Acetate Dose-Dependently Stimulates Milk Fat Synthesis in Lactating Dairy Cows1–4

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Abstract

Background: Acetate is a short-chain fatty acid (FA) that is especially important to cows because it is the major substrate for de novo FA synthesis. However, the effect of acetate supply on mammary lipid synthesis is not clear.

Objective: The objective of this experiment was to determine the effect of increasing acetate supply on milk fat synthesis in lactating dairy cows.

Methods: Six multiparous lactating Holstein cows were randomly assigned to treatments in a replicated design to investigate the effect of acetate supply on milk fat synthesis. Treatments were 0 (control), 5, 10, and 15 mol acetate/d continuously infused into the rumen for 4 d. Rumen short-chain FAs, plasma hormones and metabolites, milk fat concentration, and milk FA profile were analyzed on day 4 of each treatment. Polynomial contrasts were used to test the linear and quadratic effects of increasing acetate supply.

Results: Acetate increased milk fat yield quadratically (P < 0.01) by 7%, 16%, and 14% and increased milk fat concentration linearly (P < 0.001) by 6%, 9%, and 11% for 5, 10, and 15 mol acetate/d, respectively, compared with the control treatment. Increased milk fat yield predominantly was due to a linear increase in 16-carbon FAs (P < 0.001) and a quadratic increase in de novo synthesized FAs (<16-carbon FAs; P < 0.01), indicating that there was stimulation of de novo synthesis pathways. Apparent transfer of acetate to milk fat was 33.4%, 36.2%, and 20.6% for 5, 10, and 15 mol/d, respectively. Acetate infusion linearly increased the relative concentration of rumen acetate (P < 0.001) before feeding, but not after feeding. Acetate linearly increased plasma β-hydroxybutyric acid by 29%, 50%, and 78%, respectively, after feeding compared with the control treatment (P < 0.01).

Conclusions: Increasing acetate supply to lactating cows increases milk fat synthesis, suggesting that nutritional strategies that increase ruminal acetate absorption would be expected to increase milk fat by increasing de novo FA synthesis.

Keywords: acetate, milk fat synthesis, mammary lipogenesis, short-chain fatty acids, nutrient partitioning

Introduction

Milk fat is the major energy component of milk and is important to growth and satiety in nursing mammals and also to the efficiency and economics of dairy production. There is a broad range in milk fat concentration between mammals (e.g., in horses, ~1.9%; in cows and humans, ~3.8%; in rabbits, ~18%; and in seals, ~50%), and there are also drastic differences in mammary physiology, with some species predominantly using preformed FAs from the diet and tissue stores and others relying heavily on de novo synthesis (1). In the well-fed cow, approximately one-half of the milk fat originates from de novo synthesis in the mammary gland and one-half originates from preformed FAs taken up from plasma (2).

Milk fat concentration is the most variable component of milk in dairy cows, and it is affected by many factors, including season, stage of lactation, and genetics, but the largest impact is from diet composition and management (3). The most common example of dietary regulation of milk fat synthesis in the dairy cow is biohydrogenation-induced milk fat depression (MFD)6. During biohydrogenation-induced MFD, bioactive FAs derived from rumen biohydrogenation of dietary linoleic acid directly reduce de novo lipogenesis in the mammary gland, resulting in up to a 50% decrease in milk fat yield, with the greatest

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4 Supplemental Tables 1–6 are available from the “Online Supporting Material” link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.
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7 Abbreviations used: ACC, acetyl CoA carboxylase; BHB, β-hydroxybutyric acid; GPR, G protein–coupled receptor; MFD, milk fat depression; NEL, net energy of lactation.

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reduction occurring in de novo synthesized FAs. Although drastic changes in lipid metabolism occur, there is no change in milk or milk protein yield (3).

The main substrate for de novo synthesis of FAs in the dairy cow is acetyl-CoA originating from acetate. Acetate provides the majority of the carbon and approximately one-half of the reducing equivalents (NADPH) needed for de novo lipogenesis through the isocitrate pathway (isocitrate dehydrogenase), with the remaining NADPH coming from glucose metabolism through the pentose phosphate pathway (4). Also, close to 70% of the cow’s energy requirement is met through SCFAs (<5 carbons), and acetate represents ~60% of total SCFAs absorbed and 45% of the energy arising from SCFAs metabolism (5). Indeed, acetate availability is fundamental for meeting energy requirements and for milk fat synthesis in the dairy cow.

