



Effect of dietary supplementation of sodium acetate and calcium butyrate on milk fat synthesis in lactating dairy cows

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ABSTRACT

Acetate is a major source of energy and substrate for milk fat synthesis in the dairy cow. We recently reported a linear increase in milk fat yield and greater than a 30% net apparent transfer of acetate to milk fat with ruminal infusion of neutralized acetate. Additionally, ruminal acetate infusion linearly increases plasma β -hydroxybutyrate. The objective of the current study was to investigate the ability of acetate and butyrate fed in a diet to increase milk fat synthesis. Twelve multiparous lactating Holstein cows were randomly assigned to treatments in a 3×3 Latin square design with 14-d periods that included a 7-d washout followed by 7 d of treatment. Cows were fed ad libitum a basal diet with a low risk for biohydrogenation-induced milk fat depression, and treatments were mixed into the basal diet. Treatments were 3.2% NaHCO_3 (control), 2.9% sodium acetate, and 2.5% calcium butyrate (carbon equivalent to acetate treatment) as a percent of diet dry matter. Feeding sodium acetate increased dry matter intake by 2.7 kg, had no effect on milk yield, and increased milk fat yield by 90 g/d and concentration by 0.2 percentage units, compared with control. Calcium butyrate decreased dry matter intake by 2.6 kg/d, milk yield by 1.65 kg/d, and milk fat yield by 60 g/d, compared with control. Sodium acetate increased concentration and yield of 16 carbon mixed source fatty acids (FA) and myristic acid, while decreasing the concentration of preformed FA, compared with control. Calcium butyrate had no effect on concentration of milk FA by source, but increased concentration of *trans*-10 C18:1 in milk by 18%, indicating a shift in rumen biohydrogenation pathways. Our data demonstrate that milk fat yield and concentration can be increased by feeding sodium acetate at 2.9% of diet dry matter, but not by feeding calcium butyrate at an equivalent carbon mass.

Key words: acetate, butyrate, dairy cow, milk fat synthesis

INTRODUCTION

Milk fat production is important to the dairy industry because of its high market value and the properties milk fat imparts to dairy products. However, of all milk components, milk fat is the most variable and is highly influenced by diet nutrient composition (Palmquist et al., 1993). Therefore, dietary strategies aiming to increase milk fat synthesis are of special interest.

Milk fatty acids (FA) originate either from de novo synthesis in the mammary gland or from uptake of plasma FA from dietary absorption or endogenous reserves (preformed FA). Acetate is the predominant substrate for de novo FA synthesis in the dairy cow because it serves as a 2-carbon donor for synthesis of malonyl-CoA and for NADPH synthesis through the isocitrate pathway (Bauman et al., 1970; Ingle et al., 1973; Smith, 1983). Additionally, in the well-fed cow, acetate is the major VFA produced in the rumen and provides 45% of the energy arising from VFA metabolism (Baldwin and Smith, 1971; Bergman, 1990). Therefore, acetate is fundamental for meeting energy requirements and for milk fat synthesis in the dairy cow.

Studies examining the role of acetate supply on milk fat synthesis were mostly conducted over 30 yr ago [1955 to 1978; summarized in Urrutia and Harvatine (2017b)]. A meta-analysis primarily based on these early studies reported that acetate infusions linearly increased milk fat yield and concentration (Maxin et al., 2011), but these studies were small and used lower producing cows (14.3 ± 3.2 kg/d) fed diets very different from contemporary rations, therefore limiting extrapolation.

We recently reported that ruminal infusion of acetate neutralized to pH 6 increases milk fat yield (Urrutia and Harvatine, 2017a,b). Specifically, in a 4-d dose response study, acetate linearly increased milk fat concentration and quadratically increased milk fat yield, with milk fat yield increasing 100, 217, and 185 g/d

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when providing 300, 600, and 900 g/d of sodium acetate respectively, compared with a sodium chloride control (Urrutia and Harvatine, 2017b). Acetate infusion also increased plasma BHB linearly at a rate of 5% per mole of acetate, possibly through conversion of acetate to butyrate in the rumen and rumen wall, and subsequent conversion to BHB (Sutton et al., 2003). The BHB provides about half of the initial carbons to make the 4 carbon primer for de novo FA synthesis (Palmquist et al., 1969); therefore, it is possible that a portion of the increase in milk fat synthesis observed during rumen acetate infusions is due to increased plasma BHB. In the meta-analysis of Maxin et al. (2011), a greater milk fat response was observed for butyrate than acetate infusion; milk fat yield increased 127 g/d (adjusted $R^2 = 0.68$) and concentration increased 7.96 g/kg for each kg of additional butyrate ($P < 0.001$, $n = 6$). These data support a role for butyrate in stimulation of milk fat synthesis, but also provide a mechanism for increased milk fat synthesis observed during rumen infusion of acetate. The objective of the current experiment was to investigate the ability of the milk fat precursors acetate and butyrate to increase milk fat synthesis when fed in a TMR.

MATERIALS AND METHODS

Experimental Design and Treatments

All experimental procedures were approved by the Pennsylvania State University Institutional Animal Care and Use Committee. Twelve multiparous lactating Holstein cows (183 ± 45 DIM; 45 ± 4.7 kg of milk/d) were randomly assigned to treatments in a 3×3 Latin square design with 14-d periods, which included a 7-d washout and 7-d treatment period. Cows were housed in a tiestall barn located at the Pennsylvania State University Dairy Production Research and Teaching Center.

