Effects of acetic acid or sodium acetate infused into the rumen or abomasum on feeding behavior and metabolic response of cows in the postpartum period

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ABSTRACT

Effects of continuous isomolar infusions of acetic acid (AcA) or sodium acetate (NAc) infused into the rumen (RU) or into the abomasum (AB) on feeding behavior, dry matter intake (DMI), and metabolic response of cows in the early postpartum period were evaluated. Six rumen-cannulated multiparous Holstein cows (11.8 ± 3.9 d in milk; mean $\pm$ SD) were utilized in a 6×6 Latin square design experiment balanced for carryover effects with a 2×3 factorial arrangement of treatments. Treatments were AcA and NAc, with sodium chloride (CON) as a control, infused at a rate of ~ 0.75 mol/h (0.5 L/h) into the RU or AB for the first 8 h following feeding, with a rest day between infusion days. Treatment sequences were assigned randomly to cows. Feeding behavior was recorded by a computerized data acquisition system and blood was sampled at 0, 4, and 8 h relative to the start of infusion. We hypothesized that AcA is more hypophagic than NAc, and that infusion into the AB is more hypophagic than infusion into the RU. Dry matter intakes (DMI) for the CON treatments were similar at 6.2 kg/8 h for RU and 6.1 kg/8 h for AB, and the AcA and NAc treatments interacted with site of infusion to affect DMI. The NAc-RU treatment did not reduce DMI (7.0 kg/8 h), whereas AcA-RU (2.6 kg/8 h), AcA-AB (3.7 kg/8 h), and NAc-AB (4.0 kg/8 h) decreased DMI compared with CON. Following infusions of AcA compared with NAc, there was a residual effect on DMI for the remainder of the day, but treatments did not affect DMI during the rest day. Treatments increased plasma acetate and β -hydroxybutyrate concentrations over time (interaction) and decreased plasma insulin concentration compared with CON. Plasma glucose concentration decreased over time after AcA-AB infusion compared with other treatments and

CON. Plasma nonesterified fatty acid concentration increased over time for AcA compared with NAc and CON, suggesting an increase in lipolysis to compensate the decrease in DMI. In contrast to the other treatments, NAc-RU did not decrease DMI compared with control but we cannot determine the reason for this from the data available from the current study.

Key words: acetic acid, sodium acetate, infusion, metabolic response

INTRODUCTION

Acetic acid is the primary product of ruminal fermentation, typically comprising over 50% of the fermentation acids produced in the rumen on molar basis. It is used for fatty acid synthesis in adipose and mammary tissue and as an energy source by various tissues. Intraruminal infusions of acetic acid (**AcA**) or sodium acetate (**NAc**) have been used to test their effects on yields of milk and milk components and feed intake of lactating cows (Rook and Balch, 1961; Reynolds et al., 1979; Sheperd and Combs, 1998; Urrutia and Harvatine, 2017). Although some studies have infused AcA intraruminally (**RU**) without detrimental effects on DMI (Rook and Balch, 1961; Choi and Allen, 1999), others have reported a reduction in DMI (Montgomery et al., 1963; Wilson et al., 1967) or adverse physiological effects on the animals (e.g., cessation of rumination, decrease in ruminal pH, and symptoms of metabolic acidosis; Montgomery et al., 1963; Sheperd and Combs, 1998).

Our laboratory has used AcA alone or partially neutralized with sodium hydroxide intraruminally as control treatments to study the effects of propionate on feeding behavior of lactating cows. Infusions up to 28 mol of NAc (1.56 mol/h) or ~ 20 mol of AcA (0.75 mol/h) had no detrimental effect on the animals or reduction on feed intake (Choi and Allen, 1999; Oba and Allen, 2003a,b,c; Stocks and Allen, 2012, 2013). We considered that propionate is hypophagic compared with acetate because propionate is anaplerotic and

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stimulates hepatic oxidation of acetyl CoA in the tricarboxylic acid cycle, whereas acetate does not (Allen et al., 2009). Feeding behavior is affected by osmotic changes in the rumen so we rationalized that isosmotic infusions of acetate were appropriate controls for the propionate treatment. All previous experiments, to our knowledge, have infused AcA alone or partially neutralized with sodium hydroxide into the rumen. However, we recently observed adverse effects of abomasal (AB) infusion of 20.3 mol/d of AcA (4.2 Mcal/d) at a rate of 0.5 L/h (0.92 mol/h) to cows in the postpartum (PP) period (Gualdrón-Duarte, 2017) in which AcA decreased DMI 64% compared with control (no infusion). This was not expected because intraruminal infusions of AcA at this rate did not decrease DMI in previous experiments.

The objective of this experiment was to evaluate the effects of continuous isomolar infusions of AcA or NAc infused into the rumen or the abomasum on feeding behavior, DMI, and metabolic responses of cows in the PP period. We hypothesized that AcA would be more hypophagic than NAc, and that infusion into the AB would be more hypophagic than infusions into the RU.

