among cows, we chose to conduct crossover design experiments to evaluate effects of PG and G on plasma glucose, NEFA, BHBA, and insulin concentrations only and not their actual effectiveness as ketosis treatments, which would require a larger number of

Figure 1. Responses to glucose precursors (GP): propylene glycol (PG) or glycerol (G), and infusion sites (IS): abomasum (A) or cranial reticulorumen (R) treatments in experiment 1 on (panel A) plasma glucose (SEM = 2.33); (panel B) plasma insulin (log-transformed SEM = 0.097); (panel C) plasma NEFA (log-transformed SEM = 0.119); and (panel D) plasma BHBA (SEM = 0.863). Treatments were G-A (dashed gray line), G-R (solid gray line), PG-A (dashed black line), and PG-R (solid black line). Interactions detected for glucose response were GP $\times$ IS $\times$ time ($P = 0.009$), and GP $\times$ time ($P = 0.11$). Interactions detected for insulin response were GP $\times$ IS $\times$ time ($P < 0.0001$), GP $\times$ time ($P = 0.002$), and IS $\times$ time ($P = 0.13$). No interactions were detected for NEFA response. Interactions detected for BHBA response were GP $\times$ time ($P = 0.003$). Plasma NEFA and insulin concentrations were log-transformed for statistical analysis and back-transformed for interpretation purposes. Values are LSM, and different letters within a time point indicate a significant difference at $P \leq 0.05$.

animals. In Exp. 1, we expected G and PG to be absorbed unaltered when infused in the A. In contrast, we expected PG and G to be at least partially metabolized by ruminal microbes, before exiting the rumen through absorption or passage, when infused in the R. In Exp.

2, we were interested in comparing the recommended treatment of 300 mL of PG administered as a drench with G, 2G, and a combination of G and PG infused in the R. The treatments G and PG were infused to confirm results observed in Exp. 1. The treatment 2G was

Table 3. Dry matter intake, yields of milk and milk components, milk composition, and plasma glucose, insulin, NEFA, and BHBA responses to gluconeogenic precursor treatments infused in cranial reticulorumen in experiment 2¹

Item	Infusion treatment					Significance, <i>P</i> -value			
	PG	G	2G	GPG	SEM	Trt	Contrast		
							PG vs. G	PG vs. 2G	PG vs. GPG
Production response									
DMI, kg/d	17.3	18.6	17.7	18.0	1.18	0.10	0.02	0.41	0.16
Yield, kg/d									
Milk	43.1	42.3	42.6	42.3	0.89	0.90	0.54	0.68	0.51
Fat	2.10	1.88	1.99	1.96	0.14	0.19	0.04	0.27	0.17
Protein	1.22	1.24	1.24	1.23	0.05	0.83	0.45	0.50	0.84
Lactose	2.05	1.99	2.06	2.08	0.04	0.50	0.30	0.94	0.58
3.5% FCM	52.7	48.6	50.9	50.6	2.37	0.14	0.03	0.27	0.20
Milk composition									
Fat, %	4.90	4.48	4.66	4.52	0.32	0.38	0.13	0.37	0.17
Protein, %	2.86	2.97	2.90	2.84	0.13	0.06	0.03	0.38	0.62
Lactose, %	4.76	4.79	4.75	4.83	0.03	0.30	0.42	0.85	0.12
SCC, × 1,000/mL	93.2	64.5	78.9	52.8	39.3	0.49	0.31	0.60	0.16
MUN, mg/dL	10.8	10.1	10.1	9.36	0.38	0.14	0.24	0.24	0.03
Metabolic response									
Glucose									
Baseline, mg/dL	50.5	52.2	51.2	50.4	1.71	0.36	0.14	0.54	0.91
Delta, ² mg/dL	14.0	5.35	10.7	11.9	1.41	<0.01	<0.001	0.09	0.26
AUC, min × mg/dL	1,444	94.3	1145	1326	281	<0.001	<0.001	0.26	0.65
24 h postinfusion, mg/dL	51.5	51.4	51.9	52.8	1.72	0.62	0.93	0.77	0.28
Insulin									
Baseline, µIU/mL	1.82	1.73	1.73	1.20	0.31	0.46	0.85	0.83	0.16
Delta, ² µIU/mL	7.59	1.11	3.65	6.73	1.71	0.03	<0.01	0.07	0.68
AUC, min × µIU/mL	326	6.58	148	371	86.6	0.01	<0.01	0.10	0.66
24 h postinfusion, µIU/mL	1.73	1.66	1.52	1.39	0.30	0.85	0.86	0.62	0.43
NEFA									
Baseline, µEq/L	540	657	559	573	67.7	0.23	0.06	0.75	0.58
Delta, ³ µEq/L	-243	-225	-187	-311	47.7	0.27	0.77	0.37	0.28
AUC, min × µEq/L	18,231	-12,811	-12,344	-17,367	19,242	0.44	0.21	0.22	0.16
24 h postinfusion, µEq/L	484	501	599	536	69.1	0.22	0.77	0.06	0.37
BHBA									
Baseline, mg/dL	17.1	14.8	17.6	20.1	5.19	0.21	0.34	0.85	0.22
Delta, ³ mg/dL	-10.3	-4.21	-8.18	-11.4	2.42	<0.01	<0.01	0.25	0.56
AUC, min × mg/dL	-1578	-1.42	-922	-1831	468	<0.01	<0.01	0.16	0.57
24 h postinfusion, mg/dL	17.6	16.8	14.9	13.5	4.64	0.30	0.73	0.26	0.09

