Interaction of potassium carbonate and soybean oil supplementation on performance of early-lactation dairy cows fed a high-concentrate diet

A. R. Alfonso-Avila,* E. Baumann,* É. Charbonneau,* P. Y. Chouinard,* G. F. Tremblay,† and R. Gervais*¹

*Département des Sciences Animales, Université Laval, Québec, QC, Canada, G1V 0A6

†Québec Research and Development Centre, Agriculture and Agri-Food Canada, Québec, QC, Canada, G1V 2J3

ABSTRACT

Potassium carbonate supplementation is known to improve milk fat synthesis and to modify milk mineral composition in dairy cows. The objective of the current experiment was to evaluate the effect of K_2CO_3 on production performance, biohydrogenation of fatty acids (FA), and mineral composition of milk in early-lactation dairy cows fed a high-concentrate diet with or without soybean oil (SBO), as a source of polyunsaturated FA. Twenty-eight ruminally fistulated Holstein cows were used in a randomized complete block design. The experiment lasted 33 d, including a 5-d pretreatment collection period used as a covariate. Experimental treatments were arranged as a 2×2 factorial with 0 or 1.5% K_2CO_3 and with 0 or 2% SBO, and balanced to contain 40% forage (57% corn silage + 43% grass silage) and 60% concentrate. Preplanned orthogonal contrasts were used to assess the effects of K_2CO_3 , SBO, and their interaction. Feeding K_2CO_3 did not affect milk yield, but tended to increase 4% fat-corrected milk and fat yield when combined with SBO. However, adding SBO to diets increased milk yield. Dietary K_2CO_3 supplementation did not affect milk fat concentration of *trans*-10 18:1 or any other identified biohydrogenation intermediates. Soybean oil supplementation decreased milk fat concentration of C16 and de novo synthesized FA, and increased preformed FA. Among the other effects of SBO supplementation observed, concentrations of *cis*-9,*trans*-11 18:2 increased, as well as most of the *cis* and *trans* isomers of 18:1 and 18:0. Milk urea N decreased in cows fed K_2CO_3 as compared with unsupplemented diets. A positive relation was established between milk Cl concentration and milk yield, suggesting that the equilibrium of this ion is linked to the efficiency of lactogenesis. The effect of K_2CO_3 on this mineral equilibrium in the mammary gland remains to

be established. Overall, results have shown that potential effect of K_2CO_3 on milk fat synthesis is dependent on the levels of dietary polyunsaturated FA.

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

INTRODUCTION

Previous research suggests that the decrease in milk fat synthesis observed in dairy cows fed high-concentrate diets can be alleviated by increasing DCAD and dietary K concentration (Harrison et al., 2012). Using continuous fermenters, Jenkins et al. (2014) evaluated the effect of 3 levels of added K in the form of K_2CO_3 with 2 concentrations of soybean oil (**SBO**, 0 or 3.4% of DM) on ruminal biohydrogenation of fatty acids (**FA**). The authors reported that the addition of K_2CO_3 alone or with SBO increased 18:0 and *cis*-9,*trans*-11 18:2, whereas daily outflow of *trans*-10 18:1 and *trans*-10,*cis*-12 18:2 was decreased. These results indicate that supplementing K_2CO_3 promoted fermentation and biohydrogenation pathways that are consistent with greater milk fat concentration (Bauman and Griinari, 2003). However, in vivo studies focusing on the effects of dietary K_2CO_3 supplementation on lactation performance, when a source of PUFA is added to the diet, are scarce. On the other hand, milk mineral concentration can be affected by K_2CO_3 supplementation (Alfonso-Avila et al., 2017). Ions in mammary epithelial cells are well known to be implicated in milk synthesis through the nutrient transport into and out of the cells (Shen-nan and Peaker, 2000). It is possible to consider mineral concentrations in milk as indicators of homeostasis in the mammary gland, given that they are recognized to reflect their cell levels (Holt, 1985).

Therefore, the objective of the current study was to assess the effect of K_2CO_3 on the performance, appearance of biohydrogenation intermediates in milk, and milk mineral composition of early-lactation dairy cows fed a high-concentrate diet with or without SBO, as a source of PUFA.

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¹Corresponding author: rachel.gervais@fsaa.ulaval.ca

MATERIALS AND METHODS

Cows, Experimental Design, and Treatments

The experiment was carried out at the Centre de Recherche en Sciences Animales de Deschambault (Deschambault, QC, 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). Twenty-eight ruminally fistulated Holstein cows (20 multiparous and 8 primiparous) averaging 39 ± 9 DIM (mean $\pm$ SD), 633 ± 68 kg of BW, and producing 38.7 ± 7.3 kg/d of milk were used in a randomized complete block design (7 blocks). Cows were blocked by expecting calving date within parity (primiparous and multiparous). Blocks were completed successively from January to July 2014 and consisted of groups of 4 cows calving over an interval of 41 ± 8 d. Within each block, cows were randomly assigned to the 4 treatment diets. The experimental design included a 5-d pretreatment collection period that was used as a covariate. During this pretreatment period, cows were fed a diet with a forage-to-concentrate ratio of 52:48 (Table 1). The treatment period lasted 28 d, of which the first 23 d were used for adaptation and the last 5 d for data and sample collection. All cows were housed in individual tie stalls and had free access to water at all time.

Experimental treatments were arranged in a 2×2 factorial design with 0 or 1.5% K_2CO_3 sesquihydrate in the form of DCAD Plus (Church & Dwight Co. Inc., Princeton, NJ) and with 0 or 2% SBO and with 0 or 2% SBO. Basal diets for all treatments were balanced to contain 40% forage (including 57% corn silage and 43% grass silage) and 60% concentrate (total diet containing $26 \pm 1\%$ of starch and $30 \pm 2\%$ of NDF assayed with a heat-stable α -amylase, amylase-treated NDF), on a DM basis (Table 1). Based on initial feed ingredient composition, diets were formulated to meet or exceed the NRC (2001) requirements (Table 1). The TMR were offered once daily at 1000 h, and the amount of presented feed was adjusted to maintain 10% refusals. Forages were sampled weekly and dried for 3 d in a forced-air oven at $55^\circ C$ to determine DM concentration. The as-fed proportions of forages were adjusted accordingly to maintain the desired proportions of the ingredients on a DM basis. Cows were milked twice daily at 0700 and 1700 h.