A meta-analysis based on papers published >30 y ago (1955–1978) reported that acetate infusions linearly increased milk fat yield and concentration (6). However, the experiments in the meta-analysis included very little animal replication, used lower-producing cows (14.3 ± 3.2 kg/d), and fed diets that were very different from contemporary rations, therefore limiting the robustness of the conclusions that could be drawn. Other short- and long-term studies reported variable responses in milk fat yield and concentration, and the dose-response relation is not clear. Briefly, Sheperd and Combs (7) observed a 24% increase in milk fat yield (280 g) and a 20% increase in milk fat concentration (3.41–4.08%) when they ruminally infused 2162 g neutralized acetate/d for 21 d. More recently, we observed a 20% increase in milk fat yield (177 g/d) when we provided 424 g acetate/d for 4 d (NL Urrutia, M Baldin, Y Ying, KJ Harvatine, unpublished data, 2015). However, Maxim et al. (8) did not observe increased milk fat yield when they ruminally infused 1500 g acetate/d.

Acetate is also the most abundant SCFA produced by colonic and cecum microbiota in hindgut fermenters, in which SCFAs represent a modest energy source [≤9%, 30%, and 38% of the maintenance energy requirement for humans, pigs, and rabbits, respectively (5, 9)]. An increasing interest in the microbiome and the interplay between fermentation products (SCFAs) and host metabolism provide additional interest in understanding the effect of acetate on fat synthesis. In addition, SCFAs have been demonstrated to have bioactive properties because they have the ability to modify gene expression and cellular physiology (10, 11). To our knowledge, little data exist on the effect of acetate on metabolic regulation in the cow; however, Jacobs et al. (12) recently reported that acetate increased the expression of lipogenic enzymes [stearyl CoA desaturase 1 and acetyl CoA carboxylase (ACC)] in a bovine mammary cell line (MAC-T). This suggests that acetate may stimulate lipid synthesis by increasing the expression of lipogenic enzymes and it provides a mechanism beyond simply substrate supply for FA synthesis.

Taken together, this evidence supports an important role of acetate as a substrate, as a lipid synthesis regulator and in determining the level of milk fat synthesis, which has been largely ignored in basic and applied research over the past 3 decades. The objective of this experiment was to investigate the effect of increasing acetate supply on milk fat synthesis in dairy cows. Importantly, the mammary gland of the dairy cow has a high rate of lipid synthesis that can be directly quantified and provides a robust model to investigate the effect of specific nutrients on lipid metabolism. To our knowledge, this work is novel in extending our understanding of dietary regulation of milk fat synthesis beyond biohydrogenation-induced MFD, challenges the current dogma that acetate supply does not affect milk fat yield, and provides insights into the effect of SCFAs on lipid synthesis.

**Methods**

**Experimental design and treatments.** All experimental procedures were approved by the Pennsylvania State University Institutional Animal Care and Use Committee (no. 41727). Six ruminally cannulated multiparous Holstein cows (168 ± 106 d postpartum) were randomly assigned to 1 of 6 treatment sequences in a replicated design (Supplemental Table 1). Cows were housed in a tie-stall barn located at the Pennsylvania State University Dairy Production Research and Teaching Center. Experimental periods were 11 d in length and included 4 d of treatment and a 7-d washout. Treatments were 0 (control) and 5, 10, and 15 mol ruminally infused acetate/d.

A stock solution of acetate (3 M) was prepared by diluting glacial acetic acid in distilled water followed by adjustment to pH 6.1 with the use of sodium hydroxide (JT Baker). A stock solution of SSC (3 M) was prepared in distilled water. Treatments were prepared by mixing the stock solutions to achieve the following concentrations: 0 M acetate and 3 M SSC for the control treatment, 1 M acetate and 2 M SSC for 5 mol/d, 2 M acetate and 1 M SSC for 10 mol/d, and 3 M acetate and 0 M SSC for 15 mol/d.

