Increased anaplerosis of the tricarboxylic acid cycle decreased meal size and energy intake of cows in the postpartum period

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ABSTRACT

The objective of this study was to determine the effects of anaplerosis of the tricarboxylic acid cycle on feeding behavior and energy intake of cows in the postpartum period. We infused propionic acid (PA) and glycerol (GL) continuously into the abomasum and hypothesized that PA will decrease meal size and energy intake compared with GL because PA enters the tricarboxylic acid cycle, likely stimulating oxidation of acetyl CoA and satiety compared with GL. Three experiments (Exp.) were conducted using 20 Holstein cows between 3 and 22 d postpartum (8 cows in Exp. 1 and 6 cows each for Exp. 2 and 3). Treatments were compared using isoenergetic (Exp. 1, 193 kcal/h) and isomolar (Exp. 2, ~0.5 mol/h) continuous infusions of PA (99.5%) and GL (99.7%) to the abomasum using a double crossover design with four 2-d infusion periods each, and 2 mol of PA or GL pulse-dosed to the abomasum using a crossover design (Exp. 3). Treatment sequences were assigned alternately to cows based upon date of parturition. Feeding behavior was recorded by a computerized data acquisition system for Exp. 1 and 2. Propionic acid decreased dry matter intake (DMI) compared with GL 16.7 and 23.4% in Exp. 1 and 2, respectively. The decrease in DMI was because PA decreased meal size compared with GL by 12.6 and 15.3% in Exp. 1 and 2, respectively. Propionic acid decreased total metabolizable energy intake (diet plus treatment infusions) compared with GL for both experiments. Compared with PA, GL increased plasma glucose and insulin concentrations for Exp. 2 only. In Exp. 3, PA decreased hepatic acetyl CoA content 34%, whereas GL increased hepatic acetyl CoA content 32%, resulting in lower hepatic acetyl CoA content for PA compared with GL at 30 min (18.0 vs. 36.9 nmol/g), which persisted at 60 min after dosing (21.9 vs. 32.8 nmol/g). Consistent with our hypothesis, the obligatory anaplerotic metabolite PA decreased meal size,

DMI, and total metabolizable energy intake compared with GL, likely because of differences in their hepatic metabolism.

Key words: control of feed intake, acetyl CoA, anaplerosis, hepatic oxidation theory

INTRODUCTION

The hypophagic effects of propionate are well established but the mechanism by which it affects feeding is uncertain (Allen, 2000). Propionate decreased DMI by decreasing meal size compared with acetate when infused intraruminally in lactating cows, both continuously (Oba and Allen, 2003a) as well as at spontaneous meals (Choi and Allen, 1999). The reduction in DMI was greater than the energy infused so that energy intake was decreased by propionate compared with acetate (Oba and Allen, 2003a). In addition, propionate infusion was more hypophagic than acetate when infused in cows in the early postpartum (PP) period than in mid lactation (Oba and Allen, 2003b), likely because cows in the PP period are in a lipolytic state with both elevated plasma nonesterified fatty acid (NEFA) concentration and hepatic acetyl CoA (Ac-CoA) content (Allen et al., 2009). Anaplerosis of the tricarboxylic acid (TCA) cycle can stimulate oxidation of the existing pool of AcCoA increasing hepatic energy charge and generating a satiety signal according to the hepatic oxidation theory (HOT; Allen, 2014). In support of this, the hypophagic effect of propionate, an anaplerotic metabolite, compared with acetate was greater for cows with higher hepatic AcCoA content (Stocks and Allen, 2012, 2013). In addition, cows with more intensive body fat mobilization during the PP period had lower DMI and increased anaplerosis, TCA cycling, and mitochondrial oxidation of AcCoA (Schäff et al., 2012).

Propionic acid (PA) and glycerol (GL) are both 3-carbon glucose precursors with similar gross energy contents but they are metabolized differently in the bovine liver. Although both are anaplerotic metabolites, GL is not always anaplerotic but PA is. When insulin is low and gluconeogenesis is upregulated in the PP

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period, GL enters the gluconeogenic pathway as triose phosphate in the cytosol and is therefore not anaplerotic under those conditions. In contrast, propionate is an obligatory anaplerotic metabolite and must always enter the TCA cycle to be metabolized. The objective of this research was to challenge the HOT by evaluating effects of abomasal infusions of PA and GL on feeding behavior and metabolic responses of cows in the immediate PP period. We hypothesized that PA will be more hypophagic than GL because it is more likely to stimulate oxidation of the existing pool of AcCoA in the liver.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee at Michigan State University approved all experimental procedures for these experiments.

