



## Effects of sodium bicarbonate and calcium magnesium carbonate supplementation on performance of high producing dairy cows

R.E. Rauch<sup>a,\*</sup>, P.H. Robinson<sup>b</sup>, L.J. Erasmus<sup>a</sup>

<sup>a</sup> Department of Animal and Wildlife Sciences, University of Pretoria, Pretoria, South Africa

<sup>b</sup> Department of Animal Science, University of California, Davis, CA 95616, USA

### ARTICLE INFO

#### Article history:

Received 4 October 2011

Received in revised form 13 August 2012

Accepted 24 August 2012

#### Keywords:

Buffer

DCAD

Holstein cows

### ABSTRACT

Sodium bicarbonate (SB) is a common dairy feed supplement, although recent research on its rumen buffering efficacy in contemporary dairy diets with low starch levels is limited. In California, and other areas of the world, new environmental regulations aim to minimize the amount of fixed solids (FS), including Na, which are discharged from dairy farms. Our aim was to determine effects of SB or calcium magnesium carbonate (CMC; a potential alternative buffer which does not contribute to Na discharge), on performance of early lactation high producing Holstein cows. The study was a Latin square design with 3 periods of 28 d, 3 treatments (*i.e.*, control (C), SB, CMC) and 3 pens of ~310 cows. The total mixed ration was supplemented with 8 g/kg dry matter (DM) of SB or CMC, and contained 519 g/kg DM and 158 g/kg crude protein (CP), 334 g/kg aNDF (*i.e.*, neutral detergent fiber assayed with a heat stable amylase expressed inclusive of residual ash) and 160 g/kg starch (DM basis). The dietary cation anion difference (DCAD) for the C, SB and CMC supplemented diets was 375, 456 and 381 mEq (Na + K – Cl)/kg DM, respectively. The DM intake for C, SB and CMC cows did not differ (28.2, 28.5, 28.6 kg/d, respectively), but the SB supplemented diet tended ( $P=0.053$ ) to reduce DM digestibility (637 versus 656 g/kg DM) and increase ( $P=0.09$ ) fecal pH (6.65 versus 6.60) compared to C. The CMC supplemented cows had higher ( $P<0.001$ ) fecal pH than C cows (6.76 versus 6.60), but digestibility did not differ. SB supplemented cows had lower ( $P<0.01$ ) milk yield (45.2 versus 46.2 kg/d) and higher ( $P<0.01$ ) milk fat (35.6 versus 34.3 g/kg), but milk fat yield did not differ (1.60 versus 1.58 kg/d) compared to C. The C and CMC supplemented cows did not differ in milk yield (46.2 versus 45.7 kg/d) or composition. Changes in body condition score were similar for C and SB supplemented cows, but tended ( $P=0.08$ ) to be lower for CMC versus SB supplemented cows (–0.07, –0.09, –0.03 units/30 d for C, SB and CMC cows, respectively), and net energy (NE<sub>i</sub>) output (172.4, 170.9, 173.2 MJ/d), and diet NE<sub>i</sub> concentration (6.12, 6.00, 6.06 MJ/kg DM) for C, SB and CMC supplemented diets did not differ. Results suggest that SB buffered the rumen and/or improved acid base balance by increasing DCAD, and that CMC buffered the abomasum and lower gastrointestinal tract. However, for diets and conditions comparable to this study, use of neither SB nor CMC is supported due to similar animal performance.

© 2012 Elsevier B.V. All rights reserved.

**Abbreviations:** ADF, acid detergent fiber; ADICP, AD insoluble CP; BW, body weight; C, control treatment; CMC, calcium magnesium carbonate; CP, crude protein; DCAD, dietary cation anion difference; DDGS, dried distillers' grains with solubles; DIM, days in milk; DM, dry matter; daNDF<sub>30</sub>, digestible aNDF after 30 h *in vitro* incubation; FPR, milk fat:protein ratio; FS, fixed solids; ME, metabolizable energy; NDF, neutral detergent fiber; NE, net energy; NRC, National Research Council; OM, organic matter; SB, sodium bicarbonate; SCC, somatic cell count; TMR, total mixed ration; VFA, volatile fatty acid.

\* Corresponding author. Tel.: +27 81 321 2921; fax: +27 15 516 1163.

E-mail address: [rauch.rainer@gmail.com](mailto:rauch.rainer@gmail.com) (R.E. Rauch).

## 1. Introduction

Addition of sodium bicarbonate (SB) to diets of high producing dairy cows as a rumen buffer has become a standard procedure in many parts of the world, although locally relevant contemporary research is generally lacking. Results from 30 studies published between 1980 and 1999, summarized by Hu and Murphy (2005), showed that effects of SB addition to the total mixed ration (TMR) of lactating cows depends on forage type in the diet, with beneficial effects of SB being limited to corn silage based diets. However forage type confounded with dietary acid detergent fiber (ADF) levels and different responses to SB relative to the main dietary forage may be partly due to differences in the fiber content of the forages. Contemporary dairy farms often do not conform to conditions of these prior studies, important differences being forage type and inclusion rate, dry matter (DM) intake level and milk yield of cows. For example, average milk yield and DM intake of cows in the studies summarized by Hu and Murphy (2005) was 29.3 and 19.5 kg/d, compared to mean milk yield and DM intake of 42.0 and 26.2 kg/d reported by Swanepoel et al. (2010) in a survey of 16 California (USA) dairy 'high' groups. In addition, ADF and corn silage contents of the diets were 170.6 versus 214.9, and 295 versus 159 g/kg DM, as reported by Hu and Murphy (2005) and Swanepoel et al. (2010) respectively.

Apart from its buffering capability, dietary SB addition affects the dietary cation anion difference (DCAD) due to the Na ion. In contrast, supplementation with calcium magnesium carbonate (CMC) does not affect DCAD as Ca and Mg do not elicit important biological effects on the anion/cation balance. Recent research points to substantial effects of the DCAD value, defined as milliequivalents (mEq) of Na + K – Cl per unit DM, on performance of lactating dairy cows. In a meta-analysis of 12 studies published between 1984 and 1997, Hu and Murphy (2004) reported that milk yield and DM intake increased quadratically with DCAD, peaking at ~34–40 mEq/100 g DM, respectively. Blood pH and HCO<sub>3</sub> concentrations also increased with dietary DCAD level, which points to an improved acid–base balance of the cows (Hu and Murphy, 2004).

New environmental regulations in California limit the amount of fixed solids (FS) which may be discharged from dairy farms (California Regional Water Control Board Central Valley Region (2007)). While Na, as well as Ca and Mg, classify as FS, high levels of Na negatively affect ground and surface water for human and livestock drinking purposes and irrigation (Berg et al., 2010), while contributing to soil degradation which results in reduced biomass yield (Mengel and Kirkby, 2001). In contrast, Ca does not negatively affect soil quality or water quality for drinking and irrigation purposes (Berg et al., 2010).

While SB contains 270 mg/kg Na, milk and body tissue Na levels are regulated such that the quantity of Na entering, and subsequently discharged from, the dairy farm increases with use of SB in dairy cow rations. Kellogg et al. (2001) reported that 0.79 of high producing dairy farms ( $n = 133$ ) surveyed in the USA use SB in their diets, although it is difficult to quantify benefits in a practical context because of large variations in measured traits such as milk yield and milk fat level. Finding alternative buffers which do not contribute to Na discharge, such as CMC, provided that they have similar beneficial effects on productivity as SB, may help dairy farmers reduce the negative environmental impacts of Na based buffers.