Experimental Measurements and Sampling

Cows were weighed at 0930 h on the last 3 d of each collection period. Samples of TMR and orts were taken on the last 5 d of each period and frozen. Samples were

composited, dried, and analyzed for analytical DM, ADF, amylase-treated NDF, ether extract, ash, and starch as described by Alfonso-Avila et al. (2017). Additionally, subsamples of TMR and orts were digested to complete the oxidation of OM for further determination of CP and mineral (except Cl) composition. Chlorine in TMR and orts was extracted using a method adapted from Liu (1998). Concentrations of P, K, Ca, Mg, Na, and S were determined using inductively coupled plasma optical emission spectrometry (Optima 4300DV, Perkin Elmer, Shelton, CT), whereas ion chromatography (model ICS-2100, Dionex Corp., Sunnyvale, CA) was used to determine Cl concentration (Alfonso-Avila et al., 2017). Dietary FA were directly transmethyated and FAME were analyzed by GC with a flame ionization detector (Villeneuve et al., 2013).

On the last 5 d of each period, milk yield was recorded and samples collected at each milking using calibrated milk meters (Flowmaster Pro, DeLaval, Tumba, Sweden). Daily samples were composited on a milk production basis. A first subsample was stored at $4^\circ C$ with bronopol (2-bromo-2-nitropropane-1,3-diol) until further determination of milk fat, protein, lactose, and MUN according to 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 on the same sample at Valacta using a Fossomatic FC (Foss). A second aliquot of milk was frozen without preservative for subsequent determination of mineral concentrations and FA composition. Concentrations of milk P, K, Ca, Mg, Na, S, and Cl were determined according to the procedure described above.

Milk FA were extracted and methylated according to Chouinard et al. (1997). Analysis of the milk FA composition was carried out with a GC (Agilent Technologies, Santa Clara, CA) equipped with a 100-m CP-Sil-88 capillary column (Agilent Technologies Canada Inc., Mississauga, ON, Canada) and a flame ionization detector (Boivin et al., 2013). Glycerol in milk fat was calculated as described by Stamey et al. (2010).

Rumen fluid was collected from the ventral sac of the rumen on 2 consecutive days of each collection period at 0, 1, 2, 4, 6, and 8 h postfeeding, using a sampler tube (Bar-Diamond Inc., Parma, ID). Following an immediate pH measurement (pHTestr 30, Oakton Instruments, Vernon Hills, IL), a 10-mL aliquot was stabilized with 0.2 mL of H_2SO_4 (50%, vol/vol) and stored at $-20^\circ C$ until determination of VFA and ammonia-N (NH_3 -N) concentrations. A second aliquot (15 mL) was stored at $-20^\circ C$ before analyses for mineral composition. Ruminant cation-anion difference (**RCAD**) was calculated as follows (Alfonso-Avila et al., 2017):

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

At the time of analysis, acidified samples of rumen fluid were thawed and centrifuged at $16,060 \times g$ for 15 min at 4°C. The supernatant was analyzed for $\text{NH}_3\text{-N}$ 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 using GC according to Alfonso-Avila et al. (2017). The second sample of

rumen fluid was thawed and centrifuged at $13,000 \times g$ during 30 min at 4°C, the supernatants were then composited by period within cow and day, for further determination of P, K, Ca, Mg, Na, S, and Cl concentrations as described previously.

On the last d of each collection period, blood samples were taken at 1400 h 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,

Table 1. Ingredients and chemical composition of the experimental diets

% of DM (unless otherwise stated)	Pretreatment	Treatment			
		0% K_2CO_3		1.5% K_2CO_3	
		0% SBO ¹	2% SBO	0% SBO	2% SBO
Ingredient					
Grass silage	37.5	17.4	17.4	17.1	17.1
Corn silage	14.2	23.4	23.4	23.0	23.0
Ground corn	32.3	11.6	9.6	11.4	9.4
Ground barley	—	30.4	30.4	29.9	29.9
Soybean meal	9.0	10.0	10.0	9.8	9.8
Corn gluten meal	4.5	5.2	5.2	5.1	5.1
Mineral-vitamin mix ²	2.6	2.1	2.1	2.0	2.00
K_2CO_3 ³	—	—	—	1.5	1.5
Soybean oil	—	—	1.9	—	2.0
Chemical composition					
DM, % as fed	45.90	46.07	46.70	46.57	46.65
OM	93.88	93.96	93.62	94.07	93.56
CP	13.87	15.20	16.15	15.69	16.24
Amylase-treated NDF	35.40	29.60	30.50	29.70	29.10
ADF	23.46	18.33	19.80	18.27	18.63
Starch	23.26	26.37	24.56	26.54	25.41
NE_L , ⁴ Mcal/kg of DM	1.64	1.64	1.70	1.64	1.69
Na	0.39	0.20	0.21	0.21	0.19
K	1.38	1.12	1.11	1.94	1.94
Cl	0.55	0.47	0.48	0.48	0.44
S	0.24	0.25	0.24	0.24	0.24
Ca	0.70	0.58	0.58	0.59	0.54
Mg	0.37	0.25	0.25	0.24	0.23
P	0.47	0.51	0.51	0.51	0.49
DCAD ⁵					
Formulated, mEq/kg of DM	212	96	95	316	316
Measured, mEq/kg of DM	296	166	172	378	379
Fatty acid, mg/g of DM					
12:0	0.06	0.06	0.06	0.06	0.06
14:0	0.10	0.10	0.12	0.10	0.11
16:0	4.03	4.13	6.28	4.16	6.04
16:1	0.08	0.07	0.08	0.07	0.07
17:0	0.06	0.05	0.07	0.05	0.07
18:0	0.54	0.51	1.27	0.55	1.18
<i>cis</i> -9 18:1	4.56	3.55	6.45	3.53	6.33
<i>cis</i> -11 18:1	0.20	0.20	0.40	0.20	0.39
18:2	9.54	8.28	15.84	7.94	15.26
18:3	2.83	1.71	2.82	1.57	2.59
Total	22.00	18.65	33.40	18.21	32.10

¹SBO = soybean oil.

