Ruminal and Intermediary Metabolism of Propylene Glycol in Lactating Holstein Cows

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ABSTRACT

Four lactating Holstein cows fitted with ruminal cannulas and permanent indwelling catheters in the mesenteric artery, mesenteric vein, hepatic portal vein, and hepatic vein were used in a cross-over design to study the metabolism of propylene glycol (PG). Each cow received 2 treatments: control (no infusion) and infusion of 650 g of PG into the rumen at the time of the morning feeding. Propylene glycol was infused on the day of sampling only. Samples of arterial, portal, and hepatic blood as well as ruminal fluid were obtained at 0.5 h before feeding and at 0.5, 1.5, 2.5, 3.5, 5, 7, 9, and 11 h after feeding. Infusion of PG did not affect ruminal pH or the total concentration of ruminal volatile fatty acids, but did decrease the molar proportion of ruminal acetate. The ruminal concentrations of PG, propanol, and propanal as well as the molar proportion of propionate increased with PG infusion. The plasma concentrations of PG, ethanol, propanol, propanal, glucose, L-lactate, propionate, and insulin increased with PG and the plasma concentrations of acetate and β -hydroxybutyrate decreased. The net portal flux of PG, propanol, and propanal increased with PG. The hepatic uptake of PG was equivalent to 19% of the intraruminal dose. When cows were dosed with PG, the hepatic extraction of PG was between 0 and 10% depending on the plasma concentration of PG, explaining the slow decrease in arterial PG. The increased net hepatic flux of L-lactate with PG could account for the entire hepatic uptake of PG, which suggests that the primary hepatic pathway for PG is oxidation to L-lactate. The hepatic uptake of propanol increased with PG, but no effects of PG on the net hepatic and net splanchnic flux of glucose were observed. Despite no effect of PG on net portal flux and net hepatic flux of propionate, the net splanchnic flux of propionate increased and the data suggest that propionate produced from hepatic metabolism of propanol is partly released to the blood. The data suggest that PG

affects metabolism of the cows by 2 modes of action: 1) increased supply of L-lactate and propionate to gluconeogenesis and 2) insulin resistance of peripheral tissues induced by increased concentrations of PG and propanol as well as a decreased ratio of ketogenic to glucogenic metabolites in arterial blood plasma.

Key words: alcohol, dairy cow, metabolism, propylene glycol

INTRODUCTION

It has been recognized for decades that propylene glycol (PG) has glucogenic effects in ruminants (Johnson, 1954). Today, PG is commonly administered to dairy cows in early lactation to decrease concentrations of NEFA and BHBA in peripheral blood (for review see Nielsen and Ingvarsten, 2004; Overton and Waldron, 2004). Early data on PG metabolism suggested that PG was mainly absorbed intact from the rumen and metabolized in the liver and, to a lesser extent, fermented into propionate before absorption (Emery et al., 1967). However, work from our laboratory showed that the rate of PG metabolism was greatly decreased in dairy cows under washed rumen conditions (Kristensen et al., 2002) and only a low hepatic extraction ratio (8%) of PG was observed in steers intravenously infused with PG (Raun et al., 2004). These observations along with the consistent ruminal propionate response to PG intake (e.g., Cozzi et al., 1996; Christensen et al., 1997) indicate that ruminal fermentation might be of considerable importance in cattle. In vitro incubations of ruminal microbes with PG indicated that propanal (propionaldehyde) and propanol might be important products or intermediates in ruminal PG fermentation (Czerkawski and Breckenbridge, 1973; Czerkawski et al., 1984); these observations are supported by similar findings in other ecosystems that degrade PG; for example, soil (Veltman et al., 1998) and silage (Driehuis et al., 1999). Furthermore, we have consistently observed a sweet odor resembling that of propanol from cows dosed intraruminally with PG that cannot be explained by increasing appearance of propionate or by the weak odor of PG itself. We hypothesized that intraruminally dosed PG would be extensively fermented into volatile

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components other than VFA and that these volatiles might be of major importance for understanding PG metabolism in dairy cows. The aim of the present study was to investigate the metabolism and metabolic effects of PG dosed intraruminally in lactating dairy cows.

MATERIALS AND METHODS

The present experiment complied with the Danish Ministry of Justice Law no. 382 (June 10, 1987), Act no. 726 (September 9, 1993) concerning experiments with animals and care of experimental animals.

Animals and Feeding

Four lactating (21 ± 1 kg of milk/d) Danish Holstein cows (615 ± 21 kg of BW) fitted with ruminal cannulas (#1C, Bar Diamond, Parma, ID) and permanent indwelling catheters in the mesenteric artery ($n = 4$), mesenteric vein ($n = 4$), hepatic portal vein ($n = 4$), and hepatic vein ($n = 3$; patency of one hepatic catheter was lost before samplings in the present study were initiated) were used in the study. Surgery was done according to the procedures described by Huntington et al. (1989) with slight modifications of the catheters as previously described (Kristensen et al., 2007). Cows underwent surgery at 255 ± 11 DIM, and first samplings in the present study were 70 ± 21 d after surgery. The experimental treatments (control = no infusion; PG treatment = intraruminal infusion of 650 g of PG at time of feeding at 0700 h) were arranged in a cross-over design and separated by at least 14 d.