MATERIALS AND METHODS

Animals and Care

All experimental procedures were approved by the Institutional Animal Care and Use Committee at Michigan State University, East Lansing. Cows were ruminally cannulated at least 45 d before parturition and housed in individual tiestalls for the 12-d duration of the experiment. All cows were fitted with a single jugular catheter 2 d before the beginning of the experiment, according to Bradford et al. (2006). Catheter patency was checked daily until the catheter was removed at the end of the experiment. Cows were withheld from feed at 0800 h and were not allowed access to feed until all blood samples were collected. Cows were fed once daily (0900 h) at 125% of expected ad libitum intake and milked twice daily (0530 and 1730 h). The amounts of feed offered andorts were weighed for each cow daily.

Experimental Design and Treatment Diets

Six ruminally cannulated multiparous, lactating Holstein cows (11.8 ± 3.9 DIM; mean $\pm$ SD) at the Michigan State University Dairy Teaching and Research Center were used in a 6×6 Latin square design experiment with a 2×3 factorial arrangement of treatments. Cows were assigned randomly to stalls and treatment

sequence balanced for carryover effects. Infusion periods were 8 h long with continuous isomolar infusions (0.75 mol/h) of AcA (pH 2.3, >99.7%, Sigma Aldrich Chemical Co., St. Louis, MO), NAc (pH 5.9, AcA + NaOH), or NaCl (>99.5%, Sigma Aldrich Chemical Co.) as a control (CON), infused into the AB or RU at a rate of 0.5 L/h. Concentration of solutions and duration of infusions were 0.75 mol/h and 8 h, respectively, to limit potential adverse effects. Solutions were infused into the RU or AB using Baxter Flo-Gard 6201 infusion pumps (Baxter Medical Products, Deerfield, IL) through 2 vinyl tubes (0.95 cm outside diameter, 0.71 cm inside diameter). The first tube connected to a Nalgene bottle (3.8 cm diameter, 8.5 cm long), placed into the AB, and held by a semi-hard flexible rubber flange as described in Gualdrón-Duarte and Allen (2017), and the second tube was placed into the RU. A recovery period of 40 h was included between infusions.

All cows received a common experimental diet from parturition through the end of the experiment. The experimental diet was composed (DM basis) of corn silage (30%), ground corn (20%), soybean meal (16%), alfalfa silage (15%), alfalfa hay (14%), soy hulls (1.4%), and a vitamin and mineral mix (2.0%, containing 25.6% NaCl, 10.0% Ca, 2.0 Mg, 2.0% P, 30 mg/kg Co, 506 mg/kg Cu, 20 mg/kg I, 2,220 mg/kg Fe, 2,080 mg/kg Mn, 15 mg/kg Se, 2,030 mg/kg Zn, 300 kIU/kg vitamin A, 50 kIU/kg vitamin D, 1,500 kIU/kg vitamin E), sodium bicarbonate (1.1%), and limestone (1.1%), formulated to meet requirements for absorbed protein, minerals, and vitamins (NRC, 2001). The diet contained 52.4% DM and 28.7% NDF, with 24.3% NDF from forage, 22% starch, 17.4% CP, and 8.9% ash (DM basis).

Sample and Data Collection

Feed offered and refused was measured daily. Cows were blocked from feed from 0800 to 0900 h daily. Samples (0.5 kg) of all dietary ingredients and the TMR were collected daily throughout the experiment, andorts for each cow were taken at the end of each infusion period and stored in plastic bags at -20°C until processed. Feeding behavior data (feed disappearance and water intake) were recorded continuously for 22 h daily for each cow during infusion days via computer every 5 s, and size, length, and frequency of meals, intermeal interval, total eating time, and water intake were calculated (Dado and Allen, 1995).

Milk yield was recorded and milk samples were collected daily at each milking and stored with preservative (Bronopol tablet, D&F Control Systems, San Ramon, CA) at 4°C for component analysis (Universal

Lab Services, East Lansing, MI). Milk samples were analyzed for fat, true protein, lactose, and MUN by Michigan DHIA (AOAC International, 1997).

Blood samples were collected immediately before and at 4 and 8 h following the start of infusions. Blood was sampled from a jugular catheter and collected into 3 evacuated tubes, 2 containing K₂EDTA 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. Before and 4 h after the start of infusions, blood samples were taken in a heparinized syringe, transported to the laboratory and analyzed with a blood gas analyzer.

Analysis of Samples

Diet ingredients and Orts 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 CP, starch, NDF, and ash. Crude protein was determined according to Hach et al. (1987). Starch was analyzed using an enzymatic method (Karkalas, 1985) after samples were gelatinized with NaOH; glucose concentration was measured by a glucose oxidase method (Sigma Chemical Co., St. Louis, MO). Content of NDF was analyzed according to Mertens (2002) with the inclusion of amylase and Na sulfite. Ash content was determined after 6 h of oxidation at 500°C in a muffle furnace. 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.