Our objectives were to determine effects of SB and CMC on DM intake, intake patterns, digestibility, fecal pH, body condition score (BCS), milk yield and milk composition of high producing dairy cows fed a contemporary relatively low starch diet, and determine if CMC could substitute for SB in the diet to maintain the anticipated benefits of SB on productivity.

## 2. Materials and methods

### 2.1. Dairy, animals and management

The study was conducted on a commercial dairy farm near Hanford in the Central Valley of California (USA), which milks ~4900 Holstein cows. Three 'high' group (*i.e.*, cows which had cleared the fresh pen but were not yet confirmed in calf) free-stall pens of about 310 multiparity cows, each with an average of  $63 \pm 39.3$  days in milk (DIM) at the start of the study, were selected. Cows entered the pens by random assignment from a single fresh pen at ~15 DIM. Cows moved in and out of the treatment pens weekly and this averaged about 0.04 of all cows/wk. Only cows which remained in their originally assigned pen from the start to the end of the study were used for statistical analysis. All cows were milked three times daily in a double 35 point herringbone parlor and fed twice daily at ~06:30 and ~11:30 h.

### 2.2. Experimental design

The experiment was a  $3 \times 3$  Latin square design with 3 treatments and 3 pens in 3 periods of 28 d. The treatments were control (C), SB and CMC addition, where diets were identical in formulation among treatments except for addition of 8 g/kg (DM) of buffer to the respective treatment diets.

### 2.3. Sample and data collection

#### 2.3.1. Feeds and TMR

Feed sampling was completed twice during the last week of each experimental period (*i.e.*, days 21 and 27). Most feeds were sampled by hand by retrieving random samples from each feed, while hays were sampled with a 'golf club' style hay probe (Seifert Analytical, Lodi, CA, USA), of ~15 samples from different bales. All feeds were frozen immediately after sampling at  $-20^{\circ}\text{C}$ . Loads of a premix were prepared daily by combining almond hulls, canola meal, wheat straw, a mineral

premix, distillers' dried grains with solubles (DDGS), pima cottonseed, tallow and molasses. This premix was later added as an ingredient during final TMR mixing.

The TMR load weights were recorded 4 times/pen during the final week of each experimental period, and TMR was sampled twice during the last week of each period (*i.e.*, days 21 and 27) according to Robinson and Meyer (2010).

### 2.3.2. Dry matter intake

The total TMR intake for each pen in the final 7 d of each period was corrected for the total orts removed and then multiplied by the average DM of the two corresponding TMR samples, which was divided by the average number of cows in the pen the final week of the period to give DM intake (kg)/cow/d.

### 2.3.3. Feed intake pattern determination

Feed intake patterns were determined by weighing 3 pre-selected equally spaced sections of each pen's feedbunk three times during a morning feeding, each section representing a subsection of the feed bunk equal to 10 headlocks. The TMR was manually removed, weighed on a portable scale and returned. Each section was weighed directly after TMR was delivered from the feed truck while cows were still at milking (*i.e.*, T0) where the midpoint of the time when the first and last cow arrived back from milking was considered to be T0. The same sections were weighed again at 110 (T110) and 240 (T240) min after T0. Thus, T0 to T110 corresponded to ~07:00–08:50 h, while T110 to T240 represented ~08:50–11:00 h.

T110 was selected because this was the approximate time that cows were released from lock based on preliminary observations. The number of cows in each section were recorded, with a range of 7–10 cows/section (*i.e.*, capacity was 10 headlocks/section). TMR intake on a per cow basis for T0–T110 was calculated as TMR disappearance divided by the number of cows in the section.

T240 was selected as this was the approximate time just before the second feeding. As TMR intake (*i.e.*, disappearance of TMR) recorded between T110 and T240 represented any number of cows from a theoretical 0 to 10 cows/section, as headlocks were open, a correction factor was used which was the ratio of the number of cows in the pen to the number of available headlocks. Intake/cow in each section was divided by this correction factor to make it fully representative of the number of cows in the pen at the time (*i.e.*, the more cows that were in the pen, the lower the TMR intake/cow/feeding space).

### 2.3.4. Milk production

Dairy Herd Improvement Association personnel from Hanford (CA, USA) collected milk samples at the end of each experimental period. Milk samples from a subsample (*i.e.*,  $n = 49$ ) of cows were collected and preserved with Bronolab-W II and frozen for analysis of Na, Ca and Mg. This group of cows was a representative subsample (*i.e.*, according to DIM) of the cows that were used for BCS evaluation.

### 2.3.5. Body condition scoring

BCSs were determined according to Edmonson et al. (1989) by a single trained scorer. To create a similar average and spread of DIM among cows within pen, cows between 20 and 48 DIM at the start of the study were selected from each pen. Only cows which remained in their respective pens for the duration of the study were used for statistical analysis (*i.e.*,  $n = 112$  cows scored at the study start and end of each period). Differences in BCS for each period within cow were calculated by subtracting the initial value from the final value, and adjusting to 30 d.

### 2.3.6. Fecal

Fecal collection was completed to measure fecal pH and to determine digestibility. The same 49 cows were used for fecal collection at the end of each period, which were the same cows as those used for milk subsampling.

### 2.3.7. In vitro gas production and $\text{daNDF}_{30}$ determination

The *in vitro* gas procedure used was that of Blümmel and Ørskov (1993) with calibrated 100 ml piston pipettes of 31 mm internal diameter (Model Fortuna, Häberle Labortechnik, Lonsee-Ettlenschieß, Germany). Each sample was 200 mg of ground and dried TMR incubated in 30 ml of buffered rumen liquor, collected from a dry cow fed an all hay diet, placed in a water bath maintained at 39 °C. Readings were made at 0, 1, 2, 4, 6, 8, 10, 12, 14 and 16 h, after which readings were every 8 h through 48 h. The 4 h readings are indicative of the rapidly fermentable fraction of the ration (Groot et al., 1996), while 24 and 48 h gas production is indicative of the metabolizable energy (ME) value of the diet (Menke and Steingass, 1988) and the diets' practical extent of *in vitro* digestibility (Robinson et al., 2004), respectively. All 18 TMR samples were used, and each was repeated to create 36 total samples. Digestible neutral detergent fiber (NDF) at 30 h *in vitro* incubation ( $\text{daNDF}_{30}$ ) was according to Goering and Van Soest (1970), and samples were removed at 30 h and assayed for aNDF (*i.e.*, NDF assayed with a heat stable amylase and expressed inclusive of residual ash). Digestibility was determined by the proportional difference in aNDF between samples at 0 and 30 h, and reported as g/kg aNDF.

*In vitro* pH buffering capacity of SB and CMC was determined using the same method as described above to prepare piston pipettes with rumen liquor, in duplicate runs. The SB or CMC was placed in the piston pipettes before collecting rumen fluid

and 50 ml of rumen fluid was aliquoted into each piston pipette, and pH was recorded every 10 min until 60 min, mixing lightly between measurements.