The feed was offered at a level of approximately 85% of ad libitum intake to ensure similar feed intake between sampling days. The ration contained (% of DM) corn silage, 54.4; grass hay, 18.1; rapeseed cake, 12.4; dried sugar beet pulp, 12.1; urea, 1.4; mineral premix, 1.0; sodium chloride, 0.2; sodium sulfate, 0.2; and vitamin premix, 0.2. The mineral and vitamin premixes have been described previously (Kristensen et al., 2007). The nutrient composition of the ration was (g/kg of DM): OM, 939; NDF, 382; CP, 168; starch, 160; and ether extract, 43. The daily ration was divided into 2 equal-size meals offered at 0700 and 1900 h. Cows were milked at 0600 and 1600 h.

Experimental Samplings

On sampling days, continuous infusion of *p*-aminohippuric acid (pAH; 30 ± 1 mmol/h) into the mesenteric vein was initiated at 0530 h. The pAH infusate was a 250 mM solution of pAH (4-aminohippuric acid 99%, Acros, Geel, Belgium) adjusted to pH 7.4, filtered (Vacu Cap 0.8/0.2 μ m, Pall Corp., Ann Arbor, MI), and auto-

claved. For the PG treatment, 650 g of propylene glycol (Bie and Berntsen, Rødovre, Denmark) dissolved in 10 L of warm tap water was dosed into the ventral sac of the rumen at 0700 h using a funnel connected to an oro-ruminal probe (Geishauser, 1993). The oro-ruminal probe was introduced via the ruminal cannula and infusion was completed within 3 to 5 min. Cows were only infused with PG on the day of sampling and cows in the control group did not receive infusions. Ten sets of ruminal and blood samples were obtained at 0.5 h before feeding and at 0.5, 1.5, 2.5, 3.5, 5, 7, 9, and 11 h after feeding. Blood was sampled by simultaneously drawing blood from the artery, hepatic portal vein, and hepatic vein into 20-mL syringes and immediately transferring it to sodium heparin vacuettes (Greiner BioOne GmbH, Kremsmünster, Austria). Plasma was harvested by centrifugation at $3,000 \times g$ for 20 min and stored at -20°C until analysis. Separate blood samples were obtained in 1-mL heparinized syringes for blood-gas measurements immediately before collection of the main blood samples. Ruminal fluid was sampled from the ventral ruminal sac using a suction strainer (Bar Diamond) and a 50-mL syringe. Ruminal fluid pH was measured immediately after sampling (IQ 150 pH meter; IQ Scientific Instruments Inc., Carlsbad, CA). Immediately after reading pH, a subsample of ruminal fluid was stabilized with 5% meta-phosphoric acid and frozen at -20°C .

Analytical Procedures

Blood sampled in 1-mL syringes was immediately taken for blood gas and oximetry analysis (ABL 520, Radiometer A/S, Copenhagen, Denmark). Hematocrit was determined on heparin-stabilized arterial samples by centrifugation of capillary tubes at $13,000 \times g$ for 6 min.

Propylene glycol in plasma and ruminal fluid was determined according to the method originally described by Needham et al. (1982) with modifications as described by Raun et al. (2004). Plasma was analyzed for glucose (D-glucose oxidase) and L-lactate (L-lactate oxidase; YSI 7100, YSI Inc., Yellow Springs, OH). Plasma concentrations of propanal, ethanol, and propanol were determined by headspace GC/MS as previously described (Kristensen et al., 2007). Plasma concentrations of pAH were determined using the method described by Harvey and Brothers (1962), modified to run on a Cobas Mira autoanalyzer (Triolab A/S, Brøndby, Denmark). D-3-Hydroxybutyrate (BHBA) was determined in plasma using a Cobas Mira autoanalyzer and a kit based on D-3-hydroxybutyrate dehydrogenase (McMurray et al., 1984; Ranbut; Randox Laboratories Ltd., Crumlin, UK).

Volatile fatty acids in ruminal fluid were analyzed by GC (Kristensen et al., 1996). Alcohols and esters in ruminal fluid were determined using headspace GC/MS (see above). Glucose and L-lactate were determined in ruminal fluid using the YSI analyzer as described above. Feed samples were analyzed as previously described (Kristensen et al., 2007).

Calculations and Statistical Procedures

Calculations of net portal flux, net hepatic flux, net splanchnic flux, and hepatic extraction ratio (efficiency of the liver in removing metabolites from hepatic inflow via the hepatic portal vein and the hepatic artery) were performed as previously described (Kristensen et al., 2007).

Data for ruminal variables, arterial variables, and blood and plasma fluxes were considered as repeated measures and analyzed using the autoregressive order 1 structure in PROC MIXED of SAS (SAS Institute, 2001). The model included fixed effects of treatment, sampling time, and sampling time by treatment interaction as well as the random effect of cow. Data for daily DM intake and milk yield were analyzed by one-way ANOVA using PROC GLM of SAS. The data set was complete except for one cow missing the hepatic catheter. Significance was declared at $P < 0.05$, with a tendency at $0.05 \leq P < 0.10$. Data are presented as means $\pm$ SE.