²15% Ca, 5% P, 4% Mg, 10% Na, 15% Cl, 0.2% K, 0.2% S, 2,900 mg/kg of Fe, 1,860 mg/kg of Mn, 510 mg/kg of Cu, 260 mg/kg of Zn, 45 mg/kg of I, 25 mg/kg of Se, 20 mg/kg of Co, 332,000 IU/kg of vitamin A, 88,000 IU/kg of vitamin D, and 1,700 IU/kg of vitamin E.

³ K_2CO_3 sesquihydrate in the form of DCAD Plus (Church & Dwight Co. Inc., Princeton, NJ).

⁴Calculated according to NRC (2001).

⁵DCAD = $[\text{Na} + \text{K}] - [\text{Cl} + \text{S}]$.

Denmark) containing 80 IU of electrolyte-balanced heparin. Blood hematocrits, concentrations of electrolytes (Na^+ , K^+ , Ca^{2+} , and Cl^-) and HCO_3^- , anion gap ($\text{Na}^+ + \text{K}^+ - \text{Cl}^- - \text{HCO}_3^-$), as well as 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, blood parameters, and minerals in milk and rumen fluid were analyzed as a randomized complete 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 4), B_j is the random effect of block ($j = 1$ to 7), C is the covariate adjustment for each cow ($C = 1$ to 28), and ε_{ij} denotes the residual error. 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 4), H_j refers to fixed effect of sampling time ($j = 0, 1, 2, 4, 6,$ and 8 h postprandial), $(T \times H)_{ij}$ is the fixed effect of the interaction, $A_{(k)l}$ is the random effect of cow ($k = 1$ to 4) within block ($l = 1$ to 7), B_l is the random effect of block, C is the covariate adjustment for rumen parameters of each cow ($C = 1$ to 28), and ε_{ijkl} denotes the residual error. Cow within block was included in the model as the subject of the repeated statement. The spatial covariance structure was used to estimate covariances. Because no treatment $\times$ sampling time interaction was observed for rumen pH, and concentrations of $\text{NH}_3\text{-N}$ and VFA, values were combined by day and analyzed according to the model previously mentioned. Preplanned orthogonal contrasts were used to assess the effects of K_2CO_3 , SBO, and the $\text{K}_2\text{CO}_3 \times$ SBO interaction. Simple linear regressions were conducted using the REG procedure of SAS to determine relationships between milk or lactose yield and milk mineral concentrations. Differences were declared significant at $P \leq 0.05$ and tendencies at $0.05 < P \leq 0.10$.

The risk β (power test) was determined when $\alpha < 0.05$. The hypothesis of equality was accepted if $1 - \beta > 0.8$ (Leclercq-Perlat et al., 2000). Considering 4 treatments, a post-hoc power test (risk β) was calculated using G*Power version 3.1.9.2 (<http://www.gpower.hhu.de/>) and the final sample size required to achieve 80% power was less than the sample size used in the current experiment ($n = 25$), except for the following variables: protein yield (required $n = 36$), lactose concentration ($n = 60$), milk Cl ($n = 52$) and Na ($n = 36$) concentrations, rumen pH ($n = 28$), as well as total VFA ($n = 36$), and butyrate ($n = 40$) concentrations, and blood K concentration ($n = 36$). The final power for those analyses varied between 36 and 75%. Results should be interpreted accordingly, keeping in mind the allowable difference for all analyses and the power level for the probability of ruminal and blood parameters as well as milk performance.

RESULTS AND DISCUSSION

Three cows were removed from the trial for health reasons (displaced abomasum, $n = 1$; mastitis, $n = 2$), and data from these animals were not used in the statistical analysis.

Diets, DMI, and BW

The actual DCAD averaged 169 and 379 mEq/kg of DM for diets with or without K_2CO_3 , respectively (Table 1), confirming the effectiveness of DCAD modulation through mineral supplementation.

Dry matter intake and BW were not affected by K_2CO_3 , SBO, or their interaction (Table 2). In agreement with these results, other studies reported no difference in DMI when 1.25 or 1.21% of K_2CO_3 was added to the diets of dairy cows (West et al., 1987; Iwaniuk et al., 2015). Noteworthy is the similar forage-to-concentrate ratio (40:60) between these previous studies and the current experiment. In contrast, an increase in DMI was observed when 2.46% of K_2CO_3 was included in diets with a forage-to-concentrate ratio of approximately 70:30 (Iwaniuk et al., 2015). Discrepancies among studies could be due to difference in basal diet composition and levels of K_2CO_3 supplement.

The similar DMI observed in the current study regardless of SBO supplementation is surprising given that SBO increased dietary NE_L concentration, and that fat supplementation, at a concentration similar to the one used in the current study (+2%), has been associated with a hypophagic effect in dairy cows (Rabiee et al., 2012). However, other studies also reported no significant effect of SBO on DMI (Bu et al., 2007;

Table 2. Dry matter intake, BW, milk yield, and composition of early-lactation cows receiving high-concentrate diets with or without K₂CO₃ and soybean oil (SBO)

