Ruminal and blood responses to propylene glycol during frequent feeding

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ABSTRACT

The objective of the current experiment was to study the responses of ruminal and blood metabolites of Holstein dairy cows to propylene glycol (PG) under different methods of delivery during frequent feeding. By providing the same amount (200 mL or 200 g) of PG, delivery methods for PG were assessed: 1) control treatment: no PG; 2) dietary treatment: 200 g of PG as a dry product (65% purity; corresponded to 308 g of the dry product) mixed into the TMR; 3) oral-drench treatment: 200 mL of liquid PG (100% purity) orally drenched; and 4) rumen-drench treatment: 200 g of PG as a dry product drenched via the rumen cannula to mimic top dressing. Eight multiparous (lactation = 3 ± 1.1 SD) ruminally cannulated Holstein dairy cows (DIM = 204 ± 104.5 SD) were fed PG for 4 d (d 11 to 14) in a replicated 4×4 Latin square design with an experimental length of 14 d for each period. On the last day of each period, serial blood samples were removed from an indwelling catheter placed in the right jugular vein immediately before and for 4 h after PG administration. Cows were fed at $12 \times$ feeding/d for 2 d before entering the serial sampling period to minimize postprandial influences on blood metabolites. Ruminal content was also sampled hourly for 4 h on d 14. Milk was sampled from 2 consecutive milkings on d 13 during each period. Dry matter intake and milk yield were not affected by PG. Percentages of milk lactose were increased by PG delivered by all methods tested in the current experiment. Ruminal concentrations (as percentages of total volatile fatty acids) of acetate were decreased and concentrations of propionate and isovalerate were increased by PG, regardless of the delivery method; however, total volatile fatty acid concentration was not affected by PG. Ruminal concentrations of butyrate were decreased and concentrations of valerate were increased by PG drench, via either an oral or ruminal drench. The degree of reduction in butyrate concentration or increase in valerate concentration was affected by PG dose. Serum insulin peaked more rapidly

and at a greater concentration for cows receiving PG via drenching, but not when PG was provided as a part of the TMR. Plasma glucose, however, tended to peak more rapidly at a greater concentration for cows receiving PG, regardless of the delivery method. Propylene glycol for the amount drenched (orally or ruminally) or fed (incorporated into the ration) shifted ruminal fermentation toward a more glucogenic environment. Drenching demonstrated a better efficacy than feeding PG because of the amount of PG that was available to the animal at the time of sampling. Effects of drenching dry PG into the rumen were comparable with orally drenching liquid PG.

Key words: propylene glycol, ruminal fermentation, plasma glucose, serum insulin

INTRODUCTION

Propylene glycol (PG) via drenching or feeding was demonstrated by Johnson (1954) to treat ketosis effectively in dairy cows because of its glucogenic property (Hanzlik et al., 1939). Propylene glycol has been shown to increase the concentration of propionate and decrease the ratio of acetate to propionate, thereby resulting in a ruminal VFA pattern that is more glucogenic (Clapperton and Czerkawski, 1972; Czerkawski and Breckenridge, 1972). Supplementation of PG to cows has been shown to increase blood insulin and glucose concentrations (Studer et al., 1993; Grummer et al., 1994; Christensen et al., 1997). A drench dose of approximately 500 mL/d or more is often used as a prophylactic treatment for clinical ketosis in dairy cows (Herdt and Emery, 1992). A lower amount of PG (e.g., 118 mL/d), however, is often drenched to cows after parturition as a preventive for subclinical ketosis. Replacing routine drenching with feeding can reduce stress on the cow and on the person who does the drenching, reduce the cost of labor, and ultimately improve the overall health and production of dairy cows. Results from our previous experiments (Chung et al., 2009a,b) showed that supplementing 162.5 g/d (as a preventive dose) of PG either as a top dressing or as a part of the TMR (by mixing PG into the TMR) reduced plasma BHBA concentrations without changing serum insulin and plasma glucose. Bassett (1975) suggested that the

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magnitude of the response in blood insulin concentration is highly dependent on the amount of food ingested. Armentano et al. (1984) reported that cattle fed 12× feeding/d were in a steady state for glucose production and utilization. Therefore, to minimize postprandial effects on blood insulin and glucose responses to PG, a 12× feeding/d frequent feeding schedule was used in the current experiment. Opportunities exist for a dry form of PG to be fed or drenched to cows. A dry form of PG is easier to store on the farm and maintains the consistency of the diet if it is top dressed or mixed into the ration compared with PG in liquid form. It was hypothesized that a dry form of PG provided as a top dressing would be as effective as liquid PG provided as a drench in stimulating blood insulin and glucose responses. To ensure complete consumption of dry PG by cows, dry PG was ruminally drenched. The objectives of the current experiment were to study the effects of PG, as a dry product, via different methods of delivery on serum insulin and plasma glucose concentrations and ruminal VFA patterns for cows during frequent feeding.