## 2.4. Sample preparation and assays

### 2.4.1. Feed

The air DM content of wet feed and TMR samples were determined by gravimetric loss of free water by heating to 55 °C for 48 h, after which they were allowed to air equilibrate for 24 h. Analytical DM was determined from gravimetric loss of weight by heating to 105 °C for 3 h (NFTA, 2001). Starch, glucose, fructose and sucrose were determined according to Johansen et al. (1996), and ash analysis was according to method #942.05 of AOAC (2005b). aNDF analysis used the sodium sulfite method and a heat stable  $\alpha$  amylase (#2002-4; AOAC, 2006a), and ADF and lignin(sa) were determined using method #973.18 of AOAC (1997). Total N and AD insoluble N analyses were according to #990.03 of AOAC (2005a) while crude fat analysis used method #2003.05 of AOAC (2006b). The Se and Cl analyses were according to Tracy and Moeller (1990) and Jones (2001), respectively, while P, K, S, Ca, Mg, Zn, Mn, Cu, Mo and Na analyses were by Meyer and Keliher (1992).

### 2.4.2. Fecal

Fecal samples were measured for pH ( $n = 49$  cows) with an ISFET miniLab IQ128 pH meter (Hach Company, Loveland, CO, USA) immediately after collection by mixing equivalent volumes of fecal material and double deionized water, similar to Bach et al. (2005b).

Fecal samples were immediately frozen at  $-20^{\circ}\text{C}$  and later oven dried at  $55^{\circ}\text{C}$  for 48 h, after which they were ground to pass a #4 Wiley Mill with a 1 mm screen. One composite sample per treatment within period was created by pooling individual cow samples by weight.

### 2.4.3. Milk

Milk fat, true protein and lactose concentrations, as well as somatic cell counts (SCC), were determined using infrared spectroscopy at the Dairy Herd Improvement Association laboratory in Hanford (CA, USA). Milk Ca, Mg and Na analysis used the method of Meyer and Keliher (1992).

## 2.5. Calculations

DCAD was calculated in two ways as:

$$\text{DCAD (mEq/kg)} = ((\text{g/kg Na}/0.0023) + (\text{g/kg K}/0.00391)) - ((\text{g/kg Cl}/0.0035) + (\text{g/kg S}/0.00321)(\times 2)) \text{ [Jackson et al., 2001]}$$

$$\text{DCAD (mEq/kg)} = ((\text{g/kg Na}/0.0023) + (\text{g/kg K}/0.00391)) - (\text{g/kg Cl}/0.0035) \text{ [Hu and Murphy, 2004]}$$

Energetics were calculated according to Tyrrell and Reid (1965) for NE of milk and NRC (2001) for NE value of maintenance and BCS changes.

Nutrient digestibilities were calculated as:

$$\text{Digestibility} = 1000 - (1000 \times ((\text{g/kg lignin(sa)}_{\text{TMR}} \times 0.95/\text{g/kg lignin(sa)}_{\text{Feces}}) \times (\text{g/kg nutrient}_{\text{Feces}}/\text{g/kg nutrient}_{\text{TMR}})))$$

Assuming lignin(sa) in the TMR is 950 g/kg indigestible (Cochran et al., 1986; Stensig and Robinson, 1997).

For *in vitro* gas production, ME was calculated according to Robinson et al. (2004).

## 2.6. Statistical analysis

For milk, BCS and fecal pH analyses, only those cows which were in their originally assigned pens for the entire duration of the study were used (*i.e.*, 430, 112 and 49 cows recorded at the end of each period for milk, BCS and fecal pH data, respectively). Data were analyzed using the MIXED option of SAS (2000) using cow nested within pen as a random effect and period, pen and treatment as fixed effects.

For assessment of DM intake, ingredient chemical analysis, TMR nutrient profile (including DCAD), TMR ingredient composition and digestibility, the GLM option of SAS was used, with period, pen and treatment as effects. Chemical analyses were based on a total of 18 TMR samples (*i.e.*, 2 samples/TMR/period) and 6 samples/ingredient (*i.e.*, 2 samples/ingredient/period), where the samples were collected on days 21 and 27 of each period. Digestibility data were based on fecal samples of the same 49 cows recorded at the end of each period, which were combined by weight to create one composite sample per treatment within period. As DM intake was calculated on a pen basis, the number of observations was 9 (*i.e.*, 3 treatments  $\times$  3 periods).

The *in vitro* fitted extent of fermentation ( $B$ ) and rate of gas production ( $k$ ) was determined using the nonlinear regression (nlin) procedure of the Gauss-newton model of SAS, by TMR, as:

$$\text{gas} = b \times (1 - e(-k \times h))$$

**Table 1**

Chemical composition (g/kg DM) including standard deviation (SD) of sodium bicarbonate (SB) and calcium magnesium carbonate (CMC) supplemented to the experimental diets.

	SB	CMC
Dry matter (g/kg)	725	1000
Na	274.0	0.4
SD	0.18	0.02
Ca	8.0	222.7
SD	0.07	4.10
Mg	4.0	120.0
SD	0.42	3.18

*In vitro* gas production used GLM of SAS for the 4, 24 and 48 h values. Period, pen and treatment were class variables. Analysis of *in vitro* pH determination used the MIXED model of SAS with run, material, level, tube and time as class variables and run as random effect.

Significance of differences among all treatments was determined using the PDIFF function in SAS, with  $0.05 < P < 0.10$  accepted as a tendency and  $P < 0.05$  as a significant difference.

### 3. Results

#### 3.1. Ration evaluation and *in vitro* fermentation

The SB supplement contained 274.0 g Na/kg DM, with minor quantities of Ca and Mg (8.0 and 4.0 g/kg DM, respectively), while CMC contained 222.7 and 120.0 g/kg DM Ca and Mg, respectively, with minor quantities of Na (0.4 g/kg DM; [Table 1](#)).

The nutrient profiles of the feeds are reported in [Tables 2 and 3](#). The ingredient composition of the diet ([Table 4](#)) was similar to that of the dairy farms reported by [Swanepoel et al. \(2010\)](#). Notable exceptions were a lower relative incorporation of corn silage (104.0 versus 169.5 g/kg DM) and alfalfa hay (91.2 versus 161.2 g/kg DM), which is partly due to the higher number of ingredients in our diet compared to those of [Swanepoel et al. \(2010\)](#); 18 versus a mean of 10 ingredients, respectively).

The TMR was very similar in nutrient composition among treatments ([Table 5](#)). The only differences judged to be biologically relevant were Na, DCAD, Ca and Mg, all due to supplementation with SB and CMC, respectively.

During *in vitro* incubation, the CMC supplemented diet had a higher gas production compared to C diet at 4 h (78.0 versus 73.1,  $P = 0.05$ ), while there was a higher gas production for CMC and SB supplemented diets compared to the C diet at 24 h (227.9 and 225.4 versus 212.2 ml/g OM,  $P = 0.01$  and  $P = 0.02$ , respectively). At 48 h, the SB supplemented diet had a tendency ( $P = 0.06$ ) toward a higher gas production compared to the C diet. There were no differences in  $\text{daNDF}_{30}$ , or predicted ME and NE among diets, but there was a trend ( $P = 0.08$ ) for higher *in vitro* pH for the SB versus C diet (6.66 versus 6.44; [Table 6](#)).

