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Using organic acids to control subacute ruminal acidosis and fermentation in feedlot cattle fed a high-grain diet[1,2]

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ABSTRACT: The objective of this study was to determine whether supplementing organic acids can prevent incidences of subacute ruminal acidosis (SARA) in beef heifers fed a diet consisting of 8% barley silage and 92% barley grain–based concentrate (DM basis). Ten ruminally cannulated Hereford crossbred heifers (484 ± 25 kg BW) were used in a replicated 5 × 5 Latin square design with 14-d periods including 10 d for dietary adaptation and 4 d for measurements. Dietary treatments included no supplementation (Control), low fumaric acid (61 g/d), high fumaric acid (125 g/d), low malic acid (59 g/d), and high malic acid (134 g/d). Organic acid supplementation had no effect on DMI ($P = 0.77$). Similarly, no effects were observed on mean ($P = 0.74$), minimum ($P = 0.64$), and maximum ($P = 0.27$) ruminal pH measured continuously for 48 h. Moreover, area under the curve for pH thresholds 6.2 ($P = 0.97$), 5.8 ($P = 0.66$), 5.5 ($P = 0.55$), and 5.2 ($P = 0.93$) was similar for all treatments. However, malic acid supplementation lowered the amount of time that ruminal pH was <6.2 compared with the Control ($P = 0.02$) and fumaric acid treatments ($P < 0.01$). No effects were observed on total VFA concentrations with organic acid supplementation ($P = 0.98$) compared with the Control, but greater total VFA concentrations were observed with fumaric acid compared with the malic acid treatments ($P = 0.02$). The population of total culturable bacteria 3 h after feeding was reduced with supplemental malic acid compared with the Control ($P = 0.03$) and fumaric acid treatments ($P = 0.03$). However, no effects were observed with organic acid supplementation on lactic acid–utilizing bacteria ($P = 0.59$). In conclusion, under the conditions of the present study, organic acid supplementation did not have any significant effects on ruminal fermentation parameters compared with the Control and were not effective in preventing SARA in beef cattle fed high-grain diets.

Key words: feedlot cattle, fumaric acid, malic acid, organic acid, rumen pH

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INTRODUCTION

Subacute ruminal acidosis (**SARA**) is a prominent digestive disorder of feedlot ruminants fed high-grain diets (Owens et al., 1998) resulting in reduced animal performance and profitability of beef production systems (Martin, 1998). Dietary strategies to prevent SARA have focused on using antimicrobial compounds (Russell and Strobel, 1989), but increased concern over the use of antibiotic additives has prompted interest in alternatives including dicarboxylic acids (Castillo et al., 2004).

Dicarboxylic acids such as malic and fumaric acid provide an effective alternative to improve animal health and productivity by manipulating rumen microbial ecology (Castillo et al., 2004). Fumarate and malate are key intermediates in the succinate–propionate pathway used by some anaerobes, predominantly *Selenomonas ruminantium*, to synthesize succinate and propionate (Gottschalk, 1986). Moreover, both fumarate and malate were shown to

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stimulate lactate uptake by *S. ruminantium* (Martin, 1998). However, in vivo responses to organic acid supplementation remain inconclusive. Ruminal pH was increased with dietary malate in steers fed corn-based finishing diets (Martin et al., 1999) and with dietary fumarate in wethers fed lucerne-based diets (Molano et al., 2008). On the contrary, no effects were observed with fumarate supplemented to high-forage diets fed to steers (McGinn et al., 2004) and malate supplemented to mixed diets fed to beef heifers (Foley et al., 2009). To our knowledge, no study has investigated rumen fermentation responses to fumarate and malate when supplemented in barley grain–based diets. Because SARA is often prevalent in finishing beef cattle fed barley grain–based diets, we hypothesized that supplementation with dicarboxylic acids may be an effective means of elevating ruminal pH. Therefore, the objective of this study was to assess feed intake and ruminal fermentation responses to fumarate and malate supplementation in beef heifers fed high-grain diets.

MATERIALS AND METHODS

The study received approval of the institutional animal care committee of Agriculture and Agri-Food Canada, Lethbridge Research Centre, Lethbridge, AB, Canada, and was conducted in accordance with the guidelines of the Canadian Council on Animal Care (2009).