RESULTS

Dry matter intake did not differ between the 2 treatments (16.2 ± 0.5 kg/d; $P = 0.30$). Milk yield did not differ between treatments (21 ± 1 kg/d; $P = 0.23$).

Ruminal Variables

All ruminal variables except ruminal glucose were affected ($P = 0.04$ to $P < 0.01$) by sampling time. Treatment by time interactions ($P < 0.01$) were observed for ruminal concentrations of PG, propanol, and propanal, reflecting increased ruminal concentrations of these compounds following intraruminal infusion of PG (Figures 1, 2, and 3). Treatment by time interactions ($P = 0.04$ to $P < 0.01$) were observed for molar proportions of acetate, propionate, isovalerate, valerate, and caproate (Table 1). The profiles of molar proportions of acetate and propionate in the rumen were similar although reciprocal with nadir and zenith observed 5 h after infusion of PG and apparent separation of treatments immediately after PG infusion (Figure 4 shows the profile for propionate). In contrast, the increased molar proportions of isovalerate, valerate, and caproate with

Figure 1. Ruminal concentration of propylene glycol at 0.5 h before feeding, and at 0.5, 1.5, 2.5, 3.5, 5, 7, 9, and 11 h after feeding in cows receiving an intraruminal infusion of 650 g of propylene glycol in 10 L of water at the time of feeding (○) or no infusion (□). Effects in the model included fixed effects of treatment ($P < 0.01$), sampling time ($P < 0.01$), and interaction between treatment and sampling time ($P < 0.01$) as well as the random effect of cow. Each data point is the mean of 4 observations $\pm$ SE.

PG infusion were delayed 2.5 to 6.5 h, and the largest numerical differences between treatments were observed 5 to 8 h after dosing. Ruminal pH and total concentration of ruminal VFA as well as the ruminal concentration of ethanol, ethyl acetate, and glucose were not affected by treatment. The ruminal concentra-

Figure 2. Ruminal concentration of propanal at 0.5 h before feeding, and at 0.5, 1.5, 2.5, 3.5, 5, 7, 9, and 11 h after feeding in cows receiving an intraruminal infusion of 650 g of propylene glycol in 10 L of water at the time of feeding (○) or no infusion (□). Effects in the model included fixed effects of treatment ($P < 0.01$), sampling time ($P < 0.01$), and interaction between treatment and sampling time ($P < 0.01$) as well as the random effect of cow. Each data point is the mean of 4 observations $\pm$ SE.

Figure 3. Ruminal concentration of propanol at 0.5 h before feeding, and at 0.5, 1.5, 2.5, 3.5, 5, 7, 9, and 11 h after feeding in cows receiving an intraruminal infusion of 650 g of propylene glycol in 10 L of water at the time of feeding (○) or no infusion (□). Effects in the model included fixed effects of treatment ($P < 0.01$), sampling time ($P < 0.01$), and interaction between treatment and sampling time ($P < 0.01$) as well as the random effect of cow. Each data point is the mean of 4 observations $\pm$ SE.

Figure 4. Molar proportion of propionate at 0.5 h before feeding and at 0.5, 1.5, 2.5, 3.5, 5, 7, 9, and 11 h after feeding in cows receiving an intraruminal infusion of 650 g of propylene glycol in 10 L of water at the time of feeding (○) or no infusion (□). Effects in the model included fixed effects of treatment ($P < 0.01$), sampling time ($P < 0.01$), and interaction between treatment and sampling time ($P < 0.01$) as well as the random effect of cow. Each data point is the mean of 4 observations $\pm$ SE.

tion of L-lactate tended to decrease with PG infusion ($P = 0.05$).

Arterial Variables

Arterial blood pH increased with PG ($P < 0.01$), but hematocrit and concentrations of O_2 and CO_2 in arterial blood were not affected by treatment (Table 2). No ef-

fects of sampling time were observed for arterial blood pH, hematocrit, and CO_2 concentration ($P = 0.14$ to $P = 0.55$). Arterial concentration of O_2 tended to decrease after feeding ($P = 0.06$). Treatment by time interactions were observed ($P = 0.04$ to $P < 0.01$) for arterial plasma concentrations of PG, ethanol, propanol, propanal, glucose, acetate, propionate, isovalerate, and insulin (Table 2), reflecting increased concentrations after PG infusion except for the concentration of acetate that de-

Table 1. Ruminal variables

Item	Treatment ¹		SEM	P-value ²		
	Control	PG		Trt	Time	Trt $\times$ Time
Ruminal pH	6.60	6.63	0.07	0.67	<0.01	0.59
Propylene glycol, mM	—	10.6	1.0	<0.01	<0.01	<0.01
Ethanol, mM	1.45	1.16	0.64	0.37	<0.01	0.22
Propanol, mM	0.24	3.03	0.55	<0.01	<0.01	<0.01
Ethyl acetate, mM	0.25	0.21	0.12	0.34	<0.01	0.13
Propanal	<0.01	0.35	0.04	<0.01	<0.01	<0.01
Total VFA, mM	97	93	3	0.34	<0.01	0.62
Acetate, mol/100 mol	65.1	61.6	0.5	0.02	<0.01	<0.01
Propionate, mol/100 mol	21.5	24.9	0.5	<0.01	<0.01	<0.01
Isobutyrate, mol/100 mol	0.7	0.8	<0.1	0.41	<0.01	0.64
Butyrate, mol/100 mol	9.6	9.1	0.3	0.25	0.04	0.97
Isovalerate, mol/100 mol	1.4	1.4	0.1	0.78	<0.01	<0.01
Valerate, mol/100 mol	1.2	1.5	<0.1	0.01	<0.01	0.04
Caproate, mol/100 mol	0.5	0.7	<0.1	<0.01	<0.01	<0.01
Glucose, mM	0.03	0.04	0.01	0.88	0.20	0.36
L-Lactate, mM	0.45	0.20	0.20	0.11	0.01	0.05