¹Treatments (Trt) were 300 mL of propylene glycol (PG), 300 mL of glycerol (G), 600 mL of PG (GPG), all infused in the reticulorumen. AUC = area under the curve.

²Delta = maximum value – baseline value.

³Delta = minimum value – baseline value.

Figure 2. Responses to glucose precursor (GP) treatments infused in cranial reticulorumen: 300 mL of propylene glycol (PG; solid black line), 300 mL of glycerol (G; solid gray line), 600 mL of G (2G; dashed gray line), and 300 mL of G + 300 mL of PG (GPG; dashed black line) in experiment 2 on (panel A) plasma glucose (SEM = 1.55); (panel B) plasma insulin (log-transformed SEM = 0.084); (panel C) plasma NEFA (log-transformed SEM = 0.086); and (panel D) plasma BHBA (log-transformed SEM = 0.118). The interaction between GP treatment and time was significant for glucose ($P = 0.03$), insulin ($P = 0.003$), NEFA ($P = 0.03$), and BHBA ($P < 0.001$) responses. Plasma NEFA, BHBA, and insulin concentrations were log-transformed for statistical analysis and back-transformed for interpretation purposes. The variance-covariance structure used to analyze insulin was first-order auto-regressive with heterogeneous variance. Values are LSM, and different letters within a time point indicate a significant difference at $P \leq 0.05$.

used to evaluate if an increased dose of a gluconeogenic precursor would have similar effects compared with PG due to increased production of glucogenic VFA in the reticulorumen, by overwhelming the metabolic capacity

of the rumen microbial population, increasing passage of glycerol from the reticulorumen to the abomasum, or from a combination of both. The combination of G and PG was used to evaluate if providing G in addition to

PG would increase glucose production compared with the maximum recommended dose of PG alone.