Animals, Housing, and Diets

Three experiments (**Exp.**) were conducted using a total of 20 ruminally cannulated multiparous Holstein cows ($n = 8$, 4 ± 1.8 d PP for Exp. 1; $n = 6$, 3.8 ± 0.8 d PP for Exp. 2; and $n = 6$, 15.2 ± 7.7 d PP for Exp. 3). Cows were ruminally cannulated at least 45 d before calving and housed in individual tie stalls for the duration of each experiment. Animals were fed a TMR once daily (1200 h) at 110% of expected intake and received a common experimental diet from parturition through the end of the experiment. The experimental diet (Table 1) for Exp. 1 and Exp. 2 consisted of corn silage, alfalfa silage, alfalfa hay, ground shelled corn, soybean meal, and a vitamin and mineral mix formulated to meet requirements according to NRC (2001). The diet for Exp. 3 was composed of corn silage, alfalfa silage, alfalfa hay, ground corn, grass silage, soybean hulls, soybean meal, and a vitamin and mineral mix and was formulated to contain 17% CP, 30% NDF 25% forage NDF, and 27% starch.

Experimental Design and Treatments

Experiments 1 and 2 used a double crossover design with four 2-d infusion periods. Multiparous Holstein cows were between 2 and 6 d PP. Treatments for both experiments were PA (food grade, 99.5%, Kemin Industries Inc., Des Moines, IA) and GL (US Pharmacopoeia, 99.7%, Chemworld, Roswell, GA) infused continuously into the abomasum. Cows were assigned randomly to treatment sequence PA-GL-PA-GL or GL-PA-GL-PA. In Exp. 1, isoenergetic concentrations of PA (1.0 M) and GL (0.92 M) were infused at 528 mL/h for 22 h/d,

providing 4.24 Mcal of ME/d. Metabolizable energy content of treatments were assumed to equal their gross energy (**GE**) of 365.0 kcal/mol for PA (Labedeva, 1964) and 395.4 kcal/mol for GL (Bastos et al., 1988). In Exp. 2, isomolar concentrations (1.0 M) of each treatment were infused at ~ 521 mL/h for 22 h/d, providing 4.22 Mcal of ME/d for PA and 4.52 Mcal of ME/d for GL. The solutions were infused into the abomasum using Baxter Flo-Gard 6201 volumetric infusion pumps (Baxter Medical Products, Deerfield, IL) through vinyl tubing (0.95-cm outside diameter, 0.71-cm inside diameter) connected to a Nalgene bottle (3.8 cm diameter, 8.5 cm long). A hole was drilled through the bottle lid to allow the tubing to pass through. A nylon bushing (6-mm outside diameter, 3-mm inside diameter, 3.5 cm long) was inserted into the tubing and kept in place by a stainless steel hose clamp. This secured the tubing in the bottle and helped keep large particles of digesta from blocking the tubing orifice. Nine 7-mm holes were drilled into the bottle to allow fluid exchange. A semi-hard flexible rubber flange (approximately 12 cm in diameter) was placed behind the bottle and inserted into the abomasum to hold the device in place.

Experiment 3 was a crossover design with two 1-d periods. Treatments were 2 mol/500 mL of PA or GL pulse-dosed to the abomasum of Holstein cows ($n = 6$) between 8 and 23 d PP. Cows were assigned randomly to treatment sequence PA-GL or GL-PA. Treatments were pulse-dosed within 1 min, 1 h before feeding and tubing was held in place manually during dosing.

Table 1. Ingredients and nutrient composition of experimental diet (% of dietary DM except for DM)

Item	%
Ingredient	
Corn silage	32.3
Ground shelled corn	20.6
Alfalfa silage	19.8
Soybean meal	15.3
Alfalfa hay	9.9
Vitamin and mineral mix ¹	2.0
Sodium bicarbonate	1.1
Limestone	1.1
Nutrient composition	
DM	46.9
OM	91.9
Starch	27.4
NDF	30.0
Forage NDF	26.5
CP	15.8

¹Vitamin and mineral mix contained 25.6% NaCl, 10.0% Ca, 2.0% Mg, 2.0% P, 30 mg/kg Co, 506 mg/kg of Cu, 20 mg/kg I, 2,220 mg/kg Fe, 2,083 mg/kg Mn, 15 mg/kg Se, 2,025 mg/kg Zn, 300 kIU/kg vitamin A, 50 kIU/kg vitamin D, and 1,500 kIU/kg vitamin E.

Data and Sample Collection

Throughout Exp. 1 and 2, cows were blocked from feed from 1000 to 1200 h daily, and the amount of feed offered and orts were weighed for each cow daily. Samples (0.5 kg) of all dietary ingredients and the TMR were collected once per week, and orts for each cow were taken for the second day of each infusion period and stored in plastic bags at -20°C until processed. For Exp. 1 and 2, feeding behavior was monitored continuously for 22 h daily for each cow with a computerized data acquisition system (Dado and Allen, 1995). Feed disappearance and water consumption were recorded for each cow every 5 s, and meal size, interval between meals, and meal frequency were calculated. Hunger ratio was calculated as the weight of meal divided by pre-meal interval and satiety ratio was calculated as the weight of meal divided by post-meal interval (Forbes, 2007).

Cows were milked twice daily at 0530 h and 1730 h in their stalls. Milk yield was recorded daily at each milking, and milk samples were collected from each milking and stored with preservative (Bronopol tablet; D&F Control Systems, San Ramon, CA) at 4°C . Milk samples were analyzed for fat, true protein, lactose, SCC, and MUN by Michigan DHIA (AOAC International, 1997).