¹Treatments were no infusion (control) or intraruminal infusion of 650 g of propylene glycol (PG) in 10 L of warm tap water at the time of morning feeding.

²Probability for no effect of treatment (Trt), sampling time (Time), and interaction between treatment and sampling (Trt $\times$ Time).

Table 2. Arterial variables in whole blood and plasma

Item	Treatment ¹		SEM	P-value ²		
	Control	PG		Trt	Time	Trt × Time
Whole blood						
pH	7.44	7.46	0.01	<0.01	0.55	0.71
Hematocrit, %	27.2	26.2	1.0	0.29	0.14	0.85
O ₂ , mM	5.40	5.15	0.27	0.10	0.06	0.18
CO ₂ , mM	25.8	27.1	1.2	0.17	0.22	0.94
Blood plasma						
Propylene glycol, mM	<0.01	3.61	0.20	<0.01	<0.01	<0.01
Ethanol, mM	0.025	0.164	0.028	0.01	<0.01	0.01
Propanol, mM	0.001	0.490	0.126	0.01	<0.01	<0.01
Propanal, mM	0.0005	0.0026	0.0002	<0.01	<0.01	0.02
Glucose, mM	3.953	4.300	0.100	0.04	<0.01	<0.01
L-Lactate, mM	0.611	0.815	0.051	0.02	<0.01	0.14
BHBA, mM	0.502	0.443	0.055	0.05	<0.01	0.33
Acetate, mM	1.729	1.313	0.088	<0.01	<0.01	0.02
Propionate, mM	0.045	0.091	0.013	<0.01	<0.01	0.01
Isobutyrate, mM	0.004	0.003	0.001	0.67	<0.01	0.85
Butyrate, mM	0.021	0.015	0.003	0.08	<0.01	0.27
Isovalerate, mM	0.004	0.005	0.001	0.34	0.01	0.04
Valerate, mM	0.001	0.001	0.001	0.68	0.23	0.32
Caproate, mM	<0.001	<0.001	0.001	0.30	0.57	0.94
Insulin, pM	78	136	13	<0.01	<0.01	<0.01

¹Treatments were no infusion (control) or intraruminal infusion of 650 g of propylene glycol (PG) in 10 L of warm tap water at time of feeding in the morning.

²Probability for no effect of treatment (Trt), sampling time (Time), and interaction between treatment and sampling (Trt × Time).

creased with PG. The arterial concentration of L-lactate increased ($P = 0.02$) with PG infusion. The arterial concentration profile of PG (Figure 5) was different from the ruminal profile (Figure 1) with a relatively slow

Figure 5. Arterial blood plasma concentration of propylene glycol at 0.5 h before feeding and at 0.5, 1.5, 2.5, 3.5, 5, 7, 9, and 11 h after feeding in cows receiving an intraruminal infusion of 650 g of propylene glycol in 10 L of water at the time of feeding (○) or no infusion (□). Effects in the model included fixed effects of treatment ($P < 0.01$), sampling time ($P < 0.01$), and interaction between treatment and sampling time ($P < 0.01$) as well as the random effect of cow. Each data point is the mean of 4 observations $\pm$ SE.

decrease of the concentration in arterial blood plasma after the infusion.

Blood Flow and Net Portal Flux

No effects ($P = 0.23$ to $P = 0.72$) of treatment or time were observed for portal and hepatic arterial blood flow (Table 3). The hepatic vein blood flow increased with PG ($P = 0.04$). The net portal flux of O₂ was not affected by time or treatment, and the tendency ($P = 0.09$) observed for a treatment by time effect on net portal flux of CO₂ did not reflect any obvious systematic pattern in the data. Treatment by time effects ($P = 0.04$ to $P < 0.01$) were observed for net portal fluxes of PG, ethanol, propanol, propanal, and insulin reflecting increased flux with the PG treatment except for the net portal flux of ethanol that was lower with PG compared with control. The net portal flux of PG was apparently biphasic with a positive net portal flux 0.5 to 2.5 h after PG infusion and an apparent negative net portal flux 3.5 to 9.5 h after PG infusion (Figure 6). The net portal flux of glucose and L-lactate were not affected by time or treatment ($P = 0.12$ to $P = 0.74$). Net portal fluxes of BHBA and all VFA were affected by time of sampling ($P = 0.02$ to $P < 0.01$). The net portal flux of acetate was lower ($P = 0.02$) with PG compared with control; however, no effects of treatment ($P = 0.16$ to $P = 0.57$) were observed for the other VFA and BHBA.

