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Liquid molasses interacts with buffers to affect ruminal fermentation, milk fatty acid profile, and milk fat synthesis in dairy cows fed high-concentrate diets

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ABSTRACT

We aimed to evaluate the effects of feeding sugarcane liquid molasses (LM) with or without a commercial buffer mix (BFM) on ruminal fermentation parameters, milk fatty acid (FA) profile, and milk yield and composition in dairy cows fed high-concentrate diets (35:65 forage-to-concentrate ratio). Eight multiparous Holstein cows (4 ruminally cannulated) averaging 165 ± 12 d in milk at the beginning of the study were randomly assigned to a replicated 4 × 4 Latin square design with a 2 × 2 factorial arrangement of treatments. Each period lasted 21 d with 14 d for diet adaptation and 7 d for data and sample collection. Cows were fed the following diets: (1) no LM or BFM supplementation (CTRL), (2) LM without BFM supplementation (MOL), (3) BFM without LM supplementation (BUF), and (4) LM plus BFM supplementation (COMBO). These 4 isonitrogenous and isoenergetic diets were formulated by replacing (dry matter basis) 5% ground corn with LM, whereas BFM replaced wheat bran at 0.8% of the diet. Significant LM × BFM interactions were observed for the duration of ruminal pH below 5.8, molar proportion of propionate, acetate-to-propionate ratio, milk proportions of *trans*-10 18:1 and total *trans* FA, and concentration and yield of milk fat. Feeding MOL and BUF alone were effective on reducing the time that ruminal pH remained below 5.8 compared with the CTRL treatment, and the COMBO diet decreased it further. A similar pattern was observed for the ruminal molar proportion of propionate. The milk proportions of *trans*-10 18:1 and total *trans* FA dropped significantly with BFM or LM supplementation versus cows fed CTRL, and the COMBO diet decreased these variables further. Note, however, that these changes elicited by the COMBO diet were not in the same magnitude as those caused by MOL or BUF fed alone. The ruminal molar proportion of acetate increased with the BUF diet and that of butyrate increased in cows fed MOL, but mean ruminal pH was not affected by treatments. Diets with LM resulted in increased concentrations of short- and medium-chain FA in milk fat. The yield of 3.5% fat-corrected milk increased significantly in cows fed MOL or BUF due to the improved concentration of milk fat. A trend and a significant increase for energy-corrected milk were observed with feeding MOL or BUF, respectively. Overall, inclusion of LM and BFM appears to reduce milk *trans*-10 18:1 FA and total *trans* FA by modulating ruminal pH and volatile FA profile in cows fed high-concentrate diets.

Key words: milk fat depression, soluble carbohydrate, ruminal pH, *trans* fatty acid

INTRODUCTION

High-producing dairy cows usually receive TMR with low forage-to-concentrate ratio to increase dietary energy intake and maximize milk yield. However, excessive intake of rapidly fermentable carbohydrates can reduce ruminal pH, inhibit cellulolytic activity in the rumen, and cause milk fat depression due to changes in ruminal biohydrogenation (**BH**) pathways toward production of *trans* fatty acid (**FA**) intermediates such as *trans*-10 18:1 and *trans*-10,*cis*-12 18:2 (Kleen et al., 2003; Zened et al., 2012). Decreased dietary starch concentration can minimize the incidence of ruminal acidosis and mitigates negative effects of *trans* BH intermediates on milk fat synthesis (Kleen et al., 2003).

One strategy to decrease dietary starch concentration while maintaining lactation performance is to replace starch with sugars. Previous research showed that sugar sources such as sucrose or molasses can increase feed intake (Broderick and Radloff, 2004; Broderick et al.,

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2008) and milk fat yield (Penner and Oba, 2009; Razzaghi et al., 2016). Replacing starch with sugars in diets of dairy cows did not increase the risk of ruminal acidosis (Penner and Oba, 2009) or induce milk fat depression (Martel et al., 2011), despite sugar fermentation being faster in the rumen than starch (Chamberlain et al., 1993). Martel et al. (2011) reported that molasses may promote de novo FA synthesis in the mammary gland of cows fed high-energy rations by regulating ruminal pH and altering BH pathways. Complete ruminal BH of UFA can mitigate the potential adverse effects of *trans* FA intermediates on milk fat synthesis (Shingfield et al., 2010). Thus, sugar sources may be used to lessen milk fat depression in dairy cows fed high-concentrate diets.

Buffer supplementation has been extensively studied in dairy cows with the goal of stabilizing ruminal pH (Marden et al., 2008; Cruywagen et al., 2015) while improving the efficiency of fiber digestion (Rogers et al., 1982). Shire and Beede (2013) suggested that supplementation of cationic salts may improve lactational performance by affecting several biological processes including ruminal buffer capacity and pH, as well as lower the ruminal production of *trans* FA intermediates. However, results regarding the effects of buffers and alkalinizing agents on DMI and milk yield and composition have not been consistent in the literature (Erdman et al., 1982; Cruywagen et al., 2015; Hu and Murphy, 2005). Saliva production and absorption of VFA through the ruminal wall are involved in processes that control acidity in the rumen (Dijkstra et al., 2012). Intake of buffers and soluble sugars could also affect the buffering capacity and absorptive mechanisms associated with decreased ruminal acidity (Dijkstra et al., 2012; Gao and Oba, 2016). Moreover, dietary inclusion of sucrose and buffers decreased the proportion of total *trans*-18:1 FA in milk fat (Kalscheur et al., 1997; Razzaghi et al., 2016) and increased milk fat concentration (Broderick et al., 2008; Iwaniuk et al., 2015). Nevertheless, to our knowledge, data on how sugar sources and buffer supplementation could interact to change ruminal fermentation and milk FA profile are lacking.

We hypothesized that liquid molasses (**LM**) and buffer could interact to reduce the time that ruminal pH spent <5.8, resulting in a decreased proportion of *trans*-10 18:1 in milk fat of Holstein cows fed high-concentrate diets (35:65 forage-to-concentrate ratio). Our objective was to investigate the effects of feeding diets containing different total sugar concentrations (~4.4 vs. 8.8% of the diet DM without or with LM) supplemented or not with a commercial buffer mix (**BFM**) on ruminal fermentation parameters, milk FA profile, and milk yield and composition in dairy cows fed high-concentrate diets.

MATERIALS AND METHODS

The experiment was conducted at the Research Farm of the Faculty of Agriculture, Ferdowsi University of Mashhad (Iran) in 2015. All the animal procedures were approved by the Animal Care Committee of Ferdowsi University of Mashhad following the guidelines of the Iranian Council of Animal Care (1995).