Five liters of treatment solutions were infused for 22 h/d through the rumen cannula with the use of acid-resistant tubing (Norpore L/S 14; Cole-Parmer) and peristaltic pumps (Masterflex L/S drive 7520–35; Cole-Parmer) in a manner similar to that in the study by Sheperd and Combs (7). Pumps were turned off and lines detached for 1 h 2 times/d to allow milking in the parlor (0500 and 1700 h).

**Feed sampling and analysis.** Cows were fed individually 1 time/d (0800) at 110% of expected intake, and intake was recorded daily and feed analysis conducted as described by Rico and Harvatine (13) (Supplemental Tables 2 and 3).

**Milk sampling and analysis.** Cows were milked 2 times/d at 0500 and 1700, and milk yield was determined by an integrated milk meter (A&Milk; SAE Afkim). The parlor milk weights were calibrated weekly with the use of a stall deviation calculated with data from the entire herd (200 cows) over 7 d. Stall deviations were determined by modeling the effect of day, milking (0500 or 1700), cow, and stall, excluding observations of experimental cows during treatment periods. Milk was sampled daily during the treatment periods. One milk subsample was stored at 4°C with preservative (Bromolab-WII; D&S Control Systems) until analysis for fat and protein concentration by Fourier transform infrared spectroscopy [Fossomatic 4000 Milko-Scan and 400 Fossomatic; Foss Electric (2000); Dairy One].

FA composition was determined on a second sample by GC as described by Rico and Harvatine (13) for the characterization of FA by source [<16-carbon are de novo synthesized, >16-carbon are taken up from plasma (preformed), and 16-carbon are of mixed origin (de novo and preformed)] and trans intermediates concentration, which indicates changes in rumen fermentation.

**Blood sampling and analysis.** Key plasma metabolites and hormones were analyzed to provide insight into changes in whole cow metabolism and energy balance. Blood samples were collected from the tail vein at 0600 and 1800 on day 4 to represent before feeding and the maximal intake period of the day. Sampling and analysis was conducted as described by Niu et al. (14) with slight modification. Briefly, blood was collected in potassium EDTA-coated tubes, and harvested plasma samples were analyzed for insulin (Mercodia Bovine Insulin ELISA; Mercodia), glucose, glucagon [RIA kit catalog no. GL-32K; EMD Millipore Corporation, as in the study by Bradford and Allen (15)], nonesterified FAs, β-hydroxybutyric acid (BHB) (procedure no. 2440; Stanbio Laboratory), and plasma TGs (L-Type TG M; Wako Chemicals USA).

**Rumen sampling and analysis.** Rumen SCFAs and pH were determined to characterize the effect of acetate infusions on rumen environment and acid load. Rumen samples were collected at 0600 and 1800 on days 3 and 4 to represent before feeding and near maximal rumen fill and...
were analyzed for SCFA concentration as described by Ying et al. (16) and acidity with the use of a pH meter (model M90; Corning).

**Apparent mass and energy transfer to milk fat.** The transfer of infused acetate to milk was evaluated with the use of 2 approaches. In the first approach, the apparent transfer of acetate mass (grams) to milk fat was calculated as the response in milk fat yield (grams increase) for each treatment with respect to the control treatment divided by grams of acetate infused (0, 300, 600, and 900 g acetate/d). In the second approach, the effect of acetate infusions on milk energy content was estimated with the use of the following equation that describes energy requirements for milk synthesis in dairy cows as net energy of lactation (NEL) in megacalories per day (17):

\[
\text{NEL} = \text{Milk yield}(\text{kg/d}) \times [((0.0929 \times \text{fat\%}) + (0.0547 \times \text{protein\%}) + 0.192]
\]