Item	Treatment				SEM	Effect, <i>P</i> -value		
	0% K ₂ CO ₃		1.5% K ₂ CO ₃			K ₂ CO ₃	SBO	Interaction
	0% SBO	2% SBO	0% SBO	2% SBO				
DMI, kg of DM/d	24.73	24.64	24.25	25.46	1.48	0.88	0.64	0.57
BW, kg	645	642	640	654	12.09	0.73	0.66	0.43
Milk yield, kg/d								
Actual	39.61	41.14	37.78	42.56	1.36	0.86	0.02	0.18
Fat-corrected ¹	35.69	35.57	33.93	39.05	1.52	0.50	0.08	0.06
Energy-corrected ²	35.78	34.98	33.89	38.34	1.80	0.62	0.27	0.09
Milk fat								
Concentration, %	3.37	3.04	3.40	3.42	0.19	0.21	0.36	0.31
Yield, kg/d	1.32	1.27	1.29	1.46	0.07	0.18	0.39	0.09
Milk protein								
Concentration, %	3.25	2.96	3.22	3.10	0.09	0.50	0.02	0.28
Yield, kg/d	1.30	1.22	1.22	1.31	0.07	0.96	0.90	0.20
Lactose								
Concentration, %	4.62	4.63	4.63	4.73	0.10	0.54	0.56	0.61
Yield, kg/d	1.84	1.92	1.74	1.98	0.10	0.81	0.09	0.36
MUN, mg/dL	17.22	15.79	15.31	12.61	0.95	0.01	0.04	0.47
SCC, ×1,000/mL	35.69	35.94	35.08	38.76	1.63	0.39	0.18	0.19
Milk mineral, mmol/L								
Na	17.30	17.68	16.55	16.90	0.69	0.21	0.57	0.98
K	45.44	46.67	49.11	45.74	1.69	0.37	0.49	0.14
Cl	18.78	18.93	19.70	17.54	1.58	0.54	0.87	0.44
S	10.84	10.03	11.24	10.22	0.47	0.43	0.06	0.82
Ca	29.64	28.25	31.09	29.72	1.43	0.28	0.32	0.99
Mg	4.87	4.50	4.85	4.37	0.21	0.71	0.04	0.76
P	34.42	34.20	36.44	34.39	1.07	0.26	0.29	0.39

¹4% FCM = (0.4 × milk yield in kg/d) + (15 × fat yield in kg/d) (Gaines, 1928).

²ECM = [(23.8 × protein yield in kg/d) + (38.9 × fat yield in kg/d) + (16.3 × lactose yield in kg/d)]/3.14 (Madsen et al., 2008).

AlZahal et al., 2008). It is possible that, in the present experiment, the unsupplemented diets did not reach the energy requirements. Hence, cows were able to use the extra energy provided by SBO to increase their milk yield.

Rumen pH, Fermentation Attributes, and Minerals

In the current experiment, rumen pH was not affected by treatments. Dietary supplementation of K₂CO₃ decreased rumen molar concentration of acetate by 1.59% and increased propionate by 3.16%, which resulted in a lower acetate-to-propionate ratio (Table 3). Because the equivalence point (pK_a) of K₂CO₃ is near rumen pH, this mineral supplement has been widely used as a dietary buffer in dairy cows (Erdman, 1988). Consequently, dietary K₂CO₃ supplementation is typically associated with an increase in rumen acetate and a decrease in rumen propionate (Fraleley et al., 2015). However, one should not ignore that substantial variations exist in response to high-concentrate diets among cows (Gao and Oba, 2014, 2016), and that the absorption rates of acetate and propionate could be affected

(Penner and Oba, 2009). In the current study, a small number of animals was assigned to each treatment ($n = 7$), which could have affected the statistical power of the experiment.

As expected, K₂CO₃ supplementation increased K concentration (+29%) and decreased Na (−16%) and P (−21%) concentrations in the rumen. Bennink et al. (1978) found that a greater concentration of K in the rumen was associated with reduced Na concentration. More recently, Catterton and Erdman (2016) observed that, when cows were fed dietary K, rumen Na tended to decline from 4 to 12 h postfeeding. These authors also observed an inverse relationship between Na and K, where a 1 mEq/L increase in rumen Na corresponded to a 0.53 mEq/L decrease in rumen K.

In ruminants, P absorption is carried out mainly in the small intestine and is regulated by endocrine and nutritional factors such as mineral concentration of the diet and the form of P supplementation (inorganic, phytic acid, and organic; Feng et al., 2015). However, it is suggested that rumen microbes could affect P metabolism because their requirements of P may be greater than for the host animal and take priority (Kincaid and

Rodehutschord, 2005). Nevertheless, to our knowledge, information about P metabolism in the rumen is scarce, which prevents us from further speculating on the interrelationship observed between P and K ions when cows received K_2CO_3 .

Soybean oil supplementation resulted in a decrease of 52% in rumen Ca concentration. It is well recognized that more rumen Ca may be used in the formation of insoluble FA soaps when lipids are added to diets. Indeed, Palmquist et al. (1986) observed a 50% reduction in ruminal concentration of ionic Ca when tallow was added to diets of dairy cows. Noteworthy, these authors specify that, in the rumen, Ca soap formation is dependent on the amount of long-chain FA, but independent of Ca supplementation. No interaction was present between K_2CO_3 and SBO on mineral concentration in the rumen. Despite the effect of K_2CO_3 on DCAD (Table 1), RCAD was not affected by K_2CO_3 supplementation. These results are in contrast with previous studies (Catterton and Erdman, 2016; Alfonso-Avila et al., 2017), where a greater RCAD was reported when DCAD was increased. In the present experiment, and as mentioned above, rumen concentrations of K and Na were negatively correlated. Given that K was the only dietary ion modified with K_2CO_3 supplementation, the interrelationship between K and Na could explain the similar RCAD among treatments.

Systemic Acid-Base Status

No effect was observed of SBO or $K_2CO_3 \times$ SBO interaction on any blood parameters related to systemic acid-base status. However, adding K_2CO_3 to cow diets decreased the blood anion gap by 18%, and increased HCO_3^- and pCO_2 by 6.38 and 6.31%, respectively (Table 4). Anion gap represents the difference between the concentration of unmeasured cations and anions in serum ($K^+ + Na^+ - Cl^- - HCO_3^-$; Constable, 2000). The increase in blood pCO_2 and HCO_3^- when cows were fed K_2CO_3 suggests an altered acid-base regulation, probably related to the increase in dietary supply of CO_3^{2-} via mineral supplementation. As CO_2 in blood can be combined with water to form H_2CO_3 (Rose and Post, 2001), it is possible that cows receiving K_2CO_3 compensated by decreasing their respiration rate, allowing an elevation in pCO_2 , and consequently increased H_2CO_3 to maintain the acid-base balance.

