J. Dairy Sci. 97:975–984
http://dx.doi.org/10.3168/jds.2013-7164

Addition of potassium carbonate to continuous cultures of mixed ruminal bacteria shifts volatile fatty acids and daily production of biohydrogenation intermediates

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ABSTRACT

A recent study reported a 0.4 percentage unit increase in milk fat of lactating dairy cattle when dietary K was increased from 1.2 to 2% with potassium carbonate. Because milk fat yield has been associated with ruminal production of certain conjugated linoleic acid (CLA) isomers, 2 studies were conducted to determine if increasing potassium carbonate in the rumen would alter patterns of fermentation and biohydrogenation. In experiment 1, 5 dual-flow continuous fermenters were injected just before each feeding with a 10% (wt/wt) stock potassium carbonate solution to provide the equivalent of 1.1 (K1), 2.2 (K2), and 3.3 (K3) % of diet dry matter (DM) as added K. One of the remaining fermenters received no K (K0) and the last fermenter (NaOH) was injected with adequate NaOH stock solution (10%, wt/wt) to match the pH observed for the K3 treatment. For experiment 2, 6 dual-flow continuous fermenters were used to evaluate 6 treatments arranged in a 2 × 3 factorial to examine 2 levels of soybean oil (0 and 3.64% of diet DM) and added K at 0, 1.6, and 3.3% of diet DM. In both experiments, fermenters were fed 55 to 57 g of DM/d of a typical dairy diet consisting of 1:1 forage (10% alfalfa hay and 90% corn silage) to concentrate mix in 2 equal portions at 0800 and 1630 h, and fed the respective diets for 10-d periods. Potassium carbonate addition increased pH in both experiments. Acetate:propionate ratio and pH in experiment 1 increased linearly for K0 to K3. Acetate:propionate ratio was lower for NaOH compared with K3 but the pH was the same. The *trans*-11 18:1 and *cis*-9,*trans*-11 CLA production rates (mg/d) increased linearly from K0 to K3, but K3 and NaOH did not differ. Production of *trans*-10 18:1 decreased and that of *trans*-10,*cis*-12 tended to decrease from K0 to K3, but production of *trans*-10,*cis*-12 CLA remained high for NaOH. Addition of K to the cultures in experiment 2 decreased propionate and increased acetate and acetate:propionate ratio for the 0% fat diet but not for the 3.64% fat diet. Addition of K increased stearic acid and *cis*-9,*trans*-11 CLA but decreased daily production of *trans*-10 C18:1 and *trans*-10,*cis*-12 CLA. The results indicate that increasing potassium carbonate in the diet shifts both fermentation and biohydrogenation pathways toward higher milk fat percentage in dairy cows, but the effects are only explained in part by elevation of pH.
Key words: potassium carbonate, biohydrogenation, mixed ruminal bacteria, continuous culture

INTRODUCTION

Diet-induced milk fat depression (**MFD**) continues to have a major economic impact in the dairy industry and finding solutions for MFD remains a priority. The biohydrogenation theory links MFD with the formation of several active conjugated linoleic acid (**CLA**) isomers produced by the rumen microbial population, including *trans*-10,*cis*-12 CLA (Baumgard et al., 2000), *trans*-9,*cis*-11 CLA (Perfield et al., 2007), and *cis*-10,*trans*-12 CLA (Saebø et al., 2005). These 3 isomers, and many others, arise from the biohydrogenation of linoleic (Lee and Jenkins, 2011a) and linolenic acids (Lee and Jenkins, 2011b). Formation of the CLA isomers that cause MFD has been associated with several dietary risk factors, including excessive fat intake and low rumen pH. Solutions to solving MFD are complicated by interactions that often exist among two or more risk factors, making the process of reversing MFD slow and frustrating.

Some studies examining the effects of DCAD on lactation performance have reported improvements in milk fat. For example, in a study by Hu et al. (2007), increasing DCAD from a combination of calcium chloride, sodium bicarbonate, and potassium carbonate increased milk fat up to 0.77 percentage units in dairy cows. A more recent study (Harrison et al., 2012) increased DCAD using only potassium carbonate ses-

Received June 20, 2013.
Accepted November 2, 2013.
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quihydrate and increased milk fat 0.4 percentage units (from 4.0 to 4.4%).

The objective of this study was to determine if the improvement in milk fat percentage reported previously might have been caused by potassium carbonate influencing the type of CLA produced in the rumen. An initial experiment was done in continuous culture to establish whether CLA production was altered when the cultures were exposed to an increasing dosage of potassium carbonate and also to evaluate the possible influence of pH. After observing a CLA shift resulting from potassium carbonate addition in the first experiment, we conducted a second experiment to determine if the CLA changes caused by potassium carbonate were dependent on fat concentration in the diet.

MATERIALS AND METHODS

Experiment 1

Treatments consisted of 4 dosage levels of a 10% K_2CO_3 (wt/wt) stock solution (0, 10.6, 21.2, and 32 mL) injected directly into the fermenters twice daily immediately after each feeding. Distilled water was also injected (32, 21.4, 10.8, and 0 mL, respectively) to maintain a total injection volume of 32 mL/d for all treatments. The K was supplied at 0, 0.6, 1.2, and 1.8 g/d or 0 (K0), 1.1 (K1), 2.2 (K2), or 3.3% (K3) of the daily feed DM. Because aqueous solutions of K_2CO_3 are strongly alkaline, pH was expected to increase with increasing dosage of K_2CO_3. To determine if any changes in biohydrogenation and fermentation could be attributed to effects on pH, a fifth treatment (NaOH) consisted of injection of sufficient 10% NaOH (wt/wt) each day to match the pH of K3.

