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The effect of buffering dairy cow diets with limestone, calcareous marine algae, or sodium bicarbonate on ruminal pH profiles, production responses, and rumen fermentation

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ABSTRACT

Six ruminally cannulated Holstein cows were used to evaluate the effect of 2 dietary buffers on rumen pH, milk production, milk composition, and rumen fermentation parameters. A high concentrate total mixed ration [35.2% forage dry matter (DM)], formulated to be potentially acidotic, was used to construct 3 dietary treatments in which calcareous marine algae (calcified remains of the seaweed *Lithothamnium calcareum*) was compared with limestone (control) and sodium bicarbonate plus limestone. One basal diet was formulated and the treatment diets contained either 0.4% of dietary DM as Acid Buf, a calcified marine algae product (AB treatment), or 0.8% of dietary DM as sodium bicarbonate and 0.37% as limestone (BC treatment), or 0.35% of dietary DM as limestone [control (CON) treatment]. Cows were randomly allocated to treatments according to a double 3 × 3 Latin square design, with 3 treatments and 3 periods. The total experimental period was 66 d during which each cow received each treatment for a period of 15 d before the data collection period of 7 d. Rumen fluid was collected to determine volatile fatty acids, lactic acid, and ammonia concentrations. Rumen pH was monitored every 10 min for 2 consecutive days using a portable data logging system fitted with in-dwelling electrodes. Milk samples were analyzed for solid and mineral contents. The effect of treatment on acidity was clearly visible, especially from the period from midday to midnight when rumen pH dropped below 5.5 for a longer period of time (13 h) in the CON treatment than in the BC (8.7 h) and AB (4 h) treatments. Daily milk, 4% fat-corrected milk, and energy-corrected milk yields differed among treatments, with AB being the highest, followed by BC and CON. Both buffers increased milk fat content. Treatment had no effect on milk protein content, but protein yield was increased in the AB treatment. Total rumen volatile fatty acids and acetate concentrations were higher and propionate was lower in the AB treatment than in CON. The molar proportion of acetate was higher in AB than in CON, but that of propionate was lower in both buffer treatments than in CON. The acetate:propionate ratio was increased in the AB and BC treatments compared with CON. Lactic acid concentration was higher in the CON treatment than in the buffer treatments. Treatment had no effect on rumen ammonia concentrations. Results indicated that buffer inclusion in high concentrate diets for lactating dairy cows had a positive effect on milk production and milk composition. Calcareous marine algae, at a level of 90 g/cow per day, had a greater effect on rumen pH, milk production and milk composition, and efficiency of feed conversion into milk than sodium bicarbonate at a level of 180 g/cow per day.
Key words: Acid Buf, buffer, rumen metabolism, milk production

INTRODUCTION

To meet the energy demands for lactation and reproduction, animals of improved genetic merit are commonly fed highly digestible diets containing large amounts of readily fermentable carbohydrates (Plaizier et al., 2008). Providing such diets with limited amounts of effective fiber may result in changes in rumen VFA profiles that may increase rumen acidity (Krause and Oetzel, 2006), thus increasing the risk of SARA (Plaizier et al., 2008). Excess rumen acidity may also result in inconsistent DMI, poor feed and fiber utilization and diarrhea, (Allen, 1997; Nocek, 1997), milk fat depression, laminitis (Nocek, 1997), and death (Plaizier et al., 2008).

Although most of the VFA produced in the rumen are absorbed via the rumen wall, more than 30% are neutralized by salivary sodium bicarbonate and phosphate buffers, the flow of which is stimulated by chewing and consequently by the level of dietary effective NDF (Allen, 1997; Beauchemin, 2007). Sodium carbonates

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have been used as dietary buffers to complement this endogenous supply for high yielding animals on concentrate rich diets since the 1960s (Russell and Chow, 1993). Dietary buffers reduce rumen acidity (Erdman, 1988) and provide a more favorable environment for microbial activity (Harrison et al., 1989). They have been successful in alleviating the symptoms of SARA, and especially milk fat depression (Enemark, 2008).

Sodium bicarbonate has been the most popular dietary buffer (Chalupa et al., 1996). It has been shown to effectively neutralize rumen acidity and stabilize rumen pH (Thomas et al., 1984; Solorzano et al., 1989) and to improve the efficiency of fiber digestion, microbial protein synthesis (Rogers et al., 1982), and OM utilization (Mackie and White, 1990). It could furthermore increase milk fat content and feed intake (Enemark, 2008). However, as a soluble buffer, sodium bicarbonate is short lived in the rumen (Van Soest, 1994) and cannot effectively buffer ongoing production of acids in the rumen.