Cows were fed once daily at 0800 h at approximately 110% of expected intake and refusals were recorded daily. Treatments were sodium bicarbonate control (3.2% of diet DM), sodium acetate at 2.9% of diet DM (targeting 10 mol of acetate/d; **NaAc**; Food grade anhydrous sodium acetate, Kemin Industries Inc., Des Moines, IA), and calcium butyrate at 2.5% of diet DM (**CaBu**; Kemin Industries Inc.). The control was designed to provide equal moles of sodium as NaAc, and CaBu was designed to provide the same mass of carbon as NaAc. The basal TMR was balanced to have a low risk for biohydrogenation-induced milk fat depression as it contained adequate NDF and few rumen-available

UFA sources (Table 1). The basal TMR was mixed in a Kuhn RC 250 mixer and the treatments were mixed into the basal TMR in portable mixer cart (Rissler TMR mixer 1050, Rissler, Mohnton, PA) before delivery.

Feed Sampling and Analysis

Forage and base diet DM concentration was determined weekly for diet adjustment and DMI determination (72 h in a forced-air oven at 55°C). All individual feed ingredients were sampled by period and forages and a representative composite of the grains was analyzed for CP, NDF, and ADF by wet chemistry procedures as described by Rico et al. (2014). The composition of each TMR differed slightly due to the small differences in treatment inclusion rates. This approach was selected because the treatments are water soluble and were not expected to change the fermentability or physical fill of the diet.

Milk Sampling and Analysis

Cows were milked twice daily at 0600 and 1800 h and milk yield was determined by an integrated milk meter (AfiMilk; SAE Afikim, Israel) as described by Urrutia and Harvatine (2017b). Milk was sampled at both milkings the day before treatment feeding and on d 3, 6, and 7 of treatment feeding. Samples were composited within day and analyzed for milk fat and protein by FTIR and milk FA profile by GLC as described by Urrutia and Harvatine (2017a). Milk composition on d 6 and 7 of feeding was averaged within period.

Blood Sampling and Analysis

Blood samples were collected from a coccygeal vessel using potassium EDTA tubes (Greiner Bio-One North America Inc., Monroe, NC) at 0500 and 1700 h on the last day of treatments to represent a low intake and high intake period of the day, respectively. Blood was immediately placed on ice, centrifuged within 30 min at $1,300 \times g$ for 15 min at 4°C, and plasma was harvested and stored at -20°C until laboratory analysis. Plasma samples were analyzed for glucose [PGO Enzyme procedure no. P 7119, Sigma-Aldrich, St. Louis, MO (Raabo and Terkildsen, 1960)], nonesterified fatty acids [**NEFA**; Wako HR Series NEFA-HR kit, Wako Chemicals USA, Richmond, VA, as modified by Ballou et al. (2009)], BHB (procedure #2440, Stanbio Laboratory, Boerne, TX), and BUN (procedure #2050, Stanbio Laboratory).

Table 1. Ingredient and nutrient composition of experimental diets comparing sodium acetate to sodium bicarbonate DCAD control and equivalent carbon mass of butyrate as calcium butyrate¹

Item	Treatment ²		
	Control	NaAc ³	CaBu ⁴
Ingredient, % of DM			
Corn silage	35.9	36.0	36.2
Alfalfa haylage	12.9	13.0	13.0
Cracked corn	10.9	11.0	11.0
Canola meal	10.5	10.6	10.6
Bakery byproduct	4.68	4.70	4.72
Roasted soybeans	4.68	4.70	4.72
Molasses	4.68	4.70	4.72
Whole cottonseed	4.29	4.30	4.32
Grass hay	3.90	3.91	3.93
Ground corn	2.34	2.35	2.36
Mineral-vitamin mix ⁵	1.61	1.61	1.61
NPN ⁶	0.39	0.39	0.39
Sodium bicarbonate	3.21		
Sodium acetate		2.87	
Calcium butyrate			2.47
Nutrient composition, % of DM ± SD			
CP	14.6 ± 0.7	14.6 ± 0.7	14.7 ± 0.7
NDF	30.7 ± 0.7	30.8 ± 0.7	30.9 ± 0.7
ADF	12.5 ± 0.1	12.5 ± 0.1	12.6 ± 0.1
Starch	18.7 ± 0.9	18.7 ± 0.9	18.8 ± 0.9
Fatty acids (FA)	4.31 ± 0.6	4.33 ± 0.6	4.35 ± 0.6
FA, % of FA			
C16:0	7.40 ± 1	7.43 ± 1	7.46 ± 1
C18:0	2.87 ± 0.2	2.88 ± 0.2	2.89 ± 0.2
C18:1n-9	10.2 ± 1.5	10.2 ± 1.5	10.2 ± 1.5
C18:2n-6	18.8 ± 2.6	18.8 ± 2.6	18.9 ± 2.6
C18:3n-3	2.46 ± 0.09	2.47 ± 0.09	2.48 ± 0.09
DCAD, mEq/100 g	5.22 ± 0.17	4.78 ± 0.11	1.24 ± 0.002

¹Cows were fed the same basal TMR diet (1.67 Mcal/kg of NE_L), and treatments were mixed into the basal diet.

²Treatments were sodium bicarbonate control (3.2% of diet DM), sodium acetate at 2.9% of diet DM (targeting 10 mol of acetate/d; NaAc; food-grade anhydrous sodium acetate, Kemin Industries Inc., Des Moines, IA), and calcium butyrate at 2.5% of diet DM (CaBu; Kemin Industries Inc.) to provide an equivalent carbon mass as the NaAc treatment.

³Acetate contained in NaAc provided 2.09 Mcal of NE_L/d.

⁴Butyrate contained in CaBu provided 2.65 Mcal of NE_L/d.