Each treatment was randomly assigned to 1 of 5 continuous fermenters and run for a 10-d period with 7 d for adaptation and 3 d for sample collection. Four periods were run for each treatment. Each period began with a clean fermenter inoculated with fresh ruminal contents from a fistulated cow. A total of 54.5 g of diet DM (Table 1) was fed to each fermenter daily in 2 equal portions at 0800 and 1630 h.

Experiment 2

Six treatments were examined in experiment 2 arranged as a 2 × 3 factorial with 2 levels of added soybean oil and 3 levels of added K. Fat levels were no added fat (low fat; **LF**) or 3.64% of DM (high fat; **HF**) to bracket the intermediate fat level (2.19%) used in experiment 1. Potassium was introduced by injection of a 10% K_2CO_3 (wt/wt) stock solution (0, 16, and 32 mL/d) directly into the fermenters twice daily immediately after each feeding. Distilled water was also injected (32, 16, and 0 mL/d, respectively) to maintain

Table 1. Diet composition and nutrient inputs into fermenters in experiments 1 and 2

		Experiment 2[1]	
Item	Experiment 1	LF	HF
Ingredient, % of DM			
Corn silage	45.3	45.2	45.1
Alfalfa hay	5.2	5.1	5.1
Ground corn	19.1	21.1	19.6
Soybean oil	2.19	0.0	3.64
Soybean meal	11.9	12.1	11.2
Soybean hulls	12.9	13.1	12.2
Calcium phosphate	1.33	1.37	1.27
Limestone	0.64	0.65	0.61
Trace mineral salt	0.61	0.62	0.58
Sodium bicarbonate	0.81	0.81	0.75
Nutrient input per fermenter, g/d			
DM	54.6	54.6	56.9
CP	8.6	8.9	9.1
K	0.59	0.61	0.61
Ca	0.45	0.47	0.47
P	0.29	0.31	0.30
FA, mg/d	2.42	1.64	3.75
16:0	312	219	439
18:0	83	51	154
18:1	477	316	801
18:2	1,185	714	1,793
18:3	158	88	223
Total	2,427	1,637	3,750

[1]Nutrients supplied by the low-fat (LF) and high-fat (HF) diets in experiment 2 were equalized by adjusting diet composition and amounts fed to provide the same nutrient input (except FA) into each fermenter per day.

a total injection volume of 32 mL/d for all treatments. The K was added at 0, 0.9, and 1.8 g/d or 0 (K0), 1.6 (K1.5), or 3.3% (K3) of feed DM. A total of 54.6 and 56.9 g of DM were fed for the LF and HF diets (Table 1), respectively, in 2 equal portions at 0800 and 1630 h. The HF composition and amount fed was calculated so that soybean oil was supplied without changing the daily input of basal nutrients.

Each treatment was randomly assigned to 1 of 6 continuous fermenters and run for a 10-d period with 7 d for adaptation and 3 d for sample collection. Four periods were run for each treatment. Each period began with a clean fermenter inoculated with fresh ruminal contents from a fistulated cow.

Continuous Culture Conditions

Whole ruminal contents were collected from a ruminally fistulated Holstein cow 2 h after being fed a 50% forage:50% concentrate diet. All surgical and animal care protocols were approved by the Clemson University Animal Care and Use Committee. Within 20 min of collection, large particles were removed from the whole ruminal contents by filtration through 2 layers of cheesecloth, and the filtrate containing the microbial population was transferred immediately to the laboratory in a sealed container. With constant stirring, the filtered ruminal inoculum was diluted 1:1 with buffer (Slyter et al., 1966) and then added to completely fill (approximately 800 mL) each dual-flow continuous culture fermenter, which was modified in construction and operation from the design described by Teather and Sauer (1988). The main modifications were a reconfigured overflow sidearm that angled downward at approximately 45° to facilitate emptying; a faster stirring rate (45 rpm) that still allowed stratification of particles into an upper mat, a middle liquid layer of small feed particles, and a lower layer of dense particles; and a higher feeding rate (60 g/d). The culture was maintained for 10 d (7 d for adaptation and the last 3 d for sampling).

A buffer solution (Slyter et al., 1966) was delivered continuously using a peristaltic pump to achieve a 0.10/h fractional dilution rate. Buffer delivered to all fermenters was adjusted each day with sufficient 6 *N* NaOH or 3 *N* HCl so that the a.m. pH for the control diet was maintained at approximately 6.5. The pH was then allowed to fluctuate on its own as treatment or time after feeding permitted. The fermenter was continuously gassed with CO_2 at a rate of 20 mL/min to maintain anaerobic conditions. The temperature of the fermenter was held at 39°C by a circulating water bath.

Culture pH was monitored daily by taking pH readings (Hanna Instruments Inc., Woonsocket, RI) just before each feeding. A 4-mL sample of culture contents were taken on d 10 of each period at 0 (before the 0800 feeding), 2, and 4 h after feeding for analysis of VFA. On d 7, 8, and 9 of each period, overflow from each fermentor was collected in a 2-L Erlenmeyer flask kept in a covered ice bath. After recording the total volume, a 20% sample of the overflow was composited over sampling times and immediately frozen. Frozen samples were later thawed and a subsample was lyophilized. Culture contents were thoroughly mixed (155 rpm) before pH readings or sampling. Overflow contents were mixed continuously by using a magnetic stir bar during sampling.