Acid Buf (also known as Calmin; Celtic Sea Minerals, Cork, Ireland) is the skeletal (calcified) remains of the seaweed *Lithothamnion calcareum*, harvested off the Irish and Icelandic coasts. Calcareous marine algae consists mainly of calcium carbonate that occurs in 3 different calcium structures, viz. calcite (65%), aragonite (23%), and vaterite (12%). Aragonite and vaterite are polymorphs of calcite (Railsback, 2006). Calcium (300 g/kg) is the major mineral of calcareous marine algae, and other minerals include Mg (55 g/kg), K (7 g/kg), Fe (800 mg/kg), P (500 mg/kg), Mn (50 mg/kg), I (30 mg/kg), Cu (10 mg/kg), Zn (10 mg/kg), B (10 mg/kg), Mo (0.2 mg/kg), Se (1.8 mg/kg), and Co (0.1 mg/kg), according to Celtic Sea Minerals (2014). The honeycomb structure of calcareous marine algae results in a slow release of minerals in an acid environment. When Acid Buf was included at 0.3% in a TMR for lactating Holstein cows, Cruywagen et al. (2004) found that it had a positive influence in the rumen and that milk yield, milk fat, 4% FCM, and milk protein content improved. Other than this, information regarding the effect of calcareous marine algae on rumen metabolism and milk production in dairy cows is limited. The objectives of the current study were to compare the effects of limestone, calcareous marine algae, and sodium bicarbonate on production and rumen metabolism parameters in dairy cows fed potentially acidotic diets.

MATERIALS AND METHODS

Animals and Housing

Six multiparous, ruminally cannulated Holstein cows, 129 ± 9.2 (SE) DIM and weighing 732 ± 10.3 (SE) kg, were used in the trial. The cows were kept at the Welgevallen Experimental Farm of the Stellenbosch University, Western Cape Province, South Africa. Cows were housed individually in 6 × 4 m pens in a well-ventilated, semi-open barn with a cement floor. Each cow had access to a sand-bedded sleeping crate, a feeding trough, and fresh water via a ball-valve-controlled water bowl. The trial protocol was approved by the Stellenbosch University's Animal Ethics Committee (reference: 2005B03001).

Experimental Design and Treatments

Cows were randomly assigned to treatments according to a double 3 × 3 Latin square design with 3 treatments and 3 periods. All cows received all 3 treatments during the course of the trial. The trial duration was 66 d. Each period (22 d) consisted of a 15-d adaptation period, followed by a 7-d data collection period.

All 3 treatments had the same basal diet, which was formulated to be potentially acidotic (Table 1). The differences between treatments were attributed to the inclusion of different buffers. The buffers used in this trial included limestone, calcareous marine algae (in the form of Acid Buf, also known as Calmin), and sodium bicarbonate. Experimental diets were in the form of TMR and were mixed by Nova Feeds (Malmesbury, Western Cape Province, South Africa). Diet formulation was done with CPM Dairy, version 3 (Cornell University, Ithaca, NY; University of Pennsylvania School of Veterinary Medicine, Kennett Square, PA; The William H. Miner Agricultural Research Institute, Chazy, NY; and The University of Maryland, College Park, MD), using the chemical composition of the ingredients as determined via NIR by Nova Feeds. After mixing each TMR, samples were taken and analyzed immediately for NDF and CP via NIR at the Nova Feeds laboratory, to confirm mixing efficiency. The treatment diets contained either 3.5 g of limestone/kg of DM (**CON**), replaced by 4 g of Acid Buf/kg of DM (**AB**), or by 3.7 g of limestone/kg plus 8 g of sodium bicarbonate/kg of DM (**BC**). Treatments were formulated assuming a DMI of 23 kg/d; thus, the marine algae product was included at a level to ensure a daily intake of 90 g/cow, whereas the sodium bicarbonate was included at a level to ensure a daily intake of 180 g/cow. Cows were fed twice daily at 0700 h (40% of the daily allowance) and at 1600 h (60% of the daily allowance) at a level of approximately 5% in excess of appetite.

Data Collection and Chemical Analyses

Feed intake was recorded daily during each 7-d data collection period by recording the amount of feed sup-

Table 1. Ingredient and chemical composition of the experimental diets

Item	Treatment[1]		
	AB	BC	CON
Ingredient (% of DM)			
Oat hay	17.6	17.6	17.6
Alfalfa hay	17.6	17.6	17.6
Wheat bran[2]	4.8	4.0	4.8
Soybean meal	7.4	7.4	7.4
Cottonseed meal	3.7	3.7	3.7
Fish meal	2.6	2.6	2.6
Ground corn	40.0	40.0	40.0
Urea	0.4	0.4	0.4
Molasses	3.0	3.0	3.0
Megalac	2.0	2.0	2.0
Salt	0.3	0.3	0.3
Trace mineral/vitamin premix[3]	0.2	0.2	0.2
Limestone	—	0.4	0.4
Acid Buf[4]	0.4	—	—
Sodium bicarbonate	—	0.8	—
Chemical composition[5] (% of DM)			
CP	17.2	17.2	17.2
RUP (% of CP)	37.4	37.4	37.4
NDF	26.2	26.0	26.3
peNDF	20.5	20.4	20.5
ADF	16.8	16.7	16.8
NFC	47.1	47.1	47.1
EE	5.6	5.6	5.6
Ash	6.1	6.1	6.1
Ca	0.84	0.87	0.87
Mg	0.24	0.23	0.23
P	0.45	0.45	0.45
K	1.34	1.33	1.34
Na	0.26	0.46	0.26
Calculated ME, MJ/kg of DM	12.2	12.2	12.2

[1]AB = calcareous marine algae (Acid Buf, Celtic Sea Minerals, Cork, Ireland); BC = sodium bicarbonate; CON = control treatment.