⁵Contained (%; as-fed basis): 45.8 dried corn distillers grains with solubles; 35.8 limestone (38% Ca); 8.3 magnesium oxide (54% Mg); 6.4 salt; 1.73 vitamin ADE premix; 1.09 selenium premix (0.06% selenium); and 0.88 trace mineral mix.

⁶Nonprotein nitrogen fed as a slow-release urea (259% CP, DM basis; Optigen, Alltech Inc., Lexington, KY).

Statistical Analysis

Data were analyzed as repeated measures in SAS (version 9.3, SAS Institute Inc., Cary, NC) and the model included the random effect of cow, period, and treatment sequence and the fixed effects of a covariate (observed value for variable of interest on the day before treatment feeding), treatment, day of treatment, and their interaction. Subject was cow by period and the CS covariance structure was used. Denominator degrees of freedom were adjusted with the Kenwood Rogers method. Data points with Studentized residuals outside of ±3.5 were considered outliers and excluded from analysis. A protected least significant difference

separation was used within each time point to compare treatment means when the treatment or treatment by time interaction was significant. Differences were declared significant at $P \leq 0.05$ and tendencies at $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

A treatment by time interaction was observed for DMI, as NaAc increased DMI over 2.5 kg/d and CaBu decreased DMI over 2.6 kg/d during treatment periods, compared with control ($P < 0.05$; Figure 1A). Reduced DMI in the CaBu treatment may have been due to palatability issues (Huhtanen et al., 1993); however,

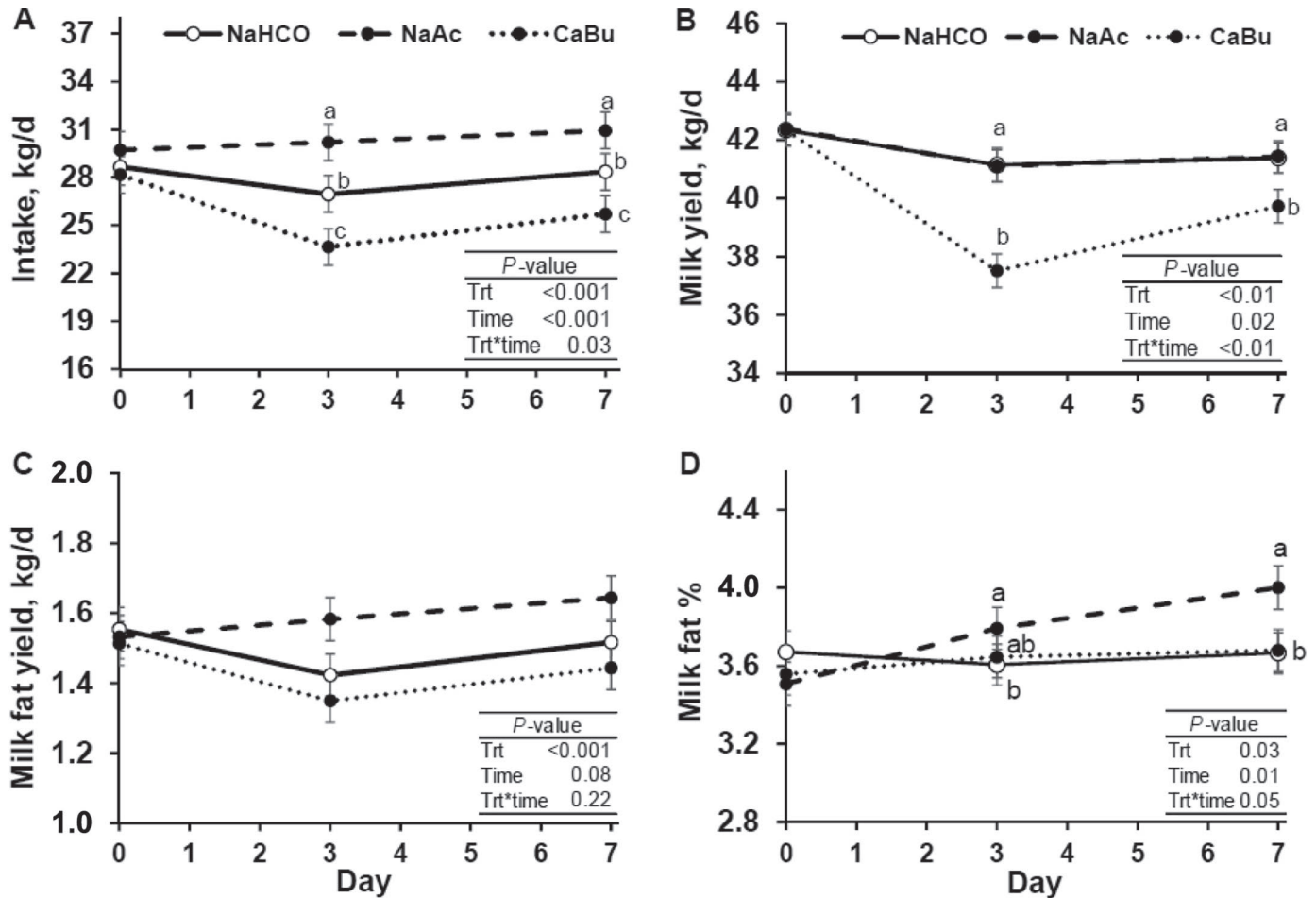


Figure 1. Time course of DMI (panel A), milk yield (panel B), milk fat yield (panel C), and milk fat concentration (panel D) of cows fed sodium bicarbonate at 3.2% of diet DM (control; NaHCO), sodium acetate at 2.9% of diet DM (NaAc), and calcium butyrate at 2.5% of diet DM (CaBu; equal carbon mass of NaAc; $n = 12$ per treatment). Least squares means and SE are shown, and means that do not share a letter (a–c) differ within time point ($P < 0.05$). Trt = treatment.

in previous research where butyrate was incorporated into pelleted concentrates as calcium salts (Balch et al., 1967) or infused ruminally as sodium salts (~ 700 g of butyrate/d; Herrick et al., 2018), DMI was not affected. Providing butyric and acetic acid as diluted acids has reduced feed intake, presumably due to stimulation of satiety signals through chemoreceptors in the rumen wall, liver, or portal system (Rook et al., 1965; Simkins et al., 1965).