Sample Analysis

Forage, concentrate, and dried overflow samples were ground in a centrifugal mill through a 0.5-mm sieve. Ground samples were analyzed for DM (100°C), Kjeldahl N (AOAC, 1990), NDF (Van Soest et al., 1991), and FA. Culture samples for VFA analysis were pipetted into polycarbonate tubes containing 1 mL of 25% (wt/wt) metaphosphoric acid, mixed, and then centrifuged at 40,000 × *g* for 20 min at 4°C. After centrifugation, 1 mL of the supernatant was combined with 0.1 mL of internal standard (86 μmol of 2-ethylbutyric acid/mL). Samples were injected into a Hewlett-Packard 5890A gas chromatograph (Hewlett-Packard, Palo Alto, CA) equipped with a Nukol fused-silica capillary column (30 m × 0.25 mm) with 0.25-μm film thickness (Supelco Inc., Bellefonte, PA).

Long-chain FA in ground feed and overflow samples were converted to methyl esters by direct transesterification in sodium methoxide and methanolic HCl (Jenkins, 2010). An internal standard (2 mg of heptadecanoic acid) was added at the start of methylation to quantify FA masses. Quantities of individual FA present in the cultures were determined on a Hewlett-Packard 5890A gas chromatograph equipped with a P-2380 fused-silica capillary column (100 m × 0.25 mm) with 0.2-μm film thickness (Supelco Inc.). The conditions used were initially 140°C for 3 min, increasing at 3.7°C/min up to 220°C and holding for 20 min. Helium was used as the carrier gas at 20 cm/s. Identities of peaks were determined by comparison of retention times to known standards.

Statistics

The analysis of response variables from experiments 1 and 2 (except pH and VFA) was based on a model for a randomized block design with repeated measures across periods. The blocks were based on periods and fermenters. In experiment 1, the model included fixed

Table 2. Average VFA and pH by treatment for samples taken from continuous culture flasks on d 10 averaged across all sampling times (0, 2, and 4 h after feeding; experiment 1)

	Treatment[1]					
Item	K0	K1	K2	K3	NaOH	SE
VFA, mol/100 mol						
Acetate[a,b]	48.2	48.7	52.0	52.1	48.7	1.0
Propionate[a,b]	36.2	35.6	32.2	32.9	36.7	1.4
Isobutyrate	0.65	0.73	0.75	0.75	0.80	0.06
Butyrate	8.8	8.9	9.9	8.9	7.9	0.9
Isovalerate	2.1	2.3	1.8	2.0	2.1	0.4
Valerate	4.1	3.8	3.4	3.3	3.9	0.4
Acetate:propionate[a,b]	1.34	1.37	1.66	1.60	1.33	0.09
Total VFA, mmol/L	103.5	95.2	98.4	95.1	95.4	6.0
pH at d 8–10[a]	6.01	6.22	6.25	6.38	6.29	0.12

[a]Linear response of K0 through K3 ($P < 0.05$).

[b]K3 and NaOH differ ($P < 0.05$).

[1]K_2CO_3 injected into culture flasks to provide the equivalent of 0, 1, 2, and 3% added K. The NaOH treatment used injections of NaOH into fermentation flasks to maintain the same pH as the K3 treatment.

effects for treatment (K level and NaOH) and random effects for period and fermenter. In experiment 2, the model included fixed effects for soybean oil, K, and the interaction of soybean oil and K, and the random effects of period and fermenter. The models for pH and VFA data included the terms above as well as fixed effects for subsampling times and time interactions, and the additional random effect treatment and period interaction. Contrasts were used in experiment 1 to determine if responses to levels of added K were linear, quadratic, or cubic in nature. Quadratic and cubic contrasts were not significant for any variable. A contrast was also used to compare the K3 and NaOH treatments. In experiment 2, the soybean oil and K interaction was significant for some of the variables. In these cases, the interaction was further investigated by testing the simple effects of the K means within each fat level. Null hypotheses were rejected and results considered statistically significant when $P \leq 0.05$. All calculations were performed with SAS (version 9.2; SAS Institute Inc., Cary, NC) using Proc Glimmix for generalized mixed models with random effects.

RESULTS

Experiment 1

Average pH values in fermenter contents across d 8 to 10 and across all times after feeding increased linearly ($P \leq 0.05$) from 6.01 (K0) to 6.38 (K3; Table 2). On d 10, the fermenter pH decreased by 2 and 4 h after feeding (Figure 1), but decreased less with higher potassium carbonate addition, resulting in a treatment by time interaction ($P \leq 0.05$). As designed, pH values were not different for K3 and NaOH (Table 1).

Addition of potassium carbonate to the fermenters had no effect on total VFA concentration, but potassium carbonate did cause differences in relative proportions of individual VFA (Table 2). The addition of potassium carbonate caused a linear increase ($P \leq 0.05$) in acetate and a linear decrease ($P \leq 0.05$) in propionate proportions. As a result, we observed a linear decrease ($P \leq 0.05$) in acetate:propionate ratio from K0 to K3. Proportions of isobutyrate, butyrate, isovalerate, and valerate were not affected by treatment.