Cows, Experimental Design, and Treatments

Eight multiparous Holstein dairy cows averaging (mean ± SD) 165 ± 12 DIM, 630 ± 24 kg of BW, and 32 ± 1.5 kg/d of milk at the beginning of the study were used. Animals were assigned randomly to treatment sequences in a replicated 4 × 4 Latin square design with a 2 × 2 factorial arrangement of treatments, with 4 ruminally cannulated cows allocated to square 1 and the remaining 4 animals to square 2. Cows in square 1 averaged (mean ± SD) 174 ± 7 DIM, 628 ± 12 kg of BW, and 32.5 ± 0.55 kg of milk yield, and those in square 2 averaged (mean ± SD) 156 ± 11 DIM, 632 ± 16 kg of BW, and 31.5 ± 1.21 kg of milk yield. This resulted in a homogeneous distribution of cows in each square regarding DIM, milk yield, and BW. Within each square, treatment sequences were balanced for carryover effects in subsequent periods. Each experimental period lasted 21 d with 14 d for diet adaptation and 7 d for data and sample collection. A high-concentrate TMR (35:65 forage-to-concentrate ratio; DM basis) formulated to be potentially acidotic was fed (Stone, 2004). Cows were offered 1 of the following 4 treatments: (1) no LM or BFM supplementation (control = **CTRL**), (2) LM without BFM supplementation (**MOL**), (3) BFM without LM supplementation (**BUF**), and (4) LM plus BFM supplementation (**COMBO**). A portion of ground corn was replaced with 5% of diet DM as sugarcane LM, whereas the BFM supplement replaced wheat bran at 0.8% (diet DM basis). The BFM supplement (pHmax, Beihagh Nutri Paya Science-Based Co., Mashhad, Iran), which was composed by buffer and alkalinizing agents (i.e., a blend of sodium bicarbonate, sodium bentonite, calcium carbonate, potassium carbonate, and magnesium oxide supplying 87 g/kg of Na, 93 g/kg of Mg, 12 g/kg of K, and 106 g/kg of Ca) was fed at a daily intake rate of 180 g/cow. All diets were formulated to be isoenergetic and isonitrogenous using the Cornell-Penn-Miner System (CPM-Dairy, Version 3.0.8; Cornell University, Ithaca, NY; University of Pennsylvania, Kennett Square, PA; and William H. Miner Agricultural Research Institute, Chazy, NY) to meet or exceed the nutritional requirements of a typical lactating dairy cow in our herd weighing 630 kg and producing 40 kg of milk with 3.5% fat and 3.0% true

Table 1. Ingredient and chemical composition of the experimental diets

Item	Treatment[1]			
	CTRL	MOL	BUF	COMBO
Ingredient, % of diet DM				
Corn silage	28	28	28	28
Alfalfa hay	7	7	7	7
Ground corn	37	32	37	32
Sugarcane liquid molasses	—	5	—	5
Soybean meal	9	9	9	9
Fish meal	3	3	3	3
Canola meal	5	5	5	5
Cottonseed meal	5.5	5.5	5.5	5.5
Wheat bran	2.5	2.5	1.7	1.7
Megalac[2]	1.3	1.3	1.3	1.3
Vitamin and mineral premix[3]	1	1	1	1
Limestone	0.5	0.5	0.5	0.5
Buffer mix[4]	—	—	0.8	0.8
Salt	0.2	0.2	0.2	0.2
Chemical composition, % of DM (unless otherwise noted)				
NE_L,[5] Mcal/kg of DM	1.66	1.66	1.65	1.66
DM, % as fed	65.2	64.4	64.8	64.7
Ash	6.3	6.7	7.1	7.4
CP	17.3	17.1	17.1	17.0
NDF	29.8	29.7	29.5	29.3
ADF	16.2	15.9	16.1	15.9
NFC[6]	45.6	46.0	45.3	46.2
Ether extract	5.6	5.7	5.6	5.7
Starch[7]	34.2	31.6	34.0	31.4
Total ethanol-soluble carbohydrates[7]	4.4	8.6	4.3	8.8

[1]CTRL = control (no liquid molasses or commercial buffer mix); MOL = liquid molasses without commercial buffer mix; BUF = commercial buffer mix without liquid molasses; and COMBO = liquid molasses plus commercial buffer mix.

[2]Contained 96.5% DM, 84% fat, and 9% Ca (Church and Dwight Co. Inc., Ewing, NJ).

[3]Each kilogram of the vitamin-mineral premix contained (DM basis): vitamin A (50,000 IU), vitamin D_3 (10,000 IU), vitamin E (0.1 g), calcium (196 g), phosphorus (96 g), sodium (71 g), magnesium (19 g), iron (3 g), copper (0.3 g), manganese (2 g), zinc (3 g), cobalt (0.1 g), iodine (0.1 g), and selenium (0.001 g).

[4]A cation-based product contained a blend of sodium bicarbonate, sodium bentonite, calcium carbonate, potassium carbonate, and magnesium oxide (pHmax, Beihagh Nutri Paya Science-Based Co. Center of Innovation, Ferdowsi University of Mashhad, Mashhad, Iran).

[5]According to CPM-Dairy (version 3.0.8; Cornell University, Ithaca, NY; University of Pennsylvania, Kennett Square, PA; and William H. Miner Agricultural Research Institute, Chazy, NY).

[6]NFC = 100 − (NDF + CP + ether extract + ash).

[7]Determined according to Hall et al. (1999).

protein concentrations and consuming 23 kg of DM/d. Cows were housed in a tiestall barn and allowed to exercise for 1 h every afternoon. Animals were fed individually at 0800, 1600, and 2400 h for a targeted refusal rate of 5% to allow for ad libitum intake. The ingredient and nutritional composition of the experimental diets are presented in Table 1, and the FA profile of the diets is shown in Table 2.

Sampling Procedures

Feed intake and milk yield were measured daily in the last 7 d of each measurement period. Feed refusals were collected before the morning feeding and weighed daily throughout the experiment. Composite samples of the TMR were oven-dried (55°C, 48 h), ground to pass through a 2-mm screen (Wiley mill standard model 4; Arthur H. Thomas Co., Philadelphia, PA), and stored for later analysis. Cows were milked 3 times daily at 0700, 1500, and 2300 h. Milk samples were collected from all 3 daily milkings and pooled by cow according to milk weights. Samples were obtained for 7 consecutive days of each period and divided into 2 subsamples; the first subsample was mixed with potassium bichromate and stored at 4°C for analysis of milk fat, true protein, and lactose, whereas the second subsample was stored at −20°C without preservative for later determination of FA using GC.