Experimental Design, Animals, Diets, and Housing

Ten ruminally cannulated Hereford crossbred heifers (484 ± 25 kg average BW for the experiment) were randomly assigned to 2 groups and used in an experiment designed as a replicated 5 × 5 Latin square with 5 dietary treatments and 14-d periods including 10 d for dietary adaptation and 4 d for measurements. Heifers were fed a basal diet representative of finishing diets typically offered to feedlot cattle in western Canada (Table 1) and formulated to provide adequate ME and MP for 500-kg growing and finishing beef cattle with an ADG of 2 kg/d (NRC, 2000). Heifers were adapted to the high-grain basal diet before the start of the experiment by gradually transitioning over 28 d from a forage-based diet. Dietary treatments included no supplementation (**Control**), low fumaric acid (77 g/d; Bartek Ingredients Inc., Stoney Creek, ON, Canada), high fumaric acid (153 g/d), low malic acid (89 g/d; Bartek Ingredients Inc.), and high malic acid (177 g/d). Low doses of fumaric and malic acid were equivalent to 0.67 mol/d whereas high doses of both organic acids were equivalent to 1.32 mol/d. High doses of fumaric and malic acid were determined based on previous in vitro study where 12 m*M* of supplemental fumarate and malate was effective in improving ruminal pH (Callaway and Martin, 1996). Similarly, the most effective dose of supplemental malate observed in steers fed a high-grain diet (Martin et al., 1999) was used for determining the low dose of malic acid in the present study, whereas the low dose of supplemental fumarate was determined based on the amounts used by McGinn et al. (2004). Organic acids were supplemented by mixing homogenously with the total mixed ration (**TMR**) by hand each day. An ionophore was not included in the diet.

Feed was offered once daily (1000 h) for ad libitum intake and refusals were recorded (0930 h) daily for each animal. Samples of the barley silage, concentrate (comprising rolled barley grain and pelleted supplement), and diet were collected 5 times weekly and composited. Dry matter was determined on a portion of each weekly composite feed sample, and the DM contents were used to adjust the silage to concentrate ratio of the diet, when necessary. Weekly samples of the barley silage, concentrate, and diet were then composited by period and retained for chemical analysis. Samples of orts were collected daily and composited by animal for each period. Samples were dried, and

Table 1. Composition of the basal diet

Item	Percent DM
Ingredient	
Barley silage[1]	8.0
Barley grain, dry rolled[2]	85.2
Supplement[3,4]	6.8
Barley grain, ground	3.34
Canola meal	1.52
Urea	0.23
Limestone	0.76
Mineralized salt	0.53
Molasses	0.28
Canola oil	0.12
Vitamins A, D, and E	0.01
Chemical composition[5]	
DM, %	81.9 ± 1.76
OM, % of DM	95.9 ± 0.26
CP, % of DM	13.4 ± 0.69
NDF, % of DM	17.3 ± 1.03
ADF, % of DM	5.25 ± 0.53

[1]Composition (% of DM; mean ± SD): 38.5 ± 2.25 DM (% as fed), 93.3 ± 0.12 OM, 11.7 ± 0.66 CP, 42.3 ± 0.75 NDF, and 22.2 ± 0.57 ADF.

[2]Composition (% of DM; mean ± SD): 91.1 ± 1.19 DM (% as fed), 97.8 ± 0.18 OM, 14.0 ± 0.65 CP, 17.8 ± 0.82 NDF, and 4.5 ± 0.51 ADF.

[3]Composition (% of DM; mean ± SD): 93.6 ± 0.49 DM (% as fed), 66.3 ± 1.44 OM, 9.6 ± 0.31 CP, 10.4 ± 0.55 NDF, and 3.17 ± 0.12 ADF.

[4]Supplied per kilogram of dietary DM: 51 mg of Zn, 24 mg of Mn, 13 mg of Cu, 0.25 mg of Se, 0.17 mg of Co, 8,600 IU of vitamin A, 850 IU of vitamin D, and 90 IU vitamin E.

[5]Based on 5 composite period samples.

DMI for each heifer was calculated based on the feed DM offered and orts DM refused. Feed DM was determined by oven-drying at 55°C for 48 h.

The animals were housed in individual tie stalls bedded with wooden shavings, provided access to water, and let outside daily into a dry lot for exercise, except during pH measurements. Body weight was measured without feed restriction at the beginning of Period 1 and the end of Period 5 and averaged for the experiment.

Rumen Measurements

Ruminal fluid was collected on d 13 and 14 of each period at 0, 3, 6, and 9 h after feeding. The whole contents (250 mL) were taken from 4 sites in the rumen and then immediately squeezed through a 355-μm polyester fabric (PECAP; Sefar Canada, Ville St. Laurent, QC, Canada) to obtain the filtrate. A volume (5 mL) of filtrate was mixed with 1 mL of 25% metaphosphoric acid (wt/vol) for VFA, lactate, and succinate analyses. All samples were stored frozen at –20°C until analysis.