Experiment 1

When absorbed intact from the gastrointestinal tract, glycerol is metabolized in the liver and enters the cytosolic gluconeogenic pathway as glyceraldehyde-3-phosphate without the need of entering the tricarboxylic acid (TCA) cycle, and therefore, skipping metabolic steps with limiting enzymes (Johnson, 1954). When PG is infused abomasally, it is absorbed intact and metabolized to lactate in the liver (Kristensen and Raun, 2007) before entering the TCA cycle via pyruvate, stimulating gluconeogenesis. When PG is infused into the reticulorumen, it can be absorbed intact through its wall or small intestine if it leaves the rumen intact, or it can be metabolized to propionate and propanol, and to a lesser extent to propanal, and then absorbed through the wall of the reticulorumen (Nielsen and Ingvarsen, 2004; Kristensen and Raun, 2007). If metabolized to propionate, PG will enter the TCA cycle through succinyl CoA. Anapleurosis of the TCA cycle will ultimately increase glucose synthesis (Nielsen and Ingvarsen, 2004). The more direct way G enters the gluconeogenic pathway when absorbed intact (infused in the A) compared with PG is consistent with abomasal infusions of G increasing plasma glucose response compared with the other treatments in the current experiment. However, PG infused in the R increased plasma insulin compared with abomasal infusion of G. Moreover, and even though glucose response to PG infused either in the R or the A was similar, infusion of PG in the R also increased plasma insulin response compared with infusion of PG in the A. It has been established that propionate is insulinotropic (Brockman, 1982), and our results indicate that propionate was likely more insulinotropic than glucose in these cows. Kristensen and Raun (2007) infused 650 mL of PG in the rumen of lactating cows and suggested that PG increases plasma glucose concentration despite an increase of plasma insulin concentration not only by increased supply of gluconeogenic precursors but also by induction of insulin resistance, determined by PG and its byproducts of ruminal metabolism, propanol and propanal. Even though the increase in plasma insulin and glucose concentration in the current experiment was rapid and not sustained over time, a temporal effect of PG and its byproducts on insulin resistance of peripheral tissues cannot be excluded.

Most research indicates G is rapidly metabolized in the rumen (Garton et al., 1961; Wright, 1969). Even though DeFrain et al. (2004) showed that G fed as a topdress is mostly metabolized to propionate, and that

it decreases the acetate to propionate ratio in dairy cattle, products of metabolism of glycerol have varied among experiments in vitro. Garton et al. (1961) conducted an in vitro study with sheep ruminal inocula and indicated that the predominant VFA produced from G metabolism was propionic acid. However, less than 50% of the total G metabolized was accounted for in that study. Wright (1969) incubated carbon 14-labeled G in cattle ruminal contents and recovered 87% of added carbon-14. Their results indicated that acetic acid was the predominant end product of G metabolism; the distribution of radioactivity from labeled G was the following: acetic acid (31.4%), propionic acid (20.6%), carbon dioxide (17%), butyric acid (7.6%), microorganisms (6.5%), and lactic acid (3.5%). Wright (1969) concluded that their results differed from those presented by Garton et al. (1961) probably due to differences in diets and microbial populations. Inclusion rates might also play a role in fermentation profiles. In our experiment, we would have expected a sharper increase in plasma insulin concentration if G infused in the R was mostly metabolized to propionate, consistent with the insulin response observed after PG infusions. Therefore, we suggest that G infused in the R was likely mostly metabolized to acetate in the current experiment, consistent with results reported by Wright (1969). The increase in insulin observed might have been from the portion of G metabolized to propionate or to the increase in plasma glucose observed, whereas the increase in plasma glucose might have been from the glucose-sparing effect of acetate, a portion of G being metabolized to propionate, or from partial absorption of glycerol and increased gluconeogenesis.

