J. Dairy Sci. 100:1751–1765
https://doi.org/10.3168/jds.2016-11776

Potassium carbonate as a cation source for early-lactation dairy cows fed high-concentrate diets

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ABSTRACT

Previous studies reported that addition of K_2CO_3 to high-concentrate diets improved milk fat synthesis, although the mechanism is yet to be established. The objective of the current experiment was to investigate the effects of dietary cation-anion difference (DCAD), cation source, and buffering ability of the mineral supplement on rumen biohydrogenation of fatty acids and production performance in dairy cows fed a high-concentrate diet. Thirty-five early-lactation Holstein cows (25 multiparous ruminally fistulated and 10 primiparous nonfistulated) were used in a randomized complete block design (7 blocks) with 33-d periods, including a 5-d pre-treatment collection period used as a covariate. Diets were (1) control, a basal diet [47% nonfibrous carbohydrates, DCAD (Na + K – Cl – S) = 65 mEq/kg of dry matter (DM)] containing 40% forage (including 60% corn silage) and 60% concentrate, (2) K_2CO_3 (control + K_2CO_3, 1.8% of DM, DCAD = 326 mEq/kg of DM), (3) $KHCO_3$ (control + $KHCO_3$, 2.6% of DM, DCAD = 324 mEq/kg of DM), (4) KCl (control + KCl, 2.0% of DM, DCAD = 64 mEq/kg of DM), and (5) Na_2CO_3 (control + Na_2CO_3, 1.4% of DM, DCAD = 322 mEq/kg of DM). Pre-planned orthogonal contrasts were used to assess the effects of K_2CO_3 (control vs. K_2CO_3), buffering ability (K_2CO_3 vs. $KHCO_3$), DCAD (K_2CO_3 vs. KCl), and cation type (K_2CO_3 vs. Na_2CO_3). Supplementing K_2CO_3 in a high-concentrate diet did not improve milk fat yield or 4% fat-corrected milk yield. Milk fat concentration was greater in cows fed K_2CO_3 compared with control (4.03 vs. 3.26%). Milk yield tended to decrease (34.5 vs. 38.8 kg/d) and lactose yield decreased in cows fed K_2CO_3 as compared with KCl (1.64 vs. 1.87 kg/d). Milk fat concentration of *trans*-10 18:1 was increased when cows were fed Na_2CO_3 as compared with K_2CO_3. A positive relationship was observed between concentrations of *anteiso* 15:0 and *trans*-10,*cis*-12 18:2 in milk fat from cows receiving K_2CO_3. Milk Na concentration was increased, whereas milk Cl was decreased with K_2CO_3 as compared with $KHCO_3$ or KCl. A positive relationship was established between milk Cl concentration and milk yield (R^2 = 0.34) across all dietary treatments. Cation-anion difference (Na + K – Cl – S) in ruminal fluid was increased with K_2CO_3 as compared with control or KCl. Blood pH tended to decrease in cows fed KCl compared with K_2CO_3. Our results suggest that mineral supplementation tends to affect milk and milk fat synthesis and that factors other than DCAD, potassium ion, or buffer ability may be implicated. The variations observed in mineral composition of milk suggest an allostatic process to maintain an ionic equilibrium in mammary epithelial cells in response to mineral composition of the diet.

Key words: dietary cation-anion difference, potassium carbonate, milk fat synthesis, chlorine

INTRODUCTION

Negative energy balance typically appears in early-lactation dairy cows as a consequence of a reduction of DMI, as well as an increase in energy demand for milk production (Grummer, 1993). To compensate for this energy deficit, cows are fed high-concentrate diets. However, highly fermentable carbohydrates introduced in diets can result in a decreased rumen pH, and consequently lead to subacute ruminal acidosis (Nocek, 1997; Krause and Oetzel, 2006). Under these conditions, the pattern of fermentation is altered, increasing rumen appearance of biohydrogenation intermediates derived from dietary PUFA, such as *trans*-10,*cis*-12 CLA (Baumgard et al., 2000) and *trans*-9,*cis*-11 CLA (Perfield et al., 2007), which are recognized for their inhibitory effects on milk fat synthesis.

Davis and Brown (1970) characterized "low-fat milk syndrome" as a consequence of diets with a high ratio of readily digestible carbohydrates to fibrous constituents that can create unfavorable conditions within the

Received July 25, 2016.
Accepted October 23, 2016.
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rumen. Based on these observations, different types of minerals were proposed to help stabilize the rumen pH and thus reduce the incidence of milk fat depression (Emery and Brown, 1961). More recently, it has been demonstrated that increasing the DCAD through mineral supplementation of diets containing 20 or 40% (DM basis) of concentrates increased the synthesis of milk and milk fat (Apper-Bossard et al., 2010). The authors suggested that responses were related to the capacity to maintain blood pH via an increase in blood HCO_3^-, as well as a localized rumen buffering effect. In vitro studies have shown that the addition of K_2CO_3 promotes the predominant pathway of fatty acid (**FA**) biohydrogenation, which is characterized by the formation of *trans*-11 18:1 and *cis*-9,*trans*-11 CLA as intermediates (Jenkins et al., 2014). Moreover, K_2CO_3 supplementation reduced outflows of isomers used as markers for altered rumen biohydrogenation pathways (*trans*-10 18:1), or linked directly with milk fat depression (e.g., *trans*-10,*cis*-12 CLA; Jenkins et al., 2014). Likewise, it has been reported that K_2CO_3 may counteract the negative effects of high-concentrate diets on milk fat synthesis (West et al., 1986; Harrison et al., 2012). Our hypothesis is that the effects observed on milk fat synthesis when K_2CO_3 is fed to dairy cows could originate from changes in the rumen environment and the acid-base status of the animal, given that supplementation of dairy cow diets with K_2CO_3 increases K ion concentration, DCAD, and buffer ability. However, the mechanism by which K_2CO_3 supplementation might decrease the effect of high-concentrate diets on milk fat depression is yet to be established.

The objective of the current study was to investigate the effects of K_2CO_3, buffering ability of the mineral supplement, DCAD, and cation source on milk production and composition in early-lactation dairy cows fed a high-concentrate diet.

MATERIALS AND METHODS

Cows, Experimental Design, and Treatments

The experiment was carried out at the Centre de Recherche en Sciences Animales de Deschambault (Deschambault, Quebec, Canada) and all the procedures with cows were approved by the animal care committee of Université Laval in accordance with the guidelines of the Canadian Council on Animal Care (1993).

Thirty-five lactating Holstein cows (25 multiparous ruminally fistulated and 10 primiparous nonfistulated) averaging 37 ± 13 DIM (mean ± SD), 618 ± 59 kg of BW, and producing 39.6 ± 8.0 kg/d of milk were used in a randomized complete block design. Cows were blocked by expecting calving date, parity (primiparous and multiparous), and cannulation. Blocks were completed successively from January to July 2013 and consisted of groups of 5 cows calving over an interval of 25 ± 18 d. Within each block, cows were randomly assigned to the experimental diets. The experiment started with a 5-d pretreatment collection period, used as a covariate. During this time, cows were fed a diet with a forage-to-concentrate ratio of 53:47 (Table 1). The treatment period lasted 28 d, of which 23 d were for adaptation and the last 5 d were used for data and sample collections. All cows were housed in individual tie stalls and had free access to water at all time.

Treatment diets consisted of (1) a control basal diet formulated to contain, on a DM basis, 40% forage (including 60% corn silage) and 60% concentrate (total diet containing 47% NFC and 24% amylase-treated NDF, with a DCAD of 65 mEq/kg of DM; control); (2) the control diet + 1.8% K_2CO_3 with a DCAD of 326 mEq/kg of DM (**K_2CO_3**); (3) control diet + 2.6% $KHCO_3$ with a DCAD of 324 mEq/kg of DM (**$KHCO_3$**); (4) the control diet + 2.0% KCl with a DCAD of 64 mEq/kg of DM (**KCl**); and (5) the control diet + 1.4% Na_2CO_3 with a DCAD of 322 mEq/kg of DM (**Na_2CO_3**). Based on initial feed ingredient composition, diets were formulated to meet or exceed the NRC (2001) requirements (Table 1). Diets were fed as TMR at 1000 h daily and the amounts of feed offered were adjusted at 110% of expected intake according to the previous day consumption. The forages were sampled every week and dried for 3 d in a forced-air oven at 55°C to determine DM concentration and adjust the as-fed forage proportions in the diets.

Experimental Measurements and Sampling

In each collection period, BW was registered at 0930 h for the last 3 d. Samples of TMR and orts were collected for the last 5 consecutive days during the pretreatment and the experimental periods and stored at −20°C. Prior to analysis, samples of TMR and orts were thawed at room temperature and dried in a forced–air oven for 72 h at 55°C to determine DM concentration. Dried samples were ground to 2 mm using a Wiley mill (model 4, Arthur M. Thomas Co., Philadelphia, PA), pooled by cow and period, ground again to 1 mm using a Cyclotec Sample Mill (model 1093, Tecator Inc., Höganäs, Sweden), and kept frozen at −20°C until further analyses.