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 nonesterified fatty acids (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 acetate, propionate, and lactate concentrations were analyzed by HPLC (Waters Corp., Milford, MA). Plasma (600 µL) was mixed with 600 µL of 3.6 M Ca(OH)₂ and 300 µL of 0.5 M CuSO₄ 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% H₂SO₄ 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 H₂SO₄. Detection was by refractive index (Waters 410; Millipore Corp., Milford, MA). Blood chemistry and gas analysis was performed with a Stat Profile pHOX Ultra Analyzer (Nova Biomedical Corp., Waltham, MA) by Michigan State University Diagnostic Center for Population and Animal Health (East Lansing) for direct measurement of pH (hydrogen ion selective glass membrane); partial pressure of CO₂ (modified hydrogen ion selective glass membrane); partial pressure of O₂ (gas-permeable membrane); saturation percentage of O₂ (reflectance photometry system); Na⁺, K⁺, Cl⁻, Ca²⁺, Mg²⁺ (ion selective membrane); glucose, lactate, and creatinine (platinum electrode); and BUN (ammonium ion selective membrane). Values for HCO₃⁻, total CO₂, base excess blood, anion gap, osmolality, and standard bicarbonate were determined indirectly by calculation.

Statistical Analysis

Feeding behavior, intake, yields of milk and milk components, concentration of milk components, and plasma composition were analyzed using the Fit Model procedure of JMP (version 12.0, 2015; SAS Institute Inc., Cary, NC) using the following model:

$$Y_{ijk} = \mu + T_i + P_j + C_k + e_{ijk},$$

where Y_{ijk} = the dependent response variable of interest, μ = overall mean, T_i = fixed effect of treatment ($i = 1$ to 6), P_j = fixed effect of period ($j = 1$ to 6), C_k = random effect of cow ($k = 6$), and e_{ijk} = residual, normally distributed.

Plasma concentrations of glucose, NEFA, BHB, acetate, propionate, lactate, and insulin were analyzed using the MIXED procedure of SAS (version 9.4, 2013; SAS Institute Inc.) with repeated measures, using the following model:

$$Y_{ijkl} = \mu + T_i + P_j + H_k + T_iH_k + C_l + e_{ijkl},$$

where Y_{ijkl} = the response variable; μ = overall mean; T_i = fixed effect of treatment ($i = 1$ to 6); P_j = fixed effect of period ($j = 1$ to 6); H_k = fixed effect of time ($k = 1$ to 3); T_iH_k = interaction of treatment and time;

C_l = random effect of cow ($l = 6$); and e_{ijkl} = residual. Time was included in the repeated statement. Covariance structure was first-order autoregressive. Treatment differences at each time point were analyzed using the SLICE option. Cow by treatment interaction was implicitly used as the error term for treatment. Denominator degrees of freedom were estimated by using the Between-Within option. For all statistical models used, residuals were normally distributed as determined by the Shapiro-Wilk test.

Blood chemistry results at 4 h following the start of infusion were analyzed using the Fit Model procedure of JMP (version 12.0, 2015; SAS Institute Inc.) with the following model:

$$Y_{ijk} = \mu + T_i + P_j + C_k + Cov + e_{ijk},$$

where Y_{ijk} = the dependent response variable of interest, μ = overall mean, T_i = fixed effect of treatment ($i = 1$ to 6), P_j = fixed effect of period ($j = 1$ to 6), C_k = random effect of cow ($k = 6$), Cov = effect of covariate, and e_{ijk} = residual, normally distributed. Data for samples preceding each infusion were included as covariates to account for differences in baseline measurements.

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 errors of the means, unless otherwise specified. Means were compared with preplanned contrasts.

RESULTS

Feed Intake and Feeding Behavior

Effects of treatments on feed intake and feeding behavior parameters are shown in Table 1. Dry matter intake for the CON treatments were similar at 6.2 kg/8 h for RU and 6.1 kg/8 h for AB, and the AcA and NAc treatments interacted with site of infusion to affect DMI. The NAc-RU treatment did not reduce DMI (7.0 kg/8 h), whereas AcA-RU (2.6 kg/8 h), AcA-AB (3.7 kg/8 h), and NAc-AB (4.0 kg/8 h) decreased DMI compared with CON. Acetic acid decreased DMI by 32% compared with NAc infusions during the 15 h following infusions, and the effects of treatment on DMI for the day of infusion were similar to those observed during the infusion period. Treatments did not affect DMI ($P = 0.22$) during the rest day. Treatments decreased water intake 33% (31.4 L/8 h vs. 47.1 L/8 h; $P < 0.01$) compared with CON, and AcA decreased water intake during the infusion period by 56% (19.1 L/8 h vs. 43.7 L/8 h; $P < 0.01$) compared with NAc.