Diurnal pH profiles were measured using an indwelling pH electrode hardwired to a data acquisition system. Ruminal pH was measured continuously for 48 h on d 11 and 12 of each period. An electrode (model PHCN-37; Omega Engineering, Stamford, CT) was inserted into the rumen of each heifer through the cannula. A weight was attached to the electrode to ensure that it remained in the ventral sac. In addition, a protective shield with large openings that allowed ruminal fluid to percolate freely was placed around the electrode to prevent it from coming in contact with the ruminal epithelium. The electrodes were removed from the rumen approximately 1 h before feeding each day and calibrated with pH 4.0 and 7.0 standards. Thus, continuous pH measurements were made for about 23 h/d. The pH was measured every 5 s, and an average of these readings was recorded every 15 min using a data logger. Ruminal pH data were summarized daily for each heifer in each period as daily mean, minimum, and maximum pH; area under the curve (**AUC**; pH × h) below pH 6.2, 5.8, 5.5, and 5.2; and the proportion of the measurement period in which pH was below 6.2, 5.8, 5.5, and 5.2. The AUC was calculated by adding the absolute value of negative deviations in pH from pH 6.2, 5.8, 5.5, and 5.2 for each 15-min interval. The proportion of time during which pH was below the particular threshold value was calculated using the actual duration that pH was measured for that animal. Ruminal pH < 6.2 was chosen as the benchmark based on in vitro observations that ruminal microbial activity is compromised when ruminal pH drops below 6.2 (Russell and Wilson, 1996; Beauchemin et al., 2003b). The occurrence of SARA was determined using 2 thresholds, 5.8 for total SARA and 5.5 for severe SARA (Dohme et al., 2008), because pH < 5.8 is harmful to fiber-degrading bacteria (Russell and Wilson, 1996) and pH < 5.5 is unfavorable for VFA absorption and detrimental to ruminal epithelium (Gäbel et al., 2002; Dohme et al., 2008). Duration and AUC for pH < 5.2 was considered indicative of acute ruminal acidosis (Owens et al., 1998). The AUC and the pH threshold indicates the severity of acidosis, whereas the duration that pH remained below the threshold indicates the duration of acidosis.

Rumen Evacuation

Rumen contents were manually removed from each animal 5 h after the feeding, before the beginning of Period 1, and after the end of Period 5. Rumen contents were stored in insulated tubs to minimize aerobic and temperature shock to the microorganisms. The total ruminal contents were weighed to obtain an estimate of rumen volume required to assess the actual molarity of the treatments. After thorough mixing, two 1-kg samples were removed and were used for determination of DM content by oven-drying at 55°C for 48 h. Contents were then immediately returned to the rumen of the animal.

Microbiological Analysis

Total culturable and lactic acid–utilizing bacteria were determined in the ruminal contents sampled on d 13 and 14 of each period at 3 h after feeding. Ruminal contents were obtained from 4 sites within the rumen (reticulum, dorsal and ventral sac, and the mat), blended anaerobically under oxygen-free CO_2, and strained through a polyester monofilament fabric (PECAP; pore size 355 μm; Sefar Canada). Blended, strained ruminal contents were serially diluted in 0.1% (wt/vol) anaerobic buffered peptone. Diluted samples were inoculated (0.5 mL/tube) in triplicate into Hungate tubes for enumeration of total (10^{-6} to 10^{-9} dilution) and lactate-utilizing bacteria (10^{-6} to 10^{-8} dilution) using the roll-tube technique. For enumeration of the total culturable bacterial population, medium 10 (Caldwell and Bryant, 1966) was used with glucose, maltose, cellobiose, DL-lactic acid solution, and starch at 0.1% (wt/vol) and 1.8% agar. The tubes were immediately spun on ice to solidify the agar as a layer onto the inside surface and resultant roll tubes were incubated at 39°C for 2 to 3 d. Similarly, for enumeration of lactate-utilizing bacteria, a selective carbohydrate agar was used based on medium 10 of Caldwell and Bryant (1966) containing 0.5% (wt/vol) 50 m*M* DL-lactic acid as the main energy source for lactate-utilizing bacteria (Beauchemin et al.,

2003a). Visible colonies were counted and population of the original sample volume was determined as cfu using following formula: cfu/mL = colonies counted/(dilution factor × volume (mL) inoculated).