Propylene glycol drenches not only increase plasma glucose and insulin concentrations (Studer et al., 1993; Grummer et al., 1994; Christensen et al., 1997) but also usually decrease plasma NEFA and BHBA concentrations (Pickett et al., 2003; Rizos et al., 2008). Even though several experiments fed G by top-dressing rations, few administered G as a drench. Osman et al. (2008) administered an oral dose of 400 mL of pure G once daily during the first 14 d postpartum and showed that G increased plasma glucose and decreased plasma NEFA and BHBA concentrations, but did not affect plasma insulin concentration compared with the control group. Kass et al. (2013) evaluated an oral drench of 500 g of glycerol (82.6% pure) administered once daily before feeding during the first 3 wk postpartum and observed a decrease in plasma NEFA concentration early in lactation, but no effect on other metabolites or insulin concentrations. When top-dressed once daily, increasing amounts of 99.8% pure G increased glucose linearly, and decreased NEFA and BHBA linearly (Wang et al., 2009). In the current experiment, all

treatments reduced plasma NEFA concentration similarly, and therefore, the increase in insulin observed for all treatments was likely sufficient to decrease lipolysis temporarily. In addition, plasma NEFA concentrations started to increase when insulin concentrations started to decrease after infusions, and the longer the cows were off feed the more plasma NEFA increased, which explains positive values of AUC. Plasma insulin and NEFA concentrations are inversely related within days (Allen, 2014), and plasma insulin concentration is normally lowest and plasma NEFA concentration normally highest before feeding.

Propylene glycol decreased plasma BHBA the most despite the lack of an effect on plasma NEFA concentration. The greater reduction in plasma BHBA concentration was likely because PG stimulated oxidation of AcCoA, reducing its availability for ketogenesis (Nielsen and Ingvarsen, 2004). Abomasal infusions tended to reduce plasma BHBA concentration more than ruminal infusions likely because of partial metabolism of both PG and G by ruminal microbes to acetate or other nonglucogenic metabolites.

Experiment 2

Infusions of 300 mL of PG, either alone or in combination with 300 mL of G, were the most effective at eliciting metabolic responses consistent with ketosis treatments, followed by 2G and then by G. However, we did not detect a benefit from combining G and PG as we expected and G would clearly not be as effective as a ketosis treatment as PG when administered to the R from our results. Both PG and GPG increased plasma insulin greatly compared with G, and even though GPG decreased plasma NEFA the most compared with the other treatments, PG was the most efficient treatment in decreasing plasma BHBA. Similar to Exp. 1, BHBA response to PG infusion was likely related to increased oxidation of AcCoA and to its removal from the ketogenic pathway (Nielsen and Ingvarsen, 2004). Area under the curve for BHBA response after PG infusion was not different from 2G and GPG, likely because all 3 increased ruminal propionate production to some extent, increasing oxidation of AcCoA in the liver. The numerical difference between BHBA AUC responses to PG or GPG and 2G might be from some PG being absorbed intact and entering the TCA cycle as pyruvate after its conversion to lactate, increasing oxidation of AcCoA.

The tendency for an effect of PG on DMI observed in the current experiment and not Exp. 1 might be because cows in Exp. 2 were slightly earlier in lactation (22 vs. 26 DIM) and in a more lipolytic state as indicated by higher baseline plasma NEFA and BHBA con-

centrations. That PG decreased DMI compared with G might indicate that PG increased oxidation of AcCoA whereas G did not, consistent with the hepatic oxidation theory (Allen et al., 2009). As lactation progresses, distention by rumen fill begins to be a more dominant signal controlling feed intake (Allen and Piantoni, 2013), and therefore, control by oxidation of fuels in the liver might have diminished for cows in Exp. 1. In Exp. 2, and compared with G, the negative effect of PG on milk protein concentration was likely related to the decrease in DMI, whereas its positive effect on milk fat yield is consistent with a temporal increase in insulin resistance as suggested by Kristensen and Raun (2007).

CONCLUSIONS

Dosing 300 mL of PG to the R is likely a more effective ketosis treatment than dosing twice the quantity of G, and adding G to the recommended dose of PG is unlikely to provide additional benefits. Infusion of G into the A was more effective than infusion in the R at increasing plasma glucose concentration, likely because of extensive metabolism of G by ruminal microbes to metabolites that are not glucogenic. Although the cost of G has recently been highly competitive with PG and G has been increasingly included in some commercial ketosis treatment mixtures, we suggest treating with PG only based on this research.

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