All TMR and orts were analyzed as described by Fauteux et al. (2016), including ash (method 942.05; AOAC International, 2000) and starch (Hall, 2009).

Additionally, subsamples of TMR and orts were subjected to a digestion process in HNO_3 (70%) + H_2O_2 (30%), according to a procedure adapted from Mills

Table 1. Ingredients and chemical composition of the experimental TMR

Item, % of DM (unless otherwise stated)	Pretreatment	Dietary treatment				
		Control	K_2CO_3	$KHCO_3$	KCl	Na_2CO_3
Ingredient						
Grass silage	32.5	14.8	14.5	14.4	14.4	14.5
Corn silage	20.3	24.5	24.3	24.1	24.2	24.2
Ground corn	30.4	24.9	23.8	23.6	23.7	23.9
Ground barley	—	24.8	24.6	24.4	24.5	24.7
Soybean meal	8.6	—	—	—	—	—
Corn gluten meal	5.1	9.4	9.3	9.2	9.3	9.3
Mineral-vitamin mix[1]	3.1	1.1	1.2	1.1	1.1	1.2
NaCl	—	0.2	0.3	0.3	0.3	0.3
Limestone	—	0.4	0.4	0.4	0.4	0.4
K_2CO_3	—	—	1.8	—	—	—
$KHCO_3$	—	—	—	2.6	—	—
KCl	—	—	—	—	2.0	—
Na_2CO_3	—	—	—	—	—	1.4
Chemical composition						
DM, % as fed	40.95	41.07	40.35	41.47	41.56	39.89
OM	95.37	95.54	95.83	95.81	95.65	95.84
CP	16.30	15.15	14.84	14.55	14.69	14.79
Amylase-treated NDF	27.08	24.00	24.41	23.84	23.33	25.32
ADF	18.93	14.51	14.83	14.04	13.98	14.81
Starch	27.23	34.67	32.27	32.87	30.94	29.96
NE_L,[2] Mcal/kg of DM	1.65	1.64	1.63	1.63	1.63	1.63
Minerals						
Na	0.23	0.28	0.23	0.24	0.24	0.79
K	1.37	0.97	1.70	1.83	1.80	0.91
Cl	0.33	0.44	0.42	0.43	1.11	0.41
S	0.24	0.22	0.21	0.21	0.22	0.21
Ca	0.74	0.61	0.58	0.61	0.66	0.57
Mg	0.33	0.21	0.17	0.18	0.17	0.17
P	0.40	0.39	0.38	0.39	0.39	0.38
DCAD,[3] mEq/kg of DM						
Formulated	240	65	326	324	64	322
Actual	209	107	285	320	120	326
Fatty acids, mg/g of DM						
12:0	0.05	0.03	0.03	0.03	0.03	0.04
14:0	0.08	0.08	0.09	0.08	0.08	0.08
15:0	0.02	0.02	0.02	0.02	0.02	0.02
16:0	4.17	4.44	4.32	4.23	4.34	4.30
16:1	0.06	0.07	0.07	0.07	0.07	0.08
17:0	0.04	0.04	0.04	0.04	0.04	0.04
18:0	0.80	1.30	1.28	1.12	1.25	1.20
cis-9 18:1	6.60	7.00	6.83	6.50	6.97	6.67
cis-11 18:1	0.25	0.34	0.33	0.29	0.35	0.31
18:2	15.56	14.49	13.50	13.44	14.48	13.44
18:3	3.61	1.87	1.75	1.66	1.94	1.73
Total	31.24	29.71	28.25	27.49	29.58	27.93

[1]Optima 20–4, La Coop, St-Romuald, QC, Canada.
[2]Calculated according to NRC (2001).
[3]DCAD = [Na + K] − [Cl + S].

and Jones (1996) using a digestion block (DigiPREP MS, SCP Science, Baie d'Urfé, QC, Canada) for CP and mineral (except Cl) determinations. Subsequently, CP (N × 6.25) was assessed using an autoanalyzer (QuikChem 8000 Lachat Zellweger Analytics Inc., Lachat Instruments, Milwaukee, WI; method 13–107–06–2–D; Lachat Instruments, 2013). Concentrations of P, K, Ca, Mg, Na, and S were determined by inductively coupled plasma optical emission spectrometry (ICP-OES, Optima 4300DV, Perkin Elmer, Shelton, CT). Finally, Cl in TMR and orts was extracted using a method adapted from Liu (1998). Briefly, 0.25 g of dried and ground samples were mixed with 20 mL of H_2SO_4 (0.007 *M*) for 60 min and then centrifuged at 32,570 × *g* for 30 min at room temperature. The concentration of Cl in the last supernatant was determined using an ion chromatograph (model ICS-2100, Dionex Corp., Sunnyvale, CA) equipped with an AS11 HC capillary column (Dionex

Corp.). Determination of dietary FA composition was carried out by gas chromatography according to the method described by Fauteux et al. (2016).

Cows were milked twice daily at 0700 and 1700 h, and milk yield was determined using calibrated milk meters (Flowmaster Pro, DeLaval, Tumba, Sweden) for the last 3 d in each collection period. Milk samples were composited daily from evening and morning milking proportionately to respective milk yields. A first composited subsample was stored at 4°C with bronopol (2-bromo-2-nitropropane-1,3-diol) until further determination of milk fat, protein, lactose, and MUN by the method 972.160 (AOAC International, 2012) at Valacta (Ste-Anne-de-Bellevue, QC, Canada), using a Foss MilkoScan FT 6000 (Foss, Hillerød, Denmark). Somatic cell count was also determined at Valacta using a Fossomatic FC (Foss). A second composite milk subsample was pooled by cow and period without preservative for subsequent determination of mineral and FA composition.

Lipid extraction of milk samples and methylation of FA were performed according to procedures described by Chouinard et al. (1997). Milk FA profile was determined according to Boivin et al. (2013), using a gas chromatograph (Agilent 7890A, Agilent Technologies, Santa Clara, CA) equipped with a 100-m CP-Sil-88 capillary column (0.25 mm i.d., 0.20 mm film thickness; Agilent Technologies Canada Inc., Mississauga, ON, Canada) and a flame ionization detector.

Milk concentrations of P, K, Ca, Mg, Na, and S were determined according to methods described above for feed and ort samples. For Cl concentration, a 1-mL sample was ashed in a muffle furnace at 550°C for 5 h and then solubilized in 1 mL of 0.5 *N* HNO_3 and diluted with distilled water to a final volume of 50 mL. Milk concentration of Cl was determined as described by Gaucheron et al. (1996), using an ion chromatograph (model ICS-2100, Dionex Corp., Sunnyvale, CA) equipped with an AS11 HC capillary column (Dionex Corp.).

Rumen fluid was collected from the ventral sac of rumen fistulated cows on 2 consecutive days of each collection period at 0, 1, 2, 4, 6, and 8 h relative to feeding time. Rumen fluid was collected and filtered using a sampler tube (Bar-Diamond Inc., Parma, ID). Following an immediate pH measurement (pHTestr 30, Oakton Instruments, Vernon Hills, IL), 10 mL of rumen fluid was stabilized with 0.2 mL of H_2SO_4 (50%, vol/vol) and stored at −20°C until determination of VFA and NH_3-N concentrations. A second sample (15 mL) of rumen fluid was collected and stored at −20°C before analyses of mineral composition. Ruminal cation-anion difference (**RCAD**) was calculated as follows:

$$\text{RCAD (mEq/L)} = [\text{Na} + \text{K}] - [\text{Cl} + \text{S}].$$

At the time of analysis, acidified samples of rumen fluid were thawed and centrifuged at 16,060 × *g* for 15 min at 4°C. The supernatant was analyzed for NH_3-N with the indophenol-blue method (Novozamsky et al., 1974) using a spectrophotometer (Spectronic 1201, Milton Roy Company, Miami, FL) at 630 nm. The profile of ruminal VFA was determined with a gas chromatograph (Clarus 680, Perkin Elmer, Waltham, MA) equipped with a polar capillary column (HP-Innowax 30-m length, 0.320 mm i.d., 0.25 μm film thickness; Agilent Technologies Canada Inc.) and a flame ionization detector. The split ratio was 25:1. At the time of the sample injection, the column temperature was 80°C, maintained for 0.5 min followed by a first increase to 180°C at 10°C/min, and a second increase to 220°C at 30°C/min. The temperature was then maintained at 220°C for 2 min. The second sample of rumen fluid was thawed and centrifuged at 13,000 × *g* for 30 min at 4°C, the supernatants were then composited by day and cow, and concentrations of P, K, Ca, Mg, Na, and S were determined according to the same procedure used for milk and dietary samples. Prior to the determination of Cl concentration in rumen fluid, samples were centrifuged a second time at 32,570 × *g* for 30 min at 4°C and diluted in water with a 1:3 rumen fluid-to-water ratio (Adriano and Doner, 1982; Johnson and Fixen, 1990). Determination of Cl was performed using ion chromatography, as described above.