Chemical Analysis

All chemical analysis was performed on each sample in duplicate, and when the CV for the replicate analysis was >5%, the analysis was repeated. Analytical DM content of the samples was determined by drying at 135°C for 2 h (AOAC, 1995; method 930.05) followed by hot weighing. The OM content was calculated as the difference between DM and ash contents (AOAC, 1995; method 942.05). The NDF and ADF contents were determined by the methods described by Van Soest et al. (1991) with amylase and sodium sulfite used in the NDF procedure. Samples were reground using a ball ground mixer (Mixer Mill MM2000; Retsch, Haan, Germany) for determination of N. The concentration of CP (N × 6.25) in feed was quantified by flash combustion with gas chromatography and thermal conductivity detection (Carlo Erba Instruments, Milan, Italy)

Ruminal VFA, lactic acid, and succinic acid were quantified using crotonic acid as the internal standard and gas–liquid chromatography (model 5890; Hewlett-Packard, Little Falls, DE) with a capillary column (30 m by 0.25 mm i.d., 1-μm phase thickness, bonded polyethylene glycol, Supelco Nukol; Sigma-Aldrich Canada, Oakville, ON, Canada) and flame ionization detection. The oven temperature was 100°C for 1 min, which was then ramped by 20°C/min to 140°C and then by 8°C/min to 200°C and held at this temperature for 5 min. The injector temperature was 200°C, the detector temperature was 250°C, and the carrier gas was helium.

Statistical Analysis

Data were analyzed using the mixed model procedure of SAS (SAS Inst. Inc., Cary, NC) to account for the random effects of square, heifer within square, and period within square and the fixed effect of treatment. Effect of treatment was partitioned into contrasts to examine the effects of the Control versus organic acid treatments, the Control versus fumaric acid, the Control versus malic acid, and fumaric acid versus malic acid treatments (low fumaric acid and high fumaric acid vs. low malic acid and high malic acid). For mean hourly ruminal pH, time was also included in the model as a repeated measure. Covariance structure was modeled using the options of autoregressive order one, compound symmetry, and unstructured order one. The best covariance structure was selected based on the lowest Akaike and Bayesian information criteria. Data are presented as least squares means ± SEM. Statistical significance was declared at $P \leq 0.05$.

Table 2. Rumen evacuation measurements and calculation of molarity of organic acid treatments

Rumen evacuation	Mean	SD	Minimum	Maximum
Total contents, kg[1]	40.0	8.19	34.3	50.3
DM content, %	17.3	1.86	15.2	19.2
Total DM, kg	6.87	1.43	5.64	8.31
Total fluid, kg	33.1	7.02	28.2	41.9
Dose[2]				
Low fumaric acid, m*M*	6.27	0.84	5.21	7.75
High fumaric acid, m*M*	12.89	1.71	10.67	15.89
Low malic acid, m*M*	5.28	0.76	4.36	6.79
High malic acid, m*M*	11.93	1.59	9.90	14.74

[1]Calculated values are average of the data observed during rumen evacuations performed before the start of Period 1 and after the end of Period 5.

[2]Values presented are the estimated plateau concentrations of organic acids calculated based on total intake of 61 or 125 g/d of fumaric acid and 59 or 134 g/d of malic acid for the low and high doses, respectively, and assuming hourly liquid ruminal turnover rate of 11% and constant influx of the respective organic acid. The molecular weights are 116.07 and 134.09 g/mol for fumaric acid and malic acid, respectively.

RESULTS

Total ruminal contents, measured during ruminal evacuation, ranged from 34.3 to 50.3 kg with DM contents ranging from 15.2 to 19.2% (Table 2). Organic acids were homogenously mixed with the TMR and, because the offered TMR was not entirely consumed, the actual consumption of low and high doses of organic acids was 61 and 125 g/d of fumaric acid and 59 and 134 g/d of malic acid, respectively. Based on the measured rumen fluid volume, and assuming constant ruminal influx of organic acid over the 24-h duration and hourly liquid turnover rate of 11%, the doses of organic acids corresponded to a mean plateau concentration of 6 and 13 m*M* of fumaric acid and 5 and 12 m*M* of malic acid, respectively (Table 2).