Milk Production and Composition

Dietary SBO supplementation resulted in a 7.5% increase in milk yield. Dietary K_2CO_3 tended to increase milk fat yield, but only when combined with SBO supplementation. These results explain the tendency for an interaction between K_2CO_3 and SBO on yields of 4%

Table 3. pH, NH_3 -N, VFA, and minerals from rumen fluid of early-lactation cows receiving high-concentrate diets with or without K_2CO_3 and soybean oil (SBO)

Item	Treatment				SEM	Effect, <i>P</i> -value		
	0% K ₂ CO ₃		1.5% K ₂ CO ₃			K ₂ CO ₃	SBO	Interaction
	0% SBO	2% SBO	0% SBO	2% SBO				
pH	6.21	6.28	6.30	6.29	0.05	0.29	0.51	0.41
NH ₃ -N, mg/dL	11.04	11.22	12.41	10.40	0.81	0.66	0.16	0.09
Total VFA, mM	87.47	85.10	89.72	89.70	3.06	0.20	0.66	0.68
VFA, mol/100 mol								
Acetate	64.01	64.12	62.92	63.20	0.54	0.01	0.63	0.83
Propionate	22.87	22.42	23.52	23.20	0.43	0.02	0.20	0.84
Butyrate	9.35	9.40	9.63	9.56	0.20	0.17	0.93	0.71
Isobutyrate	0.98	1.06	1.00	1.02	0.03	0.73	0.05	0.26
Valerate	0.95	1.03	1.06	1.03	0.03	0.06	0.32	0.06
Isovalerate	1.51	1.60	1.59	1.63	0.05	0.19	0.14	0.53
Caproate	0.30	0.33	0.36	0.31	0.03	0.49	0.79	0.15
Acetate:propionate	2.91	3.00	2.77	2.88	0.08	0.03	0.09	0.87
Mineral, mM								
Na	90.02	85.00	75.37	72.41	4.40	<0.01	0.30	0.78
K	20.63	21.13	30.56	27.91	1.69	<0.01	0.50	0.35
Cl	9.00	8.78	9.38	7.99	0.59	0.70	0.15	0.28
S	0.73	0.74	0.75	0.73	0.10	0.94	0.91	0.86
Ca	1.59	0.82	1.42	0.61	0.15	0.18	<0.01	0.88
Mg	2.61	2.20	2.43	2.20	0.40	0.81	0.37	0.83
P	12.10	12.70	9.57	10.04	1.01	0.01	0.56	0.95
RCAD, ¹ mEq/L	101.60	95.88	95.30	92.50	5.75	0.35	0.39	0.76

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

FCM and ECM. Recently, Ma et al. (2017) reported an increase in milk fat concentration when cows received 1.2% of K_2CO_3 and 1.8% SBO as compared with cows receiving SBO only. However, differences among treatments for milk fat yield were not significant.

Dietary K_2CO_3 did not affect the concentration of *trans*-11 18:1 in milk fat (Table 5). This lack of effect is contrary to the results of Ma et al. (2017) who reported a 23% increase in milk fat concentration of *trans*-11 18:1 when cows were fed a diet supplemented with both K_2CO_3 and SBO as compared with SBO only. They suggested that K_2CO_3 promotes the predominant *trans*-11 biohydrogenation pathway rather than the *trans*-10 pathway in the rumen. In the current experiment, milk fat concentration of *trans*-10 18:1, which can be used as a marker for milk fat depression (Jenkins et al., 2014), was increased by 73% when cows received SBO as compared with unsupplemented cows (Table 5). However, in contrast with Ma et al. (2017), dietary K_2CO_3 did not affect milk fat concentration of *trans*-10 18:1, or any other identified FA biohydrogenation intermediates.

As expected, SBO supplementation decreased milk fat concentration of C16 and de novo synthesized FA (−13 and −17%, respectively), and increased the concentration of preformed FA (+21%) as compared with unsupplemented cows (Table 5). It has been suggested that the reduction in de novo FA synthesis observed when feeding SBO occurs as a result of greater uptake and secretion of dietary derived FA by the mammary gland (Baumann et al., 2016). It was also observed that cows fed SBO had greater concentrations of *cis*-9,*trans*-11 18:2 (+41%), most of the *cis* and *trans* isomers of 18:1 (+19 to +58%), as well as 18:0 (+35%),

all of these FA originating from biohydrogenation of *cis*-9,*cis*-12 18:2, the major FA found in SBO.

The effect of dietary K_2CO_3 on milk FA composition was limited to a few odd- and branched-chain FA. More specifically, adding dietary K_2CO_3 decreased milk FA concentration of *iso* 17:0 (−12%) and 17:0 (−16%) as compared with unsupplemented cows. Odd- and branched-chain FA are synthesized by different rumen microbial populations, absorbed in the intestine, and taken up by the mammary gland to be incorporated in milk fat (Fievez et al., 2012). In the current study, dietary K_2CO_3 affected the ionic concentrations of K, Na, and P in the rumen, and therefore affected environmental conditions of microbial populations (Table 4). In this regard, environmental stress factors (e.g., ion concentrations, pH) are recognized to modulate the bacterial FA profile (homeoviscous adaptation) to maintain membrane fluidity (Giotis et al., 2007). Therefore, it is possible that observed changes in milk odd- and branched-chain FA profile when K_2CO_3 was incorporated in the diets were due to the effects of rumen osmolarity fluctuations on bacterial populations.

Milk protein concentration decreased by 6.2% when cows received SBO as compared with unsupplemented cows (Table 2). Fat supplementation has been previously associated with lower milk protein concentrations, regardless of the type of fat fed (DePeters and Cant, 1992). Also, early-lactation dairy cows appear more prone to fat-induced milk protein depression than those in mid to late lactation (Wu et al., 1993). These authors suggested that a greater milk yield resulting from fat supplementation increases AA requirement to maintain constant milk protein concentration. Therefore, in the current study, the decrease in milk protein

Table 4. Blood parameters of early-lactation cows receiving high-concentrate diets with or without K_2CO_3 and soybean oil (SBO)

Item	Treatment				SEM	Effect, <i>P</i> -value		
	0% K ₂ CO ₃		1.5% K ₂ CO ₃			K ₂ CO ₃	SBO	Interaction
	0% SBO	2% SBO	0% SBO	2% SBO				
pH	7.45	7.43	7.45	7.46	0.01	0.16	0.56	0.35
Electrolyte, mmol/L								
Ca ²⁺	1.14	1.23	1.20	1.19	0.04	0.68	0.21	0.15
Cl [−]	104.7	107.0	105.3	105.8	1.0	0.77	0.12	0.30
K ⁺	3.42	3.55	3.53	3.66	0.14	0.42	0.32	1.00
Na ⁺	139.1	140.0	139.4	139.3	0.8	0.79	0.54	0.53
Anion gap, ¹ mmol/L	10.98	12.08	9.71	9.25	0.68	<0.01	0.55	0.12
HCO ₃ [−] , mmol/L	26.83	24.29	28.17	27.68	1.08	0.02	0.14	0.26
Hematocrit, %	27.58	30.22	29.04	28.79	0.99	0.99	0.21	0.13
Blood gas, ² mmHg								
pCO ₂	39.50	37.68	40.85	41.52	1.51	0.01	0.49	0.14
pO ₂	39.21	43.76	37.62	42.91	3.31	0.66	0.10	0.90

¹Anion gap = $[K^+ + Na^+] - [Cl^- + HCO_3^-]$.