#### 3.2. Dry matter intake and intake patterns

There was no difference in DM intake among treatments ([Table 7](#)), but cows in the SB treatment had the highest intake of Na ( $P < 0.01$ ), while Ca and Mg intakes were higher in the CMC treatment ( $P < 0.01$ ; [Table 9](#)), differences which reflect the higher Na and Ca/Mg levels in the SB and CMC diets, respectively.

**Table 2**

Average and standard deviation (SD) of the chemical analysis (g/kg dry matter) of the forage and wet byproduct ingredients used in the experimental diets.<sup>a</sup>

	Wheat silage	Corn silage	Alfalfa fresh chop	Alfalfa hay (HQ) <sup>b</sup>	Alfalfa hay (LQ) <sup>c</sup>	Wheat straw	Carrot tubers	Citrus pulp
Dry matter	363.2	327.2	260.8	901.8	913.3	925.7	95.7	132.9
SD	25.0	23.6	43.1	6.2	2.4	8.7	5.7	19.2
Crude protein	86.5	76.9	199.8	207.5	185.8	51.6	80.6	87.3
SD	21.9	3.9	28.2	26.2	12.4	16.1	4.4	14.9
aNDF <sub>om</sub> <sup>d</sup>	480.8	459.8	335.8	335.5	390.9	691.9	186.0	220.1
SD	25.9	13.2	35.3	11.1	12.0	25.4	9.9	41.4
aNDF <sup>e</sup>	521.8	475.5	408.5	356.2	403.5	731.0	221.0	227.4
SD	22.0	13.1	51.6	18.8	11.7	26.9	1.4	45.4
Starch	118.7	236.0	11.0	25.7	21.3	12.6	34.5	10.0
SD	75.9	11.2	5.1	4.5	2.5	5.4	4.9	5.0
Ash	111.6	71.7	182.6	116.4	111.0	131.1	117.4	50.4
SD	9.8	4.7	50.5	16.3	6.3	12.5	5.1	12.3

<sup>a</sup> Average for a total of six samples, two samples collected during the last week of each of three periods.

<sup>b</sup> High quality alfalfa hay as classified by the dairy.

<sup>c</sup> Low quality alfalfa hay as classified by the dairy.

<sup>d</sup> Neutral detergent fiber assayed with heat stable amylase expressed exclusive of residual ash.

<sup>e</sup> Neutral detergent fiber assayed with heat stable amylase and expressed inclusive of residual ash.

**Table 3**Average and standard deviation (SD) of the chemical analysis (g/kg dry matter) of the concentrate ingredients used in the experimental diets.<sup>a</sup>

	DDGS <sup>b</sup>	Canola pellets	Corn grain <sup>c</sup>	Pima cotton seed	Almond hulls	Cotton seed meal	Corn gluten pellets
Dry matter	912.7	902.7	868.2	922.5	960.8	891.3	913.0
SD	7.7	9.3	6.1	4.8	3.5	6.7	12.9
Crude protein	275.7	398.8	74.6	215.0	52.6	404.2	209.5
SD	3.3	7.9	2.0	8.2	6.7	17.7	21.0
aNDFom <sup>d</sup>	284.8	285.5	84.1	389.3	279.8	376.0	372.0
SD	8.4	125.5	4.8	13.8	17.2	29.1	8.6
aNDF <sup>e</sup>	291.7	369.3	85.6	411.3	290.0	394.8	390.9
SD	8.9	219.8	4.9	14.3	17.4	31.4	11.0
Starch	53.7	34.5	742.1	6.0	18.2	<5	136.1
SD	3.6	3.4	22.8	0.8	7.4	–	8.8
Ash	50.5	76.8	12.5	49.3	73.0	72.5	62.3
SD	0.8	2.3	1.0	2.8	7.9	1.4	7.9

<sup>a</sup> Average for a total of six samples, two samples collected during the last week of each of three periods.<sup>b</sup> Dried distillers grains with solubles (corn grain).<sup>c</sup> Steam-flaked.<sup>d</sup> Neutral detergent fiber assayed with heat stable amylase expressed exclusive of residual ash.<sup>e</sup> Neutral detergent fiber assayed with heat stable amylase and expressed inclusive of residual ash.

Cows consumed more DM ( $P<0.01$ ) during the early morning (*i.e.*, 07:00 to 08:50 h) compared to the late morning (*i.e.*, 08:50 to 11:00 h; Fig. 1). However, there were no treatment effects on DM intake pattern during these time periods.

### 3.3. Digestibility and fecal pH

The DM digestibility of the SB diet tended to be lower ( $P=0.05$ ) than that of the C diet (Table 7). Fat and CP digestibility were higher ( $P=0.04$ ) for the CMC compared to the SB supplemented diet. The CMC supplemented cows had higher ( $P=0.03$ ) Na digestibilities than C cows, and SB cows tended ( $P=0.06$ ) to have a higher Na digestibility than C cows. Fecal pH (Table 7) tended to be lower in C versus SB cows ( $P=0.09$ ), while fecal pH of CMC supplemented cows was higher than SB ( $P<0.01$ ) and C ( $P<0.001$ ) cows.

**Table 4**Ingredient composition (g/kg DM) of the TMR fed to high producing dairy cows in the control (C), sodium bicarbonate (SB) and calcium magnesium carbonate (CMC) treatments.<sup>a</sup>

	Treatment			SEM	P		
	C	SB	CMC		C versus SB	C versus CMC	SB versus CMC
Wheat silage (whole crop)	165.1	163.6	163.3	0.50	0.28	0.22	0.82
Corn grain (steam flaked)	130	131	134	1.0	0.78	0.27	0.36
Almond hulls <sup>b</sup>	109.0	108.6	108.1	0.10	0.22	0.05	0.11
Corn silage (whole crop)	104.9	104.1	103.0	0.17	0.16	0.03	0.08
DDGS <sup>b,c</sup>	94.57	94.29	93.83	0.072	0.20	0.04	0.08
Canola pellets (solvent) <sup>b</sup>	61.21	61.09	60.76	0.027	0.15	0.01	0.03
Pima cottonseed (cracked) <sup>b</sup>	58.22	58.06	57.77	0.047	0.21	0.04	0.09
Alfalfa hay (HQ) <sup>d</sup>	57.5	57.5	58.2	0.22	0.87	0.27	0.23
Alfalfa hay (LQ) <sup>e</sup>	34.5	33.0	32.9	0.17	0.05	0.04	0.67
Corn gluten pellets	33.68	33.52	33.38	0.075	0.39	0.18	0.46
Alfalfa fresh chop (whole crop)	29.1	27.7	28.1	0.32	0.17	0.27	0.60
Cottonseed meal (solvent)	28.86	28.71	28.59	0.066	0.38	0.18	0.47
Whey (liquid)	23.1	24.2	24.4	0.39	0.28	0.21	0.77
Citrus pulp (orange and lemon)	19	16	16	1.1	0.30	0.31	0.98
Mineral premix <sup>b,f</sup>	14.70	14.68	14.60	0.029	0.71	0.23	0.33
Molasses (liquid) <sup>b</sup>	14.38	14.34	14.27	0.011	0.21	0.04	0.09
Wheat straw <sup>b</sup>	12.75	12.71	12.65	0.016	0.32	0.09	0.21
Sodium bicarbonate (SB)	0.0	7.9	0.0	0.17	<0.01	1.00	<0.01
Calcium magnesium carbonate (CMC)	0.0	0.0	7.6	0.30	1.00	<0.01	<0.01
Carrots (pulp/whole tubers)	5.65	5.58	5.66	0.031	0.37	0.90	0.33
Tallow <sup>b</sup>	3.66	3.65	3.64	0.0044	0.36	0.09	0.18