Dry matter intake averaged 10.1 kg/d and corresponded to 2.0% of BW daily. However, organic acid supplementation had no effect on DMI ($P = 0.77$) and mean ($P = 0.74$), minimum ($P = 0.64$), and maximum ($P = 0.27$) ruminal pH (Table 3). Moreover, AUC for pH thresholds 6.2 ($P = 0.97$), 5.8 ($P = 0.66$), 5.5 ($P = 0.55$), and 5.2 ($P = 0.93$) was similar for all treatments. However, malic acid supplementation reduced the amount of time that the ruminal pH was under 6.2 compared with the Control ($P = 0.02$) and fumaric acid treatments ($P < 0.01$). However, no effects were observed on the amount of time spent under pH

Table 3. Feed intake and ruminal pH in beef cattle fed high-grain diets supplemented with different levels of fumaric or malic acid

	Treatments						*P*-value			
		Fumaric acid[2]		Malic acid[3]						
Item	Control[1]	LF	HF	LM	HM	SEM	Control vs. organic acid	Control vs. fumaric acid	Control vs. malic acid	Fumaric acid vs. malic acid
DMI, kg/d	10.0	10.1	10.3	9.80	9.40	0.39	0.77	0.63	0.31	0.07
Ruminal pH										
Mean	5.68	5.68	5.64	5.74	5.76	0.06	0.74	0.71	0.33	0.11
Minimum	5.09	5.07	5.02	5.08	5.11	0.05	0.64	0.39	0.98	0.28
Maximum	6.37	6.39	6.39	6.44	6.53	0.06	0.27	0.71	0.11	0.12
Ruminal pH < 6.2										
AUC,[4] pH × h	12.3	12.4	13.3	11.7	11.4	1.2	0.97	0.65	0.61	0.24
Duration, h/d	20.3	20.5	20.2	18.4	17.1	2.0	0.20	0.95	0.02	<0.01
Ruminal pH < 5.8										
AUC, pH × h	5.28	5.45	6.22	5.49	5.45	0.76	0.66	0.54	0.84	0.62
Duration, h/d	14.1	14.2	14.8	13.1	12.4	1.36	0.73	0.75	0.35	0.13
Ruminal pH < 5.5										
AUC, pH × h	1.95	1.93	2.57	2.17	2.28	0.42	0.55	0.57	0.61	0.95
Duration, h/d	7.99	9.12	9.53	8.80	8.51	1.26	0.43	0.35	0.64	0.56
Ruminal pH < 5.2										
AUC, pH × h	0.40	0.21	0.58	0.33	0.45	0.14	0.93	0.96	0.92	0.95
Duration, h/d	2.32	2.32	4.18	3.50	3.55	0.82	0.28	0.29	0.17	0.69

[1]Control = no supplementation.

[2]Treatments provided a low level (low fumaric acid [LF]; 61 g/d) and a high level (high fumaric acid [HF]; 125 g/d) of fumaric acid mixed with the diets.

[3]Treatments provided a low level (low malic acid [LM]; 59 g/d) and a high level (high malic acid [HM]; 134 g/d) of malic acid mixed with the diets.

[4]AUC = area under the curve.

thresholds 5.8 ($P = 0.73$), 5.5 ($P = 0.43$) and 5.2 ($P = 0.28$) with organic acid supplementation. Hourly ruminal pH was significantly affected by the sampling time ($P < 0.01$), but treatment ($P = 0.52$) and the treatment × time interaction ($P = 0.46$) were not significant (Fig. 1).

Total VFA concentration was greater with supplemental fumarate compared with the malate treatments ($P = 0.02$; Table 4), whereas no effects were observed when compared with the Control. Similarly, fumaric acid treatments tended to reduce ($P = 0.08$) ruminal acetate proportion, whereas no effects were observed with malic acid treatments ($P = 0.38$) compared with the Control. Ruminal lactate ($P = 0.32$) and succinate ($P = 0.64$) concentrations averaged 0.22 and 0.27 m*M*, respectively, and were not altered with supplemental organic acids.

The population of total culturable bacteria, 3 h after feeding, was reduced with supplemental malic acid compared with the Control ($P = 0.03$; Table 4) and fumaric acid treatments ($P = 0.03$). However, no effects were observed with organic acid supplementation on lactic acid–utilizing bacteria ($P = 0.59$).