**Statistical analysis.** Variables that were only observed on 1 d, including rumen SCFA concentration and relative concentration, rumen pH, and plasma hormones and metabolites, were analyzed with the use of Restricted Maximum Likelihood and the fit model procedure of JMP Pro 10.0.2 (SAS Institute). The model included the random effect of cow and period and the fixed effect of treatment. When observations were made at 2 time points (e.g., from plasma collected at 0600 and 1800), data were analyzed within the time period because a time effect was expected. Preplanned polynomial contrasts were used to determine the linear and quadratic effects of acetate dose. Intake and milk production were observed each day, and the temporal response was of mechanistic interest. This time course data were analyzed with the use of the repeated-measures statement of PROC MIXED in SAS (version 9.3). The model included the random effects of cow and period and the fixed effects of day 0 as a covariate, treatment, time, and the interaction of treatment and time. The Compound Symmetry covariance structure was used, time was the repeated variable, and cow by treatment was the subject. Denominator degrees of freedom were adjusted by the Kenward-Rogers method. In the time-course analysis, preplanned contrasts were used to compare each treatment against the control treatment at each time point. Data points with Studentized residuals outside of ±3.5 were considered to be outliers and were excluded from analysis. Significance and tendencies were declared at *P* < 0.05 and *P* < 0.10, respectively, with the control treatment.

**Results**

**Performance.** The short-term infusion of acetate did not affect dry matter intake (Table 1), but increased milk yield in the 10 and 15 mol/d treatments on the last day of infusion compared with the control treatment by 3.0 and 2.5 kg, respectively (*P* < 0.05) (Figure 1).

Milk fat yield quadratically increased with increasing acetate dose (*P* < 0.01), and was 7%, 16%, and 14% higher with 5, 10, and 15 mol acetate/d, respectively, than with the control treatment. An increase in milk fat yield was detected on the first day of the infusion period (*P* < 0.05) (Figure 1) and was maintained for the duration of the 4-d treatment at the 2 highest amounts of acetate (10 and 15 mol/d). Milk fat concentration was linearly increased by acetate infusion, with a 6%, 9%, and 11% increase with 5, 10, and 15 mol acetate/d compared with the control treatment (*P* < 0.001) (Table 1). An increase in milk fat concentration was also detected in the treatment with 10 mol/d beginning on day 1 of treatment (*P* < 0.05) and in the treatment with 15 mol/d beginning on day 2 of treatment (*P* < 0.05); this continued to increase through day 3 before an increase in milk yield occurred (Figure 1).

**Milk FA profile.** The yield of milk de novo synthesized FAs (<16-carbon) was quadratically increased by acetate infusion, with a maximal increase of 57 g/d with 10 mol acetate/d (*P* = 0.01). The yield and concentration of 16-carbon FAs that originated from both de novo synthesis and uptake from plasma increased linearly by acetate (*P* < 0.001), and the concentration of preformed FAs quadratically decreased by acetate dose (*P* = 0.01) (Table 2).

**Apparent mass and energy transfer to milk fat.** Acetate infusions provided 0, 300, 600, and 900 g acetate/d in the 0, 5, 10, and 15 mol/d treatments, and milk fat yield increased by 100, 217, and 185 g/d in the 5, 10, and 15 mol/d treatments, compared with the control treatment (Table 1). Therefore, the apparent transfer of acetate mass to milk fat was 33.4%, 36.2%, and 20.6% with 5, 10, and 15 mol acetate/d, respectively. Total energy intake increased linearly from acetate dose, mainly through energy provided by the infusion (Table 3). Milk energy (NEL) also increased quadratically with acetate dose (*P* < 0.01), mainly because of increased milk fat yield, but also because of a numeric increase in milk yield.

**Rumen pH and SCFA concentration and profile.** Acetate infusions linearly increased the concentration of rumen acetate relative to total SCFAs before feeding (0600, ≤10% increase in

### TABLE 1  Effect of acetate infusion on intake, milk yield, and milk composition in lactating dairy cows1

<table>
<thead>
<tr>
<th>Acetate infusion, mol/d</th>
<th>Variable</th>
<th>Trt</th>
<th>Time</th>
<th>Trt × time</th>
<th>L</th>
<th>Q</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intake, kg/d</td>
<td>0</td>
<td>5</td>
<td>10</td>
<td>15</td>
<td>SE</td>
</tr>
<tr>
<td></td>
<td>26.6</td>
<td>26.5</td>
<td>27.3</td>
<td>26.0</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Milk yield, kg/d</td>
<td>37.7</td>
<td>38.2</td>
<td>39.2</td>
<td>38.2</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>Milk fat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yield, kg/d</td>
<td>1.37</td>
<td>1.47</td>
<td>1.59</td>
<td>1.55</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>Percentage</td>
<td>3.71</td>
<td>3.94</td>
<td>4.05</td>
<td>4.12</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>Milk protein</td>
<td>1.16</td>
<td>1.19</td>
<td>1.24</td>
<td>1.19</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>Percentage</td>
<td>3.14</td>
<td>3.17</td>
<td>3.20</td>
<td>3.15</td>
<td>0.05</td>
</tr>
</tbody>
</table>