[2]Wheat bran inclusion differed between diets to accommodate different buffer inclusion levels.

[3]Standard lactating cow premix provided by SA Premix, Burgersdorp, South Africa. Formulated to contain (per kg of DM) 100 g of Mg, 50 g of Zn, 40 g of Mn, 30 g of FE, 10 g of Cu, 0.75 g of I, 0.4 g of Co, 0.15 g of Se, 3,000,000 IU of vitamin A, 200,000 IU of vitamin D, and 3,250 IU of vitamin E.

[4]Contains calcium (300 g/kg), Mg 55 g/kg, K (7 g/kg), Fe (800 mg/kg), P (500 mg/kg), Mn (50 mg/kg), I (30 mg/kg), Cu (10 mg/kg), Zn (10 mg/kg), B (10 mg/kg), Mo (0.2 mg/kg) Se (1.8 mg/kg), and Co (0.1 mg/kg).

[5]As calculated by using CPM Dairy at Stellenbosch University, based on ingredient analyses; similar for all treatment diets.

plied and refusals weighed back. Samples of feed and refusals were also taken at these times and dried at 105°C for 24 h to determine DM to calculate DMI.

Cows were milked twice daily at 0600 and 1500 h. Daily milk yield was recorded individually at each milking throughout the data collection period. Milk samples were collected twice daily from each cow for 7 consecutive days during each data collection period. Daily milk samples were pooled per cow (proportionally according to morning and afternoon milk yields), preserved with potassium dichromate ($K_2Cr_2O_3$) in 30-mL sample bottles, stored at 4°C, and analyzed within 48 h. Milk was analyzed for fat, CP, lactose, TS, and SNF with the aid of a Milko-scan 605 analyzer (Foss Electric, Hillerød, Denmark) at the Dairy Laboratory of the Agricultural Research Council at Elsenburg, Stellenbosch.

Rumen pH was measured with the aid of WTW 340i pH data loggers and in-dwelling probes (WTW Sentinx 41 Electrodes, supplied by Merck, Cape Town). The pH loggers were housed in aluminum cases fitted on the rumen cannulas. The electrodes were housed in specially designed stainless steel capsules and attached to the cannulas via water-tight hoses and fittings. The arrangement was such that the electrodes resided more or less in the center of the rumen. On d 4 of each data collection period, the pH loggers and probes were introduced at 0630 h. All pH loggers and electrodes were removed at 0630 h on d 6 of the data collection period. Rumen pH was recorded every 10 min over 48 h and the data downloaded for processing and statistical analyses. The 6 measurements per hour were averaged to yield mean hourly pH values over 24 h per cow and treatment. The curves in Figure 1 were constructed by using mean values per treatment, whereas maximum and minimum values were extracted per cow from the hourly pH data set. Because all cows did not exhibit maximum and minimum pH values at the same time of the day, the maximum and minimum values in Table 2 would not necessarily correspond with the curves indicated in Figure 1. Time spent below pH 5.5 was calculated per cow and treatment as the total time per day (h) that pH was below 5.5 continuously for more than 1 h. Short episodes of pH below 5.5 are not considered as a cause of SARA (Krause and Oetzel, 2006).

On d 1 of each data collection period, rumen fluid samples were taken 4 and 8 h after the morning feeding and 2 h after the afternoon feeding. Samples were thus effectively taken 2, 4, and 8 h after feeding. The rumen fluid was strained through 2 layers of cheesecloth and samples (10 mL) were taken for VFA, NH_3, and lactic acid analyses. Samples were composited to obtain one daily sample per cow. For VFA and NH_3 analyses, samples were preserved with 1 mL of 1 *N* NaOH and 1 *N* H_2SO_4, respectively. No preservative was added to rumen fluid used for lactic acid analyses. All the rumen fluid samples were stored at −10°C, pending analyses. Samples for VFA and lactic acid were analyzed using a HPLC method with the aid of a Waters 717 auto sampler (Empower 2 software) equipped with a RI Detector (Waters Corporation, Milford, MA). The column used was an Aminex HPX 87H (Bio-Rad Laboratories, Hercules, CA; 65°C). Rumen fluid samples for NH_3 analysis were prepared as described by Broderick and Kang (1980) and analyzed with the aid of a spectrophotometer set at 630 nm.