Table 1. Effects of isomolar¹ intraruminal (RU) or intra-abomasal (AB) infusion (8 h/d) of sodium chloride (CON), acetic acid (AcA), and sodium acetate (NAc) on feeding behavior and water intake for cows in the immediate postpartum period

Item	Infusion						Significance, <i>P</i> -value					
	CON-RU	CON-AB	AcA-RU	AcA-AB	NAc-RU	NAc-AB	SEM	Trt ²	CON vs. AcA+NAc	NAc vs. AcA	AB vs. RU	Int ³
During infusion, /8 h												
DMI, kg	6.23	6.05	2.57	3.72	7.02	4.01	0.990	<0.001	<0.01	<0.01	0.19	0.01
Meal frequency, meals	4.67	4.67	2.83	2.46	5.12	4.08	0.772	0.15	0.14	0.03	0.40	0.69
Meal size, kg	1.37	1.34	1.45	2.04	1.39	1.22	0.315	0.41	0.46	0.13	0.48	0.19
Water intake, L	49.6	44.6	17.7	20.4	46.2	41.2	7.88	<0.01	<0.01	<0.01	0.86	0.56
Drinking frequency, bouts	7.17	5.5	2.93	3.5	7.35	5.6	1.47	<0.01	0.05	<0.01	0.50	0.19
Following infusion, /15 h												
DMI, kg	6.70	7.58	4.08	5.92	7.30	7.43	1.08	0.06	0.22	0.02	0.30	0.37
Water intake, L	31.5	30.4	26.9	27.0	32.4	28.6	6.04	0.80	0.43	0.32	0.60	0.59
Total for infusion day, /23 h												
DMI, kg/d	13.0	13.6	6.67	9.62	14.2	11.4	1.76	<0.001	0.01	<0.001	0.97	0.02
Rest day, /23 h												
DMI, kg	13.6	14.4	10.7	13.1	14.2	11.5	2.67	0.33	0.21	0.54	0.90	0.11
Water, L	64.5	78.4	66.8	69.5	62.9	72.7	11.8	0.92	0.62	0.96	0.46	0.67

¹Isomolar infusion, 0.75 mol/h.

²Trt = treatment.

³Int = interaction of acetate type (AcA vs. NAc) and site of infusion (AB vs. RU).

However, treatments did not affect water intake during the 15 h following infusions.

Milk Production and Components

At the end of the infusion period (p.m. milking), there was no effect of treatment on milk yield. Treatments interacted ($P = 0.04$) to affect fat yield and tended to interact ($P = 0.15$) to affect fat concentration with NAc increasing and AcA decreasing fat yield when infused in the RU compared with the AB (Table 2). Protein yield tended to be increased by AcA compared with NAc (0.47 vs. 0.43 kg; $P = 0.10$) but protein concentration was not affected by treatment. Lactose concentration was increased by NAc compared with AcA (4.92 vs. 4.75%; $P < 0.01$) but lactose yield was not affected by treatment. Concentration of MUN tended to be increased by AcA and NAc treatments compared with control (12.2 vs. 10.0 mg/dL; $P = 0.06$).

Effects of treatment on milk yield and composition for the a.m. milking (12 h after infusion) are reported in Table 2. The AcA and NAc treatments decreased milk yield by 15% compared with CON (13.2 kg vs. 15.5 kg; $P < 0.01$), and infusion into the AB decreased milk yield by 15% compared with infusion into the RU (12.2 kg vs. 14.3 kg; $P = 0.03$). Fat yield was affected by treatment ($P = 0.02$) with AcA-RU tending to increase fat yield compared with AcA-AB, NAc-AB, and NAc-RU (interaction, $P = 0.13$). Treatments increased fat concentration 16% compared with CON (4.9 vs. 4.1%; $P < 0.001$), and AcA increased fat concentration compared with NAc (5.6 vs. 4.2%; $P < 0.001$). Treatments decreased protein yield 14% compared with CON (0.36 vs. 0.42 kg; $P = 0.01$) and infusion into the AB decreased protein yield 14% compared with RU (0.33 vs. 0.39; $P = 0.03$). No effect of treatment on protein concentration was observed. Treatments decreased lactose yield 16% compared with CON (0.62 vs. 0.74 kg; $P < 0.01$), with a decrease of 13% for AcA compared with NAc (0.58 vs. 0.67 kg; $P = 0.05$) and a decrease of 15% for infusion into the AB compared with RU (0.57 vs. 0.67; $P = 0.03$). The AcA treatment decreased lactose concentration 3.6% compared with NAc (4.61 vs. 4.78%; $P = 0.01$). Treatments increased MUN concentration 14% compared with CON (13.9 vs. 11.9 mg/dL; $P = 0.01$), but their effects tended to differ with AcA increasing and NAc decreasing MUN concentration when infused into the RU compared with the AB (interaction $P = 0.15$).