Blood and fecal samples were collected every 6 h for 24 h ($n = 4$) during the second day of each infusion period. Blood was sampled from coccygeal vessels and collected into 3 evacuated tubes, 2 containing K_2EDTA and 1 containing potassium oxalate with NaF as a glycolytic inhibitor, and placed on ice until centrifugation. Tubes were centrifuged at $3,000 \times g$ for 15 min at 5°C immediately after sample collection. Plasma was harvested and frozen at -20°C until analysis. Fecal samples were collected and frozen at -20°C for later analysis to determine diet digestibility. Liver samples were collected by needle biopsy (Bradford and Allen, 2005) between 1130 and 1200 h (before feeding) on d 0 before infusion initiation and at the end of second day of infusions for each period for analysis of hepatic Ac-CoA content. Samples were flash frozen in liquid nitrogen and stored on dry ice for transportation. Samples were stored at -80°C until further analysis. For Exp. 3, liver tissue was biopsied and blood was collected immediately before dosing and at 30 and 60 min after dosing using the procedures mentioned above.

Analysis of Samples

Diet ingredients, orts, and fecal samples were dried in a 55°C forced-air oven for 72 h and were ground with a Wiley mill (1-mm screen; Arthur H. Thomas, Philadelphia, PA). Samples were analyzed for ash, NDF, in-

digestible NDF, CP, and starch. Feces were composited by cow by day on an equal DM basis before analysis. All nutrients are expressed as percentages of DM that was determined by drying at 105°C in a forced-air oven for more than 8 h. Ash content was determined after 6 h of oxidation at 500°C in a muffle furnace. Content of NDF was analyzed according to Mertens (2002) with the inclusion of amylase and Na sulfite. Crude protein was determined according to Hach et al. (1987). Starch was analyzed using an enzymatic method (Karkalas, 1985) after samples were gelatinized with sodium hydroxide; glucose concentration was measured by a glucose oxidase method (Sigma Chemical Co., St. Louis, MO). Indigestible NDF was determined as NDF residue after 240 h in vitro fermentation (Goering and Van Soest, 1970). Ruminal fluid was collected from a non-pregnant fistulated dry cow and flasks were re-inoculated at 120 h to ensure microbes were viable. Indigestible NDF was used to estimate fecal output and apparent total-tract digestibility (Cochran et al., 1986). All samples were analyzed for GE by bomb calorimeter according to the manufacturer's instructions (Parr Instrument Inc., Moline, IL). Digestible energy (DE) intake was calculated as GE intake minus GE output in feces. Metabolizable energy from the diet was calculated according to NRC (2001) as $\text{ME (Mcal/kg)} = 1.01 \times (\text{DE} - 0.45)$, based on measured diet digestibility. ME intake (MEI) was calculated as $\text{MEI (Mcal/d)} = \text{ME} \times \text{DMI}$ and total $\text{MEI} = \text{ME intake diet} + \text{ME infusion}$.

Plasma samples were analyzed colorimetrically with a microplate reader (SpectraMax Plus 384, Molecular Devices Corp., Sunnyvale, CA) to determine concentrations of metabolites. Commercial kits were used to determine concentrations of NEFA (NEFA HR kit, Wako Chemicals USA, Richmond, VA) and BHB (kit no. 2240, Stanbio Laboratory, Boerne, TX), and glucose was determined using a glucose oxidase method (Sigma Chemical Co.). Insulin was determined using an ELISA kit (kit no. 10-1201-01, Mercodia, Uppsala, Sweden). Plasma propionate and glycerol concentrations were analyzed by HPLC (Waters Corp., Milford, MA). Plasma (600 μL) was mixed with 600 μL of 3.6 M $\text{Ca}(\text{OH})_2$ and 300 μL of 0.5 M CuSO_4 containing crotonic acid as an internal marker in 1.7-mL microcentrifuge tubes. Samples were frozen, thawed, and centrifuged at $12,000 \times g$ for 10 min, and supernatant (1,000 μL) was mixed with 42 μL of 98% H_2SO_4 in 1.5-mL microcentrifuge tubes. Samples were frozen and thawed twice, and centrifuged at $12,000 \times g$ for 10 min to precipitate and remove protein. Supernatant was transferred to HPLC vials. The column used was Aminex HPX-87H (catalog number 125-0140; 300×7.8 mm; Bio-Rad Laboratories, Richmond, CA). Column temperature was 50°C , and the solvent was 0.015 N H_2SO_4 . Detection was by

refractive index (Waters 410; Millipore Corp., Milford, MA). Liver AcCoA was determined by HPLC following the King and Reiss (1985) procedure with modifications (Stocks and Allen, 2012). Liver AcCoA content was expressed per gram of wet liver.