1 Values are least-squares means and mean SEs, n = 6. L, linear; Q, quadratic; Trt, treatment.

2 Treatments included 4 d of continuous rumen infusion of acetate adjusted to pH 6.1.

3 Probability of a fixed effect of treatment, time, and treatment × time interaction and probability of a linear or quadratic effect of acetate dose with the use of polynomial contrasts.
The relative concentration of rumen butyrate decreased linearly before feeding (P = 0.02) (Figure 2A), and rumen propionate decreased linearly before and after feeding (P < 0.001 and P = 0.01, respectively) (Figure 2A and B). The absolute concentration of propionate butyrate was not changed at either time point by acetate infusions (Supplemental Table 4).

Mean rumen pH was 6.0 before and after feeding in the control group, and linearly increased to 6.3 after feeding with increasing acetate (P < 0.01) (Figure 2D).

**Plasma hormones and metabolites.** Plasma concentrations of nonesterified FAs, glucose, insulin, glucagon, and TGs were not affected by acetate infusion either before or after feeding (Table 4). Plasma BHB linearly increased after feeding (1800; 29%, 50%, and 78% increase at 5, 10, and 15 mol acetate/d compared with the control treatment, respectively; P < 0.01) (Table 4).

**Discussion**

Acetate is the major end product of rumen fermentation in the cow and it represents ~55% of net energy absorbed as SCFAs (18). It is well established that acetate is the major carbon source for lipid synthesis in ruminant adipose and mammary tissue (4, 19); therefore, it is logical that acetate supply could affect milk fat yield in lactating cows.

Limitations in acetate supply were proposed as a mechanism of classic diet-induced MFD, which is a reduction in milk fat yield often observed when feeding highly fermentable diets that increase propionate production (3). However, acetate deficiency theories were not supported by mechanistic investigations, and bioactive trans FAs produced in the rumen during altered rumen biohydrogenation of unsaturated FAs were identified as the causative factor of MFD (3). During biohydrogenation-induced MFD, there is an increase in rumen and milk FA alternate biohydrogenation intermediates, most clearly observed in trans 10C18:1. Importantly, the diet fed in the present experiment provided adequate fiber and was low in starch and FAs (34% neutral detergent fiber, 26% starch, and 4.1% FAs); therefore, it was a low-risk diet for biohydrogenation-induced MFD. The diet was designed to allow investigation of the effect of acetate

**TABLE 2** Effect of acetate infusion on milk FAs by source in lactating dairy cows

<table>
<thead>
<tr>
<th>Acetate infusion, mol/d</th>
<th>Yield, g/d</th>
<th>p&lt;sup&gt;2&lt;/sup&gt;</th>
<th>SE</th>
<th>Trt</th>
<th>L</th>
<th>Q</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>307</td>
<td>&lt;0.001</td>
<td>14</td>
<td>&lt;0.001</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>340</td>
<td>&lt;0.001</td>
<td>14</td>
<td>&lt;0.001</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>364</td>
<td>&lt;0.001</td>
<td>20</td>
<td>&lt;0.001</td>
<td>0.22</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>352</td>
<td>0.11</td>
<td>20</td>
<td>0.11</td>
<td>0.04</td>
<td></td>
</tr>
</tbody>
</table>

1 Concentration, g/100 g FA

1 Values are least-squares means and mean SEs, n = 6. Calculated from milk fat yield and milk FA composition. De novo synthesized was calculated as the sum of <16-carbon FAs; mixed source includes de novo and preformed FAs and was calculated as the sum of 16-carbon FAs; and preformed FAs originating from plasma were calculated as >16-carbon FAs (odd and branched FAs were excluded from all categories). L, linear; Q, quadratic; Trt, treatment.