On the last 2 d of each collection period, blood samples were taken at 0930 h (preprandial) and 1400 h (postprandial) from a jugular vein into evacuated tubes without preservative (Vacutainer 366430, Becton Dickinson, Franklin Lakes, NJ) and immediately transferred to 1.7-mL syringes (Radiometer, Copenhagen, Denmark) containing 80 IU of electrolyte-balanced heparin. Blood hematocrits, concentrations of electrolytes (Na^+, K^+, Ca^{2+}, and Cl^-) and HCO_3^-, and partial pressure of CO_2 and O_2 were immediately determined by potentiometry using a blood gas and mineral analyzer (ABL 77, Radiometer).

Statistical Analysis

Data for milk production and composition, DMI, BW, and minerals in milk and rumen fluid were analyzed as a randomized block design using the GLIMMIX procedure of SAS (version 9.3; SAS Institute Inc., Cary, NC). The model used was

$$Y_{ij} = \mu + T_i + B_j + C + \varepsilon_{ij},$$

where Y_{ij} is the variable observed, μ is the overall mean, T_i denotes the fixed effect of treatment (i = 1 to 5), B_j is the random effect of block (j = 1 to 7), C is the covariable adjustment for each cow (C = 1 to 35), and ε_{ij} denotes the residual error. Blood and rumen fermentation parameters were analyzed using the REPEATED statement in the MIXED procedure of SAS, using the following model:

$$Y_{ijkl} = \mu + T_i + H_j + (T \times H)_{ij} + A(_k)_l + B_l + C + \varepsilon_{ijkl},$$

where Y_{ijkl} is the variable observed, μ is the overall mean, T_i denotes the fixed effect of treatment (i = 1 to 5), H_j refers to fixed effect of sampling time (j = 0, and 4 h postprandial for blood parameters and j = 0, 1, 2, 4, 6, and 8 h postprandial for rumen parameters), $(T \times H)_{ij}$ is the fixed effect of the interaction, $A(_k)_l$ is the random effect of cow k (k = 1 to 5) within block (l = 1 to 7), B_l is the random effect of block, C is the covariate adjustment for blood and rumen parameters of each cow (C = 1 to 35), and ε_{ijkl} denotes the residual error. Cow within block was included in the model as the subject of the repeated statement. For blood parameters, covariance structures were selected between compound symmetry and first-order autoregressive based on the Akaike's information criterion. For rumen parameters, the spatial covariance structure was used to estimate covariances. Because no treatment × sampling time interaction was observed for blood parameters, as well as for rumen pH and VFA, values were combined by day and analyzed according to the model previously mentioned. Pre-planned orthogonal contrasts were used to assess the effects of K_2CO_3 (control vs. K_2CO_3), buffer ability (K_2CO_3 vs. $KHCO_3$), DCAD (K_2CO_3 vs. KCl), and cation source (K_2CO_3 vs. Na_2CO_3). Simple linear regressions were conducted using the REG procedure of SAS to determine relationships between milk yield and milk minerals, and the association between milk fat concentration of *trans*-10, *cis*-12 18:2, and *anteiso* 15:0. Differences were declared significant at $P \leq 0.05$ and tendencies at $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

Diets, DMI, and BW

The actual DCAD were 107, 285, 320, 120, and 326 mEq/kg of DM for control, K_2CO_3, $KHCO_3$, KCl, and $NaCO_3$ experimental TMR, respectively (Table 1). Therefore, mineral supplementation allowed a modulation of DCAD, as formulated among treatments. Also, isokalemic conditions were maintained between K_2CO_3, $KHCO_3$, and KCl treatments, as shown by the minimal variations observed in dietary K concentrations of these 3 treatments.

Supplementing cows with K_2CO_3 tended to decrease BW compared with the control diet (Table 2). However, no significant effect of mineral supplementation was observed on DMI (Table 2). Conversely, using a mineral mixture to increase DCAD from 0 to 300 mEq/kg of DM of TMR, Apper-Bossard et al. (2010) observed a linear increase in DMI up to 1.9 and 4.0 kg/d when cows received 20 and 40% of concentrate, respectively. Similarly, increasing DCAD (196 to 544 mEq/kg of DM) by increasing levels of K_2CO_3 in diets of dairy cows in established lactation (95 DIM) led to a concomitant increase in DMI up to 1.3 kg/d (Iwaniuk et al., 2015). A systematic review conducted by Hu et al. (2007a) also reported a positive relationship between DMI and the acid-base status of lactating dairy cows. On the other hand, the same group of researchers reported no effect of 2 DCAD levels (220 vs. 470 mEq/kg of DM) on DMI of cows in early lactation (1 to 47 DIM; Hu et al., 2007b). These authors attributed the discrepancies between studies due to tremendous physiological changes occurring in early lactation, which attenuated the effect of dietary treatments. Another possible implication of DCAD on DMI could be related to ion availability, because their manipulation always affects the regulation of cell volume, and consequently the transport of substrates into and out of the cell as well as regulation of osmotic pressure (McDowell, 1992; Hoffmann et al., 2009). Potassium contributes to 50% of the osmolality of intracellular fluid, whereas Na and Cl contribute to 80% of extracellular osmolality (McDowell, 1992). Although the cell has the capacity to adjust its requirements through ionic homeostasis, long-term exposure to anisosmotic conditions exerts changes that affect signaling events which control cell growth, proliferation, and death (Hoffmann et al., 2009). In this regard, it is important to notice that transport of nutrients across the rumen epithelium may be impaired. Studies support the existence of a short-chain FA transport mechanism in the epithelial cells that involves ions (Gäbel et al., 1991; Sehested et al., 2000), and this transport mechanism can be the cause of a feed-induced regulation (Sehested et al., 2000). In the current experiment, despite a variation in systemic acid-base status reflected by a tendency for lower blood pH (Table 3), cows fed KCl had a numerically higher DMI (1.9 kg/d) compared with cows fed K_2CO_3 diet. Given that increasing DCAD do not necessary implicate keeping the ideal cell ionic balance of K, Na, and Cl, we speculate that KCl as compared with K_2CO_3

Table 2. Dry matter intake, BW, and milk yield and composition of early-lactation cows receiving high-concentrate diets supplemented with different minerals

	Dietary treatment						*P*-value, K_2CO_3 vs.			
Item	Control	K_2CO_3	$KHCO_3$	KCl	Na_2CO_3	SEM	Control	$KHCO_3$	KCl	Na_2CO_3
DMI, kg/d	24.8	23.4	23.6	25.3	23.6	1.2	0.26	0.96	0.14	0.88
BW, kg	635	616	620	625	631	10.0	0.07	0.71	0.38	0.14
Milk yield, kg/d										
Actual	37.7	34.5	34.7	38.8	38.1	1.8	0.30	0.84	0.06	0.11
4% FCM[1]	33.3	34.7	33.6	36.9	36.0	2.0	0.61	0.68	0.40	0.60
ECM[2]	33.3	33.8	32.7	36.1	35.1	1.9	0.81	0.64	0.34	0.60
Milk fat										
Concentration, %	3.26	4.03	3.88	3.72	3.66	0.22	0.02	0.62	0.31	0.22
Yield, kg/d	1.22	1.39	1.31	1.43	1.38	0.1	0.21	0.55	0.80	0.95
Milk protein										
Concentration, %	3.05	3.12	2.99	3.03	2.93	0.09	0.47	0.19	0.32	0.04
Yield, kg/d	1.15	1.07	1.02	1.17	1.11	0.06	0.25	0.49	0.17	0.56
Lactose										
Concentration, %	4.84	4.75	4.84	4.80	4.83	0.04	0.09	0.11	0.31	0.12
Yield, kg/d	1.82	1.64	1.68	1.87	1.84	0.09	0.13	0.71	0.04	0.08
MUN, mg/dL	8.5	9.0	9.5	8.3	10.1	0.5	0.42	0.51	0.28	0.12
SCC, 1,000/mL	64	68	35	25	50	25.0	0.90	0.31	0.18	0.56
Milk minerals, mmol/L										
Na	15.10	16.30	15.42	14.91	15.46	0.48	0.02	0.09	<0.01	0.10
K	43.42	45.05	45.11	44.85	43.26	1.28	0.37	0.97	0.91	0.34
Cl	17.92	18.83	17.20	18.42	17.26	1.25	0.59	0.34	0.81	0.36
S	9.94	10.43	10.29	10.20	9.61	0.32	0.19	0.70	0.53	0.03
Ca	32.80	34.32	33.48	31.83	30.47	1.13	0.27	0.53	0.07	<0.01
Mg	4.95	5.25	5.21	5.16	5.01	0.18	0.17	0.85	0.67	0.27
P	36.29	37.40	38.51	37.55	35.04	1.19	0.41	0.42	0.92	0.09

[1]4% FCM = [0.4 × milk yield (kg/d)] + [15 × fat yield (kg/d)].