A treatment by time interaction was observed for milk yield, as NaAc had no effect on milk yield, but CaBu decreased milk yield by over 4% (-1.65 kg/d) during the treatment period compared with control (treatment by time interaction, $P < 0.01$; Figure 1B). The decrease in milk yield with CaBu is in agreement with the reduced DMI reported above.

Milk fat yield was increased 90 g/d by NaAc and decreased 60 g/d by CaBu compared with control ($P <$

0.05; Figure 1C). A similar amount of sodium acetate (10 mol/d) increased milk fat yield 217 g/d compared with a sodium chloride control when ruminally infused over 22 h/d (Urrutia and Harvatine, 2017b). The larger response with continuous infusion may be due to increased acetate availability over the entire day, while inclusion in TMR provides boluses with each meal. A treatment by time interaction was observed for milk fat concentration as NaAc increased milk fat concentration on d 3 and 7 compared with control (0.20 and 0.34 percentage points, respectively; interaction, $P = 0.05$; Figure 1D). Previous studies have shown increased milk fat concentration with rumen infusion of butyrate (Rook et al., 1965; Huhtanen et al., 1993), with only small nonsignificant reductions in DMI when providing high doses of butyric acid (Rook et al., 1965). As DMI was reduced with CaBu in the present experiment, other precursors for fat synthesis such as acetate are expected

to have also decreased and may have limited a positive effect of butyrate on milk fat synthesis.

Milk protein concentration did not differ between NaAc and control, but was decreased by CaBu compared with control ($P < 0.05$). Early and more recent research has not shown a consistent effect of increasing butyrate supply on milk protein content. For example, Huhtanen et al. (1993) when providing rumen infusion of increasing concentrations of butyrate (from 0 to 600 g/d) in isoenergetic mixture of VFA, observed a positive linear response of milk protein content (from 3.27% to 3.46% milk protein), whereas Rook and Balch (1961) observed no effect of 880 g/d of butyrate infused ruminally on milk protein content. It is likely that milk protein content was reduced in CaBu due to reduced feed intake, as feed restriction results in decreased milk and milk component yield and concentration (Wilson et al., 1967). A treatment by time interaction was observed for milk protein yield, as NaAc increased milk protein yield by 79 g/d on d 3 of treatments, whereas CaBu reduced milk protein yield over 100 g/d on d 3 and 7 of treatments, compared with control (Figure 2). Responses in milk protein yield were driven by increased milk yield for NaAc, but were a combination of reduced milk protein concentration and milk yield in CaBu. In previous research, milk protein concentration and yield were not affected by short (Urrutia and Harvatine, 2017a,b) or long-term (Sheperd and Combs, 1998) ruminal infusion of acetate.

There were treatment by time interactions for yield of de novo and mixed origin FA ($P = 0.09$ and $P = 0.02$, respectively; Figure 3). De novo FA were increased 50 g/d by NaAc on d 3 ($P < 0.05$) compared with control. Calcium butyrate decreased de novo FA by 12 g/d on d 7, compared with control. Sodium acetate also increased mixed source FA by over 50 g/d during the entire treatment period (d 3 to 7), compared with control. During ruminal infusion of neutralized acetate, Urrutia and Harvatine (2017b) reported increased yield of de novo (57 g/d) and mixed origin FA (87 g/d) in response to continuous rumen infusions of sodium acetate (10 mol/d), in a similar magnitude to the present experiment.

Treatment by time interactions were detected for the concentration of all FA grouped by source (de novo, mixed, and preformed) and for *trans*-10 C18:1 (all $P < 0.01$; Supplemental Figure S1; <https://doi.org/10.3168/jds.2018-16024>). The concentration of de novo synthesized FA (<16 C FA; g/100 g) were increased by NaAc on d 3 compared with control and by CaBu on d 7 compared with control. Concentration of mixed source FA (16 C FA) were increased by NaAc on d 3 and 7 and reduced by CaBu on d 3, compared with

control. Concentration of preformed FA (>16 C FA) were reduced by NaAc on d 3 and 7, and increased by CaBu on d 3 compared with control. The CaBu increased concentration of milk *trans*-10 C18:1 on d 3 and 7 of treatment (treatment by time interaction, $P < 0.01$; Supplemental Figure S1; <https://doi.org/10.3168/jds.2018-16024>) indicating a shift in rumen biohydrogenation toward alternate pathways that result in biohydrogenation-induced milk fat depression.