MATERIALS AND METHODS

Experimental Design, Animal Care, and Treatments

This experiment was conducted under the approval of The Pennsylvania State University Animal Care and Use Committee. Eight multiparous (lactation = 3 ± 1.1 SD) ruminally cannulated Holstein dairy cows (average DIM = 204 ± 104.5 SD) were allocated into 2 groups and used in a 4×4 Latin square design (2 replications) to study responses of ruminal and blood metabolites of Holstein dairy cows to PG under different methods of delivery. Allocation of cows was balanced for lactation number, predicted 305-d mature equivalent milk production for the current lactation, DIM, BW, feed intake, and milk yield and composition from the previous DHI testing result. Cows were housed in a tunnel-ventilated tie-stall barn located at the Pennsylvania State University Dairy Research and Education Center (University Park, PA). Cows were moved into this facility at least 1 wk before the initiation of the experiment.

The experiment consisted of 4 periods of 14 d each, with the last day (d 14) as the serial blood and ruminal sample collection day. During each experimental period, d 1 to 9 was the adaptation period, during which cows were milked twice daily at approximately 0630 and 1830 h and fed a basal lactating cow diet as a TMR (Table 1) once daily at approximately 0800 h after the morning milking. The TMR was formulated based on NRC (2001) guidelines for Holstein dairy cows at 660

kg of BW and producing 41 kg of milk/d with 3.7% of milk fat. Cows had access to an open dry lot for 2 h before each milking. Days 11 to 14 of each experimental period was the treatment period, during which treatments were administered and cows were gradually adapted to a frequent feeding schedule to minimize postprandial influences attributable to feeding. During the treatment period, cows were milked at 0500 and 1700 h and brought back to the tie-stall barn directly from the milking parlor after each milking.

By providing the same amount (200 mL or 200 g/cow per day) of PG, delivery methods for PG were assessed: 1) control treatment: no PG; 2) dietary treatment: 200 g/d of PG as a dry product (65% purity; corresponded to 308 g of the dry product) mixed into the TMR; 3) oral-drench treatment: 200 mL/d of liquid PG (100% purity) drenched orally by using a 200-mL drench gun (Nasco, Fort Atkinson, WI); and (4) rumen-drench treatment: 200 g/d of PG as a dry product drenched via the rumen cannula. For the rumen-drench treatment, dry PG was first dissolved into 500 mL of 38°C distilled water and drenched into the cavity of the reticulum via the rumen cannula by using a tubing connected to a funnel. The tubing and funnel were rinsed with 250 mL of 38°C distilled water to remove any residue. Ruminal drenching was completed within 3 to 5 min. Cows on the control treatment were not drenched or provided PG in the diet. The liquid PG used contained 99.9% of PG. The dry PG used was a nonacidogenic dry product (Gly-Tran 65, NutriLinx, LLC, Montpelier, VT; $NE_L = 2.6$ Mcal/kg) that was composed of 65% PG and 35% silicon dioxide as the carrier.

Frequent Feeding

Starting at 1000 h on d 10, one day before the start of each experimental treatment period, cows were gradually adapted to a frequent feeding schedule at 6×, 6×, 12×, 12×, and 12× feeding/d on d 10, 11, 12, 13, and 14, respectively. Cows were fed at 12× feeding/d for 2 d before entering the serial sampling period on d 14. The basal lactating cow TMR fed to each cow was weighed into smaller equal portions and fed frequently to each cow. Feed refusals from the previous day were weighed and the amount of feed offered was adjusted daily to allow a 10% refusal. Feed was pushed up to cows approximately 4 to 6 times per day. Treatments were administered accordingly immediately after the first feeding of fresh feed. After the conclusion of the serial sampling period at 1400 h on the last day of each experimental period, feed refusals were weighed and cows were returned to 1× feeding/d.

Table 1. Ingredient and chemical composition (means $\pm$ SD) of the basal lactating cow TMR

Item	Amount
Ingredient composition, % of dietary DM	
Corn silage	34.8 $\pm$ 0.01
Alfalfa haylage	14.6 $\pm$ 0.01
Alfalfa hay	3.2 $\pm$ 0.00
Cottonseed hulls	6.4 $\pm$ 0.00
Soybeans, roasted	9.3 $\pm$ 0.84
Corn, ground	8.3 $\pm$ 1.20
Canola meal	6.4 $\pm$ 0.00
Soybeans, ¹ heat treated	2.6 $\pm$ 0.38
Bakery by-product, ² dried	5.2 $\pm$ 0.41
Molasses, liquid	4.5 $\pm$ 0.06
Bicarbonate, bentonite, and mineral and vitamin premix ³	4.8 $\pm$ 0.00
Chemical composition	
DM, %	48.5 $\pm$ 1.70
CP, % of DM	16.2 $\pm$ 0.51
Soluble CP, % of DM	6.6 $\pm$ 0.53
ADF, % of DM	22.0 $\pm$ 1.16
NDF, % of DM	32.7 $\pm$ 1.45
Fat, % of DM	4.6 $\pm$ 0.38
Total digestible nutrients, % of DM	70.4 $\pm$ 0.53
NE _L , ⁴ Mcal/kg	1.6 $\pm$ 0.01
Ash, % of DM	8.6 $\pm$ 0.58
Ca, % of DM	1.2 $\pm$ 0.12
P, % of DM	0.4 $\pm$ 0.01
Mg, % of DM	0.3 $\pm$ 0.01
K, % of DM	1.6 $\pm$ 0.06
Na, % of DM	0.5 $\pm$ 0.03
Fe, mg/kg	326.3 $\pm$ 58.65
Mn, mg/kg	85.1 $\pm$ 5.84
Zn, mg/kg	103.0 $\pm$ 6.75
Cu, mg/kg	20.7 $\pm$ 1.99

¹AminoPlus (Ag Processing Inc., Omaha, NE).