[2]ECM = 0.327 × milk yield (kg/d) + 12.95 × fat yield (kg/d) + 7.2 × protein yield (kg/d).

may provide possibly a better balance of ions in the rumen, allowing fewer feed-induced regulation effects.

Iwaniuk et al. (2015) evaluated the effect of cation sources [$Na_3H(CO_3)_2$ vs. K_2CO_3] on performance of dairy cows fed TMR with similar DCAD (approximately 380 mEq/kg of DM) and observed no difference on DMI. These results are in agreement with our findings where cation sources modulated through the supply of Na_2CO_3 or K_2CO_3 did not affect DMI.

Investigating dietary buffers effects on high-producing dairy cows, Erdman (1988) compiled literature data and showed that, as opposed to $KHCO_3$, which did not affect DMI, adding K_2CO_3 did increase DMI compared with diets without K supplementation. The

Table 3. Blood parameters of early-lactation cows receiving high-concentrate diets supplemented with different minerals

	Dietary treatment						*P*-value, K_2CO_3 vs.			
Parameter[1]	Control	K_2CO_3	$KHCO_3$	KCl	Na_2CO_3	SEM	Control	$KHCO_3$	KCl	Na_2CO_3
pH	7.43	7.42	7.42	7.40	7.43	0.01	0.74	0.92	0.10	0.84
Electrolytes, mmol/L										
Na^+	139.7	140.1	140.0	140.4	139.6	0.4	0.46	0.85	0.58	0.36
K^+	3.69	4.01	3.79	3.92	3.68	0.07	<0.01	0.01	0.27	<0.01
Cl^-	105.9	106.1	106.5	108.0	105.3	0.8	0.84	0.61	0.04	0.41
Ca^{2+}	1.22	1.24	1.20	1.23	1.20	0.02	0.41	0.03	0.81	0.19
Anion gap, mmol/L	10.5	10.5	10.5	10.2	9.9	0.6	0.99	0.98	0.69	0.45
HCO_3^-, mmol/L	27.1	27.6	26.8	26.1	28.0	0.8	0.67	0.48	0.22	0.73
Hematocrit, %	27.5	27.4	27.6	26.6	27.3	0.7	0.92	0.84	0.39	0.90
Blood gas, mm Hg										
pCO_2	41.9	43.1	41.5	42.5	43.4	0.9	0.38	0.24	0.66	0.82
pO_2	36.9	37.9	38.9	36.9	38.6	1.8	0.60	0.53	0.56	0.66

[1]Anion gap = $[K^+ + Na^+] - [Cl^- + HCO_3^-]$; HCO_3^- = bicarbonate; pCO_2 = partial pressure of CO_2; pO_2 = partial pressure of O_2.

author suggested that the observed effects of mineral supplementation on DMI could be partly explained by a higher rumen pH, associated with a more favorable rumen environment, allowing greater DM digestibility. West et al. (1986) also observed a greater DMI for cows supplemented with K_2CO_3 compared with $KHCO_3$ or $NaHCO_3$. In contrast, in the current trial, DMI was similar for cows fed K_2CO_3 and $KHCO_3$ diets (23.5 ± 1.1 kg/d). This level of intake allowed a daily consumption of 3.05 mol of CO_3^{2-} ions and 6.11 mol of HCO_3^- ions for K_2CO_3 and $KHCO_3$ treatments, respectively. West et al. (1986) reported that, in the rumen, CO_3^{2-} ions contained in K_2CO_3 has twice the capacity to absorb hydrogen ions compared with HCO_3^- ions found in $KHCO_3$. Therefore, the isokalemic conditions maintained between K_2CO_3 and $KHCO_3$ treatments led to an equivalent buffer ability of these 2 diets and could explain the lack of difference in DMI between these 2 treatments.

A trend to decreased BW was observed in cows fed K_2CO_3 as compared with control. This tendency could possibly be explained in part by a numerically lower DMI (−1.4 kg/d) for K_2CO_3 as compared with the control treatment.

Rumen Fermentation Attributes and Minerals

As expected, concentration of K in the rumen was similar for cows fed K_2CO_3 and $KHCO_3$ or KCl (Table 4). However, despite that K_2CO_3, $KHCO_3$, and KCl treatments were isonatremic (Na = 0.23%, DM basis), concentration of Na in the rumen increased with K_2CO_3 as compared with $KHCO_3$ (+13%), and KCl (+17%). As expected, Cl concentration in the rumen was decreased when cows were fed K_2CO_3 compared with KCl (−41%). But surprisingly, despite similar dietary Cl concentrations, $KHCO_3$ increased Cl concentration by 19% as compared with K_2CO_3. These results provide further evidence that mineral interactions exist in the rumen, probably as a consequence of transport mechanisms of cations and anions across forestomach epithelia (Leonhard-Marek et al., 2010). Two active pathways contribute to Na absorption: an electroneutral Na^+/H^+ exchange and an electrogenic Na^+ conductance (Martens and Gäbel, 1988). By incubating ruminal epithelia of sheep, Leonhard-Marek et al. (2007) showed that the presence of Cl on the luminal side, which corresponds to the rumen cavity, increased electrogenic Na absorption. This increase was mediated via a greater Cl^-/HCO_3^- or Cl^-/OH^- luminal exchange, a higher pH in the microclimate of the epithelia surface, and a pH effect on the nonselective cation conductance responsible for Na absorption (Leonhard-Marek et al., 2007). According to this information, we speculated that lower Cl concentration observed in the rumen of cows fed K_2CO_3 might have decreased Na transport efficiency, which would explain the greater rumen Na concentrations observed with K_2CO_3 compared with KCl and $KHCO_3$ treatments.

Magnesium concentration in the rumen was significantly greater when cows received K_2CO_3 compared with Na_2CO_3 diet (Table 4). Absorption of Mg in the

Table 4. Rumen pH, volatile fatty acid (VFA), and mineral concentrations of rumen fluid from early-lactation cows receiving high-concentrate diets supplemented with different minerals

	Dietary treatment						*P*-value, K_2CO_3 vs.			
Item	Control	K_2CO_3	$KHCO_3$	KCl	Na_2CO_3	SEM	Control	$KHCO_3$	KCl	Na_2CO_3
pH	6.25	6.39	6.36	6.42	6.39	0.08	0.17	0.73	0.82	0.98
VFA, mol/100 mol										
Acetate	56.57	58.05	55.36	55.43	58.28	1.65	0.49	0.21	0.22	0.91
Propionate	24.68	23.25	23.28	25.85	22.36	1.95	0.61	0.99	0.35	0.75
Butyrate	12.49	12.60	15.21	12.00	12.40	1.15	0.94	0.08	0.70	0.89
Isobutyrate	1.06	1.05	1.06	1.10	0.97	0.14	0.95	0.91	0.70	0.60
Valerate	1.89	1.72	1.76	1.90	1.88	0.34	0.66	0.92	0.65	0.67
Isovalerate	2.73	2.22	2.54	2.46	2.78	0.47	0.37	0.57	0.68	0.31
Caproate	0.91	0.89	1.04	0.86	1.33	0.38	0.95	0.74	0.95	0.32
Acetate:propionate	2.40	2.57	2.46	2.25	2.74	0.24	0.63	0.74	0.35	0.62
Mineral, m*M*										
Na	180.76	191.20	169.49	162.87	197.26	7.96	0.34	0.05	0.01	0.58
K	37.99	63.01	61.81	62.90	34.44	4.88	<0.01	0.84	0.98	<0.01
Cl	11.88	11.60	14.32	19.80	11.56	1.05	0.80	0.02	<0.01	0.97
S	1.54	1.60	1.49	1.37	1.29	0.15	0.73	0.51	0.17	0.08
Ca	1.92	1.65	1.66	1.82	1.31	0.17	0.27	0.97	0.49	0.15
Mg	4.86	4.15	3.92	3.81	2.87	0.51	0.25	0.71	0.59	0.04
P	23.07	23.98	21.94	22.62	21.20	1.95	0.70	0.38	0.55	0.26
RCAD,[1] mEq/L	208	243	217	204	217	11.64	0.03	0.09	0.02	0.09

[1]RCAD (ruminal cation-anion difference) = ([Na + K] − [Cl + S]).

rumen epithelium and subsequent transport to blood (basolateral extrusion) occurs against an electrochemical gradient (Schweigel et al., 2000). An elevated K concentration in the rumen is associated with a decrease in Mg absorption, whereas higher absorption and transport efficiency for Mg is reported when Na concentration in the rumen is increased (Schweigel et al., 2008). No difference was observed between Mg concentration in the rumen of cows fed control and K_2CO_3 treatments. Therefore, the difference in ruminal Mg concentration observed between cows fed K_2CO_3 and Na_2CO_3 diets could possibly be a consequence of treatment differences in dietary Na rather than K concentrations.