DISCUSSION

The high-grain diet used in this study was typical of diets fed to finishing cattle in western Canadian feedlots where barley grain and barley silage are the main ingredients. Mean ruminal pH for beef cattle fed high-grain diets ranges from 5.8 to 6.2 (Nagaraja and Titgemeyer, 2007); however, as seen in the present study, consumption of a high-grain diet was successful in inducing an extended period of SARA and severe SARA as characterized by increased duration and area that pH was <5.8 and <5.5, respectively. Nevertheless, consumption of the high-grain diet did not induce acute ruminal acidosis as evident by minimal ruminal lactate concentrations, probably because animals were adapted to high-grain diets by gradually transitioning over 28 d from high-forage diets. This study was designed as a replicated Latin square with 14-d periods including 10 d for adaptation to treatments. The adaptation period could be considered short if sudden changes in the diet composition are required that stimulate adaptive responses by the ruminal epithelium (Steele et al., 2011). However, a short adaptation period might not be a limitation in the present study because dietary composition was not altered and animals were well adapted to the high-grain diet. Organic acid supplementation might have resulted in altered ruminal microbiome; however, previous studies have shown that changes in the rumen microbiome are of transient nature and return to steady state within a week of an acute acidosis challenge (Petri et al., 2013).

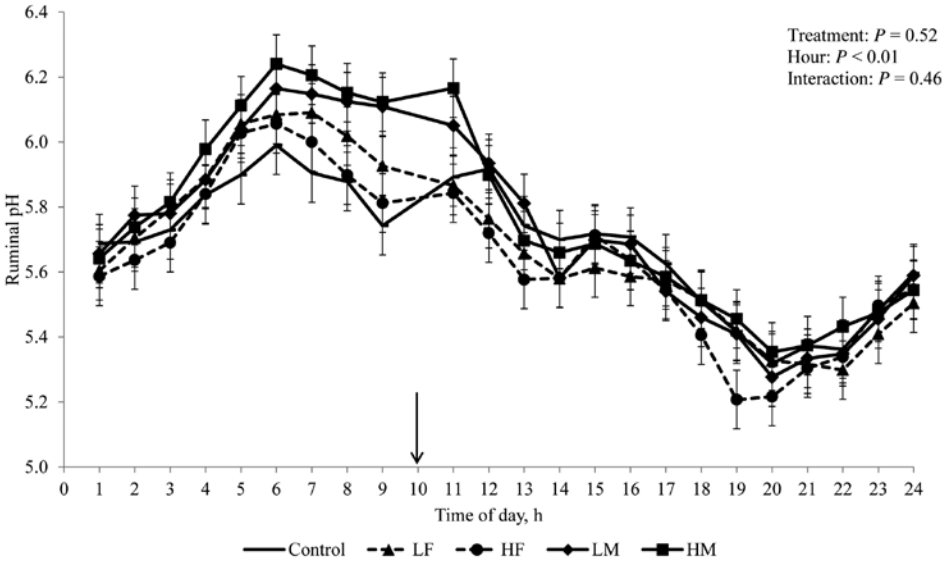

Figure 1. Diurnal pattern of ruminal pH in beef cattle fed high-grain diets supplemented with different levels of fumaric acid (low fumaric acid [LF], 61 g/d, and high fumaric acid [HF], 125 g/d) or malic acid (low malic acid [LM], 59 g/d, and high malic acid [HM], 134 g/d). The position of the arrow indicates the time of feeding, that is, 1000 h.

Dietary supplementation of organic acids has been proposed as an effective strategy to prevent SARA and to improve ruminal fermentation efficiency in beef cattle fed high-grain diets (Martin, 1998; Castillo et al., 2004). The mechanism by which organic acids influence ruminal pH and fermentation parameters is based on results observed in various in vitro studies (Martin and Streeter, 1995; Callaway and Martin, 1996; Carro et al., 1999; Gómez et al., 2005) showing that supplemental malate and fumarate elevate ruminal pH by stimulating the growth of lactate utilizers, *S. ruminantium*, thereby increasing the rate of lactic acid utilization (Nisbet and Martin, 1990, 1993, 1994). Additionally, organic acid supplementation increases the concentration of dissolved CO_2 generated as an end product of the succinate–propionate pathway utilized by *S. ruminantium*

Table 4. Ruminal volatile fatty acids and microbial populations in beef cattle fed high-grain diets supplemented with different levels of fumaric or malic acid