² pCO_2 = partial pressure of CO_2 ; pO_2 = partial pressure of O_2 .

Table 5. Composition of milk fat from early-lactation cows receiving high-concentrate diets supplemented with or without K₂CO₃ and soybean oil (SBO)

Item, %	Treatment				SEM	Effect, <i>P</i> -value		
	0% K ₂ CO ₃		1.5% K ₂ CO ₃			K ₂ CO ₃	SBO	Interaction
	0% SBO	2% SBO	0% SBO	2% SBO				
4:0	2.397	2.809	2.653	2.548	0.229	0.99	0.41	0.14
6:0	1.511	1.465	1.569	1.516	0.075	0.43	0.48	0.96
8:0	1.005	0.908	1.024	0.962	0.048	0.39	0.08	0.69
10:0	2.824	2.327	2.926	2.435	0.136	0.38	<0.01	0.98
10:1	0.229	0.180	0.244	0.184	0.013	0.35	<0.01	0.61
11:0	0.136	0.075	0.155	0.078	0.020	0.55	<0.01	0.65
12:0	3.707	2.894	3.937	2.935	0.213	0.47	<0.01	0.62
<i>iso</i> 13:0	0.033	0.034	0.031	0.034	0.004	0.90	0.52	0.79
<i>anteiso</i> 13:0	0.010	0.010	0.010	0.009	0.001	0.35	0.62	0.80
12:1	0.105	0.071	0.119	0.070	0.008	0.32	<0.01	0.26
13:0	0.199	0.137	0.227	0.138	0.023	0.49	<0.01	0.51
<i>iso</i> 14:0	0.058	0.056	0.077	0.052	0.008	0.27	0.07	0.11
14:0	11.597	10.559	11.969	10.557	0.454	0.61	0.01	0.60
<i>cis</i> -9 14:1	0.928	0.838	1.048	0.776	0.062	0.49	<0.01	0.05
<i>cis</i> -11 14:1	0.027	0.017	0.032	0.018	0.003	0.30	<0.01	0.42
<i>iso</i> 15:0	0.721	0.664	0.709	0.628	0.036	0.43	0.06	0.68
<i>anteiso</i> 15:0	0.404	0.379	0.377	0.363	0.021	0.24	0.32	0.78
15:0	1.698	1.131	1.807	1.170	0.145	0.58	<0.01	0.79
<i>iso</i> 16:0	0.140	0.154	0.197	0.178	0.031	0.17	0.93	0.57
16:0	30.597	25.042	30.546	26.462	1.150	0.52	<0.01	0.51
<i>trans</i> -9 16:1	0.026	0.046	0.028	0.042	0.004	0.83	<0.01	0.37
<i>cis</i> -9 16:1	1.423	1.263	1.386	1.088	0.143	0.42	0.11	0.59
<i>cis</i> -11 16:1	0.040	0.029	0.040	0.023	0.004	0.47	<0.01	0.42
<i>cis</i> -13 16:1	0.200	0.114	0.229	0.139	0.020	0.09	<0.01	0.90
<i>iso</i> 17:0 ¹	0.315	0.326	0.286	0.280	0.018	0.03	0.88	0.60
<i>anteiso</i> 17:0 ²	0.393	0.375	0.377	0.380	0.031	0.85	0.78	0.72
17:0	0.335	0.279	0.301	0.215	0.034	0.03	<0.01	0.46
<i>cis</i> -7 17:0	0.021	0.017	0.019	0.017	0.002	0.56	0.17	0.72
<i>cis</i> -8 17:1	0.010	0.009	0.010	0.008	0.002	0.83	0.34	0.89
<i>cis</i> -9 17:1	0.125	0.104	0.113	0.079	0.014	0.15	0.04	0.59
<i>iso</i> 18:0	0.012	0.008	0.013	0.009	0.001	0.31	<0.01	0.97
18:0	5.632	7.783	4.709	8.172	0.468	0.48	<0.01	0.07
<i>trans</i> -4 18:1	0.012	0.023	0.010	0.018	0.003	0.22	<0.01	0.55
<i>trans</i> -5 18:1	0.010	0.020	0.010	0.014	0.003	0.20	0.01	0.27
<i>trans</i> -6–8 18:1	0.142	0.419	0.146	0.274	0.067	0.27	<0.01	0.24
<i>trans</i> -9 18:1	0.115	0.314	0.101	0.202	0.051	0.19	0.01	0.30
<i>trans</i> -10 18:1	0.332	1.335	0.182	0.564	0.367	0.17	0.05	0.35
<i>trans</i> -11 18:1	0.461	0.904	0.463	0.934	0.061	0.78	<0.01	0.80
<i>trans</i> -12 18:1	0.130	0.285	0.115	0.300	0.019	0.99	<0.01	0.40
<i>trans</i> -13–14 18:1	0.172	0.406	0.140	0.446	0.039	0.91	<0.01	0.33
<i>trans</i> -15 18:1	0.189	0.368	0.175	0.376	0.011	0.78	<0.01	0.28
<i>trans</i> -16 18:1	0.151	0.253	0.142	0.294	0.017	0.28	<0.01	0.10
<i>cis</i> -6–8 18:1	0.134	0.176	0.114	0.165	0.021	0.42	0.03	0.82
<i>cis</i> -9–10 18:1	14.671	17.286	14.159	17.145	1.111	0.75	0.02	0.86
<i>cis</i> -11 18:1	0.891	0.841	0.737	0.818	0.073	0.20	0.82	0.35
<i>cis</i> -12 18:1	0.165	0.295	0.159	0.349	0.025	0.30	<0.01	0.20
<i>cis</i> -13 18:1	0.047	0.081	0.041	0.044	0.017	0.18	0.25	0.32
<i>cis</i> -14 18:1	0.032	0.066	0.030	0.051	0.006	0.10	<0.01	0.23
<i>cis</i> -15 18:1	0.033	0.076	0.039	0.203	0.052	0.18	0.04	0.22
<i>cis</i> -9, <i>trans</i> -12 18:2	0.038	0.055	0.035	0.044	0.004	0.11	<0.01	0.35
<i>trans</i> -9, <i>trans</i> -12 18:2	0.007	0.009	0.009	0.009	0.001	0.33	0.37	0.30
<i>cis</i> -9, <i>trans</i> -13 18:2	0.232	0.316	0.235	0.284	0.019	0.42	<0.01	0.34
<i>trans</i> -8, <i>cis</i> -13 18:2	0.050	0.095	0.055	0.090	0.009	1.00	<0.01	0.58
<i>trans</i> -9, <i>cis</i> -12 18:2	0.021	0.027	0.022	0.020	0.004	0.31	0.55	0.22
<i>trans</i> -11, <i>cis</i> -15 18:2	0.030	0.107	0.031	0.045	0.031	0.28	0.12	0.27
<i>cis</i> -9, 12 18:2	1.722	1.846	1.836	1.784	0.096	0.74	0.67	0.28
<i>cis</i> -9, <i>trans</i> -11 18:2	0.259	0.489	0.280	0.432	0.047	0.68	<0.01	0.37
<i>trans</i> -10, <i>cis</i> -12 18:2	0.020	0.019	0.020	0.025	0.002	0.14	0.30	0.25
<i>cis</i> -9, 12, 15 18:3	0.240	0.272	0.248	0.248	0.014	0.54	0.24	0.23
<i>cis</i> -6, 9, 12 18:3	0.035	0.031	0.034	0.030	0.002	0.46	0.04	0.86
<i>cis</i> -9, <i>trans</i> -11, <i>cis</i> -15 18:3	0.021	0.018	0.019	0.016	0.002	0.46	0.08	>0.99