Daily FA outflows of most FA from the fermenters were not affected by treatment (Table 3). Several FA not regarded as intermediates of 18:2 or 18:3 biohydrogenation included 12:0, 14:0, and 16:1, all of which increased ($P \leq 0.05$) from K0 to K3. Among the biohydrogenation intermediates, addition of potassium carbonate did not change total *trans* 18:1 outflow but did alter the production of some specific *trans* 18:1 isomers. The *trans*-6/8, *trans*-9, and *trans*-12 18:1 isomers were not affected by the potassium carbonate treatments. However, *trans*-11 18:1 outflow increased ($P \leq 0.05$) and *trans*-10 18:1 outflow decreased ($P \leq 0.05$) from K0 to K3. Similarly, K did not affect total CLA production but did alter production of specific isomers. The *cis*-9,*trans*-11 CLA and *trans*-9,*trans*-11 CLA isomers both increased ($P \leq 0.05$) from K0 to K3. Production of *trans*-10,*cis*-12 CLA was not affected by K. However, we observed a tendency ($P = 0.095$) for additional K to reduce *trans*-10,*cis*-12 CLA outflow. The NaOH and K3 treatments only differed ($P \leq 0.05$) for 16:1.

Outflow of 18:0 tended ($P = 0.091$) to change nonlinearly from K0 to K3. Outflow of 18:0 steadily increased from K0 to K2, but then declined for K3. The lowest daily outflow of 18:0 was seen for the NaOH treatment, resulting in a lower ($P \leq 0.05$) outflow of 18:0 for NaOH than for K3.

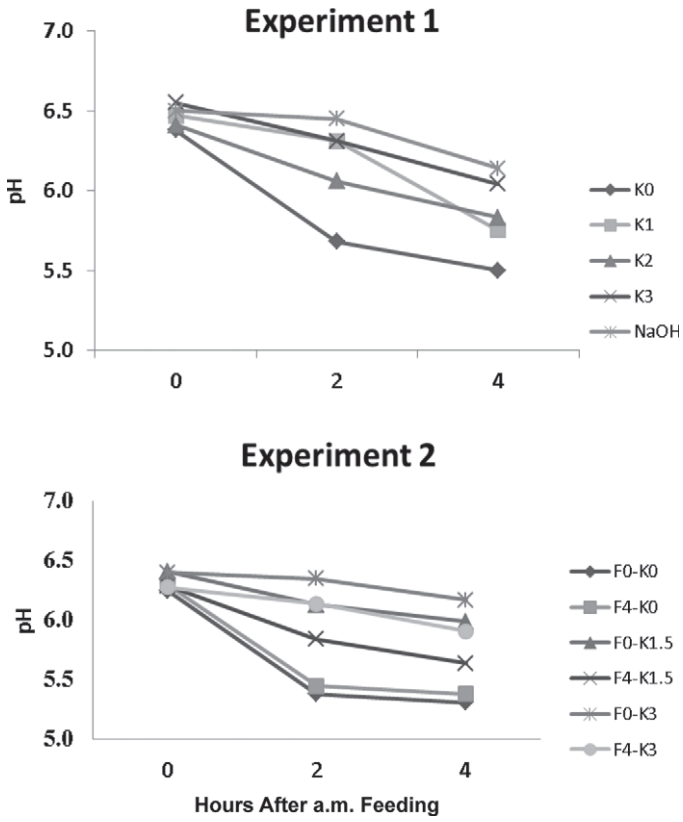

Figure 1. Changes in pH of fermenter contents in experiments 1 and 2 shown by diet and each time after the a.m. feeding on d 10. K0, K1, K2, and K3 = K supplied at 0, 1.1, 2.2, or 3.3% of the daily feed DM, respectively; NaOH = injection of sufficient 10% NaOH (wt/wt) each day to match the K3 pH. F0 and F4 = no added fat and fat added at 3.64% of DM, respectively.

Experiment 2

Culture pH values averaged across d 8 to 10 of each period were not different for HF and LF: addition of potassium carbonate increased ($P \leq 0.05$) pH regardless of fat level. We observed a treatment × time interaction ($P \leq 0.05$) for pH in culture contents on d 10 (Figure 1). The pH values were similar (average 6.5) for all treatments before the a.m. feeding (0 h) on d 10 but pH declined for the K0 treatments at 2 and 4 h postfeeding. Addition of potassium carbonate reduced the decrease in pH at 2 and 4 h after feeding.

Total VFA concentrations were not affected by treatment (Table 4). Fat × K interactions ($P \leq 0.05$) were seen for acetate, propionate, and valerate (Figure 2). Interactions occurred because the addition of potassium carbonate increased ($P \leq 0.05$) acetate and reduced ($P \leq 0.05$) propionate but only for the LF diet. As a result, potassium carbonate addition only changed the acetate:propionate ratio for LF and was higher ($P \leq 0.05$) for K1.5 compared with K0, but K1.5 and K3 did not differ. In the LF diet, valerate was lower ($P \leq 0.05$) for K1.5 and K3 compared with K0 but did not differ between K1.5 and K3. For the HF diet, valerate steadily declined ($P \leq 0.05$) from K0 to K3. Butyrate was lower ($P \leq 0.05$) for HF than for LF, but K addition increased ($P \leq 0.05$) butyrate regardless of dietary fat level.

Outflows of individual FA from the fermenters were the same for LF and HF for FA with <16 carbons (Table 5). Outflows of FA with ≥16 carbons were all higher ($P \leq 0.05$) for HF than for LF except for *cis*-9,*trans*-11 CLA. Total FA outflow was approximately 2 times higher ($P \leq 0.05$) for HF than for LF.