²Cookie meal.

³Mineral and vitamin premix contained 0.3% of S, 7.2 mg/kg of Se, 4.7 mg/kg of Co, 11.8 mg/kg of I, 21.1 mg/kg of vitamin A, 438.6 μ g/kg of vitamin D, and 685.9 mg/kg of vitamin E.

⁴Estimated based on NRC (2001).

Jugular Catheterization and Serial Blood and Ruminal Content Sampling

On the last day (d 14) of each experimental period at approximately 1 h before the administration of treatments, an indwelling catheter (Abbocath-T, 16-gauge $\times$ 140 mm, 180 mL/min; Abbott Ireland, Sligo, Ireland) was placed in the external right jugular vein of each cow. A 60-mm length of extension tubing (Cook Inc., Bloomington, IN) connected the indwelling catheter to an injection plug (Surflo SR TP2, 0.16-mL volume; Terumo Medical Corp, Elkton, MD) for blood withdrawal. Catheters and extension tubings were filled with heparinized saline (250 IU/mL of isotonic saline) after every blood sample to maintain patency. Serial blood samples were collected immediately before (designated as baseline; collected at -5 min $\pm$ 3 SD for the oral-drench group and at -6 min $\pm$ 2 SD for all other treatment groups) and immediately after (designated as time 0) the administration of treatments and again at 10, 20, 30, 40, 50, 60, 80, 100, 120, 140, 160,

180, 200, 220, and 240 min after the administration of treatments. Fifteen milliliters of blood was withdrawn each time and transferred into evacuated tubes (Becton Dickinson, Rutherford, NJ). Blood tubes for plasma collection were stored on ice before further processing. Plasma was separated from whole blood by centrifuging blood tubes at $3,000 \times g$ for 15 min at 4°C, within 1 h after collection. Blood tubes for serum collection were allowed to sit for approximately 0.5 h and centrifuged at $1,000 \times g$ for 15 min at room temperature to separate serum from whole blood. Plasma and serum were recovered and stored at -20°C until analysis of concentrations for glucose and insulin, respectively. Blood tubes for plasma collection contained potassium oxalate and 4% sodium fluoride. Blood tubes (serum separator tubes) for serum collection contained a clot activator (polymer gel with silica activator).

Ruminal contents were collected via the rumen cannula from the dorsal, ventral, and caudal areas in the rumen at 60, 120, 180, and 240 min after the administration of treatments (on d 14). Ruminal contents

were thoroughly mixed by hand, and ruminal fluid was squeezed and filtered through 4 layers of cheesecloth. The filtrate was immediately measured for pH (pH meter, model M90; Corning Inc., Corning, NY), and 15 mL of the filtrate was subsampled for determination of NH_3 and VFA concentrations. The subsamples were added with 3 mL of 25% metaphosphoric acid and with 3 mL of 0.6% 2-ethyl butyric acid as an internal standard and stored at -20°C until analyses.

Sample Collection, Measurements, and Laboratory Analyses

One sample of the basal lactating cow TMR was collected weekly and stored at -20°C for further DM and chemical analysis. Feed intake for each cow was recorded daily and adjusted for DM percentage (measured at 55°C) of the weekly TMR to calculate DMI. Body weight and milk yield were recorded at each milking. Milk from each cow was sampled from 2 consecutive milkings on d 13 of each experimental period. Evening and morning samples were analyzed separately to allow for adjustments resulting from differing yields between the evening and morning milking.

Samples of the stored TMR were thawed at room temperature and dried for 48 h at 55°C in a forced-air oven and ground in a Wiley mill (Arthur H. Thomas, Philadelphia, PA) through a 1-mm screen. Samples of the ground TMR were composited by period and analyzed for analytical DM, CP, soluble CP, ADF, NDF, fat, and minerals using wet chemistry (Cumberland Valley Analytical Services Inc., Maugansville, MD; Table 1). Analytical DM was determined by drying at 135°C for 2 h (method 930.15; AOAC, 2005). Nitrogen was determined by combustion (method 990.03; AOAC, 2005; Leco FP-528 Combustion Analyzer; Leco Instruments Inc., St. Joseph, MI) and multiplied by 6.25 to obtain CP. Soluble CP was determined using a borate-sodium phosphate buffer procedure (Roe and Sniffen, 1990). Acid detergent fiber and NDF were determined using the Ankom A200 Filter Bag Technique (Ankom Technology, Macedon, NY) according to Van Soest et al. (1991), with heat-stable amylase and sodium sulfite used in the NDF procedure. Fat was determined by using a Soxtec system (Tecator Soxtec System HT 1043 Extraction Unit; Tecator, Eden Prairie, MN; method 2003.05; AOAC, 2005). Minerals were determined using Perkin-Elmer 3300 XL and 5300 DV inductively coupled plasma instruments (Perkin-Elmer, Shelton, CT) with modifications (method 985.01; AOAC, 2000).