Table 2. Effect of calcareous marine algae (Acid Buf) and sodium bicarbonate on mean, maximum, and minimum ruminal pH values and the time that pH spent below 5.5

Item	Treatment[1]			SEM	P-value
	AB	BC	CON		
Mean daily pH	5.66	5.60	5.56	0.054	0.449
Maximum pH	6.15	6.06	6.12	0.09	0.802
Minimum pH	5.42[a]	5.37[a]	5.19[b]	0.055	0.041
Time pH below 5.5 (h/d)	4.0[c]	7.5[b]	13.8[a]	0.8842	<0.001

[a–c]Means within a row with different superscripts differ ($P < 0.05$).

[1]AB = calcareous marine algae included at 0.4% of DM; BC = sodium bicarbonate included at 0.8% of DM; CON = control diet. Acid Buf, Celtic Sea Minerals, Cork, Ireland

Statistical Analyses

The trial was executed as a double 3 × 3 Latin square design where 6 cows were randomly assigned to the rows of the squares. Results were analyzed using the REML procedure in VEPAC of Statistica (version 12, StatSoft Inc., Tulsa, OK), which is equivalent to PROC MIXED of SAS (SAS Institute Inc., Cary, NC). The following model was used to fit the data to determine the effects of dietary treatments:

$$Y_{ijkl} = \mu + S_i + P_j + C_{k(i)} + T_l + \varepsilon_{ijkl},$$

where Y_{ijkl} = the dependent variable, μ = the overall mean; S_i = the effect of square/group i, P_j = the effect of period j, $C_{k(i)}$ = the effect of cow k (within square/group i), T_l = the effect of treatment l, and ε_{ijkl} = the residual error. All terms were considered fixed, except for $C_{k(i)}$ and e_{ijkl}, which were considered random. Least squares means were separated using the LSD procedure when a significant F-test ($P \leq 0.05$) was detected.

For repeated measures on rumen pH, the following model was used with compound symmetry as covariance structure:

$$Y_{ijklm} = \mu + S_i + P_j + C_{k(i)} + T_l + \delta_m + (T \times \delta)_{lm} + \varepsilon_{ijklm},$$

where Y_{ijklm} = the dependent variable, μ = the overall mean, S_i = effect of square/group i, P_j = effect of period j, $C_{k(i)}$ = effect of cow k (within square/group i), T_l = the effect of treatment l, δ_m = effect of the mth time of measurement, and $(T \times \delta)_{lm}$ = the interaction effect of the lth treatment at the mth time.

RESULTS AND DISCUSSION

Rumen pH

The effects of treatment on rumen pH values are presented in Table 2, and diurnal pH changes are shown in Figure 1. Treatment had no significant effect on the mean or maximum pH values, but the pH nadir was lower for the CON treatment than for the buffered treatments. Rumen pH was never above 6.15, regardless of treatment. In vitro studies by Wales et al. (2004) demonstrated higher NDF and ADF digestibility when pH fluctuated around 6.1 compared with 5.6, but this difference was greatly reduced if pH was prevented from decreasing below 5.6. According to Calsamiglia et al. (1999) and Mouriño et al. (2001), reduced fibrolytic bacteria activity was observed below pH 5.8, but amylolytic bacteria are most active from 5.2 to 6 (Ishler et al., 1996). It therefore appears that rumen function may not necessarily be restricted if pH does not go above 6.1 on highly fermentable diets and that it may only become compromised if pH drops below the 5.5 margin where SARA could be induced.

The time that rumen pH remained below 5.5 continuously for longer than 1 h over a 24-h period differed between treatments. Time spent below pH 5.5 was shorter ($P < 0.001$) in the AB treatment (4 h) than in the BC treatment (7.5 h), which was shorter than in the CON treatment (13.8 h). The basal diet was formulated to be potentially acidotic in an attempt to induce SARA. An acceptable physiological pH for maintaining a well-balanced rumen population is between 5.8 and 6.4 (Ishler et al., 1996; McDonald et al., 2002). However, in high-yielding dairy cows on high-starch diets, rumen pH may decrease to levels well below 5.8. According to Krause and Oetzel (2006), SARA is caused when rumen pH remains below 5.5 for prolonged periods of time. de Veth and Kolver (2001) suggested that the period of time that ruminal pH is suboptimal may be a more critical factor for digestion than the relationship between mean daily pH and optimal pH. A linear reduction in NDF and ADF digestion was observed in a dual-flow continuous culture system as the time at suboptimal pH increased by 4-h intervals from 0 to 24 h (Cerrato-Sanchez et al., 2007). In the current trial, cows in the CON treatment were at times visibly panting and drooling and appeared to be suffering from