Table 3. Blood plasma flow in hepatic artery, portal vein, and hepatic vein and net portal flux, net hepatic flux, and net splanchnic flux of O₂, CO₂, metabolites, and insulin

Item	Treatment ¹			P-value ²		
	Control	PG	SEM	Trt	Time	Trt × Time
Blood plasma flows						
Hepatic artery, L/h	639	788	299	0.28	0.29	0.72
Portal vein, L/h	1,011	1,070	131	0.41	0.23	0.67
Hepatic vein, L/h	1,647	1,874	125	0.04	0.11	0.59
Net portal whole blood flux						
O ₂ , mmol/h	-1,580	-1,492	111	0.25	0.19	0.27
CO ₂ , mmol/h	918	1,338	131	0.10	0.03	0.09
Net portal blood plasma flux						
PG, mmol/h	0	109	48	<0.01	<0.01	<0.01
Ethanol, mmol/h	59	49	21	0.15	<0.01	0.03
Propanol, mmol/h	18	177	36	<0.01	<0.01	0.04
Propanal, mmol/h	0.2	4.7	1.8	0.08	<0.01	<0.01
Glucose, mmol/h	-40	-43	13	0.95	0.81	0.12
L-Lactate, mmol/h	125	123	23	0.97	0.53	0.74
BHBA, mmol/h	148	144	24	0.46	0.02	0.99
Acetate, mmol/h	1,916	1,564	358	0.02	<0.01	0.27
Propionate, mmol/h	638	712	127	0.31	<0.01	0.74
Isobutyrate, mmol/h	20	15	4	0.11	<0.01	0.64
Butyrate, mmol/h	102	80	21	0.23	<0.01	0.16
Isovalerate, mmol/h	10	8	2	0.38	<0.01	0.39
Valerate, mmol/h	22	22	5	0.88	<0.01	0.37
Caproate, mmol/h	20	21	5	0.72	<0.01	0.57
Insulin, nmol/h	42	60	7	0.01	0.02	<0.01
Net hepatic whole blood flux						
O ₂ , mmol/h	-2,625	-3,151	607	0.01	<0.01	0.43
CO ₂ , mmol/h	1,403	2,025	523	0.38	0.22	0.76
Net hepatic blood plasma flux						
PG, mmol/h	0	-133	57	0.48	0.04	0.04
Ethanol, mmol/h	-53	-31	27	0.36	0.01	0.32
Propanol, mmol/h	-12	-198	64	0.03	0.09	0.14
Propanal, mmol/h	-0.3	-5.5	2.3	0.17	0.10	0.09
Glucose, mmol/h	738	785	117	0.36	0.25	0.39
L-Lactate, mmol/h	-90	41	65	0.01	0.65	0.64
BHBA, mmol/h	243	220	74	0.20	0.17	0.93
Acetate, mmol/h	1,199	975	696	0.37	0.05	0.44
Propionate, mmol/h	-545	-544	168	0.89	0.03	0.97
Isobutyrate, mmol/h	-15	-12	5	0.74	0.28	0.96
Butyrate, mmol/h	-56	-50	26	0.68	0.14	0.98
Isovalerate, mmol/h	-6	-5	3	0.35	0.11	0.91
Valerate, mmol/h	-20	-20	7	0.96	<0.01	0.57
Caproate, mmol/h	-18	-21	9	0.69	<0.01	0.28
Insulin, nmol/h	-12	-14	1	0.47	0.89	0.70
Net splanchnic whole blood flux						
O ₂ , mmol/h	-4,169	-4,602	554	<0.01	<0.01	0.46
CO ₂ , mmol/h	2,238	3,389	579	<0.01	0.08	0.43
Net splanchnic blood plasma flux						
PG, mmol/h	0	-71	58	0.87	<0.01	<0.01
Ethanol, mmol/h	-10	-1	9	0.56	0.54	0.26
Propanol, mmol/h	0	-34	16	0.54	<0.01	<0.01
Propanal, mmol/h	-0.1	-0.5	0.6	0.74	0.11	0.22
Glucose, mmol/h	691	751	110	0.31	0.80	0.50
L-Lactate, mmol/h	27	150	68	0.11	0.99	0.53
BHBA, mmol/h	377	357	28	0.09	0.04	0.57
Acetate, mmol/h	3,006	2,480	327	0.03	<0.01	0.12
Propionate, mmol/h	77	131	17	0.03	<0.01	0.02
Isobutyrate, mmol/h	4.1	2.1	0.9	0.28	0.01	0.72
Butyrate, mmol/h	41	28	8	0.04	<0.01	0.13
Isovalerate, mmol/h	4.7	4.4	0.4	0.42	<0.01	0.06
Valerate, mmol/h	0.6	1.1	0.5	0.34	0.05	0.08
Caproate, mmol/h	0.3	-1.5	2	0.39	0.60	0.64
Insulin, nmol/h	32	45	9	0.09	0.04	<0.01

¹Treatments were no infusion (control) or intraruminal infusion of 650 g of propylene glycol (PG) in 10 L of warm tap water at time of morning feeding.

²Probability for no effect of treatment (Trt), sampling time (Time), and interaction between treatment and sampling (Trt × Time).