Milk samples were analyzed by Pennsylvania DHIA (University Park, PA) for content of fat, true protein, lactose, and MUN by using an infrared spectroscopic method (method 927.16; AOAC, 2005; MilkoScan 4000,

Foss Electric, Hillerød, Denmark). Plasma glucose concentration was measured according to the method of Trinder (1969; Enzymatic Glucose Kit 1075; Stanbio Laboratory Inc., Boerne, TX). Serum insulin concentration was measured using an RIA (Coat-A-Count Insulin Kit, number TKINX; Diagnostic Products Corp., Los Angeles, CA). Inter- and intraassay coefficients of variation for the insulin RIA were 6.2 and 22.3%, respectively. Ruminal fluids were thawed and centrifuged at $3,000 \times g$ for 30 min at 4°C , and the supernatants were retained and recentrifuged before analysis for NH_3 (Broderick and Kang, 1980) and VFA (Yang and Varga, 1989).

Calculations and Statistical Analyses

Net energy balance was calculated for each cow per period according to the method of Chung et al. (2007). The additional energy provided by PG was included in net energy intake. Daily measurements of DMI, BW, and milk yield that were recorded from d 11 to 14 when treatments were administered were averaged by period before statistical analysis. For serial serum insulin and plasma glucose concentrations obtained during each of the sampling periods, geometric means were calculated (areas under the curve divided by the sampling time length) to account for unequally spaced measurements; for serial ruminal characteristics, observational means were calculated because of equally spaced measurements. Maximum responses (compared with the measurement at time 0) of serum insulin and plasma glucose to PG supplementation and time required for insulin and glucose to reach the peak responses during the serial sampling period were identified for each cow at each period, and means were calculated by treatment (Matthews et al., 1990). Dependent variables were analyzed by using PROC MIXED (SAS Institute, 1999), and the general linear mixed model included the fixed effects of square, treatment (methods of delivery), and period within each square. Cow nested within square was used in the RANDOM statement. Denominator degrees of freedom were estimated using the Kenward-Roger option in the MODEL statement. The PDIF option adjusted by the Tukey method was used for multiple-comparison tests between delivery methods. A comparison between with-PG supplementation (dietary, oral-drench, and rumen-drench treatments) or without-PG supplementation (control treatment) was preplanned. The time trends for serial rumen measurements and serum insulin and plasma glucose concentrations were analyzed as repeated measures by using PROC MIXED (SAS Institute, 1999), and the general linear mixed model included the fixed effects of treatment (methods of delivery), sampling time, and their interaction. Time

Table 2. Dry matter intake, milk yield, and energy balance for cows receiving propylene glycol (PG) as a part of the TMR (dietary treatment), as an oral drench (oral-drench treatment), or as a rumen drench (rumen-drench treatment)

Variable	Treatment ¹				SEM	Effect or contrast, ² <i>P</i> -value	
	Control	Dietary	Oral drench	Rumen drench		Treatment	Control vs. PG
DMI, ³ kg/d	25.3	24.7	24.9	24.3	0.93	0.70	0.35
BW, ³ kg	708	701	703	695	27.48	0.40	0.18
DMI, ³ % of BW	3.61	3.54	3.59	3.52	0.18	0.84	0.56
Milk yield, ³ kg/d	39.0	38.9	39.6	37.7	3.28	0.25	0.77
3.5% FCM, ^{4,5} kg/d	36.5	35.1	39.1	37.8	3.81	0.53	0.72
3.5% FCM efficiency ^{4,5}	1.45	1.45	1.48	1.56	0.10	0.52	0.45
Energy balance, ⁵ % of requirement	117	118	109	112	6.34	0.62	0.38
Energy balance, ⁵ Mcal/d	5.38	5.86	3.39	3.60	1.82	0.40	0.45

¹Treatments: control = no PG; dietary = 200 g/d of PG as a dry product (65% purity; corresponded to 308 g/d of the dry product) mixed into the TMR; oral drench = 200 mL/d of liquid PG (100% purity) orally drenched; rumen drench = 200 g/d of PG as a dry product drenched via the rumen cannula.

²Treatment = comparisons among delivery methods; control vs. PG = comparison of no PG (control) with PG (dietary, oral drench, and rumen drench).

³Data were averaged from d 11 to 14 of each experimental period (treatment period).

⁴3.5% FCM yield = $(0.4255 \times \text{milk yield}) + [16.425 \times (\% \text{ fat} \div 100) \times \text{milk yield}]$; 3.5% FCM efficiency = $\text{kg of 3.5\% FCM} \div \text{kg of DMI}$.