Statistical Analysis

Data from Exp. 1 and 2 were analyzed by ANOVA using the MIXED procedure of SAS (version 9.4, 2013; SAS Institute Inc., Cary, NC) with repeated measures. Feeding behavior, intake, milk production and milk components were analyzed using the following linear model:

$$Y_{ijkl} = \mu + C_i + P_j + T_k + D_l(P_j) + P_jT_k + C_iT_k + e_{ijkl},$$

where Y_{ijkl} = the response variable; μ = overall mean; C_i = random effect of cow i ; P_j = fixed effect of period j ; T_k = fixed effect of treatment k ; $D_l(P_j)$ = fixed effect of day within period; P_jT_k = interaction of period and treatment; C_iT_k = interaction of the random effect of cow and treatment; and e_{ijkl} = residual. Day within period was included in the repeated statement to account for repeated measures and covariance structure was first-order autoregressive. Cow by treatment interaction was implicitly used as the error term for treatment.

Effects of treatment before and after infusion, change in concentration of plasma insulin and metabolites, and liver AcCoA content were evaluated using the same model without effect of day within period. Period was used in the repeated statement to account for repeated measures, and cow by treatment interaction was implicitly used as the error term for treatment:

$$Y_{ijk} = \mu + C_i + P_j + T_k + P_jT_k + C_iT_k + e_{ijk}.$$

Within-day changes in concentrations of insulin and metabolites in plasma, and liver AcCoA content were evaluated for the second day of each period using the following model:

$$Y_{ijkl} = \mu + C_i + P_j + T_k + H_l(P_j) + P_jT_k + T_kH_l(P_j) + C_iT_k + e_{ijkl},$$

where Y_{ijkl} = the response variable; μ = overall mean; C_i = random effect of cow i ; P_j = fixed effect of period j ; T_k = fixed effect of treatment k ; $H_l(P_j)$ = fixed effect of hour after feeding within period; $T_kH_l(P_j)$ = interaction of treatment and hour after feeding within period; C_iT_k = interaction of the random effect of cow and treatment; and e_{ijkl} = residual. Period and hour were

used in the repeated statement to account for repeated measures and covariance structure was unstructured first-order autoregressive. Cow by treatment interaction was implicitly used as the error term for treatment.

For Exp. 3, effects of pulse dose of treatment on concentrations of insulin and metabolites in plasma and liver AcCoA content were evaluated using the following linear model:

$$Y_{ijklm} = \mu + B_i + C_j(B_i) + P_k(B_i) + T_l + H_m + T_lH_m + C_j(B_i)T_l + e_{ijklm},$$

where Y_{ijklm} = the response variable; μ = overall mean; B_i = fixed effect of block i ; C_j = random effect of cow within block j ; P_k = fixed effect of period within block k ; T_l = fixed effect of treatment l ; H_m = fixed effect of time m ; T_lH_m = interaction of treatment and time; $C_j(B_i)T_l$ = interaction of the random effect of cow and treatment; and e_{ijklm} = residual. Time was used in the repeated statement. Covariance structure was first-order autoregressive. Treatment differences at each time point were analyzed using the SLICE option. Cow within block by treatment interaction was implicitly used as the error term for treatment.

Denominator degrees of freedom were estimated by using the Kenward-Roger option in the MODEL statement for Exp. 1 and 2 and the Between-Within option for Exp. 3. Residuals were determined to be normally distributed by the Shapiro-Wilk test. Treatment effects were declared at $P \leq 0.05$ and tendencies for treatment effects at $P \leq 0.10$. Interactions were declared significant at $P \leq 0.10$ and tendencies for interactions at $P \leq 0.15$. All data are expressed as least squares means and standard error of the means, unless otherwise specified.

RESULTS

Feed Intake and Feeding Behavior

Feed intake and feeding behavior are reported in Table 2 for Exp. 1 (isoenergetic infusions) and Exp. 2 (isomolar infusions). Propionic acid decreased DMI 16.7% for isoenergetic infusions (12.5 vs. 15.0 kg/d; $P = 0.04$) and 23.4% for isomolar infusions (11.1 vs. 14.5 kg/d; $P = 0.02$) compared with GL. The decrease in DMI was because PA decreased meal size by 12.6% (1.19 vs. 1.04 kg; $P = 0.03$) for isoenergetic infusions and by 15.3% (1.11 vs. 0.94 kg; $P = 0.01$) for isomolar infusions. Similarly, PA decreased DE intake 15.9% for isoenergetic infusions and 21.7% for isomolar infusions and MEI 15.9% for isoenergetic infusions and 21.4% for isomolar infusions compared with GL (Table 2). Treatments provided 4.27 Mcal of ME/d and did not differ

Table 2. Effect of intra-abomasal infusion (22 h/d) of propionic acid (PA) relative to glycerol (GL) on feeding behavior and energy intake for cows in the immediate postpartum period for experiment (Exp.) 1 (isoenergetic infusions¹) and Exp. 2 (isomolar infusions²)