2 Treatments included 4 d continuous rumen infusion of acetate adjusted to pH 6.1.

3 Probability of a treatment effect and of a linear or quadratic effect of acetate dose with the use of polynomial contrasts.
supply during normal milk fat conditions in the absence of inhibitors of mammary lipogenesis. The absence of biohydrogenation-induced MFD is confirmed by low yield and concentration of trans 10C18:1, and no trans 10, cis 12 CLA in milk FAs (Supplemental Tables 5 and 6).

The long-term rumen infusion of acetate (21–28 d; 15–36 mol/d) enhanced milk fat yield and concentration in previous experiments, although variable responses were observed (6, 7, 20). In the current experiment, a clear dose-response relation between acetate supply and milk fat synthesis was observed in which milk fat concentration and yield increased linearly (P < 0.001) and quadratically (P < 0.01), respectively. The apparent transfer of infused acetate to milk fat was maximally efficient at 10 mol acetate/d, with a 36% apparent transfer. The remaining acetate was expected to have been partly utilized for NAD(P)H synthesis or may have been oxidized by other tissues or utilized for lipid synthesis in adipose tissue.

Acetate has a much lower energy value than does milk fat (3.5 compared with 9.3 Mcal/kg), and apparent transfer of energy in acetate to milk was 148%, 138%, and 61% for 5, 10, and 15 mol acetate/d, respectively (calculated from Table 3). Although nonsignificant numeric increases in dietary energy (P = 0.33; Table 3) might account for some of the increase in milk energy, acetate infusion was very efficient in increasing milk energy. The change in milk energy was higher than the energy provided in acetate infusion, which may indicate that acetate acted as a bioactive nutrient to partition more energy toward the mammary gland. In vivo isotope label experiments in the cow have shown that mammary de novo synthesis accounts for 50% of milk FAs (2, 24), and butyrate provides 30% of the first 4 carbons for de novo FA synthesis, whereas acetate provides the vast majority of the remaining carbons needed. In the current experiment, the yield of all de novo FAs significantly increased linearly or quadratically with acetate dose (Supplemental Table 5), and palmitic and myristic acid showed the greatest yield response (30% increase in palmitic acid and 18% increase in myristic acid with 15 mol acetate/d). Moreover, the milk concentration of palmitic acid increased linearly with acetate dose (linear, P < 0.001) (Supplemental Table 6), reinforcing the role of the supply of acetate, butyrate, and their active metabolites (acytely-CoA, BHB, and butyryl-CoA) on de novo milk FA synthesis.

SCFAs are known to interact with G protein–coupled receptors (GPRs), which mediate changes in cell metabolism. In humans and rodents, these receptors are highly expressed in adipose tissue, but they are also found in other tissues, including the intestine, pancreas, liver, muscle, and lymph nodes, among others (10, 11). The physiologic effects of SCFAs are only partially characterized, but ongoing work in other animal models and humans indicate that there are potential effects of SCFAs on regulation of satiety, insulin sensitivity, adipose and liver lipogenesis, and adipose lipolysis through GPRs (10, 11, 25).
adipose tissue, the liver, muscle, and mammary tissue (26, 27). Although the expression of GPR43 in the bovine mammary gland over lactation has not been characterized, in subcutaneous adipose tissue and the liver, it is reduced as lactation progresses (26). GPR41 is highly expressed in retroperitoneal adipose tissue, and it is also expressed in other visceral and subcutaneous adipose depots, but it was not detected in the mammary gland, liver, or muscle of dairy cows (26). Interestingly, the expression of GPR41 was upregulated in adipose tissue after initiation of lactation (28).

The concentration of butyrate in the blood and its supply to peripheral tissues is low because it is converted to BHB in the rumen wall and liver. In peripheral tissues, BHB interacts with another GPR, GPR109A. This receptor is an important metabolic sensor that has been shown to suppress lipolysis in adipose tissue during starvation (29). In the cow, GPR109A is abundantly expressed in adipose tissue, in which its expression was reduced as lactation progressed; it is also expressed in the liver, in which its expression increased as lactation progressed (26, 28, 29). The role of GPRs in the regulation of milk fat synthesis has not been investigated, but they provide mechanisms for acetate and butyrate to modify nutrient partitioning and mammary physiology.