Continued

Table 5 (Continued). Composition of milk fat from early-lactation cows receiving high-concentrate diets supplemented with or without K₂CO₃ and soybean oil (SBO)

Item, %	Treatment				SEM	Effect, <i>P</i> -value		
	0% K ₂ CO ₃		1.5% K ₂ CO ₃			K ₂ CO ₃	SBO	Interaction
	0% SBO	2% SBO	0% SBO	2% SBO				
<i>cis</i> -6, 9, 12, 15 18:4	0.036	0.030	0.025	0.025	0.007	0.13	0.65	0.61
19:0	0.058	0.057	0.074	0.223	0.070	0.18	0.26	0.25
20:0	0.093	0.100	0.090	0.113	0.007	0.41	0.03	0.19
<i>cis</i> -9 20:1	0.034	0.033	0.033	0.035	0.002	0.81	0.82	0.42
<i>cis</i> -11 20:1	0.033	0.041	0.034	0.032	0.005	0.35	0.57	0.24
<i>cis</i> -11, 14 20:2	0.048	0.041	0.054	0.043	0.012	0.71	0.42	0.89
<i>cis</i> -11, 14, 17 20:3	0.007	0.009	0.008	0.007	0.001	0.36	0.42	0.03
<i>cis</i> -8, 11, 14 20:3	0.095	0.092	0.090	0.092	0.008	0.56	0.78	0.59
<i>cis</i> -8, 11, 14, 17 20:4	0.006	0.007	0.006	0.009	0.001	0.49	0.16	0.52
<i>cis</i> -5, 8, 11, 14 20:4	0.143	0.131	0.140	0.124	0.010	0.55	0.08	0.79
<i>cis</i> -5, 8, 11, 14, 17 20:5	0.029	0.026	0.028	0.026	0.002	0.65	0.30	0.81
22:0	0.029	0.030	0.029	0.036	0.003	0.17	0.08	0.16
<i>cis</i> -13 22:1	0.004	0.006	0.008	0.006	0.002	0.24	0.97	0.23
<i>cis</i> -13, 16 22:2	0.011	0.011	0.010	0.012	0.001	0.80	0.62	0.37
<i>cis</i> -13, 16, 19 22:3	0.004	0.003	0.004	0.005	0.001	0.27	0.57	0.41
<i>cis</i> -7, 10, 13, 16 22:4	0.022	0.021	0.022	0.019	0.003	0.47	0.35	0.66
<i>cis</i> -7, 10, 13, 16, 19 22:5	0.035	0.044	0.041	0.037	0.007	0.94	0.56	0.22
<i>cis</i> -4, 7, 10, 13, 16, 19 22:6	0.006	0.011	0.008	0.006	0.001	0.17	0.31	0.01
24:0	0.016	0.017	0.017	0.023	0.002	0.13	0.12	0.27
24:1	0.005	0.007	0.007	0.008	0.001	0.07	0.38	0.61
Glycerol	11.532	11.466	11.638	11.458	0.070	0.39	0.05	0.32
Others ³	0.801	1.058	0.876	0.740	0.127	0.25	0.56	0.13
Sum								
De novo fatty acids ⁴	22.052	19.389	22.867	19.520	0.828	0.50	<0.01	0.63
C16	32.309	26.531	32.941	27.828	1.119	0.37	<0.01	0.76
Preformed fatty acids ⁵	30.966	38.223	29.238	37.894	1.519	0.48	<0.01	0.63
<i>trans</i> -10/ <i>trans</i> -11 18:1 ratio	0.680	1.528	0.523	0.602	0.443	0.15	0.20	0.28

¹Coelution with minor concentration of *trans*-10 16:1.²Coelution with minor concentration of *cis*-10 16:1.³Represent unidentified chromatogram peaks.⁴Sum of straight even-chain fatty acids from C6 to C14.⁵Sum 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.

concentration could be related to an inadequacy of critical AA available to the mammary gland as milk volume increased.