No effect of treatment or treatment × time interaction, under any of the studied contrasts, was observed on rumen VFA concentrations and pH (Table 4). Similarly, West et al. (1986) found no difference in rumen pH when cows were fed diets containing no buffer, 1.8% $KHCO_3$, 1.2% K_2CO_3, or 1.5% $NaHCO_3$ (DM basis). Conversely, Fraley et al. (2015) demonstrated that feeding dairy cows with diets containing up to 3.2% of K_2CO_3 increased rumen pH linearly. In the current experiment, rumen pH was depressed for all treatments from 0 until 8 h (6.59 to 6.17) postfeeding (Figure 1).

Systemic Acid-Base Status

Compared with K_2CO_3, blood K concentration was lower for cows receiving control and Na_2CO_3 diets and similar for cows fed KCl treatment (Table 3). However, despite the isokalemic nature of the diets, cows fed $KHCO_3$ had lower blood K concentration compared with cows fed K_2CO_3 diet. However, to our knowledge, no study has specifically assessed the K bioavailability from these 2 mineral supplements in lactating dairy cows.

Relative to KCl, adding K_2CO_3 in the diet tended to increase blood pH, but decreased blood Cl concentration (Table 3). It is well known that DCAD, has a direct effect on the blood acid-base status, and the metabolic condition of the cows (Block, 1994). Indeed, blood HCO_3^- concentration and pH have been shown to increase with increasing DCAD (Roche et al., 2005; Apper-Bossard et al., 2006), whereas decreased DCAD though anionic diets seems to reduce blood pH (Charbonneau et al., 2009). Therefore, in agreement with Charbonneau et al. (2008), increased dietary concentration of Cl may explain the tendency for a lower blood pH found in cows fed KCl, compared with K_2CO_3 diet. In contrast, blood anion gap ($K^+ + Na^+ - Cl^- - HCO_3^-$), partial pressure of CO_2 and O_2, concentrations of HCO_3^-, and hematocrits were similar among treatments.

Milk Production and Composition

Milk yield tended to decrease and lactose yield decreased when cows were fed K_2CO_3 compared with the KCl diet (Table 2). Because of a high metabolic rate, the cellular environment of cows in lactation tends to be acidotic (Block, 1994). Shire and Beede (2013) suggested that supplementing cationic salts might improve lactation performance by affecting several biological mechanisms such as ruminal buffer ability, blood pH, rumen microbial synthesis, bioactive intermediates of ruminal FA biohydrogenation, and reactions to environmental stressors. Also, it was reported that, when

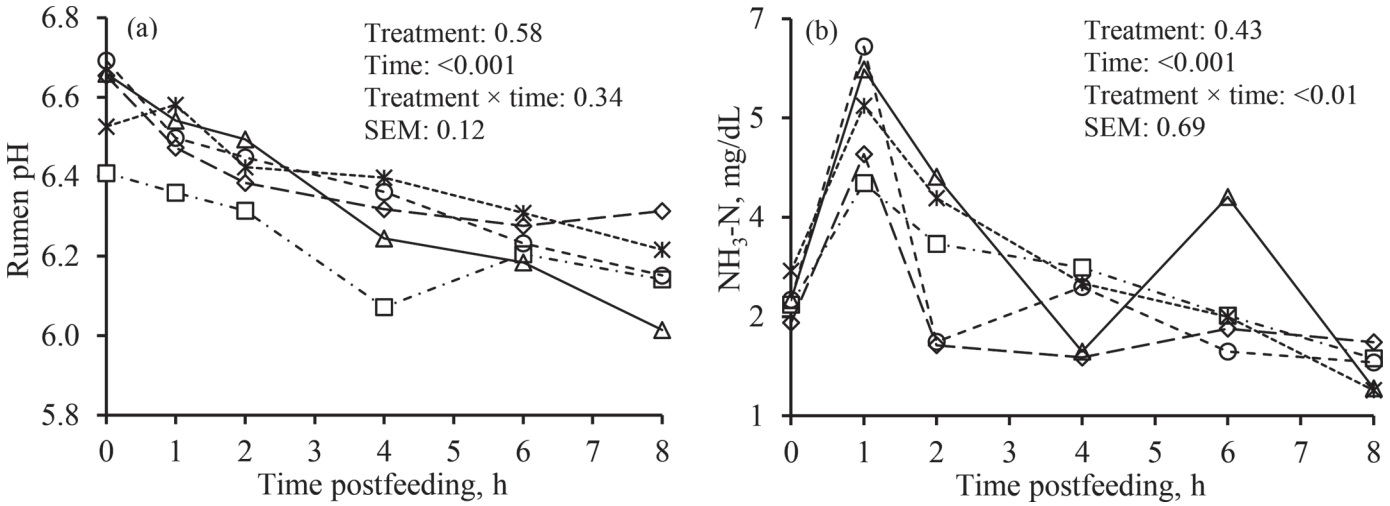

Figure 1. Postfeeding temporal pattern of rumen pH (a), and NH_3-N concentration (b) of early-lactation cows fed high-concentrate diets unsupplemented (□, control) or supplemented with different minerals (○, K_2CO_3; Δ, $KHCO_3$; ◊, KCl; x, Na_2CO_3).

compared with cows in any other stage of lactation and because they typically receive high-concentrate diets with rapidly degradable starch, early-lactation dairy cows may be more prone to metabolic acidosis (Chan et al., 2005). However, the tendency for a lower milk yield observed in the current study, when early-lactation dairy cows were fed K_2CO_3 compared with the KCl diet, suggests that the effects of mineral supplementation on milk synthesis may involve other factors than DCAD, K ion, and buffer ability. Although numerically cows fed KCl as compared with K_2CO_3 ate more (1.9 kg/d), this could be partially explain the trend to increase milk yield using KCl instead of K_2CO_3.

The cellular mechanisms involved in the transport of milk constituents out of and across the mammary secretory cell is composed of 4 transcellular and one paracellular known routes (Shennan and Peaker, 2000). These mechanisms require that a constant concentration of gradients in body fluids be maintained to support transport of substrates or metabolites in and out the cells, as well as regulation of osmotic pressure (McDowell, 1992). Mineral concentrations in milk are recognized to reflect their cellular levels (Holt, 1985). Approximately 95% of milk K and Na, and 100% of milk Cl are found in the aqueous phase, and contribute substantially to milk osmolality (Holt, 1985). However, the mammary gland is able to generate and maintain large K, Na, and Cl gradients between milk and plasma (Shennan and Peaker, 2000), suggesting that active ion movements are involved in the secretory mechanisms of milk constituents (Linzell and Peaker, 1971).

In the current study, Cl concentration was similar in milk from cows fed K_2CO_3 and KCl diets, despite a decreased blood Cl concentration in cows fed K_2CO_3 diet. This result suggests that Cl concentration in the milieu intérieur (Holmes, 1986) of mammary epithelial cells was maintained constant. The lack of difference in milk Cl concentration between K_2CO_3 and KCl treatments (Table 2) is in agreement with Bernard's constancy (Holmes, 1986), a mechanism that allows to protect cells from external (blood) conditions to maintain homeostasis of, in this case, the mammary gland.

Shennan and Peaker (2000) suggested that, similar to many secretory epithelia, intracellular accumulation of Cl^-, via Na^+-K^+-Cl^- cotransport across the basolateral membrane, is the driving force for the secretion of ions and water across the apical membrane of the mammary epithelial cell. More specifically, in mice, it has been previously observed that Cl transport in HC11 mammary epithelial cells was achieved by the coordinated action of symporters such as Na^+-K^+-$2Cl^-$ cotransporter isoform 1, cystic fibrosis transmembrane conductance regulator, or Cl^- channels 1 and 2 (Selvaraj et al., 2000; Anantamongkol et al., 2012). Accordingly, using a simple linear regression to assess the association between milk yield and milk concentrations of minerals and lactose, we observed a positive relationship between Cl concentration and milk yield (Figure 2). In contrast, milk yield was not significantly associated with milk concentrations of Na or K. Given that lactose is exclusively synthesized in mammary epithelial cells (Kuhn and Linzell, 1970), lactose synthesis could then be considered to depend mostly on the availability of precursors, the latter being in turn dependent on their transport into the cell and their metabolic fate. A positive relation was also observed between lactose yield and milk Cl concentration ($R^2 = 0.29$; Figure 2). Previous studies suggested that ions are involved indirectly (Shennan and Peaker, 2000) or directly (McManaman and Neville, 2003) in transport of nutrients across cell membranes, factors that may influence the synthesis of milk. Consequently, we hypothesized that the tendency for increased milk yield and for increased lactose yield when cows were supplemented with KCl as compared with K_2CO_3 could partly be explained by a potential role of Cl in transport of nutrients and metabolites into and out of mammary epithelial cells.