Ruminal fermentation parameters were determined using 4 ruminally cannulated cows. Ruminal fluid sam-

ples were taken by aspiration from 5 different locations in the rumen, 3 h after the morning feeding during the last 3 d of each period for determination of VFA and NH_3-N concentrations. Daily samples of ruminal fluid collected during the sampling days were composited by period, strained through 4 layers of cheesecloth, and divided into 2 subsamples; for NH_3-N analyses, samples were preserved by mixing 5 mL of squeezed ruminal fluid with 5 mL of 0.2 *N* HCl, whereas for VFA analyses 5 mL of squeezed ruminal fluid was diluted with 1 mL of 0.5 *M* H_2SO_4. All samples were stored at −20°C until analyzed. Furthermore, 50 mL of ruminal fluid was centrifuged at 2,010 × *g* for 20 min at 4°C and 5-mL subsamples of supernatant were stored at −20°C for later analysis of lactate concentration.

Ruminal pH Recording

Ruminal pH was measured continuously (1-min interval) for 72 h in each experimental period (d 18, 19, and 20) using an indwelling pH electrode (PHE-7352–6-PT100, Omega Engineering Inc., Stamford, CT) placed in the ventral sac of the rumen of 4 ruminally cannulated cows as described by AlZahal et al. (2007). On d 18 of each experimental period, the pH loggers and probes were inserted in the rumen via the ruminal cannula at 0730 h and removed at 0730 h on d 21. The 60 measurements per hour were averaged to yield mean hourly pH values over 24 h per cow and treatment. The electrode was attached to a 0.5-kg stainless steel weight to ensure that it remained in the ventral sac of the rumen and connected to the data logger (pHTemp101, Monarch Instrument, Amherst, NH) for data acquisition. All indwelling pH electrodes were calibrated using pH 4 and 7 buffer solutions (Fisher Scientific, Fairlawn, NJ) before insertion in the rumen. Mean ruminal pH, as well as minimum and maximum ruminal pH (data not shown) and duration of ruminal pH below 5.8 for each 24-h period, were measured based on AlZahal et al. (2007). A threshold pH value of 5.8 was used to define SARA according to Yang and Beauchemin (2009).

Sample Analysis

Samples of TMR were ground to pass a 2-mm screen in a Wiley mill (Arthur H. Thomas Co.) before chemical analyses. The procedures of AOAC International (2005) were used to measure DM, by drying samples in an oven at 100°C (method 934.01), ash (2 h at 600°C, method 942.05), and CP (block digestion method using copper catalyst and steam distillation into boric acid, method 2001.11) on a Kjeltec distillation unit 2100 (Foss Inc., Hillerød, Denmark). Ether extract concentration was determined using a Soxhlet Gerhardt (model SE 416, Gerhardt, Königswinter, Germany). Neutral detergent fiber and ADF were analyzed by the Fibertec System (1010 Heat Extractor, Tecator, Sweden) according to Van Soest et al. (1991), and values are reported inclusive of residual ash. Sodium-sulfite and heat-stable α-amylase (Sigma A3306, Sigma-Aldrich, Steinheim, Germany) were used during NDF analysis. Total ethanol-soluble carbohydrates and starch were determined according to the procedures of Hall et al. (1999). Fatty acid concentration analysis of feeds was performed using GC according to Sukhija and Palmquist (1988), with nonadecanoic acid as the

Table 2. Fatty acid profile (% of total FAME) of the experimental diets

	Treatment[1]			
Fatty acid	CTRL	MOL	BUF	COMBO
12:0	1.4	1.1	1.6	0.4
14:0	1.4	1.0	1.4	1.7
cis-9 14:1	0.02	0.04	0.03	0.02
16:0	28.1	29.1	28.6	29.1
cis-9 16:1	1.2	1.5	1.3	1.3
17:0	0.01	0.01	0.03	0.02
18:0	3.8	4.2	4.4	4.5
cis-9 18:1	13.9	15.4	15.1	16.3
cis-9,*cis*-12 18:2	33.7	35.1	35.8	34.7
cis-9,*cis*-12,*cis*-15 18:3	6.6	6.7	6.8	6.0
20:0	0.7	1.0	0.8	0.9
cis-9 20:1	0.0	0.7	0.5	0.4
22:0	0.5	0.3	0.5	0.4
cis-9 22:1	0.2	0.2	0.3	0.3

[1]CTRL = control (no liquid molasses or commercial buffer mix); MOL = liquid molasses without commercial buffer mix; BUF = commercial buffer mix without liquid molasses; and COMBO = liquid molasses plus commercial buffer mix.

internal standard as follows. Samples of feed (100 mg) were weighed and transferred into culture tubes. To each tube was added 1 mL of benzene containing the internal standard, 1 mL of benzene, and 3 mL of freshly made 5% methanol HCl. Solvents were added slowly without touching the side walls of the tubes. After being tightly capped, the culture tubes were vortexed for 1 min and heated for 2 h in a water bath at 70°C. Next, 5 mL of 6% K_2CO_3 was added followed by 2 mL of benzene. The contents of the tube were vortexed for 30 s followed by centrifugation at 252 × *g* for 5 min at room temperature. The upper organic phase (benzene) of the tube was transferred to a screw-capped culture tube. To the benzene extract in the culture tube was added 1 g of anhydrous sodium sulfate and the sample was vortexed for 30 s and allowed to stand for l h. The culture tubes were centrifuged at 252 × *g* for 5 min at room temperature, and the clear benzene (upper) layer containing methyl esters was transferred to a culture tube for analysis using GC.

Individual milk samples were analyzed for fat, true protein, and lactose concentrations by a Milko-Scan 605 analyzer (Foss Electric, Hillerød, Denmark). For analysis of milk FA, milk fat was extracted using the centrifugation technique described by Luna et al. (2005). The refrigerated raw milk sample was kept at 20°C for 20 min, and centrifuged at 17,800 × *g* for 30 min at the same temperature. The fat layer was transferred to a microtube and centrifuged at 19,300 × *g* for 20 min at room temperature. After the second centrifugation, the top layer was removed for analysis. Subsequently, approximately 100 mg of the milk fat was mixed with 2 mL of 1 *M* KOH followed by the addition of 5 mL of 14% boron trifluoride in ethanol (vol/vol). Samples were methylated at 100°C for 60 min and then extracted with 5 mL of hexane (ISIRI, 1997). The FAME in the hexane layer was analyzed by GC using a 3400 Varian Star instrument (Varian Inc., Palo Alto, CA) equipped with CP-SIL-88 capillary column (Chrompack, 60 m × 0.25 mm, Varian) and helium as the carrier gas. The column temperature was initially set at 50°C for 1 min and increased by 10°C/min to 190°C for another 130 min. The temperature of the injector was 280°C and that of the detector was set at 300°C. Peaks of FAME were identified by comparing their retention times with those of the standard mixture of 37 component FAME mix (Supelco 18919–1AMP, Sigma-Aldrich, St. Louis, MO) and 60 individual FAME standards (Sigma-Aldrich). Quantification of FA was based on tridecanoic acid (13:0, Sigma), which was used as the internal standard.