Item	Infusion		SEM	<i>P</i> -value
	GL	PA		Inf ³
Exp. 1: Isoenergetic infusions (n = 8)				
DMI, kg/d	15.0	12.5	0.87	0.04
Feeding behavior (22 h/d)				
Meal frequency, meals/ d	12.5	12.1	0.60	0.63
Meal length, min/meal	23.6	22.0	1.22	0.30
Meal size, kg/meal	1.19	1.04	0.05	0.03
Eating interval, min	76.9	114.2	13.5	0.09
Hunger ratio	0.027	0.021	0.002	0.04
Satiety ratio	0.034	0.025	0.003	0.01
Water intake, L/d	55.3	47.5	3.49	0.12
Drinking frequency, bouts/d	10.7	9.2	1.34	0.06
DE intake, ⁴ Mcal/d	44.7	37.6	2.72	0.04
ME intake, Mcal/d				
Diet ⁵	38.4	32.3	2.36	0.04
Infusion ⁶	4.28	4.25	0.07	0.59
Total ⁷	42.7	36.6	2.33	0.04
Exp. 2: Isomolar infusions (n = 6)				
DMI, kg/d	14.5	11.1	1.23	0.02
Feeding behavior (22 h/d)				
Meal frequency, meals/d	13.3	11.3	0.82	0.13
Meal length, min/meal	23.0	22.2	1.30	0.69
Meal size, kg/meal	1.11	0.94	0.056	0.01
Eating interval, ⁸ min	77.9	102.0	8.08	0.06
Hunger ratio	0.024	0.019	0.002	0.08
Satiety ratio	0.029	0.025	0.003	0.16
Water intake, L/d	53.0	38.6	2.84	<0.001
Drinking frequency, bouts/d	11.2	8.95	0.79	0.02
DE intake, Mcal/d	43.7	34.2	1.92	0.05
ME intake, Mcal/d				
Diet	37.4	29.4	1.65	0.05
Infusion	4.57	4.22	0.05	<0.0001
Total	42.0	33.7	1.38	0.05

¹Isoenergetic infusion, 193 kcal/h.²Isomolar infusion, 0.5 mol/h.³Inf = effect of infusion.⁴Digestible energy (DE) intake (Mcal/d) = gross energy (GE) intake – GE feces.⁵ME intake from the diet was calculated according to NRC (2001). ME (Mcal/kg) = $1.01 \times (\text{DE} - 0.45)$, based on actual digestibility of diets. ME intake (Mcal/d) = ME $\times$ DMI.⁶ME intake from the infusion was based on energy density of 0.395 Mcal/mol ME (Bastos et al., 1988) for GL and 0.365 Mcal/mol ME (Labedeva, 1964) for PA.⁷Total ME intake = ME intake diet + ME infusion.⁸Data log-transformed for statistical analysis and back-transformed for interpretation purposes.

($P = 0.59$) when infused isoenergetically but because energy per mole of GL is greater than that of propionate, GL provided more ME/d than did PA (4.57 vs. 4.22 Mcal; $P < 0.0001$) for the isomolar infusions. When the ME from the treatment infusions was added to the MEI from the diet, PA decreased total MEI 14.3% for isoenergetic infusions (36.6 vs. 42.7 Mcal/d; $P = 0.04$) and 19.8% for isomolar infusion (33.7 vs. 42.0 Mcal/d; $P = 0.05$).

Treatments did not affect meal length or meal frequency in either experiment but tended to increase the time between meals (eating interval) 48% for isoenergetic infusions (114 vs. 76.9 min; $P = 0.09$) and 31%

for isomolar infusions (102 vs. 77.9 min; $P = 0.06$) compared with GL. Propionic acid infusion decreased hunger ratio ($P = 0.04$) and satiety ratio ($P = 0.01$) for isoenergetic infusions and tended to decrease hunger ratio ($P = 0.08$) for isomolar infusions compared with GL.

Propionic acid tended to decrease drinking frequency (9.2 vs. 10.7 bouts/d; $P = 0.06$), numerically reducing water intake (47.5 vs. 55.3 L/d; $P = 0.12$) compared with isoenergetic infusion of GL, and decreased drinking frequency (8.95 vs. 11.2 bouts/d; $P = 0.02$), reducing water intake (38.6 vs. 53.0 L/d; $P < 0.001$) compared with isomolar infusion of GL (Table 2).

Table 3. Effect of intra-abomasal infusion of propionic acid (PA) relative to glycerol (GL) change in concentrations of insulin and metabolites in plasma and liver acetyl CoA (AcCoA) content over the 2-d infusion for cows in the immediate postpartum period for experiment 1 (isoenergetic infusions¹) and experiment 2 (isomolar infusions²)