Despite similar dietary concentrations (Table 1), Ca and S increased in milk when cows received K_2CO_3 as compared with Na_2CO_3 diet (Table 2). Both minerals could play an important role in the allostatic process required to maintain ionic equilibrium of the mammary epithelial cell; however, more research is needed to establish the exact mechanism.

Milk fat concentration was increased by 24% in cows fed K_2CO_3 compared with the control diet (Table 2). Consistently, Harrison et al. (2012) reported a 9% increase in milk fat concentration when cows were fed a diet supplemented with 3.2 vs. 0% K_2CO_3 (DM basis). Likewise, augmenting dietary K_2CO_3 from 0 up to 2.46% (DM basis) increased milk fat concentration by 10% (Iwaniuk et al., 2015). However, in contrast with these 2 previous studies, in the current experiment, the 4% FCM was similar in cows fed K_2CO_3 and control diets, possibly due to a numerical decrease in milk yield (−3.2 kg/d) when cows were supplemented with K_2CO_3.

When compared with the K_2CO_3, the control, $KHCO_3$, and KCl treatments did not affect milk protein concentration or yield. However, cows receiving the K_2CO_3 produced milk with a greater protein concentration as compared with cows receiving Na_2CO_3 diet. When studying mineral supplements with differing buffering capacities, Mooney and Allen (2007) did not observe any variation in milk protein concentration among cows fed NaCl, KCl, $NaHCO_3$, or $KHCO_3$ supplemented diets. However, in a more recent study by Martins et al. (2015), increasing DCAD from −71 to +290 mEq/kg of

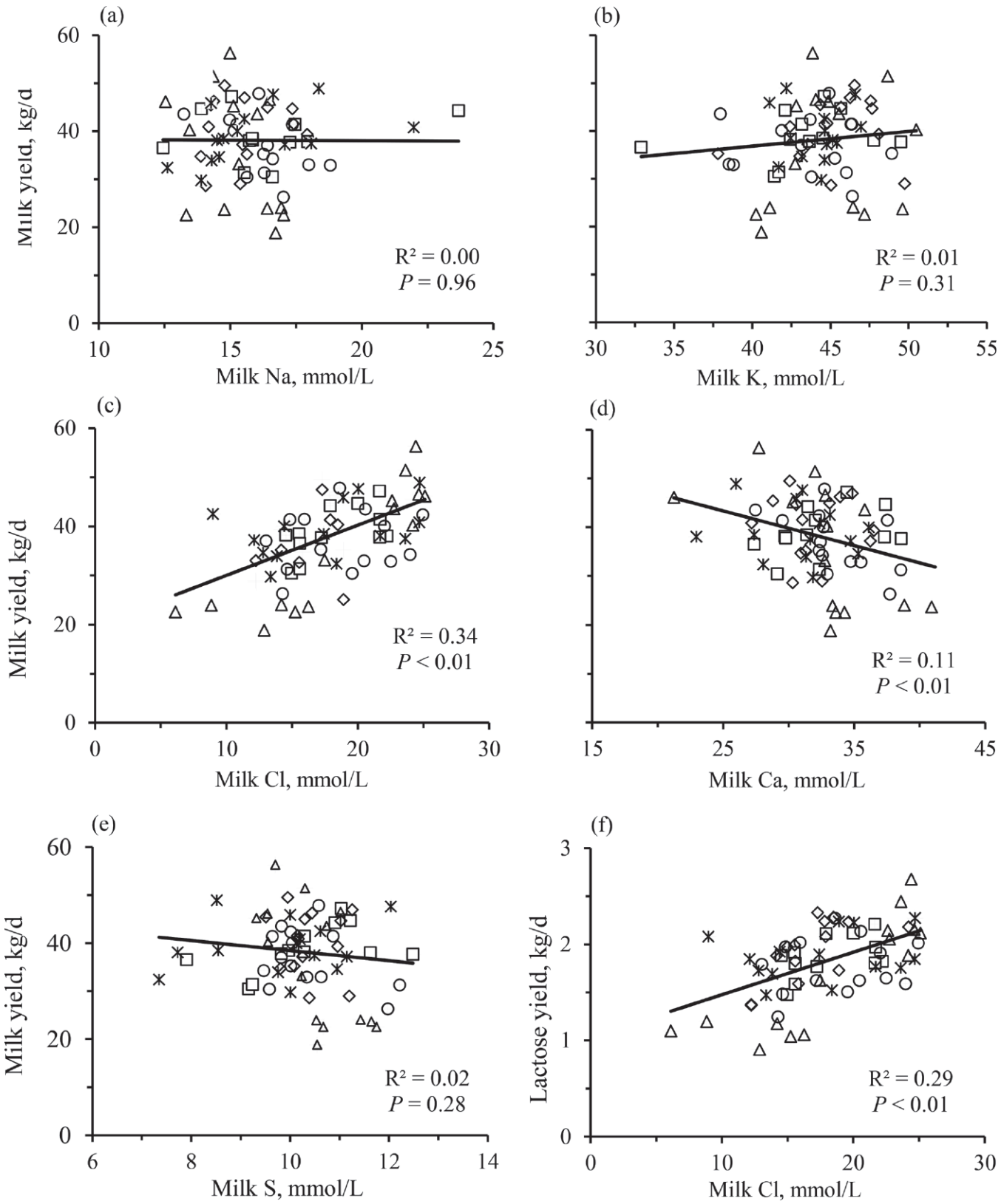

Figure 2. Association between milk yield and milk concentration of (a) sodium, (b) potassium, (c) chlorine, (d) calcium, and (e) sulfur, and between lactose yield and milk concentration of chlorine (f), in early-lactation cows fed high-concentrate diets unsupplemented (□, control) or supplemented with different minerals (○, K_2CO_3; Δ, $KHCO_3$; ◊, KCl; x, Na_2CO_3).

DM decreased milk protein concentration by 5%. These changes were related to a linear reduction of casein concentration in milk, which was also reported by Harrison et al. (2012). In the current study, DCAD did not affect milk protein synthesis. Moreover, the decrease in milk protein concentration observed with Na_2CO_3 compared with K_2CO_3 diet did not lead to a decrease in milk protein yield, suggesting a dilution effect due to a numerical increase in milk yield (+3.6 kg/d).

Also, one should not ignore that, in the current study, a small number of animals was assigned to each treatment (n = 7) and a substantial variation existed among cows in their response to high-concentrate diets. These conditions could have affected the statistical power of the current experiment, and consequently masked potential treatment effects on milk performance.

Milk Fatty Acid Composition

Dietary mineral supplementation had some effects on milk FA profile (Table 5). When Na_2CO_3 was added to the diet as compared with K_2CO_3, milk fat concentrations of *trans*-6–8 18:1, *trans*-10 18:1, and *cis*-6–8 18:1, as well as the *trans*-10/*trans*-11 18:1 ratio were increased by 18%, 140%, 55%, and 106%, respectively. A tendency for similar increases was observed for milk fat concentrations of *trans*-9 18:1, *cis*-13 18:1, and *cis*-14 18:1, whereas a tendency for a decrease in *trans*-13–14 18:1 was observed when Na_2CO_3 was compared with the K_2CO_3 diet. Ruminal bacteria are largely responsible for rumen biohydrogenation of PUFA (Harfoot, 1978). It may be possible that differences in cation source (K vs. Na) affected a niche of bacteria involved in this process, leading to differences in milk fat concentrations of various biohydrogenation intermediates. When comparing cows supplemented with KCl and K_2CO_3, along with a decrease in DCAD, milk fat concentrations of *trans*-15 18:1 (+13%), *cis*-6–8 18:1 (+44%), *cis*-13 18:1 (+60%), and *cis*-15 18:1 (+25%; tendency), as well as the *trans*-10/*trans*-11 18:1 ratio (+85%), were increased with KCl.

Moreover, a numerical (−44%) yet not significant ($P = 0.16$) decrease in milk fat concentration of *trans*-10 18:1 was observed when K_2CO_3 was compared with the KCl diet. When DCAD was increased (from 320 to 530 mEq/kg of DM) through K_2CO_3 supplementation, Harrison et al. (2012) observed a 40% decrease in concentration of *trans*-10 18:1 in milk fat of dairy cows. In contrast, other studies showed no effect on milk *trans*-10 18:1 when mineral supplements were combined to achieve different DCAD (Roche et al., 2005; Apper-Bossard et al., 2006). Discrepancies between studies could be related to different factors such as levels of mineral supplementation, basal diet composition, and individual variation in animal response to high-concentrate diets.