Ruminal NH_3-N was analyzed after distillation by the Kjeldahl method (method 984.13; AOAC International, 2005) on a Kjeltec Auto 1030 Analyzer (Tecator, Höganäs, Sweden) and the concentrations of VFA were determined by GC using a Chrompack instrument (model CP-9002, Chrompack, EA Middelburg, the Netherlands) equipped with a 50-m (0.32 mm ID) silica-fused column (CP-Wax Chrompack Capillary Column, Varian, Palo Alto, CA). Helium and crotonic acid (*trans*-2-butenoic acid) were used as the carrier gas and internal standard, respectively. Oven initial and final temperatures were set at 55 and 195°C, respectively, and detector and injector temperatures at 250°C. The concentration of lactate in the ruminal fluid supernatant was determined using a commercially available lactate assay kit (Biomedical Research Service Center, Buffalo, NY). All samples were tested in duplicate and the lactate concentration was determined by reading the optical density values on a microplate spectrophotometer (Spectramax 190, Molecular Devices Corporation, San Jose, CA) at 492 nm based on the plasma lactate method of Ametaj et al. (2009). The lactate procedure for ruminal samples was slightly modified as samples were centrifuged at 2,010 × *g* (20 min at 4°C) rather than at 3,000 × *g* (4°C for 20 min) to separate plasma as reported by Ametaj et al. (2009). Determination of ruminal lactate concentration using a similar methodology has been reported previously by Iqbal et al. (2009).

Statistical Analysis

Before statistical analysis, all data were tested for normality of distribution by evaluating the Shapiro-Wilk statistic using the UNIVARIATE procedure of SAS (version 9.2, SAS Institute Inc., Cary, NC), and where appropriate, variables were transformed using a $\log_{10}$ transformation. Ruminal pH and fermentation parameters were analyzed as a 4 × 4 Latin square design with a 2 × 2 factorial arrangement of treatments using the PROC MIXED procedure of SAS (SAS Institute Inc.). The covariance structure for repeated measures over time was chosen using the lowest Bayesian information criterion value, with the compound symmetry covariance structure retained in the final model. The following model (n = 4 cows) was used for variables with repeated measures over time (i.e., ruminal pH):

$$\begin{aligned} Y_{ijklm} = \mu + P_i + LM_j + BFM_k \\ + (LM \times BFM)_{jk} + H_l + e1_{ijkl} + (LM \times H)_{jm} \\ + (BFM \times H)_{km} + e2_{ijklm}, \end{aligned}$$

where Y_{ijklm} is the dependent variable, μ is the overall mean, P_i is effect of period i, LM_j is the fixed effect of

Table 3. Effect of sugarcane liquid molasses (LM) and commercial buffer mix (BFM) supplementation on ruminal pH profile and fermentation characteristics

Item	Treatment[1]				SEM	*P*-value[2]		
	CTRL	MOL	BUF	COMBO		LM	BFM	LM × BFM
Ruminal pH								
Mean	5.84	5.89	5.92	5.96	0.04	0.34	0.12	0.75
Time <pH 5.8, min/d	468	258	240	198	12.81	<0.01	<0.01	<0.01
Ruminal VFA profile								
Total VFA, m*M*	99.7	103	104	106	1.05	0.18	0.06	0.53
Acetate, mol/100 mol	62.8	65.2	67.2	69.1	0.89	0.06	<0.01	0.75
Propionate, mol/100 mol	25.2	24.4	23.2	22.6	0.45	0.08	0.02	0.04
Butyrate, mol/100 mol	16.0	18.1	16.4	17.7	0.33	<0.01	0.87	0.17
Acetate:propionate	2.50	2.80	2.91	2.98	0.07	<0.01	<0.01	0.05
Valerate, mol/100 mol	0.91	0.97	0.94	0.82	0.15	0.81	0.39	0.31
Isovalerate, mol/100 mol	1.31	1.47	1.34	1.26	0.27	0.89	0.77	0.70
Ruminal NH_3-N, mg/dL	15.8	15.6	15.6	16.2	0.62	0.72	0.74	0.47
Ruminal lactate, m*M*	1.42	1.22	1.39	1.18	0.11	0.07	0.74	0.96

[1]CTRL = control (no LM or BFM); MOL = LM without BFM; BUF = BFM without LM; and COMBO = LM plus BFM.

[2]Statistical comparisons: LM effect (CTRL plus BUF vs. MOL plus COMBO); BFM effect (CTRL plus MOL vs. BUF plus COMBO); LM × BFM (the interaction effect between LM and BFM dietary levels). Significance was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.

the jth LM (0 vs. 5% LM), BFM_k is the fixed effect of the kth amount of BFM (0 vs. 180 g/cow daily), $(LM \times BFM)_{jk}$ is the fixed effect of the interaction between the jth LM and the kth amount of BFM, H_l is the fixed effect of the lth hour of measurement, $e1_{ijkl}$ is the random whole plot residual error, $(LM \times H)_{jm}$ is the fixed effect of the interaction between the jth LM effect at the mth hour of measurement, $(BFM \times H)_{km}$ is the fixed effect of the interaction between the kth BFM effect at the mth hour of measurement, and $e2_{ijklm}$ is the random subplot residual error.

Production and milk FA profile data were analyzed without repeated measures over time using the PROC MIXED procedure of SAS (SAS Institute Inc.) according to a replicated 4 × 4 Latin square design (n = 8 cows) with a 2 × 2 factorial arrangement of treatments based on the following model:

$$Y_{ijklm} = \mu + Sq_i + C_{j(i)} + P_k + LM_l + BFM_m + (LM \times BFM)_{lm} + e_{ijklm},$$

where μ is the overall mean, Sq_i is the random effect of the ith square, $C_{j(i)}$ is the random effect of the jth cow within the ith square, P_k is the fixed effect of the kth period, LM_l is the fixed effect of lth LM (0 vs. 5% LM), BFM_m is the fixed effect of the mth amount of BFM (0 vs. 180 g/cow daily), $(LM \times BFM)_{lm}$ is the fixed effect of the interaction between the lth LM and the mth amount of BFM, and e_{ijklm} is the random residual error. The following treatment comparisons were done: (1) effect of LM inclusion (CTRL plus BUF vs. MOL plus COMBO), and (2) effect of BFM (CTRL plus MOL vs. BUF plus COMBO). Significance was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.