Item	Infusion		SEM	P-value
	GL	PA		Inf ³
Isoenergetic infusions (n = 8)				
AcCoA before, ⁴ nmol/g	18.5	20.7	1.59	0.19
AcCoA after, ⁴ nmol/g	16.7	17.1	1.52	0.73
Change in AcCoA, nmol/g	-1.76	-3.64	1.58	0.54
Glucose before, mg/dL	50.9	52.0	1.26	0.29
Glucose after, mg/dL	53.5	51.6	1.32	<0.01
Change in glucose, mg/dL	2.62	-0.32	1.54	0.36
Insulin before, µg/L	0.11	0.11	0.03	0.97
Insulin after, µg/L	0.16	0.13	0.03	0.44
Change in insulin, µg/L	0.045	0.017	0.039	0.72
NEFA ⁵ before, µEq/L	746	726	55.0	0.74
NEFA after, µEq/L	585	531	39.3	0.21
Change in NEFA, ⁴ µEq/L	-162	-195	56.4	0.73
BHB before, mg/dL	8.77	9.47	0.89	0.51
BHB after, mg/dL	6.24	6.37	0.69	0.89
Change in BHB, mg/dL	-2.54	-3.11	0.94	0.75
Isomolar infusions (n = 6)				
AcCoA before, nmol/g	17.2	22.7	2.66	0.14
AcCoA after, nmol/g	18.7	15.4	2.51	0.35
Change in AcCoA, nmol/g	1.50	-7.39	3.12	0.17
Glucose before, mg/dL	48.8	52.1	1.83	0.20
Glucose after, mg/dL	54.5	52.1	1.86	0.23
Change in glucose, mg/dL	4.60	-0.01	1.91	0.24
Insulin before, µg/L	0.134	0.124	0.041	0.83
Insulin after, µg/L	0.203	0.169	0.055	0.65
Change in insulin, µg/L	0.055	0.045	0.06	0.92
NEFA before, µEq/L	703	793	56	0.14
NEFA after, µEq/L	596	622	43	0.73
Change in NEFA, µEq/L	-104	-171	60.1	0.55
BHB before, mg/dL	10.9	9.77	1.90	0.70
BHB after, mg/dL	5.81	6.46	0.51	0.38
Change in BHB, mg/dL	-5.16	-3.31	2.07	0.62

¹Isoenergetic infusion, 193 kcal/h.

²Isomolar infusion, 0.5 mol/h.

³Inf = effect of infusion.

⁴Data log-transformed for statistical analysis and back-transformed for interpretation purposes.

⁵Nonesterified fatty acids.

Milk Production and Components

Milk yield was not affected by treatment (data not shown) and averaged 35.8 kg/d for Exp. 1 ($P = 0.22$) and 34.4 kg/d for Exp. 2 ($P = 0.13$). Propionic acid decreased milk protein content ($P = 0.04$) and yield ($P = 0.05$) and tended to reduce milk lactose content ($P = 0.08$) and nonfat solids content ($P = 0.06$) in Exp. 1 but had no effect on milk components in Exp. 2 (data not shown).

Plasma Metabolites and Insulin and Liver AcCoA Contents

Treatment did not affect changes in concentrations of plasma metabolites and insulin or hepatic AcCoA con-

tent over the 48-h infusion periods (Table 3). Plasma metabolite and insulin concentrations within the second day of infusion are reported in Table 4. Plasma glucose concentration was not affected by treatment for isoenergetic infusions but was increased by GL compared with PA (53.7 vs. 49.5; $P = 0.03$) for isomolar infusions. Propionic acid increased insulin concentration to a lesser extent than GL ($P = 0.05$ for isomolar infusions). We detected no effects of treatment on concentrations of NEFA or BHB in plasma for either experiment.

In Exp. 3, treatments interacted with time to affect hepatic AcCoA content (Table 5; $P < 0.01$). At 30 min after dosing, PA decreased AcCoA content by 34%, whereas GL increased AcCoA content by 32%, resulting in lower AcCoA content for PA compared with GL at 30 min (18.0 vs. 36.9 nmol/g; $P < 0.0001$),

Table 4. Within-day effect of intra-abomasal infusion (22 h) of propionic acid (PA) relative to glycerol (GL) on plasma metabolites and insulin for cows in the immediate postpartum period for experiment (Exp.) 1 (isoenergetic infusions¹) and Exp. 2 (isomolar infusions²)

Item	Infusion			<i>P</i> -value	
	GL	PA	SEM	Inf ³	Inf × Time
Exp. 1: Isoenergetic infusions (n = 8)					
Glucose, mg/dL	50.9	48.9	2.33	0.33	0.33
Insulin, ⁴ μg/L	0.211	0.146	0.066	0.45	0.50
NEFA, ^{4,5} μEq/L	418	431	51.9	0.88	0.95
BHB, mg/dL	5.78	6.16	0.91	0.77	0.91
Exp. 2: Isomolar infusions (n = 6)					
Glucose, ⁴ mg/dL	53.7	49.5	1.45	0.03	0.71
Insulin, ⁴ μg/L	0.293	0.176	0.056	0.05	0.78
NEFA, μEq/L	419	546	56.3	0.60	0.56
BHB, mg/dL	7.30	7.13	0.479	0.75	0.57

¹Isoenergetic infusion, 193 kcal/h.²Isomolar infusion, 0.5 mol/h.³Inf = effect of infusion.⁴Data log-transformed for statistical analysis and back-transformed for interpretation purposes.⁵Nonesterified fatty acids.

which persisted at 60 min after dosing (21.9 vs. 32.8 nmol/g; $P < 0.01$). Plasma glucose concentration was not affected by treatment and increased over time ($P < 0.001$), whereas plasma insulin concentration increased similarly for both treatments from 0 to 30 min and continued to increase from 30 to 60 min for GL but decreased for PA ($P = 0.02$ interaction). Treatments tended to interact over time to affect plasma NEFA concentration ($P = 0.15$). Although plasma NEFA concentration tended ($P = 0.06$) to be lower for PA compared with GL at 30 min, it was numerically higher by 60 min when hepatic AcCoA content remained lower. Plasma BHB concentration decreased over time after infusion ($P < 0.001$) but was not affected by treatment. There was an interaction of the treatment infusions over time for glycerol ($P = 0.001$) and propionate ($P = 0.08$) concentrations in plasma. After 30 min of PA dose, propionate concentration increased ($P < 0.01$) whereas GL decreased after 60 min, with no differences compared with GL at 60 min after dose. Glycerol concentration in plasma increased ($P = 0.05$) after 30 min for GL and remained higher ($P < 0.0001$) after 60 min compared with PA dose.