Additionally, a decreased concentration of *anteiso* 15:0 in milk fat was observed when K_2CO_3 was added to the diet, compared with control, $KHCO_3$, or Na_2CO_3. Lipid membrane composition of ruminal bacteria is characterized by a large proportion of branched-chain FA (*iso* 15:0, *iso* 17:0, *anteiso* 15:0, *anteiso* 17:0; Vlaeminck et al., 2005). In particular, amylolytic bacteria are reported to have greater proportions of *anteiso* FA in their lipid membranes (Vlaeminck et al., 2006). Diets with high inclusion of concentrate, which increase the relative abundance of amylolytic bacteria in the rumen, can be expected to lead to greater proportions of rumen *anteiso* FA and, consequently, to greater concentrations of these FA in milk fat (Vlaeminck et al., 2006).

A decrease in melting point of bacterial membrane lipid composition has been previously reported as a mechanism to modulate and maintain membrane fluidity and transport functions (homeoviscous adaptation) in response to pH (Giotis et al., 2007) and osmotic stress (Chihib et al., 2003). Because *anteiso* FA have low melting points compared with *iso* FA, synthesis of *anteiso* FA is stimulated under stress conditions (Annous et al., 1997). Consequently, it could be suggested that adding K_2CO_3 to the diet, compared with other source of minerals, induced modifications in the rumen environment, which prevented bacteria to resort to modulation of their membrane FA profile to resist stressing conditions normally related to high-concentrate diets.

Of note, when cows were fed the K_2CO_3 diet, milk fat concentrations of *anteiso* 15:0 and *trans*-10,*cis*-12 18:2 were positively correlated (Figure 3). Because concentrations of milk branched-chain FA were not determined when mineral supplementation was evaluated in earlier studies, the link between *anteiso* 15:0 and *trans*-10,*cis*-12 18:2 has not been previously reported. Further research is then needed to elucidate the potential link between these 2 FA involved in rumen lipid metabolism and the effect of mineral supplementation on this relationship.

CONCLUSIONS

In the current experiment, and as opposed to previous studies, supplementing high-concentrate diets with K_2CO_3 did not increase milk or milk fat yield in early-lactation cows. Adding K_2CO_3 even led to a tendency for a decreased milk yield when compared with KCl. Overall, using K_2CO_3 as a mineral supplement to modulate DCAD, K ion, or buffer ability of diets, affected the rumen environment, but did not stimulate synthesis of milk components. Our results suggest that increasing

Table 5. Fatty acid composition of milk fat from early-lactation cows receiving high-concentrate diets supplemented with different minerals

	Dietary treatment						*P*-value, K_2CO_3 vs.			
Item, %	Control	K_2CO_3	$KHCO_3$	KCl	Na_2CO_3	SEM	Control	$KHCO_3$	KCl	Na_2CO_3
4:0	2.643	2.64	2.648	2.682	2.853	0.126	0.99	0.97	0.82	0.24
6:0	1.726	1.796	1.751	1.741	1.831	0.083	0.56	0.71	0.64	0.76
8:0	1.103	1.185	1.108	1.095	1.170	0.064	0.39	0.41	0.34	0.88
10:0	2.882	3.083	2.785	2.700	2.964	0.206	0.50	0.32	0.20	0.69
10:1	0.225	0.258	0.252	0.229	0.225	0.018	0.17	0.81	0.20	0.17
11:0	0.099	0.109	0.093	0.109	0.094	0.023	0.74	0.57	1.00	0.60
12:0	3.610	3.762	3.534	3.218	3.631	0.261	0.68	0.53	0.14	0.72
iso 13:0	0.019	0.019	0.021	0.019	0.022	0.002	0.93	0.72	0.87	0.29
anteiso 13:0	0.013	0.014	0.013	0.011	0.016	0.001	0.69	0.78	0.34	0.28
12:1	0.092	0.104	0.102	0.086	0.088	0.009	0.29	0.89	0.13	0.19
13:0	0.159	0.150	0.136	0.158	0.141	0.028	0.78	0.72	0.82	0.80
iso 14:0	0.053	0.062	0.050	0.057	0.075	0.012	0.50	0.38	0.74	0.30
14:0	11.506	10.765	10.775	10.193	11.356	0.376	0.17	0.98	0.28	0.27
cis-9 14:1	0.875	0.871	0.860	0.945	0.827	0.067	0.96	0.41	0.90	0.64
cis-11 14:1	0.023	0.026	0.021	0.024	0.022	0.002	0.36	0.58	0.17	0.24
iso 15:0	0.585	0.501	0.547	0.521	0.553	0.059	0.08	0.33	0.64	0.22
anteiso 15:0	0.357	0.307	0.375	0.333	0.383	0.017	0.03	0.01	0.26	<0.01
15:0	1.380	1.164	1.210	1.375	1.165	0.202	0.43	0.87	0.44	1.00
iso 16:0	0.156	0.164	0.164	0.193	0.173	0.033	0.82	1.00	0.42	0.79
16:0	29.428	29.351	30.932	28.757	28.48	0.894	0.94	0.15	0.58	0.43
trans-9 16:1	0.031	0.033	0.039	0.031	0.063	0.014	0.93	0.76	0.95	0.13
cis-9 16:1	1.135	1.242	1.368	1.297	1.205	0.087	0.39	0.31	0.65	0.77
cis-11 16:1	0.038	0.039	0.031	0.043	0.035	0.004	0.81	0.19	0.53	0.42
cis-13 16:1	0.185	0.206	0.203	0.179	0.169	0.019	0.34	0.89	0.23	0.11
iso 17:0[1]	0.257	0.236	0.264	0.266	0.260	0.017	0.31	0.16	0.18	0.26
anteiso 17:0[2]	0.362	0.345	0.393	0.392	0.396	0.021	0.59	0.14	0.12	0.11
17:0	0.254	0.238	0.255	0.274	0.243	0.021	0.60	0.56	0.23	0.86
cis-7 17:0	0.013	0.014	0.014	0.014	0.013	0.001	0.78	0.68	0.59	0.44
cis-8 17:1	0.009	0.010	0.009	0.010	0.010	0.001	0.39	0.43	0.79	0.88
cis-9 17:1	0.097	0.099	0.102	0.115	0.106	0.011	0.86	0.87	0.32	0.66
iso 18:0	0.017	0.015	0.017	0.019	0.021	0.002	0.75	0.59	0.41	0.20
18:0	6.463	6.638	6.015	6.775	6.541	0.523	0.78	0.32	0.82	0.87
trans-4 18:1	0.016	0.018	0.016	0.016	0.020	0.002	0.57	0.61	0.66	0.44
trans-5 18:1	0.013	0.014	0.014	0.014	0.016	0.001	0.71	0.82	0.89	0.38
trans-6–8 18:1	0.178	0.165	0.172	0.206	0.200	0.015	0.43	0.69	0.02	0.04
trans-9 18:1	0.156	0.155	0.161	0.178	0.179	0.011	0.91	0.63	0.08	0.06
trans-10 18:1	0.254	0.222	0.299	0.395	0.527	0.098	0.79	0.54	0.16	0.02
trans-11 18:1	0.528	0.502	0.548	0.562	0.552	0.085	0.68	0.47	0.35	0.43
trans-12 18:1	0.189	0.185	0.177	0.196	0.168	0.015	0.82	0.66	0.55	0.34
trans-13–14 18:1	0.318	0.306	0.292	0.282	0.220	0.034	0.79	0.58	0.75	0.06
trans-15 18:1	0.244	0.228	0.227	0.257	0.239	0.012	0.32	0.93	0.05	0.49
trans-16 18:1	0.189	0.172	0.196	0.166	0.178	0.009	0.21	0.72	0.10	0.64
cis-6–8 18:1	0.118	0.085	0.116	0.125	0.139	0.012	0.05	0.06	0.03	<0.01
cis-9–10 18:1	14.993	15.25	14.646	16.401	15.363	0.961	0.85	0.64	0.37	0.93
cis-11 18:1	0.711	0.649	0.569	0.782	0.623	0.064	0.48	0.37	0.13	0.77
cis-12 18:1	0.206	0.219	0.212	0.237	0.229	0.015	0.49	0.70	0.39	0.61
cis-13 18:1	0.049	0.049	0.045	0.081	0.075	0.011	0.98	0.78	0.03	0.07
cis-14 18:1	0.042	0.036	0.038	0.040	0.047	0.004	0.36	0.67	0.48	0.09
cis-15 18:1	0.038	0.036	0.044	0.048	0.047	0.005	0.70	0.23	0.08	0.11
cis-9,*trans*-12 18:2	0.045	0.042	0.039	0.046	0.044	0.002	0.42	0.40	0.33	0.66
trans-9,*trans*-12 18:2	0.011	0.012	0.010	0.010	0.012	0.001	0.64	0.53	0.38	0.99
cis-9,*trans*-13 18:2	0.277	0.258	0.256	0.259	0.264	0.023	0.55	0.93	0.99	0.84
trans-8,*cis*-13 18:2	0.082	0.074	0.082	0.082	0.080	0.004	0.26	0.25	0.23	0.36
trans-9,*cis*-12 18:2	0.022	0.019	0.023	0.025	0.021	0.001	0.19	0.13	0.01	0.47
trans-11,*cis*-15 18:2	0.035	0.030	0.037	0.038	0.047	0.006	0.35	0.16	0.09	<0.01
cis-9,12 18:2	2.046	2.152	2.028	2.278	2.153	0.185	0.34	0.27	0.27	0.99
cis-9,*trans*-11 18:2	0.263	0.249	0.295	0.282	0.279	0.034	0.64	0.12	0.27	0.31
trans-10,*cis*-12 18:2	0.012	0.015	0.013	0.014	0.012	0.001	0.22	0.30	0.73	0.14
cis-9,12,15 18:3	0.278	0.268	0.275	0.274	0.274	0.030	0.80	0.87	0.89	0.88
cis-6,9,12 18:3	0.032	0.036	0.031	0.031	0.033	0.003	0.17	0.12	0.12	0.33
cis-9,*trans*-11,*cis*-15 18:3	0.020	0.017	0.022	0.024	0.019	0.002	0.21	0.05	0.01	0.59
cis-6,9,12,15 18:4	0.022	0.018	0.019	0.021	0.024	0.003	0.37	0.94	0.53	0.17
19:0	0.065	0.068	0.061	0.061	0.066	0.005	0.70	0.37	0.38	0.76
20:0	0.095	0.098	0.087	0.097	0.094	0.006	0.70	0.17	0.87	0.65