⁵Calculated once for each cow per period. Energy balance = $[\text{NE}_I \div (\text{NE}_M + \text{NE}_L)]$ as Mcal/d or $[\text{NE}_I \div (\text{NE}_M + \text{NE}_L) \times 100]$ as a percentage of the requirement. Net energy intake (NE_I) = $\text{DMI} \times \text{dietary NE}_L + \text{NE}_L$ from PG. NE_M = $\text{BW}^{0.75} \times 0.08$. NE_L = $\text{milk yield (kg)} \times [(0.0929 \times \% \text{ fat}) + (0.0563 \times \% \text{ protein}) + (0.0395 \times \% \text{ lactose})]$.

of sampling was used in the REPEATED statement. Time-series covariance structure was modeled using the options of autoregressive order one, compound symmetry, unstructured, and spatial power. The best time-series covariance structure was selected based on the lowest Akaike and Bayesian information criteria. Data are presented as means $\pm$ standard deviations or least squares means $\pm$ standard errors or standard errors of the means. Statistical significance was declared at $P \leq 0.05$ and a tendency to significance was declared at $0.05 < P \leq 0.10$.

RESULTS

Supplementing lactating cow diets with PG, regardless of the delivery method, did not affect DMI, BW, milk yield, 3.5% FCM yield and efficiency, energy balance (Table 2), or milk composition (Table 3), except for milk lactose percentage. Percentages of milk lactose were increased by PG for all delivery methods

(treatment effect: $P = 0.05$; control vs. PG: $P < 0.01$); however, yields of milk lactose were not significantly affected by PG.

Dry matter intake and ruminal characteristics for 4 h after PG administration are reported in Table 4. Dry matter intakes during the 4-h serial sampling period after PG administration were similar among treatments. Ruminal pH (treatment effect: $P = 0.02$), NH_3 concentration (treatment effect: $P < 0.01$; control vs. PG: $P = 0.02$), and individual VFA concentrations as percentages of total VFA were affected by PG supplementation, delivery methods for PG, or both. Total VFA concentrations, however, were similar among treatments. Concentrations of acetate were decreased and concentrations of propionate and isovalerate were increased by PG supplementation, regardless of the delivery method (treatment effect: $P < 0.01$; control vs. PG: $P < 0.01$). Concentrations of butyrate were decreased (treatment effect: $P = 0.05$; control vs. PG: $P = 0.01$) and valerate was increased (treatment effect: $P < 0.01$).

Table 3. Percentages and yields of milk components for cows receiving propylene glycol (PG) as a part of the TMR (dietary treatment), as an oral drench (oral-drench treatment), or as a rumen drench (rumen-drench treatment)

Variable ¹	Treatment				SEM	Effect or contrast, <i>P</i> -value	
	Control	Dietary	Oral drench	Rumen drench		Treatment	Control vs. PG
Fat, %	3.68	3.41	3.60	3.43	0.22	0.47	0.20
Fat yield, kg/d	1.31	1.21	1.37	1.31	0.15	0.60	0.93
Protein, %	3.07	3.07	3.08	3.05	0.09	0.83	0.79
Protein yield, kg/d	1.08	1.07	1.18	1.15	0.08	0.28	0.34
Lactose, %	4.63	4.73	4.72	4.72	0.08	0.05	<0.01
Lactose yield, kg/d	1.65	1.67	1.85	1.78	0.15	0.18	0.16
MUN, mg/dL	13.3	12.9	13.7	12.9	0.63	0.74	0.79

¹Data were averaged from 2 consecutive milkings sampled on d 13.

Table 4. Dry matter intake and rumen characteristics of cows for 4 h after receiving propylene glycol (PG) as a part of the TMR (dietary treatment), as an oral drench (oral-drench treatment), or as a rumen drench (rumen-drench treatment)

Variable	Treatment				SEM	Effect or contrast, <i>P</i> -value	
	Control	Dietary	Oral drench	Rumen drench		Treatment	Control vs. PG
DMI, kg/4-h sampling period	3.9	4.0	3.7	4.0	0.42	0.86	0.97
Ruminal characteristic ¹							
pH	6.22 ^{ab}	6.32 ^a	6.14 ^b	6.25 ^{ab}	0.10	0.02	0.70
NH ₃ , mg/dL	9.94 ^a	8.14 ^{ab}	7.91 ^b	9.81 ^a	0.94	<0.01	0.02
Total VFA, mM	124	120	124	119	6.84	0.14	0.21
Individual VFA, % of total VFA							
Acetate (A)	62.0 ^a	61.0 ^b	58.2 ^c	58.8 ^c	0.71	<0.01	<0.01
Propionate (P)	21.9 ^c	22.9 ^b	25.5 ^a	24.9 ^a	0.61	<0.01	<0.01
Isobutyrate	0.90 ^{ab}	0.91 ^{ab}	0.88 ^b	0.94 ^a	0.03	0.07	0.69
Butyrate (B)	11.4 ^a	11.2 ^{ab}	11.1 ^{ab}	11.0 ^b	0.19	0.05	0.01
Isovalerate	1.75 ^b	1.92 ^a	1.96 ^a	1.97 ^a	0.06	<0.01	<0.01
Valerate	2.05 ^b	2.11 ^b	2.36 ^a	2.38 ^a	0.06	<0.01	<0.01
A:P ratio	2.86 ^a	2.71 ^b	2.30 ^c	2.37 ^c	0.10	<0.01	<0.01
(A + B):P ratio	3.39 ^a	3.20 ^b	2.73 ^c	2.82 ^c	0.10	<0.01	<0.01

^{a-c}Within a row, means that do not have a common superscript differ, $P \leq 0.05$.