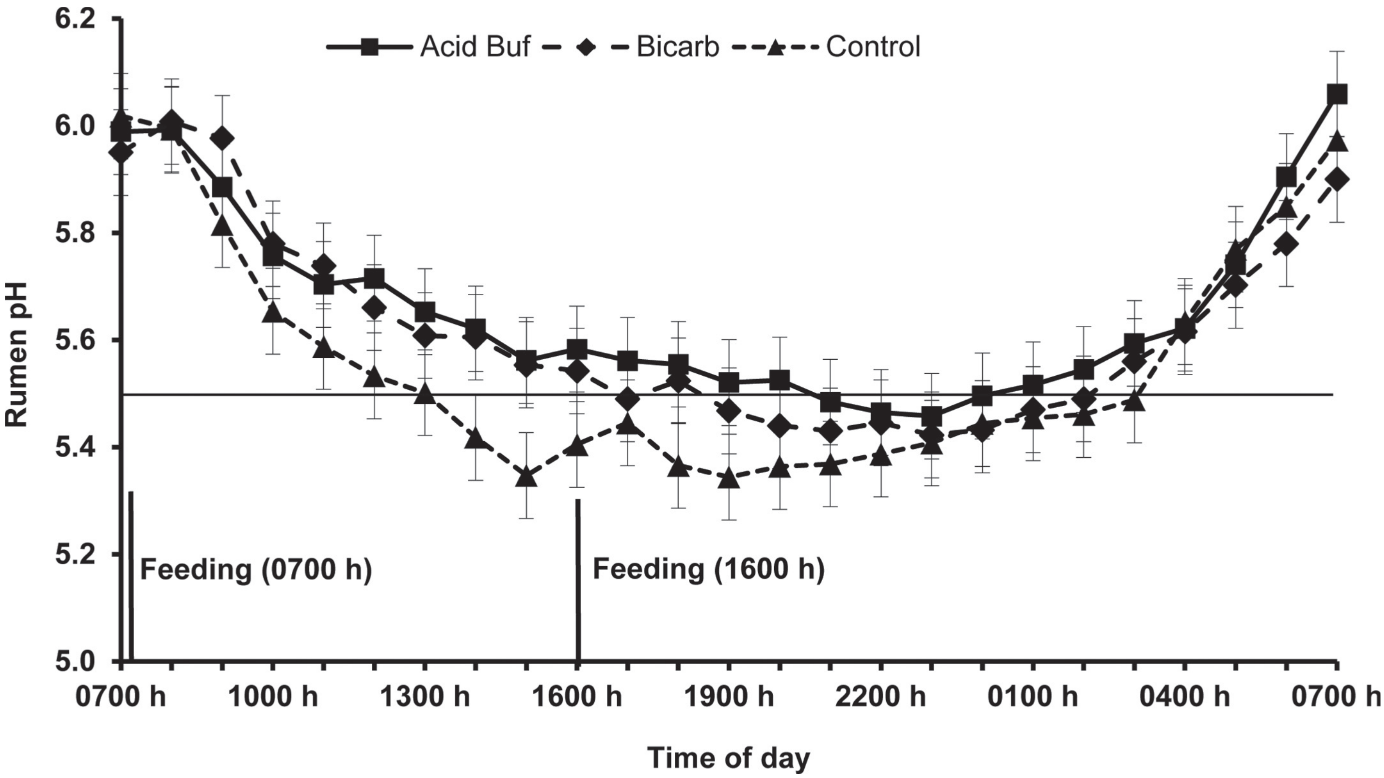

Figure 1. The effect of calcareous marine algae (Acid Buf, Celtic Sea Minerals, Cork, Ireland) and sodium bicarbonate (Bicarb) on rumen pH profiles of lactating Holstein cows. Results are means ± SEM.

problems associated with acidosis, whereas cows in the AB and BC treatments appeared to be more comfortable.

The effect of treatments on diurnal rumen pH patterns is presented in Figure 1. A repeated measures ANOVA revealed that, over a 24-h period, all 3 pH curves differed from each other (AB-BC: $P < 0.02$; AB-CON: $P < 0.001$; BC-Con: $P < 0.04$). Rumen pH decreased in all treatments after the morning feeding and the decrease was more significant ($P < 0.02$) in cows receiving the CON treatment. Although neither buffer increased pH after first feeding, the BC treatment did sustain the prefeeding pH for approximately 1 h longer than either CON or AB. This may be due to the higher solubility of sodium bicarbonate compared with that of calcareous marine algae (**CMA**). Different forms of $CaCO_3$ (calcite, aragonite, and vaterite) exist in CMA. Vaterite is more soluble than aragonite, which is more soluble than calcite (Railsback, 2006). However, the solubility of calcite is increased in the presence of Mg. Calcite is the most abundant mineral (650 g/kg) in the CMA product that was used, and the product also contains 55 g/kg of Mg. It can therefore expected that CMA is sparingly soluble in water, compared with sodium bicarbonate, which is highly soluble.