RESULTS

Ruminal Fermentation

The ruminal proportion of butyrate increased ($P < 0.01$) in cows fed LM (MOL and COMBO diets) compared with those not supplemented with LM (CTRL and BUF; Table 3). In contrast, the ruminal concentrations of total VFA and NH_3-N, as well as the molar proportions of valerate and isovalerate were not affected by inclusion of LM. Whereas the molar proportion of acetate tended ($P = 0.06$) to increase in cows fed the MOL and COMBO diets, a trend ($P = 0.07$) for decreased ruminal concentration of lactate was observed when LM was added to the diets (Table 3).

The ruminal molar proportion of acetate was greater ($P < 0.01$), and total VFA concentration tended ($P = 0.06$) to increase in cows fed diets with BFM (i.e., BUF and COMBO) versus those with LM (Table 3). However, the ruminal concentrations of NH_3-N and lactate, and the ruminal molar proportions of butyrate, valerate, and isovalerate were not affected by dietary supplementation with BFM (Table 3).

Liquid molasses × BFM supplementation interactions were observed for the time that ruminal pH spent <5.8 ($P < 0.01$), ruminal molar proportion of propionate ($P = 0.04$), and ruminal acetate-to-propionate ratio ($P = 0.05$; Table 3). In contrast, mean ruminal pH did not differ across treatments. Both MOL (−44.9%) and BUF (−48.8%) diets were effective in reducing the time

that ruminal pH spent <5.8 compared with the CTRL treatment, and a further decline was observed for cows fed the COMBO diet versus the average of MOL and BUF (−20.5%) but not at similar magnitude of these 2 diets alone (Table 3). The ruminal molar proportion of propionate decreased slightly in cows fed MOL versus CTRL (−3.2%), and it decreased more effectively with feeding the BUF diet (−7.9%); however, feeding the COMBO diet decreased ruminal propionate by 5% relative to the average value MOL and BUF diets. The ruminal acetate-to-propionate ratio increased by 12 and 16.4% when cows were fed the MOL or the BUF diet, respectively, versus the CTRL counterpart, and the additional increase of 4.4% with the COMBO diet compared with the average of MOL and BUF was not as large as either of these 2 diets alone (Table 3).

The effect of treatments on diurnal changes of ruminal pH is shown in Supplemental Figure S1 (https://doi.org/10.3168/jds.2019-17169). Cows in the CTRL diet experienced longer times of ruminal pH below 5.8 (~8 h/d) in comparison with the MOL, BUF, and COMBO diets (<4.5 h below 5.8 daily). After the first feeding at 0800 h, the ruminal pH in cows fed the CTRL diet decreased from 6.05 to 5.80 at 1300 h, after which it decreased gradually below 5.8 until 1900 h. However, 5 h after the second feeding at 1600 h, the ruminal pH in cows offered the CTRL diet decreased again and reached 5.68 at 2300 h before the third feeding at 2400 h. Furthermore, ruminal pH increased during the early morning hours in all treatments.

Milk FA Profile

Out of the 34 individual FA analyzed in milk, only 1 FA was affected by dietary inclusion of LM (Table 4). Whereas the milk proportion of 10:0 increased ($P = 0.03$) in cows fed the MOL and COMBO diets, that of *cis*-9 18:1 tended ($P = 0.09$) to decrease with feeding these 2 treatments. The milk proportions of total short- ($P < 0.01$) and medium-chain FA ($P = 0.03$) were greater in cows fed diets containing LM, whereas the inclusion of LM decreased total *trans*-18:1 FA in milk fat ($P < 0.01$). Furthermore, the proportions of total long-chain FA ($P = 0.03$) and total UFA ($P < 0.01$) were reduced with feeding the MOL and COMBO diets. Dietary inclusion of LM did not change the milk Δ^9-desaturase index, the proportions of total PUFA, or total CLA in milk fat (Table 4).

Only 1 milk FA was affected by BFM supplementation, with cows fed the BUF and COMBO diets showing the lowest proportion of *cis*-9 18:1 (Table 4). The milk proportion of 18:0 tended ($P = 0.07$) to increase, whereas that of 20:0 tended ($P = 0.08$) to decrease in cows fed diets supplemented with BFM. In addition, the milk proportions of total UFA ($P = 0.02$) and total *trans*-18:1 FA ($P = 0.08$) decreased in cows offered the BUF or COMBO treatments. In contrast, the milk proportions of short-, medium-, and long-chain FA, total CLA, and total PUFA were not affected by dietary inclusion of BFM (Table 4).

Interaction effects were observed for the proportions of some milk FA including 14:0 ($P = 0.05$), *cis*-9 16:1 ($P < 0.05$), *trans*-10 18:1 ($P < 0.01$), *trans*-9,*trans*-12 18:2 ($P = 0.01$), and total *trans* FA ($P = 0.01$) (Table 4). The milk proportions of both 14:0 and *cis*-9 16:1 increased by 9.9 and 11.4%, respectively, in cows fed the COMBO diet compared with the average of the CTRL, MOL, and BUF treatments, with these 3 diets very close to each other. Feeding MOL or BUF decreased the proportion of *trans*-10 18:1 in milk fat by 72.2 and 59.1%, respectively, and a further drop of 31.3% was observed with COMBO versus the average of MOL and BUF diets. However, this 31.3% reduction in milk *trans*-10 18:1 was not of the same magnitude compared with MOL or BUF alone. Similar patterns were observed for the milk proportions of *trans*-9,*trans*-12 18:2 and total *trans* FA. Interaction trends were observed for the milk proportions of *trans*-9 18:1 ($P = 0.09$) and *trans*-11 18:1 ($P = 0.07$) in milk fat (Table 4). The milk proportion of *trans*-9 18:1 followed the same pattern observed for *trans*-10 18:1, *trans*-9,*trans*-12 18:2, and total *trans* FA. In contrast, the milk proportion of *trans*-11 18:1 increased by 10 and 14.2% in cows fed MOL and BUF, respectively, compared with cows on the CTRL treatment, whereas the COMBO diet decreased this FA by 17.5% relative to the average proportion of MOL and BUF.

Intake and Milk Yield and Composition

Milk yield, concentrations and yields of milk true protein, lactose, and SNF, and feed efficiency were not affected by dietary inclusion of LM (Table 5). In contrast, 3.5% FCM yield increased ($P = 0.04$) by 7.2% in cows fed diets containing LM versus BFM. Moreover, DMI ($P = 0.08$) and ECM yield ($P = 0.09$) tended to increase with feeding the MOL and COMBO diets compared with the CTRL and BUF counterparts (Table 5).