Milk urea N concentration decreased when cows received dietary K₂CO₃ (−15%) as compared with nonsupplemented diets. Based on a systematic review, Spek et al. (2013) established a linear relationship between plasma urea N or MUN and urinary N, and concluded that the level of water intake could affect these relationships due to an associated increase in urine production. Urea plays an essential role in the urine-concentrating mechanism that regulates water excretion (Sands and Layton, 2014). Urea permeability increased rapidly in perfused terminal inner medullary collecting ducts of rats when osmolarity was increased, a distinct mechanism from common vasopressin or cyclic AMP regulations (Sands and Schrader, 1991). Accordingly, in the current trial, the observed decrease

in MUN when cows were fed K₂CO₃-supplemented diets could be due to the greater mineral concentration of their rations, which might have increased the urinary excretion of these animals to maintain their systemic K⁺ balance. Milk urea N concentration was also decreased by dietary supplementation of SBO (−13%). Broderick and Clayton (1997) reported that milk yield and NE_L intake are negatively related to MUN due to a dilution effect. As such, it is possible that the decrease in MUN observed in cows fed SBO may be partly explained by their greater milk yield.

It is well documented that ions are strongly implicated in cellular mechanisms that transport milk constituents or their precursors into, out of, and across the mammary secretory cells (Shennan and Peaker, 2000), and are consequently considered as determinant in milk synthesis. Moreover, given that ions in milk reflect the concentration of ions in the mammary epithelial

Figure 1. 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 with or without K_2CO_3 and soybean oil (SBO). Δ , 0% K_2CO_3 , 0% SBO; $\circ$, 1.5% K_2CO_3 , 0% SBO; $\diamond$, 0% K_2CO_3 , 2% SBO; $\square$, 1.5% K_2CO_3 , 2% SBO.

cells (Holt, 1985), milk mineral composition could be considered as an indicator of homeostasis in the mammary gland. According to these interconnections, we

previously reported that milk Cl concentration was positively associated with milk yield ($R^2 = 0.34$, Alfonso-Avila et al., 2017). This positive relationship is

confirmed by the results from the current study, as milk Cl concentration and milk yield were significantly and positively correlated regardless of dietary treatments ($R^2 = 0.40$; Figure 1).

To deal with osmotic challenges, cells will activate specific metabolic or membrane-transport processes, which will counteract swelling or shrinkage and will bring cell volume back to its normal resting state (McManus et al., 1995). In the case of mammary epithelial cells, water is drawn into the Golgi vesicles as sugar accumulates in the Golgi compartment, and consequently, the lactating mammary cells will become swollen (Neville, 2001). The translocation of osmolytes (inorganic and organic) toward the extracellular space will allow this volume adjustment to reach a new osmotic equilibrium (Franco, 2003). The swelling of the mammary epithelial cell will induce efflux of K^+ and Cl^- via K^+/Cl^- cotransport as well as separate K^+ and Cl^- channels (Shennan, 2008). When the ion composition is disturbed, cells produce organic osmolytes, molecules specifically designed to create osmolarity without compromising other cell functions (Lang et al., 1998). Indeed, even though cell volume regulation is mainly driven by ions, organic osmolytes (e.g., AA, polyalcohols, or methylamines) play a pivotal role in the cellular hydration state (Lang et al., 1998), and consequently in the mammary cell metabolism (Shennan and Thomson, 2004). Many studies have focused on the properties of volume-sensitive transport of taurine, a nonprotein β -AA abundant within cells and tissues and essentially metabolically inert, and thus being considered representative of organic osmolytes (Franco, 2003; Shennan, 2008). In relation to this, previous studies with rats reported that Cl (e.g., Cl^- channels) is largely implicated in the transport of taurine in the mammary tissue, allowing mammary cells to volume-regulate and consequently help to control mammary metabolism (Shennan and Thomson, 2004). Given that protein and lipid synthesis are sensitive to changes in the cellular hydration state (Häussinger, 1996), Cl could play a role in milk synthesis via regulation of cell volume.

Milk Mg concentration decreased (-8.8%) when cows received SBO-supplemented instead of unsupplemented diets. Magnesium is an important modulator of intracellular free Ca concentration and intracellular pH, which are the major determinants of cell contraction, secretion, and motility (Schmitz et al., 2007), factors that could be relevant to milk synthesis. It is suggested that modifications of intracellular Mg, such as those triggered by hormones and growth factors, are likely due to the modulation of specific transport mechanisms (Romani, 2007). Nevertheless, these transport processes have not been fully characterized and there is not enough evidence to explain how dietary

supplementation of SBO is linked to a decrease in milk Mg concentration in lactating dairy cows.

CONCLUSIONS

In summary, the addition of K_2CO_3 to diets did not increase milk yield, but tended to increase milk fat yield when combined with SBO. Dietary K_2CO_3 did not affect milk fat concentration of *trans*-10 18:1 or any other identified intermediates of FA biohydrogenation. Moreover, K_2CO_3 in cow diets induced a compensatory metabolic response associated with pH regulation as shown by an increase in blood HCO_3^- and pCO_2 . The increase in blood pCO_2 and HCO_3^- when cows were fed K_2CO_3 suggests an altered acid-base regulation, probably related to the increase in dietary supply of CO_3^{2-} via mineral supplementation. Also, supplementing diets with SBO increased milk yield, decreased milk fat concentrations of C16 and de novo synthesized FA, and increased milk preformed FA concentration. Among the other effects of lipid supplementation observed, concentrations of *cis*-9,*trans*-11 18:2 increased, as well as most of the *cis* and *trans* isomers of 18:1, and 18:0, all of these FA originating from biohydrogenation of *cis*-9,*cis*-12 18:2, the major FA found in SBO. Overall, effect of K_2CO_3 on milk fat synthesis was dependent on the level of dietary PUFA. A positive association between milk yield and milk Cl concentration suggests that the equilibrium of this ion is linked to the efficiency of lactogenesis. The effect of dietary K_2CO_3 supplementation on the metabolism of mammary gland remains to be established.

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