Addition of potassium carbonate had no effect on outflows of the 3 primary 18-carbon unsaturated fatty acid substrates, 18:1, 18:2, and 18:3. Accordingly, we observed no effect of potassium carbonate on outflow of total FA from the fermenters. Intermediates of biohydrogenation, however, were affected by K level. Addition of K increased ($P \leq 0.05$) daily production of *trans*-9 18:1, *trans*-10 18:1, and *trans*-11 18:1 but had no effect on *trans*-6/8 18:1 or *trans*-9 18:1. Overall, K addition increased ($P \leq 0.05$) total *trans* 18:1 outflow but no difference was found between K1.5 and K3. Total CLA outflow was not affected by K level although we did observe a shift among individual isomers. Addition of K increased ($P \leq 0.05$) outflow of *cis*-9,*trans*-11 but decreased ($P \leq 0.05$) outflow of *trans*-10,*cis*-12 CLA. Changes in daily outflows of CLA intermediates were the same for K1.5 and K3.

The only K × fat interaction for any fatty acid was for 18:0. For the LF diet, 18:0 outflow was higher ($P \leq 0.05$) for K1.5 and K3 (423.8 and 478.5 mg/d) than for K0 (87.5 mg/d). The interaction occurred because for diet HF, 18:0 outflow for K0 (291.6 mg/d) was lower ($P \leq 0.05$) than for K1.5 (459.7 mg/d) but the same as that for K3 (414.6 mg/d).

DISCUSSION

This study was prompted by previous results showing that increases in DCAD led to an improvement in milk fat percentage. For example, Wildman et al. (2007) compared the effects on lactation performance of 2 levels of DCAD each at 2 different CP percentages. The 2 DCAD levels were 25 and 50 mEq/100 g and were adjusted using sodium bicarbonate at 0.97% and potassium carbonate at 0.97% of diet DM. Cows that were consuming the diet with increased DCAD level showed an increase in milk fat percentage from 2.44 to 2.92%. In a study by Hu et al. (2007), diets fed to cows compared 3 levels of DCAD (−3, 22, and 47 mEq/100 g) that were adjusted with calcium chloride, sodium bicarbonate, and potassium carbonate. Results showed a positive linear response on milk fat

Table 3. Daily FA output and extents of biohydrogenation from continuous culture flasks injected with potassium carbonate or NaOH solutions (experiment 1)

	Treatment[1]					
Item	K0	K1	K2	K3	NaOH	SE
Outflow,[2] mg/d						
12:0[a]	5.3	7.0	6.6	7.0	6.5	0.6
14:0[a]	15.2	19.1	18.3	18.1	18.2	1.7
14:1	8.9	9.3	8.6	8.6	8.9	1.2
15:0	13.0	14.6	14.3	14.7	16.9	1.4
16:0	248.1	288.0	287.7	289.5	279.6	16.1
16:1[a,b]	2.0	2.9	2.5	3.1	3.4	0.4
18:0[b]	401.6	451.6	535.5	366.1	175.0	99.0
18:1	230.6	275.1	244.2	205.0	260.8	21.8
19:0	0.9	1.0	1.6	1.2	2.3	0.85
18:2	305.5	339.1	274.8	320.7	287.7	32.8
20:0	8.7	10.8	11.0	10.3	9.4	0.87
18:3	32.7	35.6	29.0	33.4	29.5	3.0
22:0	8.6	9.7	9.6	9.4	8.8	0.5
24:0	7.7	8.3	8.3	8.2	7.8	0.4
Biohydrogenation intermediates						
Total *trans* 18:1	537.6	499.8	461.1	538.2	575.8	38.4
trans-6/8 18:1	34.5	35.2	33.7	33.7	26.8	6.1
trans-9 18:1	17.8	18.8	18.6	17.0	13.6	3.0
trans-10 18:1[a]	416.5	317.6	234.7	295.3	355.6	42.8
trans-11 18:1[a]	46.9	106.7	145.5	169.1	165.4	20.7
trans-12 18:1	23.6	26.0	24.7	19.3	16.4	2.9
Total CLA	17.3	21.3	20.1	20.2	25.3	3.3
trans-1,*cis*-12 CLA	11.6	11.3	7.9	7.6	13.2	2.6
cis-9,*trans*-11 CLA[a]	2.3	4.9	7.1	6.8	6.8	1.1
trans-9,*trans*-11 CLA[a]	3.3	5.0	5.3	5.4	5.4	0.77
Total	2,098	2,263	2,222	2,139	2,026	130
Biohydrogenation, outflow as % of input						
18:1	51.5	42.1	43.9	43.7	45.1	5.7
18:2	74.0	71.1	75.5	72.2	75.6	3.4
18:3	79.2	77.3	80.4	78.3	81.2	2.4

[a]Linear response of K0 through K3 ($P < 0.05$).

[b]K3 and NaOH differed ($P < 0.05$).

[1]K_2CO_3 injected into culture flasks to provide the equivalent of 0, 1.1, 2.2, and 3.3% of diet DM as added K. The NaOH treatment used injections of NaOH into fermentation flasks to maintain the same pH as the K3 treatment.

[2]Individual FA abbreviations are denoted as number of carbons:number of double bonds; CLA = conjugated linoleic acid.