Analysis of individual FA identified some FA specific interactions (Table 2). No treatment or treatment by time interactions effects were observed for C4:0, C6:0, and C8:0. NaAc decreased milk concentration of C17:0, *trans*-11 C18:1, *cis*-9, *trans*-11 CLA, and total odd- and

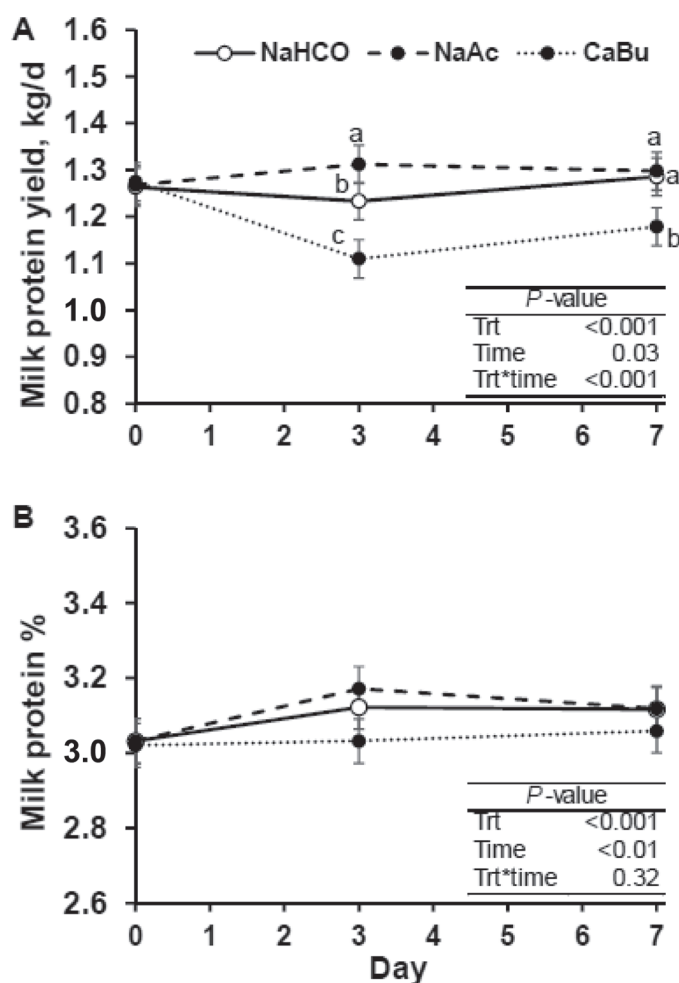


Figure 2. Time course of milk protein yield (panel A) and concentration (panel B) of cows fed sodium bicarbonate at 3.2% of diet DM (control; NaHCO), sodium acetate at 2.9% of diet DM (NaAc), and calcium butyrate at 2.5% of diet DM (CaBu; equal carbon mass of NaAc; $n = 12$ per treatment). Least squares means and SE are shown, and means that do not share a letter (a-c) differ within time point ($P < 0.05$). Trt = treatment.

branched-chain FA compared with control (all $P < 0.05$, Table 2). The CaBu increased concentration of C17:0 and C17:1, compared with control (both $P < 0.05$). Several treatment by time interactions were detected (Supplemental Table S1; <https://doi.org/10.3168/jds.2018-16024>). Importantly, NaAc increased concentration of palmitic acid (C16:0) and reduced concentration of *iso* C14:0, *trans*-9 C18:1, C18:2 n-6, and C18:3 n-3 in milk fat on d 3 and 7 of treatments, compared with control ($P < 0.05$). Also, NaAc increased concentration of C10:0, C12:0, and C14:0, and reduced concentration of *anteiso* C15:0, *anteiso* C17:0, and *cis*-9 C18:1 in milk fat on d 3, compared with control (all $P < 0.05$). Feeding CaBu increased milk concentration of *iso* C15:0, *iso* C17:0, C18:0 on d 3, and C10:0, C12:0, *anteiso* C13:0, and C14:0 on d 7, compared with control (all $P < 0.05$). Also, CaBu reduced concentration of milk C14:0, *iso* C16:0, and C16:0 on d 3 and *anteiso* C15:0, *anteiso* C17:0, and C18:0 on d 7, compared with control

(all $P < 0.05$). The CaBu increased concentration of *trans*-9 18:1 on d 3 and 7 and reduced concentration of *iso* C14:0 on d 3 and 7, compared with control ($P < 0.05$). Overall, NaAc increased milk fat synthesis primarily through an increase in synthesis of de novo and mixed FA (C10:0, C12:0; C:14; C16:0), whereas CaBu increased synthesis of C10:0 and C12:0 and modified FA of microbial origin, indicating a shift in rumen fermentation pattern (Vlaeminck et al., 2006).

Treatment by time interactions were detected for plasma NEFA and BUN (Figure 4A and 4B). The CaBu reduced plasma NEFA by 18% and increased BUN by 12% at 1700 h compared with control (both $P < 0.05$). Also, both NaAc and CaBu increased BUN by over 24% at 0500 h compared with control ($P < 0.05$). Reduced plasma NEFA in CaBu may result from a BHB-mediated lipolysis inhibition through activation of adipose nutrient sensing receptors such as the hydroxycarboxylic acid receptor 2 (Metz and Bergh, 1972;