	Treatments						*P*-value			
		Fumaric acid[2]		Malic acid[3]						
Item	Control[1]	LF	HF	LM	HM	SEM	Control vs. organic acid	Control vs. fumaric acid	Control vs. malic acid	Fumaric acid vs. malic acid
Total VFA, m*M*	135.6	138.4	138.6	134.5	128.8	3.83	0.98	0.30	0.32	0.02
Individual VFA, mol/100 mol										
Acetate	51.1	49.3	49.1	50.2	50.2	1.20	0.15	0.08	0.38	0.26
Propionate	34.7	35.2	34.9	34.3	34.4	2.33	0.98	0.91	0.89	0.78
Butyrate	9.11	9.90	10.2	10.0	10.3	0.98	0.53	0.59	0.54	0.92
Isobutyrate	0.91	0.84	0.91	0.97	0.88	0.05	0.76	0.42	0.80	0.20
Valerate	2.04	2.49	2.68	2.31	1.98	0.35	0.40	0.21	0.82	0.20
Isovalerate	1.60	1.74	1.79	1.75	1.80	0.33	0.60	0.64	0.62	0.98
Caproate	0.45	0.51	0.48	0.47	0.35	0.09	0.95	0.58	0.66	0.23
Lactate, m*M*	0.09	0.21	0.50	0.13	0.15	0.14	0.32	0.13	0.78	0.13
Succinate, m*M*	0.11	0.97	0.09	0.08	0.12	0.40	0.64	0.38	0.99	0.28
Acetate:propionate	1.59	1.61	1.54	1.60	1.68	0.21	0.95	0.94	0.85	0.73
Total culturable bacteria ($\times 10^9$), cfu/mL	5.64	5.00	4.76	4.60	3.86	0.66	0.13	0.63	0.03	0.03
Lactate utilizers ($\times 10^9$), cfu/mL	4.74	4.78	4.16	4.18	4.91	0.74	0.59	0.91	0.38	0.35

[1]Control = no supplementation.

[2]Treatments provided a low level (low fumaric acid [LF]; 61 g/d) and a high level (high fumaric acid [HF]; 125 g/d) of fumaric acid mixed with the diets.

[3]Treatments provided a low level (low malic acid [LM]; 59 g/d) and a high level (high malic acid [HM]; 134 g/d) of malic acid mixed with the diets.

(Martin, 1998). Various in vitro studies have consistently reported elevated pH, increased VFA production, and reduced acetate-to-propionate ratio, predominantly due to an increase in propionate synthesis resulting from lactate fermentation, with supplementation of fumarate (Asanuma et al., 1999; Newbold et al., 2005) and malate (Martin and Streeter, 1995; Newbold et al., 2005) to different incubated substrates.

However, contrary to our hypothesis, organic acid supplementation was not effective in preventing SARA as few treatment effects were observed on ruminal pH and fermentation parameters. The only beneficial effect observed was with malate supplementation as it reduced the duration of time spent below pH 6.2; however, no other ruminal pH parameters were altered compared with the Control.

Lack of effects observed on ruminal pH is an apparent contradiction from the consistent beneficial effects observed in in vitro studies and could be attributed to the differences in the experimental conditions (Carro et al., 2006). Ruminal DM content in the present study ranged from 15.2 to 19.2%; however, DM in in vitro systems ranges from 2 to 4% of the total volume, resulting in significant differences in the amount of organic acid supplemented when expressed as grams of organic acid per 100 g of diet (Carro et al., 2006). Previous batch culture studies provided 6.8 (Callaway and Martin, 1996), 12.7 (Carro and Ranilla, 2003), and 17.1% (Tejido et al., 2005) of organic acids on a DM basis whereas Rusitec fermenters provided 3.4 (Carro et al., 1999) and 7.1% (Gómez et al., 2005) of organic acids on a DM basis (Carro et al., 2006). The concentrations of dietary fumarate and malate supplemented in this study ranged from 0.76 to 1.48% and 0.90 to 1.88% on a DM basis, respectively. Furthermore, this calculation does not account for fluid dilution rate and passage of organic acids from the rumen in vivo, factors that do not occur in batch culture experiments (McGinn et al., 2004). Moreover, VFA concentrations measured in vitro reflect production, whereas in vivo concentrations are the balance between production and absorption (Beauchemin and McGinn, 2006). Therefore, the results observed from in vitro studies could be used to elucidate mechanisms; however, experimental conditions observed in vitro are not representative of the ruminal milieu and ruminal fermentation responses might not be generalized to in vivo studies.