The effect of the time of the second feeding on rumen pH after 1600 h is not clear in all the treatments. After the first feeding, pH in the CON treatment decreased rapidly from 6.0 to 5.35 at 1500 h, after which it started to recover. However, 1 h after the second feeding at 1600 h, pH (CON) decreased again to reach 5.34 at 1700 h before it started to increase gradually, but constantly, until 0700 h. The increase in rumen pH during the early morning hours observed in all the treatments may be related to the type and quantity of substrate retained in the rumen. We postulated that the NDF:NSC ratio would be higher during this period of time, the rate of fermentation would be lower, and that salivary buffers would aid in the recovery of rumen pH.

Milk Yield and Composition

The effects of treatment on DMI, milk yield, and milk composition is presented in Table 3. Treatment had no effect on DMI, suggesting that the different buffers did not affect palatability. When planning the trial, the different buffer inclusion levels were based on an anticipated DMI of 23 kg/d. The actual intakes were close to expected intakes. Although the mean DMI of

Table 3. The effect of calcareous marine algae (Acid Buf) and sodium bicarbonate on feed intake, milk yield, milk composition, Ca balance, and efficiency of milk production[1]

Item	Treatment			SEM	P-value
	AB	BC	CON		
DMI (kg of DM/d)	23.3	24.2	23.1	1.623	0.353
Yield (kg/d)					
Milk	31.8[a]	29.1[b]	27.6[c]	2.546	0.006
Fat	1.33[a]	1.22[b]	1.06[c]	0.091	<0.001
4% FCM	32.8[a]	29.9[b]	26.9[c]	2.350	<0.001
ECM	35.2[a]	32.0[b]	29.2[c]	2.376	<0.001
CP	1.09[a]	0.98[b]	0.93[b]	0.060	0.003
Lactose	1.46[a]	1.34[b]	1.26[b]	0.125	0.003
Milk composition (%)					
Fat	4.21[a]	4.18[a]	3.86[b]	0.097	0.014
CP	3.47	3.38	3.43	0.132	0.399
Lactose	4.58	4.59	4.57	0.053	0.838
Efficiency					
Milk/DMI (kg/kg)	1.36[a]	1.20[b]	1.19[b]	0.031	<0.001
4% FCM/DMI (kg/kg)	1.40[a]	1.23[b]	1.16[c]	0.028	<0.001

[a–c]Means within a row with different superscripts differ ($P < 0.05$).

[1]AB = calcareous marine algae included at 0.4% of DM; BC = sodium bicarbonate included at 0.8% of DM; CON = control diet. Acid Buf, Celtic Sea Minerals, Cork, Ireland.

the BC diet was 5.2% higher than the anticipated DMI, the difference between treatments was not statistically significant.

Treatment affected ($P < 0.01$) daily milk yield, 4% FCM, and ECM, which were highest for cows in the AB treatment, followed by those in the BC treatment, and lowest for cows in the CON treatment. In a trial by Kennelly et al. (1999) where cows received a high-concentrate diet (concentrate: forage 75:25) with (1.2% of DM) or without sodium bicarbonate, the buffer resulted in an increase in both milk yield and 4% FCM, which they ascribed to an increase in total VFA resulting from an increase in acetate concentration. In a similar trial by Khorasani and Kennelly (2001), sodium bicarbonate inclusion had no effect on milk yield, but 4% FCM yield was increased by the buffer as a result of increased milk fat content. In the current trial, both buffers increased all milk yield parameters, but calcareous marine algae had the biggest effect. As mentioned earlier, the CON cows experienced prolonged times of rumen pH below 5.5 (13.8 h per day) and showed signs of SARA (panting and drooling). It is well known that SARA has a negative effect on milk production (Krause and Oetzel, 2006; Enemark, 2008).

In a meta-analysis by Hu and Murphy (2005), 27 studies were reviewed where early- and mid-lactation dairy cow responses were evaluated when diets were buffered with sodium bicarbonate. The studies included a total of 30 experiments, 73 dietary treatments, and 369 cows, and the authors only included studies in which sodium bicarbonate levels were 0 (control), or ranged between 7.0 and 10.0 g/kg (moderate) and 10.5 to 15 g/kg (high). Hu and Murphy (2005) also categorized forage type as maize silage (**MS**), when it was the sole or main forage in the diet, or nonmaize silage forage (**NMS**), when alfalfa hay or silages other than maize silage were the sole or main forages. Significant interactions were observed, and they reported that milk production, protein content, and protein yield were not affected by buffer treatment, regardless of forage type. However, for MS-based diets, sodium bicarbonate increased fat content and fat yield. It should be mentioned that the fiber content of the diets included in all the studies varied widely. For example, the ADF content of the diets in the MS studies varied between 77 and 225 g/kg of DM and in only 2 of the 18 studies the ADF content was above 200 g/kg of DM. In the NMS studies, ADF content varied between 160 and 220 g/kg of DM, and in only 4 of the 9 studies, the ADF content was below 220 g/kg. In our study, the ADF content varied between 167 and 168 g/kg. As the authors concluded, differences in response to buffer addition associated with forage type might have been related to fiber content.