Continued

Table 5 (Continued). Fatty acid composition of milk fat from early-lactation cows receiving high-concentrate diets supplemented with different minerals

	Dietary treatment						*P*-value, K_2CO_3 vs.			
Item, %	Control	K_2CO_3	$KHCO_3$	KCl	Na_2CO_3	SEM	Control	$KHCO_3$	KCl	Na_2CO_3
cis-9 20:1	0.033	0.031	0.031	0.034	0.032	0.001	0.53	0.83	0.27	0.72
cis-11 20:1	0.036	0.037	0.031	0.040	0.040	0.003	0.86	0.26	0.68	0.70
cis-11,14 20:2	0.025	0.027	0.023	0.029	0.026	0.002	0.56	0.28	0.62	0.67
cis-11,14,17 20:3	0.008	0.006	0.008	0.008	0.008	0.001	0.24	0.23	0.14	0.18
cis-8,11,14 20:3	0.090	0.085	0.075	0.085	0.089	0.010	0.66	0.33	0.99	0.75
cis-8,11,14,17 20:4	0.008	0.008	0.008	0.010	0.011	0.001	0.92	0.88	0.15	0.06
cis-5,8,11,14 20:4	0.135	0.139	0.133	0.152	0.126	0.012	0.69	0.60	0.33	0.28
cis-5,8,11,14,17 20:5	0.037	0.027	0.029	0.034	0.030	0.002	0.02	0.58	0.08	0.42
22:0	0.017	0.017	0.014	0.016	0.012	0.001	0.90	0.16	0.83	0.02
cis-13 22:1	0.007	0.008	0.007	0.009	0.008	0.001	0.25	0.38	0.64	0.95
cis-13,16 22:2	0.008	0.008	0.007	0.008	0.009	0.001	0.81	0.81	0.85	0.52
cis-13,16,19 22:3	0.004	0.004	0.005	0.006	0.004	0.001	0.78	0.32	0.60	0.97
cis-7,10,13,16 22:4	0.015	0.017	0.016	0.014	0.012	0.002	0.44	0.18	0.65	0.04
cis-7,10,13,16,19 22:5	0.035	0.033	0.034	0.030	0.028	0.007	0.75	0.59	0.91	0.32
cis-4,7,10,13,16,19 22:6	0.010	0.009	0.009	0.010	0.009	0.001	0.84	0.44	0.59	0.50
24:0	0.009	0.008	0.009	0.008	0.008	0.001	0.39	0.87	0.60	0.79
24:1	0.006	0.007	0.007	0.006	0.007	0.001	0.84	0.90	0.70	0.59
Glycerol	11.598	11.618	11.546	11.607	11.658	0.065	0.83	0.91	0.44	0.65
Others[3]	0.680	0.675	0.724	0.546	0.613	0.061	0.95	0.11	0.56	0.47
Sum										
De novo fatty acids[4]	22.022	21.840	21.290	20.198	22.114	0.957	0.89	0.68	0.22	0.84
C16	30.818	30.865	32.554	30.314	29.969	0.900	0.97	0.14	0.63	0.43
Preformed fatty acids[5]	32.360	32.103	31.010	34.468	32.870	1.403	0.89	0.54	0.19	0.67
Ratio										
trans-10/*trans*-11 18:1	0.520	0.473	0.618	0.867	0.972	0.188	0.84	0.55	0.10	0.04

[1]Coelution with minor concentration of *trans*-10 16:1.

[2]Coelution with minor concentration of *cis*-10 16:1.

[3]Represent unidentified chromatogram peaks.

[4]Sum of straight even-chain fatty acids from C6 to C14.

[5]Sum of branched-chain fatty acids (*iso* 13:0, *anteiso* 13:0, *iso* 14:0, *iso* 15:0, *anteiso* 15:0, *iso* 16:0, *iso* 17:0, and *anteiso* 17:0), odd-chain fatty acids (13:0 and 15:0), and all fatty acids with a chain length of 17C or more.

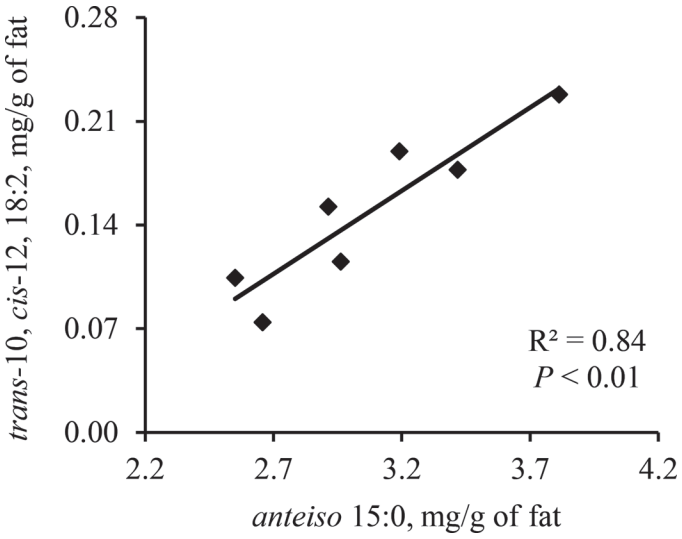

Figure 3. Association between milk fat concentration of *trans*-10, *cis*-12 18:2, and *anteiso* 15:0 in early-lactation cows fed a high-concentrate diet containing 1.8% of K_2CO_3 (DM basis).

dietary K through the addition of K_2CO_3 could lead to a disequilibrium in cellular ion composition that can impair nutrient transport into and out of the mammary epithelial cells, and consequently affect milk synthesis. Further research is needed to establish under which conditions dietary K_2CO_3 supplementation can contribute to rumen stability of early-lactation cows, and to determine how dietary mineral supplementation affects the metabolism of mammary epithelial cells.

ACKNOWLEDGMENTS

This experiment was funded through the Programme de recherche en partenariat pour l'innovation en production et en transformation laitières supported by Agriculture and Agri-Food Canada, the Fonds de Recherche du Québec–Nature et Technologies (FRQNT; Québec, QC, Canada), the Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec, and Novalait Inc. The authors thank Gilles Bélanger, Danielle Mongrain, and Mario Laterrière from Agriculture and Agri-Food Canada, (Québec, QC, Canada), as well as

Micheline Gingras and Yolaine Lebeuf from Université Laval (Québec, QC, Canada) for professional and technical assistance, Yan Martel-Kennes and Annie Dumas for their help in organizing the experiment at the farm, and André Perreault, Philippe Cantin, Denis Lefebvre, and Sébastien Coursol for all their work in the barn at the Centre de Recherche en Sciences Animales de Deschambault (Deschambault, QC, Canada).

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