<sup>a</sup> Based on two TMR samples collected per period per diet (*i.e.*, 6 samples per diet).<sup>b</sup> Ingredients used to create the premix.<sup>c</sup> Dried distillers grains with solubles (corn).<sup>d</sup> High quality alfalfa hay as classified by the dairy.<sup>e</sup> Low quality alfalfa hay as classified by the dairy.<sup>f</sup> Premix (998.2 g/kg DM) contained (as guaranteed by the supplier) 244.9 g/kg Ca, 44.6 g/kg Mg, 7.2 g/kg P, 2.0 g/kg K, 123.1 g/kg Cl, 79.6 g/kg Na, 2.8 g/kg S, 59.63 mg/kg Co, 828.22 mg/kg Cu, 59.63 mg/kg I, 1192.62 mg/kg Mn, 15.24 mg/kg Se, 3511.63 mg/kg Zn, 331.20 KIU/kg Vit A, 99.36 KIU/kg Vit D, 1.10 KIU/kg Vit E on a DM basis (Nutrius LLC, Kingsburg, CA, USA).



**Table 5**Nutrient profile of the TMR fed to high producing dairy cows in control (C), sodium bicarbonate (SB) and calcium magnesium carbonate (CMC) treatments.<sup>a</sup>

	Treatment			SEM	P		
	C	SB	CMC		C versus SB	C versus CMC	SB versus CMC
g/kg DM							
Dry matter	513	521	524	4.2	0.43	0.32	0.78
Crude protein	156.2	158.7	158.5	0.96	0.32	0.35	0.94
ADICP <sup>b</sup>	67.7	67.3	65.7	0.75	0.82	0.31	0.39
aNDF <sup>c</sup>	339	331	332	0.8	0.03	0.04	0.47
aNDFom <sup>d</sup>	324	316	316	1.1	0.06	0.06	0.92
ADF <sup>e</sup>	223	222	220	2.4	0.88	0.67	0.78
Lignin(sa) <sup>f</sup>	47	49	48	0.62	0.28	0.45	0.65
Crude fat	54.3	54.4	54.5	0.69	0.93	0.92	0.99
Starch	157	158	166	3.5	0.93	0.34	0.37
Free sugars	44	38	38	1.1	0.13	0.11	0.77
Ash	90.6	93.8	97.5	0.59	0.11	0.03	0.09
Ca	7.49	7.48	9.30	0.079	0.95	<0.01	<0.01
Mg	3.18	3.18	4.13	0.046	0.94	<0.01	<0.01
K	17.9	17.6	17.9	0.12	0.28	0.78	0.36
P	4.73	4.69	4.78	0.022	0.45	0.35	0.16
S	2.88	2.90	2.88	0.017	0.60	0.98	0.61
Na	3.0	5.0	3.1	0.050	<0.01	0.41	<0.01
Cl	7.6	7.5	7.4	0.14	0.85	0.65	0.79
DCAD <sup>g</sup>	195	276	202	5.4	0.02	0.60	0.02
DCAD <sup>h</sup>	375	456	381	4.3	0.01	0.52	0.01
mg/kg DM							
Zn	76.4	78.1	77.7	0.88	0.42	0.54	0.82
Mn	41.7	43.3	43.1	0.43	0.20	0.25	0.80
Cu	17.2	17.8	17.8	0.26	0.38	0.39	0.98
Mo	1.253	1.233	1.245	0.0098	0.42	0.74	0.59
Se	0.361	0.381	0.373	0.0056	0.22	0.38	0.57

<sup>a</sup> Based on two TMR samples collected per period per diet (i.e., 6 samples/diet).<sup>b</sup> Acid detergent insoluble crude protein expressed as g/kg CP.<sup>c</sup> Neutral detergent fiber assayed with heat stable amylase expressed inclusive of residual ash.<sup>d</sup> aNDF expressed exclusive of residual ash.<sup>e</sup> Acid detergent fiber expressed inclusive of residual ash.<sup>f</sup> Lignin assayed with sulfuric acid.<sup>g</sup> Dietary cation anion difference calculated as: milliequivalents/kg of (Na + K) – (Cl + S).<sup>h</sup> Dietary cation anion difference calculated as: milliequivalents/kg of (Na + K) – Cl.**Table 6***In vitro* gas production, digestible aNDF after 30 h *in vitro* incubation (daNDF<sub>30</sub>), predicted metabolizable energy (ME) of the diet and pH of rumen liquor for control (C), sodium bicarbonate (SB) and calcium magnesium carbonate (CMC) supplemented diets.

	Treatment			SEM	P		
	C	SB	CMC		C versus SB	C versus CMC	SB versus CMC
Gas production (ml/g OM)							
4 h	73	75	78	2.4	0.44	0.05	0.23
24 h	212	225	228	5.5	0.02	0.01	0.65
48 h	246	267	251	7.2	0.06	0.63	0.14
Fermentation kinetics							
B <sup>a</sup>	244	268	250	7.4	0.04	0.62	0.11
k <sup>b</sup>	0.092	0.086	0.094	0.0027	0.12	0.57	0.04
daNDF <sub>30</sub> (g/kg aNDF)	499	496	493	3.9	0.64	0.28	0.53
ME <sup>c</sup>							
1 × M	12.56	12.56	12.48	0.05	0.99	0.26	0.25
3 × M	11.56	11.56	11.48	0.05	0.99	0.26	0.25
NE <sup>d</sup>	7.17	7.17	7.12	0.03	0.99	0.26	0.25
In vitro pH <sup>e</sup>							
Level 1	6.44	6.60	6.47	0.023	0.11	0.46	— <sup>f</sup>
Level 2	6.44	6.66	6.46	0.023	0.08	0.56	— <sup>f</sup>

<sup>a</sup> Fitted extent of fermentation (ml/g OM).<sup>b</sup> Rate of gas production (/h).<sup>c</sup> UC Davis approach to estimate ME (MJ/kg DM) of a feed (Robinson et al., 2004). 1 × M, ME requirements for maintenance; 3 × M, ME requirements for lactation at 3 times maintenance energy.<sup>d</sup> NE of the diets using the formula: ME × 0.62 (McDonald et al., 2002).<sup>e</sup> Combined analysis of 6 pH values recorded every 10 min from 10 to 60 min after rumen liquor addition to empty piston pipettes (C), or with addition of 1 (Level 1) or 2 (Level 2) mg SB or CMC/ml rumen liquor.<sup>f</sup> Statistical analysis limited to C versus SB and C versus CMC comparisons due to experimental design.