Because the efficacy of organic acids in elevating ruminal pH is dependent on lactate uptake by ruminal organisms, it was proposed that the response in ruminal pH would be notable in cattle fed high-grain diets due to significant lactate accumulation. In agreement with this hypothesis, ruminal pH was elevated with supplemental malate in steers fed high-grain diets (Martin et al., 1999; Montano et al., 1999); however, no effects were observed with fumarate (McGinn et al., 2004; Beauchemin and McGinn, 2006) and malate (Foley et al., 2009) in animals fed high-forage or mixed diets. Nevertheless, despite the use of high-grain diets in the present study, no effects were observed on ruminal pH and fermentation parameters. The lack of effects could be attributed to the small ruminal lactate concentrations observed in this study. The accumulation of ruminal lactate occurs in feedlot animals during abrupt transition from a high-forage to a high-grain diet as lactic acid utilizers are slow to adapt to abrupt changes in the diet composition (McAllister et al., 2011). However, with gradual adaptation to high-grain diets, only small concentrations of lactic acid are observed (Burrin and Britton, 1986; Vyas et al., 2014), as shown in the present study, which could possibly explain reduced efficacy of dietary fumarate and malate in preventing SARA in the present study. However, contrary to our assumption, Martin et al. (1999) observed a linear increase in ruminal pH associated with malic acid provided at 4, 8, and 12 m*M* ruminal concentrations (assuming ruminal volume of 50 L) to steers fed a corn-based finishing diet despite small ruminal lactate concentrations. The apparent contradiction between studies is difficult to explain given that the estimated molar amount of ruminally available malic acid was similar in both studies. However, it might be attributed to the mode of supplementing organic acids. In the present study, both fumaric and malic acid were supplemented in the diet, whereas Martin et al. (1999) administered malic acid directly into the rumen 30 min after the morning feeding. Ruminal administration might have resulted in rapid availability of malic acid and probably explains the significant effects on ruminal fermentation and ruminal pH. Another possibility for inconsistent results between both studies might be due to the technique and sampling frequency used for ruminal pH measurements. Ruminal pH was measured every 5 s continuously for 48 h in the present study and is more representative of the postprandial variations as compared with the study by Martin et al. (1999) where ruminal samples were only taken between 0 and 12 h after feeding. It is quite possible that magnitude of treatment differences observed on ruminal pH would have disappeared after 12 h given that treatment differences in ruminal pH were smaller at 12 h compared with 1 h after feeding (Martin et al., 1999). However, considering lack of treatment × time interaction for hourly ruminal pH, the possibility of dilution of treatment effects on ruminal pH seems improbable.

Previous studies have reported variable effects on DMI with organic acid supplementation. In agreement with the results from our study, some authors have re-

ported no effects on DMI (Martin et al., 1999; Bayaru et al., 2001; Carro et al., 2006). In contrast, a significant drop in DMI was observed in wethers fed lucerne-based diets supplemented with fumaric acid at 4, 6, 8, and 10% of diet DM (Molano et al., 2008) or in beef heifers fed mixed diets supplemented with 2.5 and 5% of malic acid (Foley et al., 2009). Lower inclusion levels of fumarate and malate used in the present study might have prevented their negative effect on DMI. Nevertheless, reduced DMI with organic acid supplementation could reduce ruminal availability of fermentable substrates and might result in elevating ruminal pH. In support of our argument, studies that have reported increased ruminal pH in response to organic acid supplementation have also observed reduced DMI (Molano et al., 2008; Foley et al., 2009).

Furthermore, the form of organic acid supplemented could also have significant effect on ruminal fermentation (Asanuma et al., 1999; Castillo et al., 2004). Organic acids can be supplemented either as free acid or as a salt; however, sodium salts were proposed to be more effective due to the presence of sodium moiety serving as buffering agent to raise ruminal pH, an effect similarly observed with the addition of sodium bicarbonate (Chalupa, 1977; Wheeler, 1980). However, it is unclear whether using sodium salts of organic acids in the present study could have stimulated ruminal fermentation as results based on previous in vitro (Martin and Streeter, 1995) and in vivo (Castillo et al., 2007) studies do not confirm greater efficacy of sodium salts of organic acids in buffering ruminal pH relative to its free acid form.

The effects observed on total bacterial populations are consistent with an earlier study using disodium fumarate in goats (Yang et al., 2012). Similarly, the abundance of lactate utilizers observed in the present study corresponds well with a previous study where no changes were observed in the abundance of lactate utilizer *S. ruminantium* in goats given high-concentrate diets (Yang et al., 2012) despite significant changes in total VFA concentration and proportions of ruminal acetate and propionate. Nevertheless, results observed for microbial populations in this study correspond well with the lack of significant treatment effects on ruminal fermentation.

In conclusion, supplementing a barley-based grain diet with malic acid showed some beneficial effects on preventing low ruminal pH, but did not completely eliminate SARA in feedlot cattle. No response was observed on ruminal pH and fermentation parameters with fumaric acid. Based on the results from the present study, organic acid supplementation has limited potential to prevent incidences of SARA in beef cattle adapted to high-grain diets. However, it would be of interest to observe the effects of supplementing organic acids in animals not adapted to consumption of high-grain diet primarily during transition from a high-forage to a high-grain diet.

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