In our study, both milk fat yield and fat content were higher for cows receiving buffered diets compared with the control diet. When cows received high concentrate diets (75% concentrate), Khorasani and Kennelly (2001) reported milk fat content to be 4.09% when diets were buffered compared with 2.91% when diets were not buffered. Xu et al. (1994), Kalscheur et al. (1997), and Kennelly et al. (1999) also reported that dietary buffers prevented milk fat depression. Milk fat content is affected by multiple factors, including VFA ratios. In our study, prolonged episodes of ruminal pH below 5.5 were observed that may have altered VFA ratios (discussed later).

Treatment had no effect on milk protein and lactose contents, but due to differences in milk yield among treatments, the AB treatment resulted in the highest milk protein and lactose yields. In agreement with our study, Khorasani and Kennelly (2001) also reported a lack of effect of dietary buffers on milk protein and lactose contents.

Treatment had a significant effect on the efficiency of feed utilization. Cows in the AB treatment produced more milk per kilogram of DMI than cows in the other treatments. Regarding ECM, efficiencies differed between all treatments, being highest for cows in the AB treatment and lowest for the CON cows.

Rumen VFA, Lactic Acid, and Ammonia Concentrations

The results of rumen VFA, NH_3, and lactic acid concentrations are presented in Table 4. Total VFA concentration was higher for the AB treatment than for CON, with BC being intermediate. Values tended to be higher ($P = 0.074$) for the BC treatment than for CON. Both Kennelly et al. (1999) and Khorasani and Kennelly (2001) reported higher total VFA values in the rumen fluid of cows receiving high-concentrate (75%) diets that were buffered with sodium bicarbonate. They ascribed the higher VFA concentrations to an increase in acetate. The higher total rumen VFA values observed in the AB cows, and the tendency observed in the BC cows compared with CON, contributed to the higher milk yields observed in cows receiving the buffered diets.

Acetate concentration was higher for the AB treatment than for CON. Values for the BC treatment were intermediate, but tended to differ from CON ($P = 0.082$) and AB ($P = 0.061$). The molar proportion of acetate followed the same pattern as acetate concentration. The increase in acetate concentration and molar proportion of acetate that was observed with buffer inclusion in the current study is in agreement with results of Erdman et al. (1982), Xu et al. (1994), Kennelly et al. (1999), and Khorasani and Kennelly (2001). According to Calsamiglia et al. (1999) and Mouriño et al. (2001), reduced fibrolytic bacteria activity can be expected when rumen pH values decrease below pH 5.8. From Figure 1, it is evident that the rumen pH profile of the CON cows was not conducive to optimal acetate production. The higher rumen acetate values observed in cows receiving the buffered diets also manifested in higher milk fat levels.

Propionic acid concentration was higher for the CON treatment than for AB and tended ($P = 0.090$) to be higher than for the BC treatment. This is not uncommon in buffered high-concentrate diets and lower molar percentages of propionic acid have been reported by Erdman et al. (1982), Kennelly et al. (1999), and Khorasani and Kennelly (2001), using sodium bicarbonate in high-concentrate diets. Similarly, in experiment 3 of a trial by Xu et al. (1994), it was reported that the inclusion of Rumen 8 (a commercial buffer) at 2.2% of DM in a 68% concentrate diet resulted in a lower rumen fluid propionate concentration compared with control cows (no buffer). In both the AB and BC treatments, molar proportions of propionate were lower than in the CON treatment, which agrees with results of Xu et al. (1994) who buffered high-concentrate diets with a commercial buffer.

In the previously mentioned meta-analysis by Hu and Murphy (2005), where they reviewed 27 studies to evaluate dairy cow responses when diets were buffered with sodium bicarbonate, they confirmed work by others that showed a relationship between milk fat and VFA patterns. Their mixed model analyses showed a positive relationship between milk fat content and molar pro-

Table 4. The effect of calcareous marine algae (Acid Buf) and sodium bicarbonate on rumen VFA, lactate, and ammonia concentrations[1]

Item	AB	BC	CON	SEM	P
Total VFA (mM)	126.3[a]	119.8[ab]	112.9[b]	2.517	0.013
Acetic (mM)	79.9[a]	73.1[ab]	66.8[b]	2.451	0.010
Propionic (mM)	25.0[c]	26.4[ab]	28.3[a]	0.799	0.032
N-Butyric (mM)	21.4[a]	20.3[a]	17.7[b]	0.365	0.001
Acetate:propionate	3.20[a]	2.80[b]	2.36[c]	0.135	0.004
Molar proportions (mol/100 mol)					
Acetic	63.2[a]	61.0[ab]	59.2[b]	0.926	0.018
Propionic	19.9[c]	22.1[b]	25.1[a]	0.710	0.001
N-Butyric	17.0	17.0	15.7	0.379	0.074
Lactic acid (mM)	0.61[b]	0.60[b]	1.54[a]	0.234	0.008
NH_3 (mM)	11.66	11.13	11.11	0.785	0.851

[a–c]Means within a row with different superscripts differ ($P < 0.05$).