**Table 7**

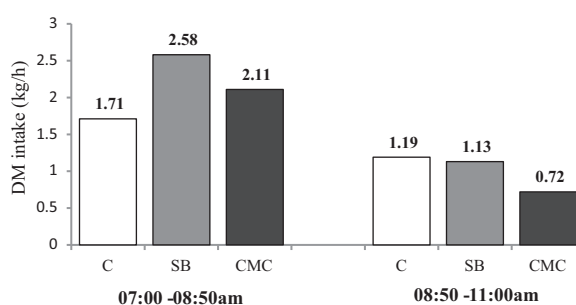
Dry matter intake, fecal pH and total tract digestibility<sup>a</sup> (g/kg) of the TMR fed to high producing dairy cows in control (C), sodium bicarbonate (SB) and calcium magnesium carbonate (CMC) treatments.

	Treatment			SEM	P		
	C	SB	CMC		C versus SB	C versus CMC	SB versus CMC
Dry matter intake	28.2	28.5	28.6	0.27	0.64	0.56	0.89
Digestibility							
Dry matter	656	637	649	2.2	0.05	0.27	0.12
aNDFom <sup>b</sup>	417	382	408	11.0	0.25	0.73	0.35
CP	609	594	621	2.7	0.12	0.16	0.04
Starch	995	1000	978	11.0	0.85	0.51	0.42
Fat	842	831	860	3.2	0.22	0.10	0.04
Na	719	765	787	5.6	0.06	0.03	0.19
Ca	332	286	300	25.1	0.45	0.59	0.80
Mg	157	111	170	27.4	0.49	0.84	0.39
Fecal pH <sup>c</sup>	6.60	6.65	6.76	0.027	0.09	<0.0001	<0.01

<sup>a</sup> Based on two TMR samples collected per treatment per period (18 samples total) and composite fecal samples pooled by pen and period.

<sup>b</sup> Neutral detergent fiber assayed with heat stable amylase expressed exclusive of residual ash.

<sup>c</sup> Fecal samples collected during the last day of each period, only using cows that remained in originally assigned pens throughout the experiment ( $n = 49$  cows).



**Fig. 1.** Dry matter intake patterns of dairy cows between 07:00 and 08:50 a.m. and 08:50 and 11:00 a.m. in the control (C), sodium bicarbonate (SB) and calcium magnesium carbonate (CMC) treatments. C versus SB:  $P=0.16$ ; C versus CMC:  $P=0.91$ ; SB versus CMC:  $P=0.15$ ; time:  $P<0.0001$ ; time  $\times$  treatment:  $P=0.17$ ; SEM = 0.516.

### 3.4. Milk yield, milk composition and performance characteristics

Milk and milk lactose yields (Table 8) were lower for SB supplemented versus C cows ( $P<0.01$  and  $P=0.02$ , respectively), and there was a tendency ( $P=0.05$ ) for SB cows to yield less milk true protein (1.34 versus 1.36 kg/d). Milk fat level was

**Table 8**

Production performance and net energy (NE) output of high producing dairy cows ( $n=430$ ) in the control (C), sodium bicarbonate (SB) and calcium magnesium carbonate (CMC) treatments.

	Treatment			SEM	P		
	C	SB	CMC		C versus SB	C versus CMC	SB versus CMC
Yield (kg/d)							
Milk	46.2	45.2	45.7	0.36	<0.01	0.16	0.19
Fat	1.58	1.60	1.58	0.018	0.41	0.90	0.34
True protein	1.36	1.34	1.34	0.010	0.0502	0.11	0.71
Lactose	2.21	2.16	2.18	0.018	0.02	0.12	0.43
Energy (MJ/d)	132.1	131.4	131.0	1.13	0.60	0.40	0.75
Composition (g/kg)							
Fat	34.3	35.6	34.7	0.31	<0.01	0.29	0.013
True protein	29.5	29.7	29.5	0.10	0.07	0.81	0.04
Fat:protein ratio	1.165	1.199	1.177	0.0098	<0.01	0.29	0.07
Lactose	47.73	47.85	47.66	0.070	0.105	0.34	0.0098
Energy (MJ/kg)	2.86	2.92	2.88	0.013	<0.01	0.39	<0.01
SCC ( $\times 1000$ cells/ml)	210	223	224	27.7	0.69	0.65	0.96
Body condition score							
BCS, units	2.31	2.33	2.33	0.034	0.37	0.29	0.88
BCS change, units/30 d	-0.07	-0.09	-0.03	0.023	0.62	0.21	0.08
Energetics							
Total NE output (MJ/d)	172.4	170.9	173.2	1.50	0.68	0.81	0.53
Diet NE concentration (MJ/kg)	6.12	6.00	6.06	0.023	0.12	0.38	0.28



**Table 9**

Effects of sodium bicarbonate (SB) and calcium magnesium carbonate (CMC) supplementation on intake, digestible intakes and milk concentration of Na, Ca and Mg of high producing dairy cows.

	Treatment			SEM	P		
	C	SB	CMC		C versus SB	C versus CMC	SB versus CMC
Intake (g/d)							
Ca	211.6	214.3	266.4	1.91	0.55	<0.01	<0.01
Mg	90	90.8	118.2	1.08	0.72	<0.01	<0.01
Na	84.2	143.5	88.2	2.71	<0.01	0.53	<0.01
Digestible intakes (g/d)							
Ca	69.2	61.5	80.1	5.63	0.56	0.44	0.24
Mg	13.9	9.6	20.0	2.86	0.53	0.40	0.21
Na	61.6	110.7	70.7	3.17	0.02	0.29	0.02
Milk concentration (mg/L)							
Ca	1190	1212	1200	24.0	0.51	0.76	0.72
Mg	104	97	100	2.5	0.05	0.29	0.34
Na	323	330	323	12.3	0.62	0.99	0.60

elevated in SB versus C ( $P<0.01$ ) and SB versus CMC ( $P=0.013$ ) cows, which corresponds to a similar trend in milk energy concentration where SB cows produced milk with a higher ( $P<0.01$ ) energy density compared to C and CMC cows. Milk protein level was elevated in SB versus CMC cows ( $P=0.04$ ), and had a tendency to increase in SB versus C cows ( $P=0.07$ ). There was a higher ( $P<0.01$ ) fat to protein ratio (FPR) in SB versus C cows, and a trend ( $P=0.07$ ) to a higher FPR in SB versus CMC cows. Cows in the SB treatment group produced milk with a higher lactose concentration than CMC cows ( $P=0.01$ ), with no difference between SB and C cows.

Milk Ca and Na concentrations were similar among treatments, but there was a tendency ( $P=0.05$ ) for a higher milk Mg concentration in C versus SB supplemented cows (Table 9). Milk Ca and Mg concentrations of our cows were similar to those reported by Lucey and Horne (2009; 1201 versus 1040 to 1280 and 100 versus 100 to 150 mg/L, respectively). However, milk Na concentrations were marginally lower in our study compared to those of Lucey and Horne (2009; 325 versus 350 to 600, respectively).