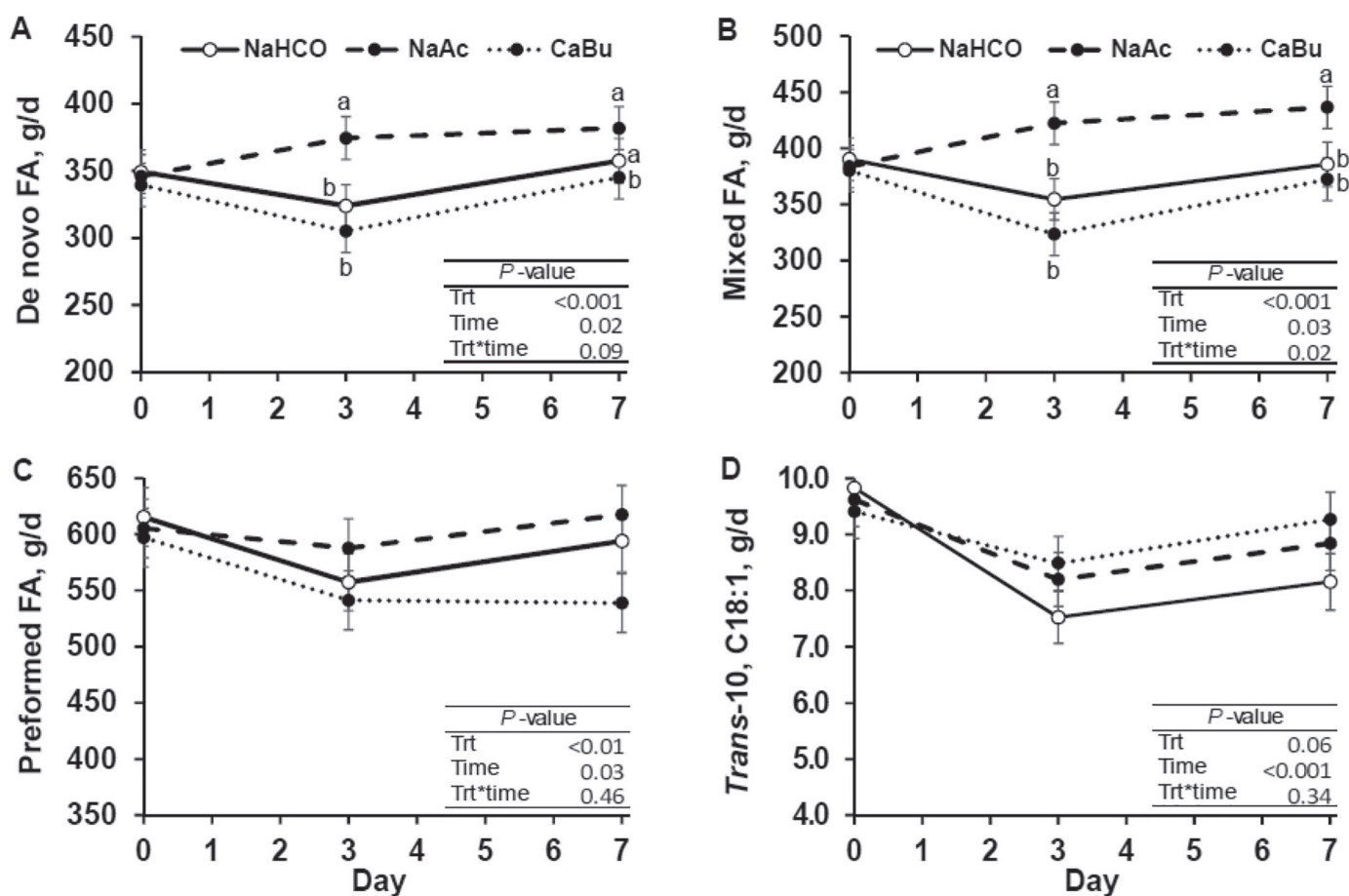


Figure 3. Time course of milk fatty acid (FA) yield of de novo (<16 carbons; panel A), mixed source (16 carbons; panel B), preformed origin (>16 carbons; panel C), and *trans*-10, C18:1 (panel D) of cows fed sodium bicarbonate at 3.2% of diet DM (control; NaHCO), sodium acetate at 2.9% of diet DM (NaAc), and calcium butyrate at 2.5% of diet DM (CaBu; equal carbon mass of NaAc; n = 12 per treatment). Least squares means and SE are shown, and means that do not share a letter (a,b) differ within time point ($P < 0.05$). Trt = treatment.

Mielenz, 2017). However, recent studies have not shown reductions in plasma NEFA in response to rumen infusion of butyrate (Herrick et al., 2018) or to high plasma BHB resulting from rumen acetate infusion (Urrutia and Harvatine, 2017b). Blood urea nitrogen is an indicator of efficiency of protein utilization and is related to energy balance and plasma NEFA (Broderick and Clayton, 1997; Rastani et al., 2006). Elevated BUN observed in CaBu may indicate inefficient dietary protein utilization or increased body protein mobilization and catabolism due to reduced feed intake.

Treatment affected plasma glucose and BHB (Figure 4). Plasma BHB concentration was 539, 650, and 853 mg/dL in control, NaAc, and CaBu treatments, respectively (SE = 125; Figure 4C). The NaAc did not affect BHB, whereas CaBu increased BHB by 58%, compared with control ($P < 0.05$). In previous studies (Urrutia and Harvatine, 2017a,b), continuous acetate infusions resulted in increased plasma BHB compared with sodium chloride control; however, in the present study,

feeding sodium acetate did not affect plasma BHB compared with a sodium bicarbonate control. Possible reasons may include a synchronized availability of rumen acetate and butyrate (made from acetate) with rumen wall energy demand after feeding in the present study, reducing transfer to blood circulation. Feeding butyrate (CaBu) in the current experiment resulted in a similar increase in plasma BHB compared with previous continuous ruminal acetate infusions (Urrutia and Harvatine, 2017b). This agrees with previous findings, as rumen butyrate, once absorbed is rapidly metabolized to BHB by rumen epithelial cells (Storry and Rook, 1965b). In the current study, both NaAc and CaBu reduced plasma glucose concentration by ~10% compared with control ($P < 0.05$; Figure 4D). Early reports have shown a role for butyrate in the regulation of glucose metabolism (Storry and Rook, 1965a), supported by in vitro studies where butyrate inhibits liver uptake of propionate (Demigné et al., 1986). Recently, Herrick et al. (2018) reported a 10% reduction