Table 4. Main effects of fat and K levels on VFA and pH in samples taken from continuous culture flasks on d 10 (experiment 2)

	Treatment[1]						
	Fat effect			K effect			
Item	LF	HF	SEM	K0	K1.5	K3	SEM
VFA, mol/100 mol							
Acetate[d]	53.3	51.0	2.1	48.6[b]	54.1[a]	53.7[a]	2.2
Propionate[d]	27.6	32.2*	1.5	34.1[a]	28.5[b]	27.2[b]	1.6
Isobutyrate	1.2	1.1	0.14	1.0	1.3	1.2	0.15
Butyrate	12.2*	9.1	0.78	9.2[b]	10.3[ab]	12.4[a]	0.91
Isovalerate	2.3	2.6*	0.37	2.4	2.5	2.4	0.38
Valerate[d]	3.5	3.9	0.28	4.9	3.3	2.9	0.31
Total VFA, mmol/L	72.7	81.6	5.0	78.3	77.8	75.3	5.7
Acetate:propionate[d]	2.05*	1.63	0.15	1.47[b]	1.96[a]	2.10[a]	0.16
pH at d 8–10	6.04	5.92	0.04	5.68[c]	6.05[b]	6.21[a]	0.05

[a–c]Denotes a significant K effect where least squares means with the same letter are not significantly different ($P < 0.05$).

[d]Denotes a significant fat × K interaction ($P < 0.05$).

[1]Treatments consisted of 2 diets containing either 0 (low fat; LF) or 3.64% added soybean oil (high fat; HF). For each diet, sufficient potassium carbonate was injected daily into the fermenter contents to provide an additional 0 (K0), 1.5 (K1.5), or 3% (K3) of the diet as added K.

*Significant fat effect ($P < 0.05$).

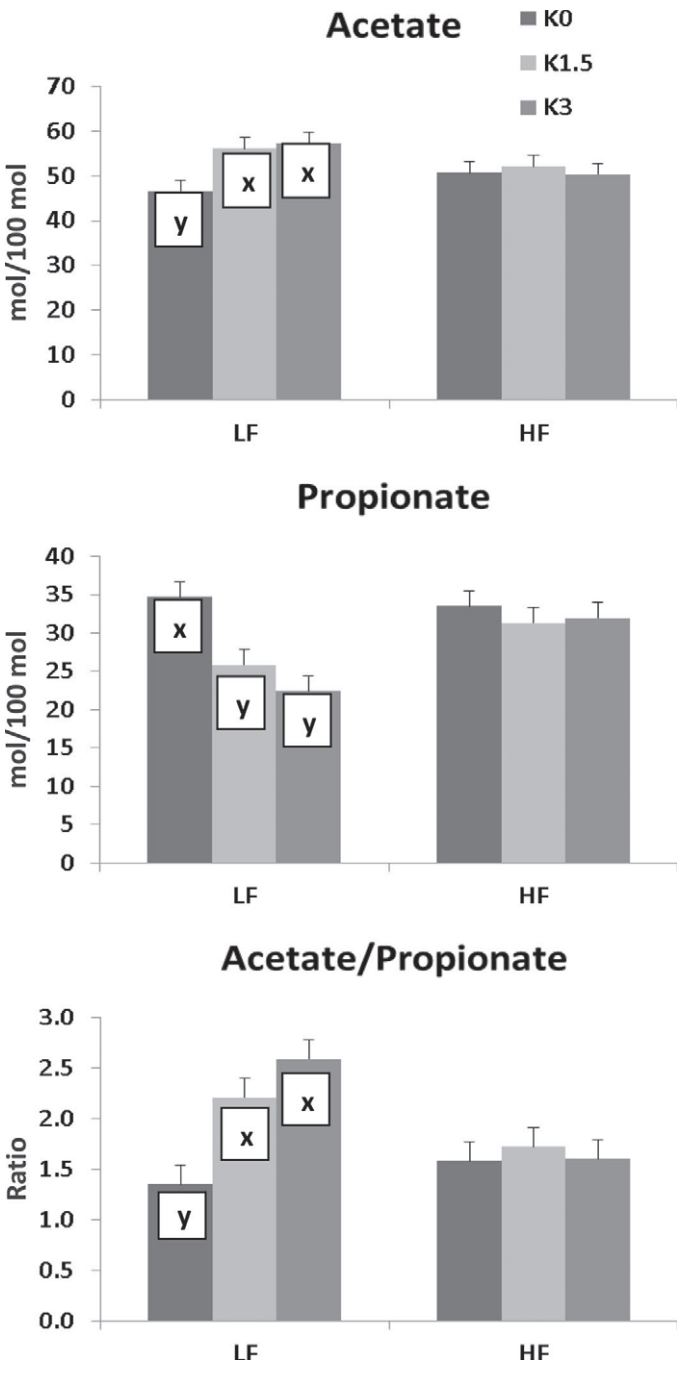

Figure 2. Fat level (low fat, LF, and high fat, HF) by K level interactions for acetate, propionate, and acetate/propionate averaged over time after feeding on d 10. K0, K1.5, and K3, = K supplied at 0, 1.6, or 3.3% of the daily feed DM, respectively. ^{x,y}K means within a fat level with different letters are significantly different ($P < 0.05$).

percentage and milk fat yield with increasing DCAD levels.

Because most DCAD studies fed a combination of mineral supplements, it is difficult to isolate the effects of K. In several studies examining lactation effects of K supplementation alone, the effects on milk fat percentage were variable. Holstein cows past the peak of lactation were fed 0.45, 0.55, and 0.66% of diet DM as KCl with no reported effects on milk yield, milk fat, or solids-not-fat content (Dennis et al., 1976). A recent study by Harrison et al. (2012) reported that additional dietary K fed as potassium carbonate sesquihydrate increased dietary K from 1.3 to 2.1% of diet DM from wk 3 to 12 of lactation and increased milk fat percentage from 4.01 to 4.38%. The positive milk fat response to potassium carbonate in previous studies led to questions about its mechanism of action, particularly if the target site was mainly the rumen or mainly systemic.