Dry matter intake ($P < 0.01$) and yields of ECM ($P = 0.05$) and 3.5% FCM ($P = 0.02$) were greater in cows fed diets supplemented with BFM compared with those without supplementation (Table 5). In contrast, BFM supplementation did not affect milk yield, concentrations and yields of milk components, and feed efficiency (Table 5).

Significant LM × BFM interactions were observed for the concentration and yield of milk fat (Table 5). Compared with the CTRL treatment, milk fat concen-

tration increased by 29 and 30.7% in cows fed MOL or BUF, respectively, with the COMBO diet increasing it further by 2% relative to the average of MOL and BUF but at a lower magnitude. The interaction response detected for milk fat yield followed the same pattern displayed by milk fat concentration (Table 5).

DISCUSSION

Nutrient Composition of Diets

All experimental diets contained similar concentrations of CP (mean = 17.1%), NDF (mean = 29.6%),

Table 4. Effects of sugarcane liquid molasses (LM) and commercial buffer mix (BFM) on milk fatty acid (FA) profile (% of total FAME)

	Treatment[1]					*P*-value[2]		
FA	CTRL	MOL	BUF	COMBO	SEM	LM	BFM	LM × BFM
4:0	1.93	1.80	1.63	2.03	0.16	0.41	0.80	0.13
6:0	1.68	1.87	1.57	1.73	0.12	0.17	0.29	0.92
8:0	1.28	1.37	1.34	1.28	0.05	0.72	0.89	0.25
10:0	2.82	3.28	2.75	2.92	0.14	0.03	0.12	0.28
cis-9 10:1	0.25	0.20	0.21	0.22	0.02	0.53	0.29	0.16
11:0	0.35	0.37	0.36	0.35	0.03	0.70	0.74	0.53
12:0	2.85	3.16	2.96	3.00	0.13	0.20	0.84	0.33
12:1	0.12	0.13	0.14	0.12	0.02	0.70	0.95	0.42
Short-chain FA (4:0–12:0)	11.1	12.0	10.7	11.5	0.44	<0.01	0.13	0.66
13:0	0.17	0.17	0.18	0.18	0.01	0.79	0.62	0.96
13:1	0.14	0.15	0.13	0.14	0.01	0.42	0.27	0.84
14:0	11.3	11.6	10.4	12.2	0.35	<0.01	0.61	0.05
trans-14:1	0.25	0.26	0.24	0.21	0.01	0.47	0.11	0.42
cis-14:1	0.53	0.59	0.52	0.58	0.04	0.17	0.80	0.93
15:0	0.74	0.72	0.71	0.70	0.03	0.70	0.38	0.98
15:1	0.24	0.25	0.25	0.27	0.02	0.41	0.45	0.81
16:0	24.3	25.6	25.5	26.0	0.77	0.27	0.36	0.57
trans-16:1	0.28	0.25	0.29	0.27	0.02	0.29	0.46	0.78
cis-9 16:1	1.92	1.82	1.80	2.06	0.07	0.31	0.41	0.02
Medium-chain FA (13:0–16:0)	40.0	41.5	40.1	42.5	0.85	0.03	0.52	0.59
17:0	0.57	0.64	0.66	0.63	0.04	0.59	0.34	0.33
17:1	0.22	0.25	0.24	0.24	0.02	0.41	0.70	0.52
18:0	11.9	12.6	13.0	13.1	0.43	0.36	0.07	0.44
cis-9 18:1	24.3	23.4	23.1	22.3	0.53	0.09	0.03	0.83
cis-11 18:1	0.61	0.66	0.64	0.60	0.03	0.72	0.96	0.14
trans-9 18:1	0.32	0.25	0.27	0.22	0.04	0.32	0.14	0.09
trans-10 18:1	2.37	0.66	0.97	0.56	0.06	<0.01	<0.01	<0.01
trans-11 18:1	1.20	1.32	1.37	1.11	0.10	0.84	0.49	0.07
cis-9,*cis*-12 18:2	2.68	2.77	2.79	2.88	0.16	0.59	0.51	0.98
trans-9,*trans*-12 18:2	1.05	0.95	0.92	0.83	0.06	0.54	0.42	0.01
cis-9,*cis*-12,*cis*-15 18:3	0.61	0.57	0.60	0.59	0.04	0.58	0.83	0.67
20:0	0.44	0.41	0.39	0.39	0.02	0.30	0.08	0.56
20:1	0.32	0.37	0.35	0.34	0.04	0.83	0.15	0.39
22:0	0.15	0.16	0.16	0.15	0.02	0.70	0.92	0.80
22:1	0.14	0.16	0.13	0.12	0.01	0.79	0.12	0.29
Total CLA	1.41	1.35	1.29	1.31	0.24	0.12	0.31	0.59
Long-chain FA (>16:0)	45.2	43.0	44.1	42.4	0.81	0.03	0.31	0.73
Total *trans*-18:1[3]	3.96	2.20	3.04	1.89	0.58	<0.01	0.08	0.34
Total UFA[4]	36.7	33.8	34.3	32.9	0.66	<0.01	0.02	0.26
Total PUFA[5]	4.34	4.17	4.31	4.54	0.18	0.87	0.37	0.31
Total *trans*-FA[6]	5.55	3.56	3.54	3.42	0.35	<0.01	<0.01	0.01
Δ^9-desaturase index[7]	0.071	0.065	0.064	0.062	0.005	0.32	0.22	0.82

[1]CTRL = control (no LM or BFM); MOL = LM without BFM; BUF = BFM without LM; and COMBO = LM plus BFM.

[2]Statistical comparisons: LM effect (CTRL plus BUF vs. MOL plus COMBO); BFM effect (CTRL plus MOL vs. BUF plus COMBO); LM × BFM (the interaction effect between LM and BFM dietary levels). Significance was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.

[3]Includes *trans*-9, *trans*-10, and *trans*-11 18:1.

[4]UFA = 10:1 + 12:1 + 13:1 + 14:1 + 15:1 + 16:1 + 17:1 + 18:1 + 18:2 + 18:3 + 20:1 + 22:1 (*cis*/*trans* isomers).

[5]PUFA = 18:2 + 18:3 (*cis*/*trans* isomers).

[6]Total *trans*-FA = *trans*-14:1 + *trans*-16:1 + *trans*-9,10,11 18:1 + *trans*/*trans*-18:2.

[7]Calculated according to Bouattour et al. (2008) as (product of Δ^9-desaturase)/(product of Δ^9-desaturase + substrate of Δ^9-desaturase), using 14:0 = 14:1/(14:1 + 14:0).