Figure 6. Net portal flux of propylene glycol at 0.5 h before feeding, and at 0.5, 1.5, 2.5, 3.5, 5, 7, 9, and 11 h after feeding in cows receiving an intraruminal infusion of 650 g of propylene glycol in 10 L of water at the time of feeding (○) or no infusion (□). Effects in the model included fixed effects of treatment ($P < 0.01$), sampling time ($P < 0.01$), and interaction between treatment and sampling time ($P < 0.01$) as well as the random effect of cow. Each data point is the mean of 4 observations $\pm$ SE.

Net Hepatic Flux

The net hepatic uptake of O_2 increased ($P = 0.01$) with PG; however, the hepatic CO_2 output was not affected ($P = 0.38$) by treatment. Net hepatic fluxes were characterized by relatively large standard errors and there were only 3 observations. A treatment by time effect ($P = 0.04$) was observed for the net hepatic flux of PG, and a tendency ($P = 0.09$) for a treatment by time effect was observed for the net hepatic flux of propanal, both reflecting increased hepatic uptake with PG infusion. Treatment effects ($P = 0.03$ to $P = 0.01$) were observed for net hepatic fluxes of propanol and L-lactate, reflecting increased uptake of propanol and a shift from net uptake to net output for L-lactate. The net hepatic fluxes of ethanol, propionate, valerate, and caproate were affected ($P = 0.03$ to $P = 0.04$) by sampling time, reflecting increased hepatic uptake after feeding except for acetate, for which increased output was observed after feeding.

Net Splanchnic Flux

The net splanchnic uptake of O_2 and the net splanchnic output of CO_2 increased ($P < 0.01$) with PG. Treatment by time effects ($P = 0.02$ to $P < 0.01$) were observed for PG, propanol, propionate, and insulin, reflecting increased splanchnic output of propionate and insulin with the PG treatment. For PG and propanol, the patterns were biphasic indicating net splanchnic output at the first samplings and net splanchnic uptake from approximately 3.5 h after PG infusion. Effects ($P = 0.04$

to $P < 0.01$) of sampling time were observed for the net splanchnic flux of BHBA, acetate, isobutyrate, and butyrate, reflecting increased splanchnic output after feeding.

Hepatic Extraction Ratio

The hepatic extraction ratio for PG was, on average, $3 \pm 1\%$ with the PG treatment (Table 4). Figure 7 shows the hepatic extraction ratio for PG across the sampling window, which indicates that the hepatic extraction ratio was very low (numerically negative) during the first samplings and increased to approximately 10% during the second half of the sampling window. Treatment by time interactions were observed ($P < 0.01$) for the hepatic extraction of propanol and propionate, reflecting decreased hepatic extraction from 1.5 to 5 h after PG dosing. The lowest hepatic extraction ratios observed for propionate and propanol after PG infusion were 46 ± 8 and $0 \pm 5\%$, respectively. The hepatic extraction of ethanol was decreased ($P = 0.02$) with PG compared with control.

DISCUSSION

Microbial Metabolism of PG

Studies from various ecosystems containing PG, such as in vitro rumen fermentation (Czerkawski and Breckenbridge, 1973), silage inoculated with *Lactobacillus buchneri* (Driehuis et al., 2001; Krooneman et al., 2002), and wastewater digesters (Veltman et al., 1998), all point to the same overall pathway of microbial PG degradation. The first step in the pathway is dehydration of PG that leads to formation of propanal (propionaldehyde). A dehydrase with relatively high affinity for PG ($K_m = 0.7$ mmol/L) has been isolated from *Lactobacillus brevis* (Radler and Zorg, 1986), and involvement of the same step in the rumen is in agreement with the transient presence of propanal after infusion of PG in previous in vitro studies (Czerkawski and Breckenbridge, 1973) and in the present study. It has been suggested that decomposition of PG in wastewater digesters leads to production of equal amounts of propanol and propionate using half the propanal as electron donor (i.e., oxidized to propionate) and half the propanal as electron acceptor (i.e., reduced to propanol) and coupling of propionate production to ATP synthesis (Veltman et al., 1998). Data from the present study indicated that the increase in net portal flux of propanol (treatment difference: 159 mmol/h) was considerable greater compared with the increase in net portal flux of propionate (numerical treatment difference: 74 mmol/h), which could indicate that a larger fraction of propanal might be diverted to propanol and thereby used as an electron

Table 4. Hepatic extraction ratios for variables with positive values

Item	Treatment ¹		SEM	P-value ²		
	Control	PG		Trt	Time	Trt × Time
Oxygen	0.34	0.38	0.04	0.14	0.11	0.78
Propylene glycol	—	0.03	0.01	—	—	—
Ethanol	0.40	0.02	0.11	0.02	0.08	0.83
Propanol	0.46	0.38	0.06	0.14	<0.01	<0.01
Propanal	0.70	0.30	0.50	0.72	0.38	0.44
Propionate	0.75	0.66	0.05	<0.01	<0.01	0.04
Isobutyrate	0.57	0.45	0.12	0.53	0.14	0.72
Butyrate	0.42	0.41	0.12	0.80	0.11	0.27
Isovalerate	0.38	0.20	0.17	0.28	0.85	0.52
Valerate	0.74	0.72	0.16	0.99	<0.01	0.55
Caproate	0.96	1.50	0.58	0.49	0.86	0.35
Insulin	0.08	0.05	0.04	0.39	0.32	0.09

¹Treatments were no infusion (control) or intraruminal infusion of 650 g of propylene glycol (PG) in 10 L of warm tap water at time of morning feeding.