¹Data were averaged from the 4 sampling time points over the 4-h period (on d 14).

0.01; control vs. PG: $P < 0.01$) by PG supplementation via drenching orally or ruminally, but not via feeding. Cows receiving PG as an oral or ruminal drench had the lowest acetate-to-propionate ratios and the lowest ketogenic (acetate plus butyrate)-to-glucogenic (propionate) ratios, followed by cows receiving PG as a part of the TMR and control cows (treatment effect: $P < 0.01$; control vs. PG: $P < 0.01$).

Serum insulin and plasma glucose concentrations for 4 h after PG administration are reported in Table 5. During the 4-h serial sampling period after PG supplementation, mean serum insulin concentration was increased (treatment effect: $P < 0.01$; control vs. PG: $P = 0.02$) by both oral and ruminal drenching of PG. Concentrations of serum insulin for cows receiving PG as an oral or ruminal drench peaked higher (treatment effect: $P < 0.01$; control vs. PG: $P < 0.01$) and more rapidly (treatment effect: $P < 0.01$; control vs. PG: $P = 0.09$) than for cows receiving PG as a part of the TMR or for control cows. Unlike serum insulin, mean plasma glucose concentrations tended (control vs. PG: $P = 0.09$) to be greater for cows receiving PG supplementation than for control cows regardless of the delivery method. Concentrations of plasma glucose for PG-supplemented cows also tended to peak higher (control vs. PG effect: $P = 0.06$) and more rapidly (control vs. PG effect: $P = 0.06$) than for control cows. Serum insulin and plasma glucose concentrations were similar among treatments immediately before the administration of treatments (baseline).

DISCUSSION

Propylene glycol, in either liquid or dry form in the current study, shifted ruminal VFA patterns toward a more glucogenic environment without affecting ruminal

fermentation and ruminal NH₃ availability. Ruminal pH observed in the current study at any sampling time points ranged from 6.05 to 6.45, which was within the normal range for ruminal pH from 5.8 to 6.8 (Bergman, 1990) and was above the pH level (<5.5) that can cause subclinical ruminal acidosis (Nocek, 1997). A decrease in pH could have been expected because of an increase in propionate concentration in the rumen from ruminal fermentation of PG, as suggested by Nielsen and Ingvarsen (2004). The effect of PG on ruminal pH, however, was not consistent in the current study. During the 4-h serial sampling period, only cows receiving PG as an oral drench consistently had lower ruminal pH compared with all other treatments. The amount (200 mL or 200 g/d) of PG drenched or fed in the current study was equal to or less than the amounts of PG used in other studies (Dhiman et al., 1993; Cozzi et al., 1996; Shingfield et al., 2002; PG ranged from 200 to 688 g/d), in which no effects of PG were found on ruminal pH for cows in midlactation. An explanation for the lack of effects of PG on ruminal pH observed in the studies mentioned previously was suggested to be related to the lack of effects of PG on ruminal total VFA production (Nielsen and Ingvarsen, 2004). A potential reduction in ruminal pH because of PG may negatively influence ruminal fermentation when cows have low ruminal pH, such as conditions of high starch load in the diet or inadequate effective fiber from forage.

Propylene glycol did not seem to have an effect on ruminal NH₃ availability. In the current experiment, although cows receiving liquid PG as an oral drench had the lowest ruminal NH₃ concentration, cows receiving dry PG as a rumen drench had a ruminal NH₃ concentration similar to the control cows or the cows receiving dry PG from the diet. These comparable

Table 5. Serum insulin and plasma glucose concentrations of cows for 4 h after receiving propylene glycol (PG) as a part of the TMR (dietary treatment), as an oral drench (oral-drench treatment), or as a rumen drench (rumen-drench treatment)

Variable ¹	Treatment				SEM	Effect or contrast, <i>P</i> -value	
	Control	Dietary	Oral drench	Rumen drench		Treatment	Control vs. PG
Serum insulin, pmol/L							
Mean ²	134 ^b	129 ^b	242 ^a	217 ^a	37.25	<0.01	0.02
Maximum ³	164 ^b	152 ^b	439 ^a	407 ^a	58.64	<0.01	<0.01
Time to reach the maximum, ⁴ min	108 ^{ab}	130 ^a	36 ^b	26 ^b	23.59	<0.01	0.09
Baseline ¹	108	115	97	104	24.95	0.87	0.86
Plasma glucose, mg/dL							
Mean ²	73.2	75.4	75.8	76.1	1.46	0.36	0.09
Maximum ³	78.3	81.0	81.3	82.7	1.60	0.22	0.06
Time to reach the maximum, ⁴ min	136	88	96	50	25.05	0.15	0.06
Baseline ¹	67.8	70.8	68.9	69.3	1.53	0.39	0.20

^{a,b}Within a row, means that do not have a common superscript differ, $P \leq 0.05$.