ADF (mean = 16%), NFC (mean = 45.6%), and NE_L (mean = 1.66 Mcal/kg of DM; Table 1). The concentration of NFC > 45% of the diet DM is explained by high dietary inclusion of ground corn (mean = 34.5%; DM basis) and the low forage-to-concentrate ratio (i.e., 35:65). While the dietary concentration of starch decreased, that of total ethanol-soluble carbohydrates increased in the MOL and COMBO diets due to the high content of sucrose in LM (Brito et al., 2015, 2017). Linoleic acid (*cis*-9,*cis*-12 18:2) was the FA with the greatest proportion in the experimental diets (mean = 34.8%) followed by 16:0 (mean = 28.7%) and *cis*-9 18:1 (mean = 15.2%; Table 2). However, the variation in dietary FA was relatively small and mostly driven by the replacement of ground corn with LM.

Ruminal pH

Feeding LM (4 vs. 8% total dietary ethanol-soluble carbohydrates concentration) and BFM (0 vs. 0.8% of the diet DM) did not change the daily mean ruminal pH, which is in agreement with observations from Chibisa et al. (2015) and Cruywagen et al. (2015). However, we observed that feeding the MOL or BUF diet reduced the time ruminal pH spent below 5.8, and the COMBO diet decreased it further although not at the same level compared with either the MOL or BUF diet alone. Collectively, these results suggest that supplementation with LM and BFM has potential to reduce the occurrence of SARA in lactating dairy cows fed acidotic-prone diets compared with the CTRL treatment. There have been concerns of triggering ruminal acidosis by replacing dietary starch with sugar because of the faster ruminal fermentation rate of sugar compared with starch sources. However, several mechanisms have been proposed regarding the role of sugars on mitigating depression of ruminal pH (Oba, 2011). In our study, LM increased the ruminal butyrate proportion, and it has been shown that butyrate stimulates blood flow to the ruminal epithelium, thereby enhancing the uptake of VFA by cells that can buffer ruminal pH (Oba et al., 2015). Alternatively, increased dietary concentration of sugars may increase the ruminal passage rate with less OM available for acid production (Penner and Oba, 2009). Another hypothesis is that sugars may be stored as glycogen by certain species of ruminal bacteria (Hall and Weimer, 2007), hence preventing sugar fermentation in the rumen. Regarding buffers, they have a direct effect on ruminal pH through chemical changes that neutralize acidity via H^+ sequestration, resulting in improved buffering capacity of the rumen (Calsamiglia et al., 2012).

Ruminal VFA and Lactate

In the present experiment, total VFA concentration in the rumen was not altered by LM inclusion, which agrees with previous studies (Penner and Oba, 2009; Razzaghi et al., 2016). Cows fed the BFM-supplemented diets showed a trend for increased total VFA concentration in the rumen. Likewise, Marden et al. (2008) observed that cows fed 150 g of sodium bicarbonate

Table 5. Effects of sugarcane liquid molasses (LM) and commercial buffer mix (BFM) on DMI and milk yield and composition

	Treatment[1]					*P*-value[2]		
Item	CTRL	MOL	BUF	COMBO	SEM	LM	BFM	LM × BFM
DMI, kg/d	22.2	22.9	23.3	23.7	0.38	0.08	<0.01	0.53
Milk yield, kg/d	31.1	30.8	30.8	31.3	0.92	0.89	0.87	0.66
ECM,[3] kg/d	29.2	31.9	32.1	32.7	1.08	0.09	0.05	0.27
3.5% FCM,[4] kg/d	28.4	32.0	32.4	33.1	1.16	0.04	0.02	0.16
Milk fat, %	2.90	3.74	3.79	3.84	0.12	<0.01	<0.01	<0.01
Milk true protein, %	3.16	3.19	3.16	3.13	0.04	0.82	0.35	0.38
Milk lactose, %	4.40	4.42	4.38	4.30	0.06	0.66	0.20	0.36
Milk SNF, %	8.16	8.20	8.14	8.06	0.12	0.82	0.46	0.52
Milk fat, kg/d	0.92	1.15	1.18	1.21	0.05	0.01	<0.01	0.05
Milk true protein, kg/d	1.01	0.98	0.97	0.98	0.03	0.57	0.41	0.53
Milk lactose, kg/d	1.41	1.36	1.35	1.34	0.04	0.49	0.28	0.61
Milk SNF, kg/d	2.61	2.53	2.51	2.52	0.07	0.57	0.44	0.49
Feed efficiency								
3.5% FCM/DMI	1.28	1.40	1.38	1.38	0.05	0.19	0.38	0.22
ECM/DMI	1.31	1.39	1.36	1.36	0.04	0.39	0.78	0.38

[1]CTRL = control (no LM or BFM); MOL = LM without BFM; BUF = BFM without LM; and COMBO = LM plus BFM.

[2]Statistical comparisons: LM effect (CTRL plus BUF vs. MOL plus COMBO); BFM effect (CTRL plus MOL vs. BUF plus COMBO); LM × BFM (the interaction effect between LM and BFM dietary levels). Significance was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.

[3]ECM yield = (kg of milk × 0.3246) + (kg of milk fat × 12.96) + (kg of milk protein × 7.04) as described by Sjaunja et al. (1991).

[4]3.5% FCM yield = (0.432 × kg of average milk yield) + (16.216 × kg of fat) (Erdman, 2011).

had 11.7% greater total VFA concentration in the rumen compared with those in the control treatment. Both MOL and BUF diets increased the proportion of acetate in the rumen. Feeding diets with high concentration of disaccharides and LM has been reported to yield inconsistent effects on ruminal acetate proportion, with previous studies showing a decrease (Ribeiro et al., 2005), an increase (Heldt et al., 1999; Martel et al., 2011), or no change in acetate (Golombeski et al., 2006; Chibisa et al., 2015). However, the increased molar proportion of ruminal acetate observed herein in response to BFM inclusion agrees with previous results (Erdman et al., 1982; Xu et al., 1994; Kennelly et al., 1999). Cows fed LM-supplemented diets had a greater molar proportion of ruminal butyrate than those not receiving LM. Similarly, Golombeski et al. (2006) and Gao and Oba (2016) reported that the ruminal proportion of butyrate increased with dietary inclusion (DM basis) of 8.6 and 5.5% of fermentable sugars and lactose or sucrose, respectively.