Lastly, ACC $\alpha$ is the first rate-limiting step in de novo lipogenesis, and it is regulated at multiple levels, including covalently, transcriptionally, and allosterically. Citrate is an important allosteric regulator because it improves the enzymatic activity of ACC by inducing polymerization to the active filament form (30). Citrate is made in the tricarboxylic acid cycle from acetyl-CoA and oxaloacetate, and it can freely cross the mitochondrial membrane (31). Therefore, it is possible that increasing the supply of acetate enhanced ACC activity through surplus intracellular citrate.

### TABLE 4

<table>
<thead>
<tr>
<th>Variable</th>
<th>Acetate infusion, $^2$ mol/d</th>
<th>$^3$</th>
<th>$^4$</th>
<th>$^5$</th>
<th>$^6$</th>
<th>$^7$</th>
<th>$^8$</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEFA, $\mu$Eq/L</td>
<td>0600 0 5 10 15 Trt L Q</td>
<td>0.76</td>
<td>0.72</td>
<td>0.35</td>
<td>0.24</td>
<td>0.83</td>
<td>0.05</td>
</tr>
<tr>
<td>Glucose, mg/dL</td>
<td>0600 0 5 10 15 Trt L Q</td>
<td>0.26</td>
<td>0.59</td>
<td>0.37</td>
<td>0.87</td>
<td>0.55</td>
<td>0.58</td>
</tr>
<tr>
<td>BHB, $\mu$M</td>
<td>0600 0 5 10 15 Trt L Q</td>
<td>0.32</td>
<td>0.59</td>
<td>0.37</td>
<td>0.07</td>
<td>0.01</td>
<td>0.98</td>
</tr>
<tr>
<td>Insulin, ug/L</td>
<td>0600 0 5 10 15 Trt L Q</td>
<td>0.36</td>
<td>0.39</td>
<td>0.16</td>
<td>0.43</td>
<td>0.15</td>
<td>0.94</td>
</tr>
<tr>
<td>Glucagon, pg/mL</td>
<td>0600 0 5 10 15 Trt L Q</td>
<td>0.23</td>
<td>0.33</td>
<td>0.12</td>
<td>0.23</td>
<td>0.33</td>
<td>0.12</td>
</tr>
<tr>
<td>TGs, mg/dL</td>
<td>0600 0 5 10 15 Trt L Q</td>
<td>0.92</td>
<td>0.22</td>
<td>0.67</td>
<td>0.02</td>
<td>0.01</td>
<td>0.25</td>
</tr>
</tbody>
</table>

$^1$ Values are least-squares means and mean SEs, $n = 6$. BHB, $\beta$-hydroxybutyric acid; L, linear; NEFA, nonesterified FA; Q, quadratic; Trt, treatment.

$^2$ Treatments included 4 d of continuous rumen infusion of 0 (control), 5, 10, or 15 mol acetate/d adjusted to pH 6.1 ($n = 6$). Rumen SCFA profile before feeding (0600) (A), rumen SCFA profile during the high intake period of the day (1800) (B), total rumen concentration of SCFAs at 0600 and 1800 (C), and rumen pH at 0600 and 1800 (D). Values are least-squares means ± SEs. *Linear effect of acetate dose on the variable, with the $P$ value for the linear effect shown above the bar (polynomial contrasts).
although investigation of this mechanism was beyond the scope of the current experiment.

In conclusion, increasing acetate supply improves milk and milk fat synthesis in dairy cows. The increase in milk fat synthesis is predominantly through an increase in yield and concentration of 16-carbon FAs, whereas the synthesis of de novo synthesized <16-carbon FAs was increased to a lesser extent. Milk yield was improved only on the last day of treatments; therefore, the effect of acetate supply on milk synthesis requires further investigation. Possible mechanisms include mass action kinetic effects of increased availability of acetate for mammary gland uptake and milk fat synthesis; greater production of BHB in rumen epithelial cells, which is a carbon source for the initiation of lipogenesis; and changes in metabolism through nutrient-sensing receptors that are ligands for SCFAs. This experiment extends our understanding of dietary regulation of milk fat synthesis, demonstrates a role for acetate in mammary lipid synthesis regulation, and provides new insights into the effect of SCFAs on mammary physiology.

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