Blood Metabolites and Insulin Concentration

Compared with CON, treatments decreased plasma lactate concentration by 8% ($P = 0.01$), decreased plas-

ma insulin concentration by 38% ($P = 0.01$), increased plasma acetate concentration by 122% ($P < 0.001$), and tended to increase propionate concentration by 21% ($P = 0.07$). Whereas no differences were detected between infusion treatments (AcA vs. NAc), site of infusion (RU vs. AB), or their interaction for any plasma metabolite or hormone, treatments interacted with time to affect plasma concentrations of glucose ($P = 0.01$), NEFA ($P = 0.02$), BHB ($P < 0.01$), acetate ($P < 0.0001$), and propionate ($P = 0.03$; Table 3). Over time, CON-AB increased, and AcA-AB decreased plasma glucose concentration; AcA (RU and AB) and increased plasma NEFA concentration; AcA-AB increased plasma BHB concentration; NAc (RU and AB) increase plasma acetate concentration; and AcA-AB increased plasma propionate concentration compared with other treatments (Figure 1).

Blood Chemistry

Effects of treatment on blood chemistry are reported in Table 4. Acetic acid decreased pH (7.42 vs. 7.44; $P = 0.04$), HCO_3^- (22.0 vs. 25.3 mmol/L; $P < 0.01$), total CO_2 (23.0 vs. 26.4 mm/L; $P < 0.01$), base excess (−1.10 vs. 1.89 mmol/L; $P < 0.01$), and osmolality (273 vs. 278 mOsm/L; $P < 0.01$) compared with NAc but interacted with site of infusion with decreases for AcA and increases for NAc when infused into the AB compared with the RU (interaction $P \leq 0.10$). Acetic acid increased anion gap (16.3 vs. 15.1 mmol/L; $P = 0.04$) and ionized magnesium (1.33 vs. 1.20 mg/dL; $P < 0.01$) and decreased sodium (139 vs. 142 mmol/L; $P < 0.01$) and tended to decrease creatinine (0.946 vs. 0.999, $P = 0.08$) and ratio of calcium to magnesium (2.32 vs. 2.43; $P = 0.08$) compared with NAc. Acetic acid decreased BUN (12.3 vs. 13.0 mg/dL) and the ratio of BUN to creatinine (12.7 vs. 13.1) when infused into the RU, but increased BUN (13.1 vs. 12.5 mg/dL) and BUN:creatinine (14.0 vs. 13.2) when infused into the AB (interaction $P = 0.02$). Acetic acid and NAc treatments decreased chloride (1.05 vs. 1.08 mmol/L; $P < 0.001$) and tended to decrease normalized calcium (4.91 vs. 5.10 mg/dL; $P = 0.06$) compared with CON. Plasma potassium concentration was not affected by treatment ($P > 0.15$).

DISCUSSION

This study was conducted to investigate the mechanism by which abomasal infusion of AcA decreased DMI by cows in the immediate PP period in a previous experiment (Gualdrón-Duarte, 2017). Our objective was to evaluate the effects of continuous isomolar

Table 2. Effects of isomolar¹ intraruminal (RU) or intra-abomasal (AB) infusion (8 h/d) of sodium chloride (CON), acetic acid (AcA), and sodium acetate (NAc) on daily and per milking milk yield and milk components for cows in the immediate postpartum period