¹Jugular blood was sampled immediately before (baseline) and immediately after (time 0) the administration of treatments and at 10-min intervals for the first hour and at 20-min intervals for an additional 3 h (total of 17 time points).

²Data were averaged from the 17 sampling time points over the 4-h period. Geometric mean = areas under the curve $\div$ sampling time length.

³The maximum response from time 0.

⁴Time required to reach the maximal level from time 0.

concentrations of ruminal NH_3 and the similar MUN between treatments suggested that the lower ruminal NH_3 concentration observed in orally drenched cows was likely not affected by PG. Ruminal NH_3 concentrations observed in the current study at any sampling time points exceeded the concentration of 5 mg/dL that was determined to maximize microbial efficiency in continuous culture (Satter and Slyter, 1974). Ammonia is the major N source for the growth of many rumen bacterial species (Bryant, 1974). The ruminal NH_3 concentrations obtained in the current study could likely indicate that the ruminal N availability was not limited or affected by PG for the maximal rate of synthesis and yield of microbial protein, at least for the amount (200 g or 200 mL/d) of PG supplemented and in the time frame monitored (4 h after administration).

Propylene glycol appeared to shift ruminal VFA patterns by producing more glucogenic VFA, such as propionate (Figure 1) and valerate, at the expense of lipogenic VFA, such as acetate (Figure 2). The effect of PG on ketogenic VFA, such as butyrate, seemed to be inconsistent and relatively small compared with the effect of PG on propionate and acetate (Nielsen and Ingvarsen, 2004). The inconsistent effect of PG on butyrate may be related to the type of diet fed and the amounts of PG infused. Propionate can provide a minimum of 50% to a maximum of 75% of the glucose requirement in ruminants (Bergman, 1990). Valerate and isobutyrate can provide carbon sources for glucose biosynthesis and thus are considered to be glucogenic, although their contributions to gluconeogenesis are relatively small compared with propionate (Leng, 1970). The relative proportion of propionate to total VFA on

a molar basis is approximately 20% and that of valerate and isobutyrate combined is 2 to 4%; therefore, approximately 25% of total VFA are glucogenic. In the current study, the total glucogenic VFA was increased from 24.9% for cows receiving no PG to 25.9, 28.8, and 28.3% for cows receiving PG mixed into the TMR, as an oral drench, and as a ruminal drench, respectively. It is obvious that 200 mL or 200 g of PG, in either liquid or dry form, by drenching shifted ruminal VFA patterns; however, PG as a dry product mixed into the TMR at as little as 24.4 g/cow (± 2.4 SE; the average amount of PG consumed during the 4-h sampling period) could also affect ruminal VFA patterns.

In the current experiment, postprandial effects appeared to be minimized by 12 $\times$ feeding/d, as indicated by minimal fluctuations in serum insulin concentrations observed in the control cows (Figure 3). Based on the assumption made by Armentano et al. (1984) that cattle fed 12 $\times$ feeding/d were in a steady state for glucose production and utilization, any responses observed in blood glucose could therefore be attributed to treatments. Plasma glucose was tightly regulated in a narrow range; however, in the current study, the mean plasma glucose levels were numerically greater for the PG-supplemented cows than for the control cows, which could indicate that PG, its metabolite(s), or both affected glucose production, utilization, or both. Kristensen and Raun (2007) suggested that glucose utilization, rather than glucose production, was affected by PG, its metabolite(s), or both because glucose production was not increased in cows infused with PG (Palmquist and Brunengraber, 1997; Kristensen and Raun, 2007). An increase in plasma glucose concentration after PG

Figure 1. Ruminal propionate concentrations of cows (fed at $12\times$ feeding/d) for 4 h after receiving propylene glycol mixed into the TMR (dietary treatment), as an oral drench (oral-drench treatment), or as a ruminal drench (rumen-drench treatment). Pooled SEM = 0.77; treatment (delivery method) effect: $P < 0.01$; time effect: $P < 0.01$; treatment $\times$ time interaction: $P < 0.01$. The dotted vertical line indicates feeding.