²Probability for no effect of treatment (Trt), sampling time (Time), and interaction between treatment and sampling (Trt × Time).

sink in the rumen. However, studies on portal recovery of propionate infused into the rumen indicate that exogenous supply of propionate decreases ruminal production, leading to portal absorption of only 50 to 60% of the infused propionate (Kristensen, 2005). Studies on portal recovery of feed alcohols indicate complete portal recovery of ingested alcohols (Kristensen et al., 2007). It is therefore likely that the ruminal metabolism of propanal leads to production of equal amounts of propionate and propanol as observed in other ecosystems. The amount of the PG dose accounted for by metabolites produced in the rumen can be estimated as between 33 and 45% using either the numerical increase in net

portal flux of propanol plus propionate or 2× increased net portal flux of propanol. The increased net portal flux of propanal accounted only for approximately 0.6% of the PG dose.

Slow intermediary metabolism of PG enabled the cow to function as a reservoir for PG. Even though the initial ruminal disappearance rate for PG was found to be high in the present study as in previous studies (Clapperton and Czerkawski, 1972; Giesecke, 1974), the intermediary metabolism of PG cannot be estimated from the initial ruminal disappearance rate because PG diffuses back into the rumen. Accumulation of PG in buffers incubated in the washed rumen of steers receiving continuous jugular infusions of PG showed that portal-drained visceral uptake of arterial PG at least partly represents transfer of PG to the lumen of the gastrointestinal tract (Raun et al., 2004). In the present experiment, the ruminal concentration of PG returned to baseline approximately 3.5 h after the intraruminal infusion; at this time, the net portal flux of PG became negative indicating that ruminal microbes metabolized part of the PG that initially escaped ruminal fermentation.

Figure 7. Hepatic extraction of propylene glycol at 0.5 h before feeding, and at 0.5, 1.5, 2.5, 3.5, 5, 7, 9, and 11 h after feeding in cows receiving an intraruminal infusion of 650 g of propylene glycol in 10 L of water at the time of feeding (○). No meaningful values were observed with the control treatment. Each data point is the mean of 3 observations ± SE.

Hepatic Metabolism of PG

In the present study hepatic uptake of PG could account for approximately 19% of intraruminally infused PG (100 × 12-h sampling window × 133 mmol/h of difference in net hepatic uptake of PG/8,543 mmol of PG infused = 19% recovery). The relatively low hepatic extraction of PG explains why the blood concentration of PG remained at a high level for hours after infusion. Low hepatic extraction of PG is in agreement with previous studies from our laboratory showing that dairy

cows infused with PG under washed rumen conditions metabolize PG very slowly (Kristensen et al., 2002) and that the hepatic extraction ratio of PG in steers was only approximately 8% (Raun et al., 2004). L-Lactate, and not glucose, appeared to be the main product of hepatic metabolism of PG, which points to PG metabolism by alcohol and aldehyde dehydrogenases as the primary hepatic pathway. This is supported by the observed decrease in the hepatic extraction ratio of ethanol and propanol, which are assumed to be competing for the same pathway in the liver. Studies in rats showed that PG-induced hyperlactatemia could be inhibited by pyrazole, a known inhibitor of alcohol dehydrogenase (Morshed et al., 1989). Metabolism of PG by alcohol dehydrogenase could lead to release of lactaldehyde into peripheral circulation and it could be speculated that this explains the side effects of PG feeding reported in the literature (Nielsen and Ingvarsten, 2004). Involvement of alcohol and aldehyde dehydrogenases might also lead to a concern when dosing ketotic cows with PG. The assumed close equilibrium between the ratios of NADH:NAD⁺ and alcohol:aldehydes:carboxylic acid in the cytosol (Krebs, 1969) could lead to the hypothesis that hepatic metabolism of PG might be decreased in ketotic animals because of the increased hepatic NADH:NAD⁺ ratio (Zammit, 1990). A consequence could be that peripheral tissues are exposed to increased concentrations of alcohols and aldehydes originating from both ruminal and hepatic metabolism of PG.

Hepatic Metabolism of Propanol and Propionate

Despite indications of ruminal production of propionate from PG, infusion of PG was not followed by increased net portal absorption of propionate. Furthermore, the hepatic uptake of propionate did not change. However, both the arterial concentration and the splanchnic output of propionate were approximately doubled and the hepatic extraction of propionate decreased with PG. It seems, therefore, as if the additional propionate released into peripheral blood was primarily produced in the liver. It is very likely that propanol taken up by the liver is oxidized to propionate and that this endogenously produced propionate is either metabolized further within the liver or released to hepatic blood. The increased splanchnic flux of propionate could account for approximately 29% of the increased hepatic uptake of propanol, indicating that the largest part of propionate potentially produced from propanol is metabolized within the liver and directly available for gluconeogenesis.