A decrease in milk *trans*-10 18:1 from feeding potassium carbonate in the current study suggested a role of the rumen. Lipid biohydrogenation in the rumen normally produces mainly *cis*-9,*trans*-11 CLA and *trans*-11 18:1 intermediates from linoleic and linolenic acids. However, biohydrogenation can deviate from its normal pathway and increase microbial production of alternate CLA that lower milk fat such as *trans*-10,*cis*-12 CLA (Baumgard et al., 2000). Because *trans*-10 18:1 originates from the reduction of *trans*-10,*cis*-12 CLA, it has a negative curvilinear relationship with milk fat yield. This prompted us to determine if potassium carbonate had any ability to shift the pathway of biohydrogenation away from the milk fat–depressing *trans*-10,*cis*-12 CLA intermediate back to the normal *cis*-9,*trans*-11 CLA intermediate and consequently support a higher milk fat percentage.

In this study, results from both experiments supported that additional K in ruminal contents promoted a biohydrogenation shift that was consistent with a higher milk fat percentage. Additional K in both experiments increased *cis*-9,*trans*-11 CLA and *trans*-11 18:1 but reduced outflows of *trans*-10,*cis*-12 CLA and *trans*-10 18:1. Because neither CLA nor *trans* 18:1 isomers were present in the feed, outflows of these biohydrogenation intermediates from the fermenters can be interpreted as daily production rates (in mg/d). Based on these results, increasing the K concentration in ruminal contents appears to downregulate microbial production of CLA isomers that cause MFD and assist in re-establishing biohydrogenation pathways back to normal intermediates. This would serve to explain why cows fed potassium carbonate sesquihydrate in previous studies averaged milk fat levels 0.4 percentage units higher than those in cows not fed potassium carbonate.

Although K altered intermediates of biohydrogenation, it had no effect on ruminal losses of unsaturated fatty acids. Extent of biohydrogenation (expressed as mg of outflow per 100 mg of input) for 18:1, 18:2, and 18:3 was not affected by addition of potassium carbonate in either experiment. Biohydrogenation was higher

Table 5. Main effects of fat level and K level on daily fatty acid output and extents of biohydrogenation from continuous culture (experiment 2)

	Treatment[1]						
	Fat effect			K effect			
Item	LF	HF	SEM	K0	K1.5	K3	SEM
Output,[2] mg/d							
12:0	6.3	7.0	0.30	6.3	6.9	6.7	0.38
14:0	16.3	17.1	1.3	14.0[b]	18.1[a]	18.1[a]	1.4
14:1	9.6	8.8	0.37	8.8	9.0	9.8	0.44
15:0	17.8	17.9	1.0	16.1[b]	18.4[ab]	19.0[a]	1.1
16:0	198.0	339.6*	5.5	246.5[b]	280.3[a]	279.5[a]	6.8
16:1	2.7	3.4*	0.21	2.9	3.1	3.1	0.17
18:0[c]	329.9	388.7	31.0	189.1[b]	441.8[a]	446.6[a]	35.7
18:1	182.0	369.4*	11.6	276.2	267.2	283.8	13.7
18:2	238.4	410.8*	25.6	335.7	315.2	322.9	28.2
20:0	9.1	13.8*	0.21	11.0	11.7	11.6	0.25
18:3	25.3	44.3*	2.3	36.3	34.2	33.9	2.6
22:0	7.6	13.3*	0.38	10.4	10.2	10.8	0.44
24:0	8.5	9.7*	0.19	8.9	9.1	9.3	0.23
Biohydrogenation intermediates							
Total *trans* 18:1	160.5	713.0*	32.9	540.3[a]	408.4[b]	361.5[b]	35.9
trans-6/8 18:1	9.0	30.2*	3.4	20.1	23.3	15.3	3.8
trans-9 18:1	4.8	14.0*	1.6	8.8	11.5	8.0	1.8
trans-10 18:1	100.6	578.8*	32.5	471.7[a]	296.8[b]	250.6[b]	36.1
trans-11 18:1	39.8	72.0*	7.8	29.1[b]	62.9[a]	75.7[a]	9.2
trans-12 18:1	6.3	17.9*	0.78	10.0[b]	13.6[a]	12.7[a]	0.9
Total CLA	12.4	71.2*	2.5	46.6	37.0	41.8	3.0
trans-10,*cis*-12 CLA	4.3	53.8*	2.1	36.3[a]	24.0[b]	26.8[b]	2.5
cis-9,*trans*-11 CLA	5.1	5.8	0.59	2.7[b]	6.0[a]	7.6[a]	0.72
trans-9,*trans*-11 CLA	2.9	11.6*	0.42	7.6	6.9	7.3	0.51
Total	1,483	2,845*	44	2,100	2,217	2,175	51
Biohydrogenation, outflow as % of input							
18:1	42.3	53.8*	2.2	46.6	48.5	49.2	2.6
18:2	66.6	77.1*	2.0	70.6	71.7	73.2	2.1
18:3	71.2	80.2*	1.3	74.4	75.4	77.1	1.5

[a,b]Denotes a significant K effect where least squares means with the same letter are not significantly different ($P \leq 0.05$).

[c]Denotes a significant fat × K interaction ($P \leq 0.05$).

[1]Treatments consisted of 2 diets containing either 0 (low fat; LF) or 3.64% added soybean oil (high fat; HF). For each diet, sufficient potassium carbonate was injected daily into the fermenter contents to provide an additional 0 (K0), 1.5 (K1.5), or 3% (K3) of the diet as added K.