DISCUSSION

According to the HOT, feeding behavior is controlled by a signal from hepatic vagal afferents that is affected by hepatic oxidation of fuels and the production of ATP (Allen et al., 2009). More rapid anaplerosis within a meal is expected to increase ATP synthesis, stimulate satiety sooner, and decrease meal size and possibly DMI. Consistent with our hypothesis, PA decreased DMI by decreasing meal size compared with

GL. Although both PA and GL are 3-carbon glucose precursors, their hepatic metabolism differs. Propionate is rapidly extracted from the blood (Reynolds et al., 2003), converted to propionyl CoA in the mitochondria, and is anaplerotic, entering the TCA cycle as succinyl CoA via methylmalonyl CoA. However, GL enters the gluconeogenic pathway in the cytosol with little or no anaplerosis because gluconeogenesis is upregulated and glycolysis is downregulated in the liver of mature ruminants (Aschenbach et al., 2010).

Oxidation of AcCoA in the TCA cycle and subsequent hepatic ATP production is dependent on availability of TCA cycle intermediates. Over time, the pool of TCA cycle intermediates is a function of the balance between anaplerosis and cataplerosis. Glucose demand is high during the PP period, and TCA cycle intermediates are shuttled to the cytosol for gluconeogenesis. The pool of TCA cycle intermediates and oxidation of AcCoA likely increase during meals as supply temporarily exceeds demand. The activity of the TCA cycle is affected by the pool size of TCA cycle intermediates and the speed of reactions, which are regulated in part by the redox state. Although reducing equivalents are abundant for cows in a lipolytic state, their utilization for the production of ATP by oxidative phosphorylation via the electron transport chain is stimulated by activity of the TCA cycle. Bicarbonate ions, produced by the carbonic anhydrase reaction from carbon dioxide, stimulates oxidative phosphorylation (Acin-Perez et al., 2009) and carbon dioxide is produced primarily by oxidation of AcCoA in the TCA cycle. Therefore, anaplerosis of the TCA cycle stimulates ATP production from the existing pool of reducing equivalents, generating a satiety signal according to the HOT.

Table 5. Effect of intra-abomasal pulse dose of propionic acid (PA) relative to glycerol (GL) on hepatic acetyl CoA (AcCoA) content and plasma metabolites for cows in the immediate postpartum period (experiment 3)

Item	Infusion ¹						P-value			
	GL			PA			SEM	Inf ²	Time	Inf × Time
	0	30	60	0	30	60				
Liver AcCoA, nmol/g	27.9	36.9	32.8	27.3	18.0	21.9	3.34	<0.001	0.99	<0.01
NEFA, ³ μEq/L	872	905	951	638	522	1,001	208	0.23	0.07	0.15
BHB, mg/dL	25.5	25.0	21.0	22.3	18.0	12.4	9.35	0.40	<0.001	0.16
Glucose, mg/dL	43.1	49.4	58.7	42.7	52.9	54.8	3.42	0.95	<0.001	0.19
Insulin, μg/L	0.125	0.235	0.376	0.127	0.281	0.149	0.06	0.09	0.01	0.02
Glycerol, mM	0.086	0.820	2.23	0.054	0.054	0.083	0.27	<0.01	<0.001	<0.01
Propionate, mM	0.198	0.262	0.214	0.231	0.555	0.227	0.07	0.16	0.12	0.08

¹Time in minutes (0, 30, or 60 min) after 2 mol of PA or GL pulse dosed to the abomasum.

²Inf = effect of infusion.

³Nonesterified fatty acids.

When treatments were administered as isoenergetic infusions in Exp. 1, PA decreased DMI ~17% compared with GL, resulting in ~14% lower total MEI, including the ME infused. However, because GL is more reduced and contains more gross energy per mole than PA (395.4 vs. 365.0 kcal/mol), we considered that treatment effects might have been because more moles of PA than GL were infused (0.528 vs. 0.486 mol/h). However, PA also decreased DMI ~23% compared with GL when isomolar solutions (0.5 mol/h) were infused in Exp. 2, decreasing total MEI ~20%. Suppression of DMI by PA compared with GL is likely because of differences in their hepatic metabolism more than the energy supply per se because ~8% less energy was infused for PA than GL in Exp. 2.