## 4. Discussion

### 4.1. Product and ration evaluation

Similar *in vivo* aNDF digestion and daNDF<sub>30</sub> values for the three experimental TMR shows that aNDF digestion was not treatment affected. The increase in 24 h *in vitro* gas production was likely due to dissociation of SB which resulted in increased gas volume due to CO<sub>2</sub> liberation. This supports use of daNDF<sub>30</sub>, rather than *in vitro* gas production, to calculate dietary NE<sub>i</sub> concentration. However increased *in vitro* gas production of CMC versus C diets is difficult to explain, as CMC is not considered soluble at normal rumen pH and does not buffer rumen fluid, as was demonstrated by a lack of change in *in vitro* pH between C and CMC diets. A low pH of the rumen fluid of the donor cow used for *in vitro* gas production is unlikely to have led to partial dissociation of CMC and an increase in CO<sub>2</sub> production because the donor cow was fed an all hay diet. A more likely explanation is that increased *in vitro* gas production of CMC versus C diets may be due to a small amount of CMC being soluble at normal rumen pH, which resulted in a slight increase in gas production and associated release of Ca and Mg. It has been reported that Mg has a positive effect on rumen microbial growth (Galbraith et al., 1971) and organic matter digestibility in sheep (Wilson, 1980), and that cellulose degradation is stimulated by Ca and Mg (Hubbert et al., 1958). An increase in cellulose digestion would result in an elevated acetate to propionate ratio, and increased gas volume, as propionate contains an extra carbon atom which would otherwise have formed a CO<sub>2</sub> molecule (Wolin, 1960), and it has been suggested that differences in molar proportions of volatile fatty acids (VFA) be accounted for when reporting gas production (Schofield and Pell, 1995). It is thus possible that slight increases in rumen liquor Ca and Mg concentrations with the CMC supplemented diet stimulated microbial activity and VFA production, or that differences in VFA proportions led to an increase in gas volume *in vitro*. However, similar *in vivo* aNDF digestibilities between C and CMC diets indicate that potential increases in Ca and Mg concentrations in rumen fluid did not occur *in vivo*, or that differences occurred without affecting microbial fermentation and fiber degradation in the cows due to a more complex interaction between feed, animal and microbial populations *in vivo*.

While the lack of treatment differences in estimated NE using daNDF<sub>30</sub> is consistent with the similar calculated *in vivo* NE among treatments, there is a disagreement in absolute NE values between the two methods, as NE predicted by daNDF<sub>30</sub> is higher than the *in vivo* calculated NE value. As *in vivo* NE prediction measures animal energy output, it seems that the daNDF<sub>30</sub> prediction overestimated the actual NE value of our diet (i.e., average daNDF<sub>30</sub> was 496 g/kg, compared to an average *in vivo* aNDF digestibility of 402 g/kg). Interestingly, Robinson and McQueen (1992) reported that ~0.86 of whole tract NDF digestion occurs in the rumen and, if this factor is used, predicted rumen aNDF digestion in our study would be 347 g/kg, which is 30% lower than the prediction based on daNDF<sub>30</sub>. Using this 'corrected' *in vivo* digestibility, predicted NE of the diets using the daNDF<sub>30</sub> formula would fall to 6.63 MJ/kg, which is closer to the calculated *in vivo* NE value of 6.06 MJ/kg,

but still high. It is therefore likely that high DM intakes of our cows resulted in relatively shorter rumen retention time of aNDF, and that the daNDF<sub>30</sub> incubation period was too long to accurately represent it. This suggests that it may be desirable to reduce the daNDF incubation time for studies with cows that have high DM intakes to a 24 h, or even shorter, incubation.

#### 4.2. Effects of sodium bicarbonate supplementation

##### 4.2.1. Gastrointestinal effects

That DM intake was not affected by SB in our study is consistent with a meta analysis by Hu and Murphy (2005), who found that SB supplementation increased intake by 1.24 kg/d for corn silage based diets, but did not affect intake in non-corn silage based diets. Based on our experimental diet's low corn silage and starch levels, the ruminal acid production potential of the ration was low, while the relatively high alfalfa and fiber levels resulted in increased dietary buffering capacity and rumination, respectively. This contrasts with numerous previous studies which used corn silage based diets with high dietary proportions of starch, in which SB supplementation resulted in substantial productive benefits (e.g., Snyder et al., 1983; Erdman et al., 1980; Rogers et al., 1985).

One of the typical responses to supplementing SB is an increase in rumen pH, but there have been reports of no effects (e.g., Hu and Murphy (2005) in non-corn silage based diets) or a decrease (Rogers et al., 1985). However, an increase in rumen pH of our cows was likely, at least based on a tendency to an elevated *in vitro* pH with the SB supplemented diet. A substantially lower numerical Mg digestibility in SB compared to C and CMC supplemented cows (111 versus 170 and 157 g/kg) may also support this possibility. As the reticulo-rumen is the main site of Mg absorption, and its rate of absorption is dependent on its concentration which increases with a decrease in rumen pH, a numerical decrease in Mg digestibility for SB supplemented cows may have been due to rumen buffering of the SB, and subsequent reduction in rumen Mg concentration and digestibility. A large variation in Mg digestibility is consistent with Lomba et al. (1968), who found that endogenous fecal Mg losses were highly variable, and this may have masked detection of statistical significance among our treatments. A tendency to lower milk Mg concentration of SB supplemented cows may be further support for this hypothesis. In addition, the large numerical difference in intake during the first 110 min of the morning between SB supplemented versus C and CMC supplemented cows, may indicate that rumen pH did not decrease to the same extent as in C or CMC supplemented cows, resulting in a delay of feed intake inhibition normally associated with a decrease in rumen pH. Furthermore, a trend to elevated fecal pH with SB cows indicates that there was a buffering effect in the gastrointestinal tract, which likely occurred in the rumen.

Rumen buffering is often associated with a shift in VFA ratios to an increased acetate:propionate ratio, and to a change in microbial activity, where fibrolytic activity is progressively reduced as pH values fall below 6.0 (Mould et al., 1983). A shift in VFA ratios has been linked to increases in milk fat concentration and yield due to propionate's lipogenic properties (i.e., decreased propionate production reduces insulin secretion and body fat deposition in favor of milk fat synthesis). However, an increase in milk fat concentration without impacting milk fat yield in our study indicates that rumen buffering may have occurred without affecting VFA ratios. This is consistent with the meta analysis of Hu and Murphy (2005), who reported that SB supplementation decreased molar proportions of propionate in SB supplemented cows fed a corn silage based diet, but did not affect acetate or propionate concentrations, or their ratios, in cows fed non-corn silage based diets. It has been suggested that rumen pH should be maintained above 6.0–6.1 to avoid inhibition of cellulolysis (Mould et al., 1983) and, as *in vivo* NDF digestibility did not differ between C and SB diets, it is likely that rumen pH was predominantly above this level. Furthermore, a relatively high and stable rumen pH would imply that rumen buffering was not required under the conditions and type of diet fed in our experiment (i.e., aNDF levels of 334 for our diets versus NRC (2001) minimum recommendation of 250 g/kg, relatively low corn silage and readily fermentable carbohydrate levels and very high producing cows). While high DM intakes may increase the risk of acidosis due to a relatively high intake of fermentable carbohydrates, fiber intakes also increase and are expected to stimulate rumination thereby enhancing saliva flow and buffering capacity. Furthermore, high milk production would sustain a large diffusion gradient of substrates (i.e., VFA) between the rumen, blood and mammary gland, therefore maintaining high rates of removal of VFA from the rumen to ensure relatively high and stable rumen pH levels.