Item	Infusion						Significance, <i>P</i> -value					
	CON-RU	CON-AB	AcA-RU	AcA-AB	NAc-RU	NAc-AB	SEM	Trt ²	CON vs. AcA+NAc	NAc vs. AcA	AB vs. RU	Int ³
Yield, kg/d												
Milk	31.8	33.6	31.0	28.6	31.3	28.1	3.45	0.09	0.03	0.98	0.07	0.78
Fat	1.54	1.48	1.41	1.49	1.46	1.29	0.173	0.42	0.31	0.22	0.39	0.35
Protein	0.860	0.889	0.862	0.781	0.846	0.744	0.094	0.24	0.15	0.60	0.08	0.82
Lactose	1.48	1.64	1.45	1.33	1.54	1.35	0.173	0.13	0.06	0.49	0.08	0.70
Composition												
Fat, %	4.79	4.37	4.88	5.16	4.68	4.63	0.324	0.17	0.14	0.09	0.58	0.42
Protein, %	2.82	2.62	2.78	2.69	2.7	2.71	0.075	0.80	0.97	0.69	0.50	0.39
Lactose, %	4.75	4.81	4.68	4.68	4.87	4.84	0.065	0.01	0.68	<0.01	0.68	0.75
MUN, mg/dL	12.1	11.9	14.5	14.4	12	13.3	0.772	0.01	0.01	0.01	0.34	0.26
p.m. milking ⁴												
Yield, kg												
Milk	16.5	18.0	17.0	17.5	16.9	14.9	1.74	0.30	0.37	0.14	0.42	0.19
Fat	0.895	0.857	0.688	0.855	0.884	0.736	0.106	0.16	0.17	0.60	0.89	0.04
Protein	0.458	0.478	0.481	0.464	0.449	0.407	0.044	0.33	0.41	0.10	0.26	0.62
Lactose	0.786	0.870	0.808	0.823	0.828	0.735	0.084	0.53	0.42	0.44	0.38	0.23
Composition												
Fat, %	5.42	4.75	4.16	4.78	5.24	4.87	0.417	0.15	0.24	0.09	0.71	0.15
Protein, %	2.84	2.65	2.83	2.65	2.69	2.78	0.084	0.42	0.85	0.95	0.56	0.08
Lactose, %	4.78	4.85	4.76	4.73	4.92	4.92	0.060	<0.001	0.44	<0.01	0.67	0.77
MUN, mg/dL	12.6	11.7	13.6	13.9	12.3	13.0	0.884	0.14	0.06	0.11	0.43	0.79
a.m. milking ⁵												
Yield, kg												
Milk	15.4	15.6	14.0	11.1	14.5	13.2	1.85	0.01	<0.01	0.14	0.03	0.38
Fat	0.646	0.624	0.775	0.601	0.584	0.56	0.087	0.02	0.88	0.02	0.04	0.13
Protein	0.424	0.409	0.382	0.306	0.393	0.35	0.049	0.02	0.01	0.33	0.03	0.59
Lactose	0.726	0.747	0.643	0.515	0.701	0.63	0.090	<0.01	<0.01	0.05	0.03	0.50
Composition												
Fat, %	4.15	3.98	5.60	5.54	4.09	4.37	0.359	<0.001	<0.001	<0.001	0.68	0.54
Protein, %	2.80	2.59	2.74	2.72	2.71	2.64	0.082	0.90	0.92	0.49	0.52	0.75
Lactose, %	4.72	4.77	4.60	4.62	4.81	4.75	0.078	0.08	0.35	0.01	0.72	0.52
MUN, mg/dL	11.7	12.0	15.4	14.8	11.6	13.7	0.966	0.01	0.01	0.01	0.41	0.15
Isomolar infusion, 0.75 mol/h.												

¹Isomolar infusion, 0.75 mol/h.²Trt = treatment.³Int = interaction of acetate type (AcA vs. NAc) and site of infusion (AB vs. RU).⁴Samples were collected immediately after 8 h of infusions.⁵Samples were collected 12 h after infusions stopped.

Table 3. Effects of isomolar¹ intraruminal (RU) or intra-abomasal (AB) infusion (8 h/d) of sodium chloride (CON), acetic acid (AcA), and sodium acetate (NAC) on plasma metabolites and insulin for cows in the immediate postpartum period

Item	Infusion						Significance, <i>P</i> -value							
	CON-AB	CON-RU	AcA-AB	AcA-RU	NAc-AB	NAc-RU	SEM	Trt ²	Time	Trt × time	CON vs. AcA+NAC	NAC vs. AcA	AB vs. RU	Int ³
Glucose, mg/dL	42.0	45.3	41.3	39.0	41.2	43.0	4.2	0.56	<0.01	0.01	0.24	0.43	0.67	0.41
NEFA, ⁴ μEq/L	601	527	676	773	604	653	137	0.34	<0.001	0.02	0.11	0.25	0.73	0.77
BHB, mg/dL	23.2	22.0	25.5	30.2	27.8	29.0	6.7	0.59	<0.0001	<0.01	0.10	0.89	0.63	0.65
Lactate, mmol	0.333	0.368	0.337	0.309	0.336	0.309	0.01	0.01	0.06	0.71	0.01	0.98	0.51	0.97
Acetate, mmol	1.46	1.26	2.33	2.98	2.82	3.95	0.54	<0.01	<0.0001	<0.0001	<0.001	0.12	0.18	0.61
Propionate, mmol	0.117	0.108	0.145	0.155	0.125	0.142	0.03	0.40	0.03	0.03	0.07	0.39	0.71	0.87
Insulin, μg/L	0.180	0.182	0.105	0.093	0.131	0.119	0.04	0.17	0.94	0.43	0.01	0.40	0.77	1.00

¹Isomolar infusion, 0.75 mol/h.

²Trt = treatment.

³Int = interaction of acetate type (AcA vs. NAC) and site of infusion (AB vs. RU).

⁴Nonesterified fatty acids.

infusions of AcA or NAC infused into the RU or AB on feeding behavior, DMI, and metabolic responses of cows in the PP period.

In agreement with our hypothesis, AcA was more hypophagic than NAC but, compared with CON, NAC-AB decreased DMI whereas NAC-RU did not. The decrease in DMI by AcA was because AcA decreased meal frequency and not meal size compared with NAC, indicating that AcA did not affect satiety within the timeframe of meals but delayed hunger. Choi and Allen (1999) reported no effect of AcA, NAC, or sodium chloride on meal size of lactating cows when infused into the rumen at spontaneous meals consistent with the results of the current experiment. However, unlike the present experiment, effects of AcA did not affect hunger as indicated by intermeal interval or DMI compared with NAC (Choi and Allen, 1999). Decreased water intake for AcA compared with CON and NAC is consistent with results reported by Choi and Allen (1999) and is likely because of differences in intakes of Na and DM. Murphy et al. (1983) reported that DMI explains most of the variation in water intake, which is also correlated with milk production, and that 1 g of additional sodium fed increases water intake by 50 ± 23 mL.