[1]AB = calcareous marine algae included at 0.4% of DM; BC = sodium bicarbonate included at 0.8% of DM; CON = control diet. Acid Buf, Celtic Sea Minerals, Cork, Ireland.

portion of acetate and acetate:propionate, whereas milk fat was negatively correlated with the molar proportion of propionate. Our study also showed positive relationships (albeit low) between milk fat content and molar proportion of acetate (0.36) and acetate:propionate (0.32). As in the study of Hu and Murphy (2005), we found a negative relationship (−0.36) between milk fat content and molar proportion of propionate. In the current study, we did not measure milk FA, but we postulate that in the CON cows, possible SARA conditions could have influenced the milk fat by modification of the biohydrogenation pathways (Bauman et al., 1999) or by changes in the VFA profiles, but this was corrected by both buffer treatments.

Butyrate concentrations in the current study were higher and molar proportions tended to be higher ($P = 0.074$) in rumen fluid of cows receiving the AB and BC treatments compared with the CON treatment. This is in agreement with results published by Kennelly et al. (1999) and Khorasani and Kennelly (2001) who used sodium bicarbonate in high-concentrate diets.

Calcareous marine algae (AB) resulted in an increased rumen acetate:propionate (**A:P**) ratio compared with the BC treatment, which had a higher ratio than the CON treatment. Rogers et al. (1982) also reported increased A:P ratios when sodium bicarbonate was supplemented to a high-concentrate diet. Higher molar acetate proportions, lower propionate proportions, and wider A:P ratios resulting from buffer addition to high-concentrate diets have been reported by Erdman et al. (1982), Xu et al. (1994), Kennelly et al. (1999), and Khorasani and Kennelly (2001).

Higher rumen lactic acid concentrations were observed in CON cows than in the cows that received buffered diets. As mentioned earlier, the CON cows experienced prolonged episodes of rumen pH below 5.5 and also showed visible signs of SARA. According to Krause and Oetzel (2006), rumen VFA have a pK_a of about 4.9, which shifts the acids to toward the undissociated (protonated) form when pH drops below 5.5. As passive VFA absorption only occurs in the protonated form, absorption is facilitated at low pH levels. However, the increase in VFA absorption can be offset by lactic acid production. Lactate is usually used at the same rate that it is produced, but at high dietary levels of starch and sugar, *Streptococcus bovis* starts to ferment glucose to lactate instead of VFA, decreasing ruminal pH even further. Compared with VFA, lactate has a much lower pK_a (3.9), meaning that at a ruminal pH of, for example, 5.0, lactate is 5.2 times less dissociated than VFA, thus accumulating in the rumen and contributing to a downward spiral (Krause and Oetzel, 2006). In the current study, the higher lactate concentrations observed in the CON cows correlates with the extended period of time when pH was below 5.5.

Treatment had no effect on rumen NH_3 values. This is in agreement with Doepel and Hayirli (2011) who fed a wheat-based diet with or without sodium bicarbonate to lactating Holstein cows. Rumen NH_3 concentrations reported by these authors were similar to those observed in the current study. However, the effect of buffers on rumen NH_3 concentrations does not appear to be consistent. Kilmer et al. (1981) and Kennelly et al. (1999) reported an increase in NH_3 concentration, whereas Khorasani and Kennelly (2001) and Doepel and Hayirli (2011) reported that dietary sodium bicarbonate had no effect on rumen NH_3 levels in dairy cows. Mees et al. (1985) found a decrease in NH_3 concentrations and increased bacterial N flow and efficiency of bacterial protein synthesis in sheep when sodium bicarbonate was included in the diet.

CONCLUSIONS

The high-concentrate diet used in this experiment resulted in some SARA-related symptoms (panting and drooling) in cows, which were relieved by the addition of buffers. Replacing limestone in the basal diet with calcareous marine algae (90 g/d) or with limestone + sodium bicarbonate (180 g/d) resulted in an improved rumen pH profile and a higher yield of milk and milk solids. The duration of rumen pH below 5.5 was reduced from 13 h per day to 4 h by the inclusion of calcareous marine algae, and to 8.7 h by sodium bicarbonate. Buffer supplementation, and especially the calcareous marine algae, improved total VFA and acetate concentrations and reduced lactate concentration. It was concluded that the response of calcareous marine algae to an increasing acid load provides an alternative to sodium bicarbonate as a means to prevent SARA. This also resulted in greater improvements in the efficiency of feed utilization for milk output and milk composition.

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