The LM × BFM interaction found for the molar proportion of ruminal propionate was likely caused by changes in substrate available to ruminal microbes and diurnal shifts in ruminal pH especially because BFM appeared to be more effective than LM at decreasing propionate in the rumen. A LM by BFM interaction was also observed for the ruminal acetate-to-propionate ratio, which followed the changes observed for acetate and propionate particularly in response to BFM supplementation. Cruywagen et al. (2015) also reported increased ruminal acetate-to-propionate ratio in dairy cows fed potentially acidotic diets supplemented (DM basis) with 0.4% of a commercial buffer or 0.8% of sodium bicarbonate.

The trend for lowered concentration of ruminal lactate by including LM in the diets may be linked to the key role of mono- or disaccharides in maintaining a high number of lactate-fermenting bacteria (Nagaraja and Titgemeyer, 2007; Firkins, 2010). In contrast, the addition of BFM did not change the ruminal concentration of lactate in the current experiment, which agrees with previous research (Khorasani and Kennelly, 2001; Marden et al., 2008).

Milk FA Profile

Increased proportion of *trans* FA in milk has been associated with milk fat depression in cows fed high-concentrate diets (Kalscheur et al., 1997; Griinari et al., 1998). Shingfield et al. (2010) reported that production of *trans* FA isomers increased with feeding a high-concentrate diet because the incomplete BH of dietary 18:2 and 18:3 FA by ruminal bacteria led to the formation of *trans*-10 18:1 and *trans*-10,*cis*-12 CLA, which are known to exert anti-lipogenic effects. Our results agreed with Martel et al. (2011) and Brito et al. (2015) who demonstrated that replacing ground corn with molasses led to decreased milk proportions of *trans*-10 18:1 and total *trans*-18:1 FA in milk fat. High-concentrate diets increased *trans*-10 18:1 at the expense of *trans*-11 18:1 in ruminal fluid, thus revealing a *trans*-11 to *trans*-10 BH shift (Zened et al., 2012). Specifically, *trans* FA isomers can reduce de novo FA synthesis and esterification of FA into triglycerides in mammary tissues (Griinari et al., 1998). Kalscheur et al. (1997) observed that the duodenal flow of total *trans*-18:1 FA doubled in cows fed a 75% concentrate diet without buffer supplementation, resulting in a 2-fold increase in total *trans*-18:1 FA concentration in milk fat. Kalscheur et al. (1997) suggested that low ruminal pH may interfere with the conversion of *trans*-18:1 to 18:0 leading to accumulation of *trans* FA intermediates in the rumen. However, neither LM nor BFM supplementation affected daily mean ruminal pH in the present experiment, although the time ruminal pH spent <5.8 appeared to be more influenced by LM or BFM fed individually than combined.

The dietary inclusion of LM increased the concentration of 10:0 and 14:0 FA, which are positively related to short- and medium-chain FA proportions in milk fat. Therefore, it appears that de novo synthesis of short- and medium-chain FA in the mammary gland was promoted in cows fed the MOL and COMBO diets as suggested by Martel et al. (2011). The Δ^9-desaturase index was not affected by the LM and BFM, indicating that the increased milk proportion of *cis*-9 16:1 may be explained by chain elongation of 14:1.

Intake and Milk Yield and Composition

It should be noted that due to the small number of cows and the relatively short experimental periods (3 wk), the present study was not designed to measure production responses and results should be interpreted cautiously. Specifically, only production variables with significant effects were discussed herein.

The effects of dietary inclusion of sugars via sucrose or molasses (dried or liquid) on DMI have been inconclusive across the literature. Previous studies conducted with lactating dairy cows reported no effect of sources rich in disaccharides on DMI either in typical (Razzaghi et al., 2016) or high-concentrate diets (Martel et al., 2011), whereas others (Broderick et al., 2008; Penner and Oba, 2009) observed increased DMI. A possible explanation for the positive effect of LM on DMI could be related to improved diet palatability (Khalili and Huhtanen, 1991). We also observed enhanced DMI in cows fed BFM-supplemented diets, which can be associated with a shorter duration that rumen pH was below

5.8 and better ruminal environment, both conducive of improved fiber digestibility in the rumen. Xu et al. (1994) observed increased DMI when a combination of sodium carbonate and sodium bicarbonate was supplemented at the rate of 1.5% of the diet DM to cows fed 40% grass silage and 60% concentrate.

Significant LM × BFM interactions were observed for milk fat concentration and yield, likely in response to enhanced short- and medium-chain FA synthesis with feeding LM and reduced milk *trans*-10 18:1 with the inclusion of BFM. Increased milk fat secretion has been reported with feeding fermentable sugars (Golombeski et al., 2006) or sucrose (Penner and Oba, 2009) compared with control diets. This response has been attributed to an increase in ruminal butyrate production and its extensive metabolism by ruminal epithelial cells during absorption to form BHB, which is a precursor for milk FA synthesis. In fact, Penner and Oba (2009) demonstrated a positive correlation ($r = 0.308$, $P < 0.001$) exists between plasma BHB and milk fat yield. Although butyrate makes up a small proportion of the FA found in milk fat, it constitutes 30% of the FA in the *sn*-3 position in milk triglycerides (Jensen, 2002). In addition, feeding BFM led to greater proportion of ruminal acetate, which together with butyrate are the key precursors for de novo synthesis of FA in the mammary gland (Jenkins et al., 2008). Khorasani and Kennelly (2001) reported that milk fat concentration averaged 4.09% when diets were buffered compared with 2.91% when diets were not buffered in dairy cows receiving high-concentrate diets (75% concentrate). It has been reported that buffer may counteract the negative effects of high-concentrate diets on milk fat synthesis by promoting the normal ruminal BH pathway of *cis*-9,*cis*-12 18:2 FA while reducing the duodenal flow of *trans*-FA isomers involved with milk fat depression (Jenkins et al., 2014). Moreover, the observed effect on milk fat synthesis when BFM was fed to dairy cows could have originated from changes in the ruminal environment and the acid-base status of the cows (Iwaniuk et al., 2015). Both yields of ECM and 3.5% FCM followed milk fat concentration and yield responses except that LM by BFM interactions were not significant for ECM and 3.5% FCM in the current study.

CONCLUSIONS

Significant LM by BFM interactions were observed for the time ruminal pH spent below 5.8, milk proportion of *trans*-10 18:1, and milk fat yield among other variables. Overall, the individual responses of either BFM or LM were more effective to reduce both the time that ruminal pH remained below 5.8 and the milk proportion of *trans*-10 18:1 in milk fat, and to increase milk fat yield than when these 2 supplements were combined with each other. However, our results should be interpreted with caution due to the low number of cows used in the present study. Future work investigating how LM and BFM affect ruminal digestion kinetics (e.g., rate of passage, degradation rate) and duodenal flow of microbial protein are warranted.

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