Studies that have evaluated effects of intraruminal infusions of acetate salts or acetic acid on DMI have used different treatment conditions, including rate of infusion, solution concentration, and duration of treatment, and cows varying in physiological state. We infused 0.75 mol/h continuously for 8 h (6.0 mol/d). Acetate production rate was reported as 57 mol/d (2.38 mol/h) for mid-lactation cows fed 12.9 kg/d of DM (Sutton et al., 2003). Cows on our control treatments had similar DMI, so we estimate that our treatments provided approximately one-third of the acetate produced in the rumen per day. In general, infusion of AcA or NAC do not alter DMI of cattle (lactating or nonlactating) when the dose is 1.2 mol/h or less for up to 6 wk (Rook and Balch, 1961; Storry and Rook, 1965; Wilson et al., 1967; Orskov et al., 1969; Reynolds et al., 1979; Huntington et al., 1983; Maxin et al., 2011; Urrutia and Harvatin, 2017), similar to the effect of NAC-RU in the current study. However, AcA decreases DMI when infused into the rumen at rates greater than 1.2 mol/h. Montgomery et al. (1963) infused AcA at 3.5 mol/h for 4 h into rumens of lactating and nonlactating cows and found that it greatly decreased DMI; however, after partial neutralization to pH 5.0 with NaOH, acetate did not alter DMI. Similarly, infusion of AcA at 1.5 mol/h into the rumens of mid-lactation cows stopped rumination and induced metabolic alkalosis within 8 h but had no adverse effects when AcA was partially neutralized (Shepherd and Combs, 1998). In the present

Figure 1. Effects of isomolar intraruminal (RU) or intra-abomasal (AB) infusion (8 h/d) of sodium chloride (CON), acetic acid (AcA), and sodium acetate (NAC) on plasma metabolites—glucose, nonesterified fatty acids (NEFA), BHB, acetate, and propionate—for cows in the immediate postpartum period. Treatments were continuous isomolar infusions (0.75 mol/h) of acetic acid (circle), sodium acetate (triangle), or sodium chloride (square) as a control; infused into the abomasum (AB, solid line and solid symbols) or rumen (RU, dotted line and open symbols) at a rate of 0.5 L/h. Asterisks within a time point indicate significant differences at * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

study, AcA reduced DMI when infused in both the RU and AB; although NAc infused into the rumen did not reduce DMI compared with CON, it did reduce DMI compared with CON when infused into the AB.

Response in feed intake to ruminal infusions of AcA might be affected by stage of lactation or site of infusion. Wilson et al. (1967) reported decreased DMI when AcA was infused at rates greater than 0.44 mol/h to cows in the PP period, consistent with results of the present experiment. Similarly, infusion of AcA into the AB of cows in the PP period at 0.91 mol/h decreased DMI by 64% (Gualdrón-Duarte, 2017). Feed intake response to infusions of acetate salts or acetic acid might be affected by site of infusion; AcA and NAc caused diarrhea and cessation of eating within 24 and 48 h, respectively, when infused into the duodenum of nonpregnant, nonlactating cows (Tyrrell et al., 1979).

Infusion of AcA and NAc increased plasma acetate over time more than did CON, as expected, but the reason for the wide range of response to AcA and NAc treatments at 8 h after infusion is not apparent. Interaction of treatment and time for plasma propionate concentration appears to be from greater propionate concentration for AcA-AB after 8 h of infusion compared with other treatments, but the reasons are not apparent. Effects of treatment on plasma insulin concentration is consistent with their effects on DMI except that an interaction of AcA and NAc and site of infusion was not observed; although NAc-RU did not decrease DMI, it did decrease plasma insulin concentration. Effects of treatment on plasma concentrations of NEFA and BHB increased over time and were generally opposite of their effects on DMI and plasma insulin concentration, as expected.

Table 4. Effect of isomolar¹ intraruminal (RU) or intra-abomasal (AB) infusion (8 h/d) of sodium chloride (CON), acetic acid (AcA), and sodium acetate (NAc) on blood chemistry for cows in the immediate postpartum period (samples taken 4 h after starting of infusions)