Table 2. Effect of feeding sodium acetate (NaAc) and an equal carbon mass of calcium butyrate (CaBu) on milk fatty acid (FA) composition

FA, g/100 g	Treatment ¹				P-value		
	NaHCO	NaAc	CaBu	SE	Trt	Time	Trt × time ²
C4:0	4.24	4.21	4.26	0.065	0.75	<0.001	0.29
C6:0	2.17	2.17	2.21	0.027	0.31	<0.001	0.82
C8:0	1.18	1.19	1.20	0.022	0.37	<0.001	0.34
C10:0	2.58	2.64	2.62	0.057	0.34	<0.001	0.05
C11:0	0.042	0.042	0.042	0.002	0.66	<0.001	0.85
C12:0	2.92	3.00	2.98	0.068	0.18	<0.001	0.03
iso C13:0	0.021	0.020	0.021	0.001	0.77	0.68	0.41
anteiso C13:0	0.056	0.056	0.059	0.001	0.16	<0.001	0.02
iso C14:0	0.102	0.091	0.082	0.004	<0.001	<0.001	0.06
C14:0	10.1	10.4	10.1	0.12	<0.01	0.12	<0.001
iso C15:0	0.199	0.201	0.202	0.003	0.39	<0.001	0.03
anteiso C15:0	0.36	0.35	0.35	0.004	0.04	0.35	0.04
C15:0	0.85	0.83	0.85	0.012	0.22	<0.001	0.75
iso C16:0	0.22	0.22	0.19	0.010	0.04	<0.001	0.01
C16:0	25.7	26.6	25.3	0.13	<0.001	0.01	<0.001
iso C17:0	0.26	0.26	0.27	0.005	0.04	<0.001	<0.001
anteiso C17:0	0.34	0.33	0.34	0.004	0.12	0.002	0.002
C17:0	0.46 ^b	0.45 ^c	0.47 ^a	0.006	0.001	<0.001	0.22
C17:1	0.128 ^b	0.124 ^b	0.133 ^a	0.002	0.01	0.001	0.11
C18:0	12.7	13.0	12.9	0.17	0.42	<0.01	<0.001
trans-9 C18:1	0.33	0.32	0.34	0.011	<0.001	0.18	<0.001
trans-10 C18:1	0.59	0.59	0.66	0.020	<0.001	0.04	<0.01
trans-11 C18:1	1.27 ^a	1.2 ^b	1.27 ^a	0.035	0.01	<0.001	0.25
cis-9 C18:1	19.5	18.8	19.4	0.56	<0.01	<0.001	<0.001
C18:2n-6	3.12	2.95	3.17	0.097	<0.001	0.06	<0.01
C18:3n-3	0.47	0.44	0.47	0.017	<0.01	0.25	0.01
cis-9,trans C18:2	0.54 ^a	0.51 ^b	0.53 ^a	0.014	0.01	<0.001	0.12
Σ OBCFA ³	3.05 ^a	2.96 ^b	3.03 ^a	0.026	<0.001	<0.001	0.32

^{a-c}Least squares means that do not share a superscript are significantly different ($P < 0.05$).

¹Treatments were on a DM basis: 3.2% NaHCO₃ (control), 2.9% NaAc, and 2.5% CaBu (carbon equivalent to acetate treatment), n = 12 per treatment.

²Main effects of treatment (Trt) when a Trt by time interaction was detected are described in the text and shown in Supplemental Table S1 (<https://doi.org/10.3168/jds.2018-16024>).

³Odd- and branched-chain FA.