infusion was due to a decrease in glucose utilization by peripheral tissues, even under increased serum insulin concentrations (Kristensen and Raun, 2007). This insulin-resistant-like effect on peripheral tissues was likely induced by an increased concentration of PG and propanol, a decreased ratio of ketogenic to glucogenic metabolites in the blood from PG metabolism, or both (Kristensen and Raun, 2007). Responses of insulin, similar to the responses of ruminal VFA, to PG supplementation were greater for the oral or ruminal drench of PG compared with mixing PG into the TMR, likely because of the amount of PG that was available to the rumen of the animal at the time of sampling. A relatively large amount of propionate produced from ruminal metabolism of PG could saturate the liver, thereby escaping hepatic removal and stimulating a pancreatic release of insulin. In the current study, by feeding cows frequently, at $12\times$ feeding/d, to minimize postprandial effects caused by feeding, oral or ruminal drenching of 200 mL or 200 g/d of PG increased the insulin level by 169 and 195% (from the level at time 0 to the maximal level), respectively, as opposed to the 102% observed in our previous experiment (Chung et al., 2009). Oral or ruminal drenching of PG in the current study also increased the mean insulin concentration during the serial sampling period, as opposed to our previous experiment (Chung et al., 2009) in which the mean insulin concentration was not increased by PG top dressing, although a pattern of insulin response similar to that in the current study was observed. When a bolus dose of PG was provided to cows, the discrepancy in increased insulin concentration between the current study (oral

and ruminal drenching) and our previous experiment (top dressing) indicated that an effect of PG on insulin was perhaps masked by feed intake (cows were fed $1\times$ feeding/d in our previous experiment).

Supplementing diets with PG tended to increase milk yield and reduce milk fat percentage for cows in early lactation, as summarized in a review by Nielsen and Ingvarsen (2004); however, an effect of PG on other milk components was not consistent in the literature. In the current study, milk yield was not affected by PG, and a clear reduction in milk fat percentage by PG was not evident. Fisher et al. (1973) reported a 0.2-percentage-unit increase in milk lactose percentage by feeding 495 g/d of PG to cows in early lactation, whereas feeding 178 to 260 g/d had no effects on milk lactose. Cozzi et al. (1996) and Shingfield et al. (2002) reported no effects of PG on milk lactose by feeding 200 to 400 g/d of PG to cows in midlactation. Supplementation of PG from 161 (± 4.1 SE; the average amount of PG that was consumed daily from TMR) to 200 g/d (drenched orally or ruminally), regardless of the delivery method, increased milk lactose percentage in the current experiment. A possible explanation for an increase in milk lactose percentage is an insulin-resistant-like effect on peripheral tissues induced by PG (Kristensen and Raun, 2007), as discussed previously. An increased concentration of blood PG and propanol, a decreased ratio of ketogenic to glucogenic metabolites in the blood from PG metabolism, or both can decrease glucose utilization by peripheral tissues; therefore, more glucose can be partitioned to the mammary gland for milk lactose synthesis.

Figure 2. Ruminal acetate concentrations of cows (fed at $12\times$ feeding/d) for 4 h after receiving propylene glycol mixed into the TMR (dietary treatment), as an oral drench (oral-drench treatment), or as a ruminal drench (rumen-drench treatment). Pooled SEM = 0.83. Treatment (delivery method) effect: $P < 0.01$; time effect: $P < 0.01$; treatment $\times$ time interaction: $P < 0.01$. The dotted vertical line indicates feeding.

Figure 3. Serum insulin concentrations of cows (fed at 12× feeding/d) for 4 h after receiving propylene glycol mixed into the TMR (dietary treatment), as an oral drench (oral-drench treatment), or as a ruminal drench (rumen-drench treatment). Pooled SEM = 33.1. Treatment (delivery method) effect: $P < 0.01$; time effect: $P < 0.01$; treatment × time interaction: $P < 0.01$. The dotted vertical lines indicate feeding.

Drenching PG by using an esophageal-stomach tube or a drench gun, as opposed to feeding PG, could likely lead to a greater portion of PG bypassing the rumen because of the closure of the reticular groove while drenching. However, in the current study, oral drenching of PG had a similar effect in stimulating insulin to rumen drenching of PG. It was likely that some of the PG by rumen drenching also bypassed the rumen through the reticular-omasal orifice. Christensen et al. (1997) reported that the ability of PG administered via oral drenching to increase serum insulin and decrease plasma NEFA concentrations was comparable with feeding PG in the concentrate. This similar glucogenic capacity of PG between drenching and bolus feeding (e.g., top dressing or feeding PG in the concentrate and fed separately from forage) could probably be explained by the diffusion of PG from the bloodstream into the rumen (Raun et al., 2004) after the PG that bypasses the rumen is absorbed from the lumen of the small intestines. Propylene glycol, as a dry form drenched into the cavity of the reticulum via the rumen cannula, was as effective as liquid PG drenched via the mouth in shifting ruminal VFA profiles and stimulating blood insulin and glucose responses.

CONCLUSIONS

Oral drenching of 200 mL of liquid PG or rumen drenching of 200 g of PG as a dry product shifted ruminal fermentation toward a more glucogenic environment and increased serum concentration of insulin. Feeding dry PG as a part of the diet (incorporating dry PG

into the diet) was not as effective as drenching PG. Dry matter intake, BW, and milk yield and composition, except for milk lactose percentage, were not affected by PG supplementation. Effects of drenching dry PG into the rumen were comparable with orally drenching liquid PG.

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