Item ²	Infusion						Significance, <i>P</i> -value					
	CON-RU	CON-AB	AcA-RU	AcA-AB	NAc-RU	NAc-AB	SEM	Trt ³	CON vs. AcA+NAc	NAc vs. AcA	AB vs. RU	Int ⁴
pH	7.43	7.42	7.43	7.41	7.43	7.45	0.009	0.06	0.29	0.04	0.96	0.05
pCO ₂ , mm Hg	35.1	34.1	33.7	33.2	36.04	37.4	1.26	0.05	0.76	0.92	0.94	0.94
pO ₂ , mm Hg	43.2	46.2	48.1	46.8	44.1	46.4	2.16	0.48	0.29	0.23	0.79	0.33
p50, mm Hg	27.1	27.0	26.7	27.0	27.1	26.9	0.23	0.62	0.32	0.37	0.90	0.23
Total CO ₂ , mmol/L	24.6	23.2	23.6	22.3	25.5	27.3	1.07	0.02	0.27	<0.01	0.77	0.09
Saturation O ₂ , %	77.6	81.2	83.6	81.2	78.4	83.2	2.54	0.30	0.22	0.46	0.56	0.10
HCO ₃ , mmol/L	23.5	22.2	22.6	21.3	24.4	26.1	1.03	0.02	0.28	<0.01	0.80	0.01
sHCO ₃ , mmol/L	24.2	23.2	23.8	22.4	24.9	26.5	0.79	0.03	0.22	<0.01	0.87	0.06
BEb, mmol/L	0.27	-1.08	-0.31	-1.88	1.09	2.69	0.93	0.03	0.60	<0.01	0.99	0.08
BEecf, mmol/L	-3.5	-1.81	-2.55	-1.04	1.86	-0.004	1.10	0.02	0.24	<0.01	0.91	0.01
Anion gap, mmol/L	14.5	14.5	16.3	16.3	15.3	14.9	0.53	0.07	0.02	0.04	0.86	0.66
Osmolality, mOsm/kg	278	279	274	271	277	279	1.42	0.01	0.05	<0.01	0.83	0.10
BUN, mg/dL	13.3	12.7	12.3	13.1	13.0	12.5	0.333	0.11	0.24	0.82	0.61	0.02
Creatinine, mg/dL	0.982	0.955	0.959	0.933	1.00	0.995	0.03	0.55	0.87	0.08	0.58	0.75
BUN:creatinine	13.6	13.7	12.7	14	13.1	13.2	0.43	0.02	0.09	0.51	0.01	0.02
Glucose, mg/dL	44.8	48.1	42.2	43.8	46.3	44.5	2.26	0.56	0.29	0.33	0.97	0.49
Sodium, mmol/L	140	141	139	138	141	142	0.78	0.02	0.14	<0.01	0.90	0.12
Potassium, mmol/L	3.97	3.86	3.86	3.85	3.86	3.51	0.13	0.18	0.19	0.18	0.16	0.18
Chloride, mmol/L	107	108	105	105	105	103	0.78	0.03	<0.001	0.27	0.36	0.43
Lactate, mmol/L	0.471	0.458	0.447	0.421	0.440	0.471	0.02	0.43	0.26	0.27	0.90	0.17
iCa, mmol/L	1.30	1.26	1.25	1.25	1.28	1.21	0.03	0.18	0.12	0.81	0.11	0.20
nCa, mmol/L	1.28	1.26	1.23	1.23	1.26	1.18	0.03	0.12	0.06	0.58	0.13	0.13
iMg, mmol/L	0.51	0.56	0.53	0.57	0.49	0.49	0.02	0.02	0.28	0.01	0.40	0.30
nMg, mmol/L	0.53	0.55	0.55	0.55	0.53	0.52	0.02	0.66	<0.001	0.02	<0.0001	<0.0001

¹Isomolar infusion, 0.75 mol/h.

²pCO₂ = partial pressure of CO₂; pO₂ = partial pressure of O₂; p50 = partial pressure of oxygen at which hemoglobin is 50% saturated; sHCO₃ = standard bicarbonate ion concentration after the blood has been equilibrated to a pCO₂ of 40 mmHg; BEb = base excess blood; BEecf = base excess extracellular fluid; iCa and iMg = ionized Ca and Mg; nCa and nMg = Ca and Mg normalized to pH 7.4, respectively.

³Trt = treatment.

⁴Int = interaction of acetate type (AcA vs. NAc) and site of infusion (AB vs. RU).

The effect of treatments on blood gases and chemistry showed differences among treatments in some of the measurements, but most were within the normal range for dairy cows according to Michigan State University Diagnostic Center for Population and Animal Health (East Lansing). However, all infusions had abnormally high values for anion gap and abnormally low osmolality and sodium concentration for lactating cows. Differences among the parameters measured could not explain the effects of treatment on DMI because all values for NAc-RU, which did not reduce DMI, were intermediate among treatments.

CONCLUSIONS

Our results indicate that infusion of Na acetate into the rumen at 6 mol/8 h does not reduce DMI of cows in the early postpartum period compared with infusion of NaCl as an osmotic control. However, infusion of acetic acid into the rumen or infusions of either acetic acid or Na acetate into the abomasum at similar rates greatly decreased DMI compared with control. Evaluation of changes in plasma concentrations of metabolites, insulin, blood gases, and blood chemistry did not reveal clues for potential mechanisms responsible for different effects of treatments, which requires further investigation.

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