Feed intake is a function of the size and frequency of meals and the decrease in DMI by PA compared with GL was primarily from a reduction in meal size in both Exp. 1 and 2. Anaplerosis gradually increases at the initiation of meals as PA is produced, primarily through the fermentation of starch. As more feed is consumed within a meal, the production, absorption, and extraction of PA from the blood by the liver increases, increasing anaplerosis and TCA cycle activity, stimulating ATP synthesis, and eventually causing satiety according to the HOT (Allen et al., 2009). Treatment effects on meal size are consistent with our hypothesis that a greater baseline of anaplerosis throughout the day by continuous infusion of PA compared with GL would stimulate satiety sooner and decrease meal size. The decreased satiety ratio for PA (numerical only in Exp. 2) indicates that the PA treatment extended satiety following meals compared with GL. These results are consistent with previous research with cows in a lipolytic state in the PP period in which continuous PA infusion compared with isomolar infusion of acetic acid, decreased DMI by decreasing meal size (Oba and Allen, 2003b; Stocks and Allen, 2012, 2013). The results of those experiments were consistent with the HOT because propionate is extracted from blood by the liver and can stimulate oxidation of AcCoA, whereas acetate is not anaplerotic and cannot stimulate oxidation of AcCoA. In addition, the ruminant liver has limited ability to extract acetate from the blood and add to the pool of AcCoA because AcCoA synthetase activity is low, as discussed by Allen et al. (2009).

Although propionate was likely the major anaplerotic metabolite within meals, other metabolites (e.g., lactate, gluconeogenic amino acids) become greater contributors to anaplerosis between meals as PA production from the previous meal subsides. The tendency of PA to increase the intermeal interval compared with GL for both experiments was likely from the continuous supply of PA, increasing anaplerosis and oxidation of

AcCoA between meals. Although PA infusion tended to extend the interval between meals compared with GL, meal frequency, although numerically lower for PA than GL, was not statistically significant. The decreased hunger ratio for PA (tendency only for Exp. 2) indicates that PA treatment decreased hunger compared with GL.

The lack of treatment effects on changes in concentrations of glucose, insulin, NEFA, and BHB in plasma over the 48-h infusion period (Table 3) was likely due to the rapidly changing metabolic states among cows in the PP period. However, numerical differences between treatments within days in both experiments (Table 4) are consistent with expectations, in that GL increased plasma glucose concentration more than PA because PA decreased DMI, and both treatments increased insulin concentration and decreased concentrations of NEFA and BHB in plasma. Greater concentrations of plasma glucose and insulin over time for GL compared with PA over the second day for isomolar infusions were likely because PA decreased DMI compared with GL.

Although the change in liver AcCoA content after 48 h of infusion was numerically lower for PA than GL, the difference was not statistically significant. However, liver biopsies were taken before feeding, when plasma concentration of NEFA is greatest within days (Allen et al., 2005) and changes within days are likely more relevant. We recently showed that hepatic AcCoA content decreased over the 4 h after feeding as plasma NEFA concentration decreased, and that the decrease in hepatic AcCoA was positively related to DMI among cows over that period (Piantoni and Allen, 2015). We were unable to determine the temporal pattern of AcCoA within a day for these experiments because the biopsy process would alter feeding behavior.

The results of our third experiment, in which PA or GL were pulse-dosed to the abomasum, were consistent with our expectation that PA would decrease liver AcCoA content compared with GL by stimulating AcCoA oxidation in the TCA cycle. As expected, both treatments increased plasma glucose concentration similarly over the short-term when they had no access to feed and PA could not reduce DMI. However, treatments interacted with time to affect plasma insulin and NEFA concentrations (tendency). While liver AcCoA content might have decreased because of lower plasma NEFA concentration ($P = 0.06$) for PA compared with GL at 30 min after dosing, plasma NEFA concentration was numerically greater and BHB concentration was numerically lower ($P = 0.16$) for PA compared with GL at 60 min after dosing, whereas liver AcCoA remained lower. The continued increase in plasma glycerol concentration over time indicates that its supply exceeded hepatic uptake. The increase in plasma propionate

concentration at 30 min and the subsequent decrease at 60 min after PA dose is likely explained by its rapid uptake by the liver and entrance to the TCA cycle during the timeframe of a meal. Therefore, it is likely that PA decreased liver AcCoA content by stimulating its oxidation in the TCA cycle.

CONCLUSIONS

Together, the results of these experiments show clearly that fuels can have very different effects on energy intake depending upon their metabolism that is independent of their energy content. These results are strongly supportive of the HOT for the control of feed intake because PA likely decreased energy intake compared with GL because it is anaplerotic and can stimulate oxidation of AcCoA in the liver, whereas GL is much less so. The pool of AcCoA in the liver is continually replenished by β -oxidation of NEFA and entry of other fuels through pyruvate, but AcCoA is particularly abundant for cows in the PP period that are in a lipolytic state. Diets vary greatly in the type and temporal pattern of fuels absorbed. Although cows in the PP period have high glucose demand to support milk production and a high requirement for glucose precursors, careful consideration must be given to select feeds and formulate rations to maximize energy intake. More research is needed to determine the effects of type and temporal absorption of fuels on feeding behavior and energy intake of cows, particularly during the PP period.

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