Glucose Metabolism

Drenching with PG has long been known to increase plasma glucose in dairy cows (Johnson, 1954; Palmquist and Brunengraber, 1997) and this was observed in the present study. However, similarly to the study of Palmquist and Brunengraber (1997), we did not detect any increase in glucose production measured as increased net hepatic flux or increased net splanchnic flux of glucose. This indicates that the increased plasma level of glucose was caused primarily by decreased glucose demand by peripheral tissues, despite an increased insulin level. Numerous changes in the supply of both ketogenic and glucogenic metabolites might be affecting the demand for glucose by muscle and other peripheral tissues. Studies in rats indicated that diols such as PG and 2,3-butanediol could be directly involved in inducing insulin resistance in muscle and adipose tissue, and that these effects were observed at one-tenth of the average plasma concentration of PG in the present study (Lomeo et al., 1988; Xu et al., 1998). The potential PG-induced insulin resistance is not assumed to be specific to PG, but rather representing a more general effect of alcohols. The potency of individual alcohols is likely related to their ability to interfere with the microenvironment of the cell membrane (Xu et al., 1998). In the present study, we observed a pronounced response in the arterial concentration of propanol to PG infusion, and propanol might be involved in the insulin resistance induced by ruminal PG dosing. Data are generally lacking on plasma levels of PG and propanol in previous studies on PG drenching and feeding to dairy cows. However, it could be speculated that one of the major differences among trials as well as the difference in effect with method of delivery of PG (Christensen et al., 1997) is related to the ability to reach plasma concentrations of PG and propanol that induce insulin resistance in the cows.

Glucose uptake by peripheral tissues could also be affected by decreased splanchnic release of acetate, butyrate, and 3-hydroxybutyrate, which decreases the availability of substrates for de novo fatty acid synthesis in the mammary gland and adipose tissue followed by a decreased need for generation of NADPH from glucose. Simultaneously, the arterial concentrations of L-lactate, propanol, and propionate increased, which also might decrease glucose uptake in peripheral tissues. Furthermore, we cannot exclude the possibility that the marked response of plasma propionate to PG infusion could be involved in the hyperinsulinemic effect of PG (Sano et al., 1993; Leuvenink et al., 1997).

It seems, therefore, that the marked improvement in glucogenic status induced by dosing PG is less likely to be because of an actual increase in glucose supply of

the cows, but rather to decreased glucose demand by peripheral tissues. Several metabolic factors might affect peripheral glucose demand but, at present, we cannot separate the effects of a decreased ratio of ketogenic to glucogenic metabolites in plasma from the effects of increasing blood levels of PG and propanol.

Metabolism of BHBA

In the present study, we observed decreased plasma concentrations of BHBA after ruminal PG infusion, in agreement with previous studies showing decreased ketone and NEFA levels with PG (e.g., Sauer et al., 1973; Studer et al., 1993; Pickett et al., 2003). The hypoketonemic effect of PG was the very reason for its initial use in treatment of ketosis (Johnson, 1954). Insulin is a key hormone in regulation of lipolysis in adipocytes (Vernon, 2005), and decreased plasma levels of ketones and NEFA point to decreasing lipolysis in adipocytes induced by the increasing insulin level.

Mode of Action for PG

Data from the present study together with literature data suggest that metabolic effects of PG involve 2 modes of action. First, supply of glucogenic substrates by increasing absorption of PG as well as propanol, propionate, and propanal originating from ruminal metabolism of PG makes PG glucogenic in the classical sense (Krebs, 1963). Second, insulin resistance induced by increased circulating levels of PG and propanol or induced by a decrease in the ratio of ketogenic to glucogenic metabolites in plasma will decrease the demand for glucose by peripheral tissues and increase insulin and glucose levels, which, in turn, will decrease lipolysis and have hypolipidemic and hypoketonemic effects. Recognition of the different modes of action of PG might be key to explaining some of the peculiarities related to using PG as a feed additive. One example is the variable response to different ways of delivering PG as previously shown by Christensen et al. (1997), in which greater metabolic effects were observed following delivery of PG as a drench or in concentrate compared with feeding PG in a TMR. It is likely that rapid delivery of PG to the rumen gives a greater plasma concentration of PG because of the competition between intraruminal metabolism and absorption. When PG has first been absorbed, slow intermediary metabolism means that high plasma levels will be maintained for a long time, maintaining the insulin resistance responsible for the hypoketonemic and hypolipidemic effects.

CONCLUSIONS

Propylene glycol infused as a single ruminal dose was extensively metabolized in the rumen leading to

increased portal absorption of propanol and propanal. Propylene glycol absorbed to the blood was metabolized to L-lactate in the liver or diffused back into the rumen after microbial metabolism had lowered the ruminal PG level to favor blood-to-lumen flux. In total, approximately 46% of the ruminally infused PG could be accounted for by increased hepatic uptake of PG, propanol, and propanal. Propylene glycol infusion did not affect hepatic or splanchnic glucose output and it is concluded that the hyperglycemic and hyperinsulinemic effects of PG most likely are caused by insulin resistance induced by increased concentrations of PG and propanol and a decreased ratio of ketogenic to glucogenic metabolites in arterial blood plasma.

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