[2]Individual FA abbreviations are denoted as number of carbons:number of double bonds; CLA = conjugated linoleic acid.

*Significant fat effect ($P \leq 0.05$).

for HF compared with LF in experiment 2, presumably because of adherence of the added soybean oil to the surface of feed particles making them more accessible to microbial enzymes than the fat located internally in basal feed ingredients. Because of the added soybean oil in experiment 1, biohydrogenation values for 18:2 and 18:3 in experiment 1 were more similar to HF values than to LF values in experiment 2.

It is not clear from this study if the shift in biohydrogenation intermediates should be attributed to K alone or to intact potassium carbonate. One argument in favor of potassium carbonate rather than K was the possibility that responses were mediated through elevation of pH. Previous studies have reported that pH is a major factor influencing the production of *trans*-C 18:1 and CLA isomers by mixed ruminal bacteria (Martin and Jenkins, 2002), and rumen pH is reported to have a positive relationship with milk fat percentage in dairy cows (Allen, 2000; Zebeli et al., 2010). Low pH conditions in continuous cultures have been shown to reduce proportions of *trans*-11 18:1 and *cis*-9,*trans*-11 CLA, but increase proportions of *trans*-10 18:1 and *trans*-10,*cis*-12 CLA (Fuentes et al., 2009, 2011).

Biohydrogenation shifts from low pH are usually attributed to changes in the composition of the bacterial community. Individual cows share a large number of bacterial species but still vary considerably among each other in relative abundances of these common species (Weimer et al., 2010). Rumen factors that cause variation in milk fat percentage also can be linked to changes in bacterial community composition (Weimer et al., 2010). For example, DNA abundance of several principal bacterial species involved in biohydrogenation (*Butyrivibrio fibrisolvens*, *Butyrivibrio hungatei*, and *Butyrivibrio proteoclasticus*) was reduced by addition of fat supplements to continuous cultures (Gudla et al.,

2012). Lowering pH in ruminal contents likewise has similar effects on shifting microbial populations because some key bacterial species involved in biohydrogenation, such as *Butyrivibrio fibrisolvens*, have pH optima between 7.0 and 7.2 (Kepler and Tove, 1967). As these pH-sensitive bacteria decline under low pH conditions, other species are given a selective advantage, including several that utilize lactate and produce *trans*-10 18:1, such as *Megasphaera elsdenii* (Kim et al., 2002) and *Propionibacterium acnes* (Wallace et al., 2007).

Particularly sensitive to low pH conditions in the rumen are the bacterial species whose contribution to biohydrogenation is limited to the second reductase step that converts *trans* 18:1 to stearic acid (Buccioni et al., 2012). Some bacteria produce the isomerase and first reductase enzymes that synthesize CLA and *trans* 18:1 but go no further (Buccioni et al., 2012). Although a multitude of bacterial species are capable of the initial steps of biohydrogenation, the best known and studied is *B. fibrisolvens.* Fewer bacterial species are responsible for the terminal step of biohydrogenation and include *Clostridium proteoclasticum and B. hungatei* (Jenkins et al., 2008). There were indications in experiment 2 that K favored bacteria responsible for the terminal step. Addition of K in experiment 2 increased outflow of 18:0 and decreased outflow of total *trans* 18:1. In experiment 1, 18:0 outflow was higher for K3 than for NaOH, suggesting that the K effect was not entirely pH related.

Experiment 1 attempted to eliminate pH as a variable by injection of sufficient NaOH to maintain the same pH as the K3 treatment. Although pH values were not different for the NaOH and K3 treatments, only the K treatment increased acetate:propionate. This suggests that VFA changes due to potassium carbonate could be explained by factors other than pH. Conversely, the *trans* 18:1 and CLA production rates were the same for NaOH and potassium carbonate, suggesting that pH did account for the biohydrogenation shift. The tendency ($P = 0.095$) for a difference in *trans*-10,*cis*-12 CLA production between the K3 and NaOH treatments in experiment 1 suggests a unique response to potassium carbonate independent of pH.

Aside from the potassium carbonate increasing the pH, an alternative explanation for its effects on biohydrogenation might be a specific effect from the K ion. Some bacterial species are sensitive to changes in ion balance and transfer across the plasma membrane, as demonstrated by ionophores. The changes in VFA profile due to K addition seen in this study support this explanation. It is plausible that high K in ruminal contents favors bacterial species that produce *cis*-9,*trans*-11 CLA and discriminates against the species producing *trans*-10,*cis*-12 CLA that causes MFD. In any event, further studies will need to define if the biohydrogenation advantages seen for potassium carbonate in this study can be duplicated by other K sources, such as potassium chloride, or by other carbonate sources, such as sodium carbonate.

CONCLUSIONS

In 2 separate experiments, the addition of potassium carbonate to continuous cultures increased culture pH and increased acetate:propionate ratio. The addition of potassium carbonate increased the daily production of *trans*-11 18:1 and *cis*-9,*trans*-11 CLA but decreased production of *trans*-10 18:1 and *trans*-10,*cis*-12 CLA. This shift in biohydrogenation intermediates supports changes in rumen lipid metabolism as an explanation for the increase in milk fat yield reported previously for cows fed potassium carbonate. Not all of the culture responses to potassium carbonate in this study could be explained by elevation of pH.

ACKNOWLEDGMENTS

This article has been approved as Technical Contribution No. 6179 of the Clemson University Experiment Station. Partial financial assistance for this project was provided by Church & Dwight Inc. (Princeton, NJ).

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