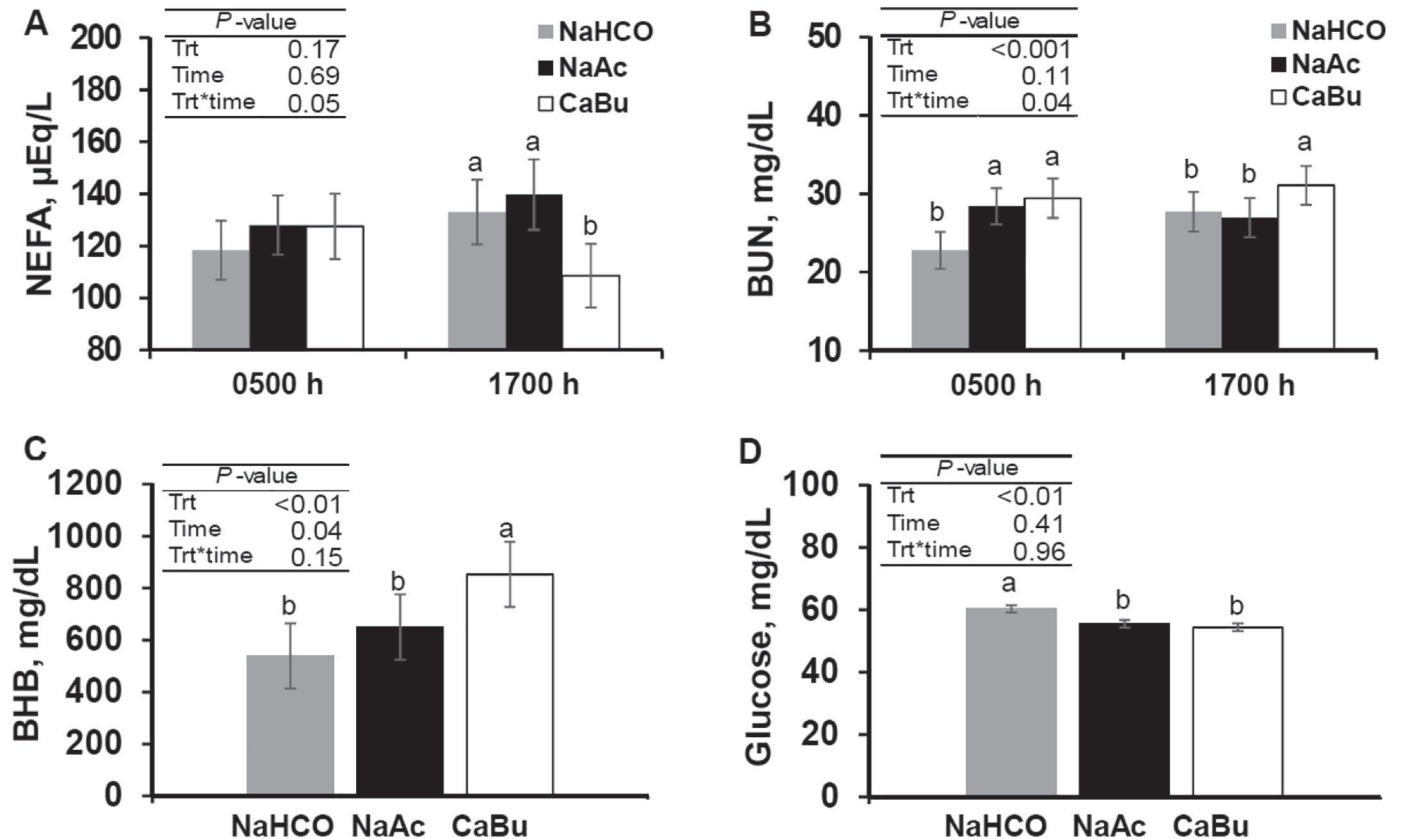


Figure 4. Effect of feeding sodium bicarbonate at 3.2% of diet DM (control; NaHCO), sodium acetate at 2.9% of diet DM (NaAc), and calcium butyrate at 2.5% of diet DM (CaBu; equal carbon mass of NaAc) on plasma nonesterified fatty acids (NEFA; panel A), BUN (panel B), plasma BHB (panel C), and plasma glucose (panel D; $n = 12$ per treatment). Least squares means and SE are shown, and means that do not share a letter (a,b) differ within time point ($P < 0.05$). Trt = treatment.

in plasma glucose when providing ~700 g/d of butyrate as a continuous ruminal infusion. Although previous studies have not shown reductions in plasma glucose in response to acetate infusion (Storry and Rook, 1965a; Sheperd and Combs, 1998; Urrutia and Harvatine, 2017a,b), it is possible that increased milk fat synthesis in NaAc resulted in an increase in glucose requirement for NADPH synthesis in the mammary gland.

Continuous sodium acetate infusions resulted in a linear increase in rumen pH compared with a sodium chloride control (Urrutia and Harvatine, 2017b), as sodium acetate infused at a pH of 6.0 provided buffering capacity to the rumen. The use of a sodium chloride control did not allow control for buffering capacity and for DCAD. Interestingly, DCAD has been reported to increase milk fat yield and concentration [0.1% and 36 g/d for every 100 mEq/kg increase in DCAD, respectively; Iwaniuk and Erdman (2015)] presumably through improved rumen buffering capacity, although the mechanism has not been demonstrated. Improved buffering capacity may result in a shift of rumen

fermentation pathways toward reduced formation of alternate biohydrogenation intermediates that induce milk fat depression such as *trans*-10 C18:1 and *trans*-10, *cis*-12 C18:2 (Harvatine et al., 2009), and therefore, an apparent increase in milk fat synthesis.

In the present experiment, a sodium bicarbonate treatment was used to provide a DCAD and buffering capacity control to sodium acetate. We observed no treatment or treatment by time interactions for NaAc on milk *trans*-10 C18:1, compared with control (Figure 3 and Supplemental Figure S1; <https://doi.org/10.3168/jds.2018-16024>), and *trans*-10, *cis*-12 C18:2 was not detected in any sample; therefore, we attribute the milk fat yield and concentration response observed in the current experiment to acetate supply and not a rumen buffering capacity or DCAD effect. Yet, the provision of sodium acetate as a feed supplement would provide an additional benefit to the increase in milk fat synthesis, as it would improve rumen buffering capacity and therefore rumen health, especially when feeding more highly fermentable diets and during the high

intake period of the day after delivery of fresh feed and during the afternoon when rumen pH is lowest (Salfer et al., 2018).

Several mechanisms may be involved in the increased milk fat synthesis observed with acetate supplementation, including simply an increase in substrate supply for milk fat synthesis or modification of metabolism as a bioactive nutrient. Molecular mechanisms exist for acetate to potentially modify metabolic signaling and regulation of gene expression (discussed by Urrutia and Harvatine, 2017b), but have not been well investigated in the cow. Feeding sodium acetate is expected to increase acetate supply more during the high intake period of the day (feeding to midnight). To determine if treatment effects differed at the morning and afternoon milking, milking time (AM/PM) and its interaction with treatment and experimental day were tested but were not significant (data not shown). This may be due to the timing of milking relative to the high intake period of the day and may require increased milking frequency to observe a temporal effect on milk fat yield.

CONCLUSIONS

In conclusion, milk fat yield and concentration can be increased by dietary supplementation of sodium acetate, and not calcium butyrate at the level investigated. Increased milk fat synthesis observed during acetate feeding may be due to either a greater substrate supply allowing greater synthesis of de novo and mixed FA, modification of metabolism by acetate, or a combination of these. Inclusion of sodium acetate into dairy cow diets will depend on the cost of a commercial supplement and market price for milk fat.

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