An alternative theory for the action of SB is increased water intake and rumen fluid dilution rate, resulting in increased starch flow from the rumen and decreased propionate production (Russell and Chow, 1993). The tendency for reduced DM digestibility with SB supplementation and numerically lower digestibility of aNDFom, CP and fat, without a change in daNDF<sub>30</sub>, may indicate an increased rate of ruminal passage, which supports an increase in feed intake capacity, although it could have been counteracted by increased water intake, which is suggested by similar DM intakes among C and SB cows. As discussed previously, a shift in propionate production, and/or VFA ratios, in our study was unlikely. However, an increased rate of passage is possible without affecting propionate production. The low starch levels of our diet (i.e., 160 g/kg DM) may have resulted in virtually complete starch fermentation in the rumen, resulting in little or no starch flow from the rumen, and an increased rate of passage would therefore not affect rumen propionate production. This hypothesis is supported by Wiedmeier et al. (1987) in which outflow of rumen fluid increased from 68 to 88 L/d without affecting concentrations of acetate or propionate, or their ratio. Although the cows used were dry cows with a low DM intake, the estimated starch content according to ingredient composition of that diet was 257 g/kg DM, which is comparable to our lower starch content (160 g/kg DM), assuming a lower intake and rate of passage in the cows used by Wiedmeier et al. (1987).

Sodium bicarbonate may elicit its physiological effects by increasing biohydrogenation of fatty acids in the rumen due to elevated pH (Fuentes et al., 2009). This reduces the amount of fatty acids absorbed from the small intestine, some of

which are known to inhibit milk fat synthesis in the mammary gland (i.e., predominantly *trans*-10 C<sub>18:1</sub> and *trans*-10, *cis*-12 C<sub>18:2</sub>). While it is possible that the rate of fatty acid biohydrogenation was increased in our study due to a higher rumen pH in SB supplemented cows, an increased rate of passage may have negated this effect and resulted in absorption of similar amounts of inhibitory fatty acids from the small intestine and equivalent milk fat yields between C and SB supplemented cows. However, based on our diets' normal fiber and low unsaturated fat levels, it is unlikely that the rumen microbial capacity to biohydrogenate unsaturated fatty acids was overwhelmed, and that amounts of inhibitory fatty acids absorbed, regardless of changes in rate of passage, were not large enough to lead to treatment differences in milk fat yield. Based on these hypotheses, it may be that the increase in milk fat concentration was largely due to a concentration of milk fat due to lower milk yield of SB supplemented cows.

An increase in the FPR of SB supplemented cows primarily reflects the increased concentration of fat, as protein concentrations were similar. A FPR of about 1.18 indicates that the cows were in a positive energy balance, at least according to Hagert (1991) and Dirksen (1994) who found that a ratio of less than 1.4 indicated optimal or positive energy balance, and those above 1.4 an energy deficit. When cows are in an energy deficit, fat mobilization from adipose tissue partially maintains milk fat synthesis, and a dietary deficiency of energy in the rumen results in reduced microbial protein synthesis and a reduction in milk protein synthesis. However, FPR is only a moderately good indicator of energy status (Hagert, 1991; Dirksen, 1994) and, as cows in our study were in negative energy balance despite having a FPR of less than 1.4, we suggest that FPR alone should not be used as an indicator of energy status. Enemark (2009) reported that a normal FPR is 1.0–1.5, with values below 1.0 being indicative of subacute rumen acidosis. As the ratio in our study was much higher than 1.0 the risk of subacute rumen acidosis for our cows was likely very low, and may partly explain the lack of benefit of SB feeding.

The tendency of SB supplemented cows to have had lower milk protein yield may be due to a combination of interrelated factors. While an increase in yield and efficiency of microbial protein synthesis occurs with increased rates of ruminal passage and liquid dilution rates (Bach et al., 2005a), microbial protein synthesis is also correlated with digestibility where, on average, 16.9 g microbial CP is synthesized/100 g apparently digested OM (Stern and Hoover, 1979). Feeding SB may have stimulated microbial protein synthesis due to a higher rate of passage but, to a larger degree, inhibited microbial protein synthesis by reducing DM digestibility and energy available to microbes, thus reducing protein flow to the small intestine and decreasing milk protein synthesis. While it has been found that microbial protein flow is higher at lower rumen pH (Bach et al., 2005a), this is unlikely to have affected our results as this is a consequence of the inverse relationship between rumen pH and dietary level of highly fermentable carbohydrates which increases the energy supply to, and thus protein synthesis by, rumen microbes (Stern et al., 2006).

#### 4.2.2. Effects on blood acid base balance, mineral metabolism and FS discharge

Apart from the buffering effect in the digestive tract, SB likely affected blood acid base balance, which occurs when there is an increase in blood pH and blood bicarbonate concentration, resulting in a compensatory respiratory effect and a quadratic increase in milk and milk fat yield (Hu and Murphy, 2004). Based on our DCAD values, DM intake and milk and milk fat yields are consistent with the meta analysis of Hu and Murphy (2004). Using DCAD equations developed during this meta analysis, DM intake of the C and SB cows would be predicted to be 19.6 and 19.6 kg/d, respectively, while milk yield of the C and SB cows would be predicted to be 24.3 and 24.2 kg/d, respectively, at a corresponding increase in DCAD of 37.5–45.6 mEq (Na + K – Cl)/100 g. It is possible that the small difference in predicted milk yield between C and SB supplemented cows would be amplified at higher rates of milk production, which would be consistent with our results.

A tendency to an elevated Na digestibility, and higher levels of Na in the SB diet, resulted in substantially higher intakes of absorbable Na compared to the C cows. As blood Na levels are tightly regulated (Hu and Murphy, 2004), excess Na must leave the blood via one or more of several pathways. One involves Na loss in saliva and sweat associated with heat stress but, as this study was conducted in the spring with average minimum and maximum temperatures of 6.7 ± 3.46 and 21.0 ± 5.09 °C, respectively, it is unlikely that cows experienced sweating or excess saliva production leading to increased Na excretion.

A second output for excess Na is bone deposition. While soft tissue contains ~600 mg/kg Na (wet mass), these levels are closely regulated by re-absorption and excretion in the kidneys via endocrine control to regulate blood pressure and fluid volume (NRC, 2001; Leheska et al., 2008). Although bone Na is ~350 g/kg total body Na, only about half of this is bound to the bone surface and is a part of the dynamic Na pool (Greene and Kleeman, 1991). This amounts to 10 mmol/kg BW and, in a 650 kg cow, this equals ~150 g of available Na. Even in the unlikely scenario of this Na reservoir being completely depleted at the onset of SB feeding, it could have been replenished within ~3 d of SB feeding, at least based on differences in digestible Na intakes between C and SB cows. Additional Na would likely have been shunted to the final and most important route of Na excretion, which is urine. Indeed, digestible intakes of Na for C and SB cows were 61.6 and 110.7 g/d (i.e., 79.7% increase in SB cows) while milk Na concentrations were not affected. This suggests a substantially higher Na discharge of SB cows. Reduced milk yield of SB supplemented cows may have been due to this increased intake of Na, consistent with previous studies which found decreases in milk yield with increased Na intake. For example, Solomon et al. (1995) reported a reduction in milk yield from 35.2 to 33.1 kg/d when Na intake from water and salt supplementation was 69.3 and 46.0 g/d in saline and desalinated water treatments, respectively, despite similar DM intakes of 22.6 and 23.0 kg/d, respectively. Jaster et al. (1978) reported a decrease in milk yield from 34.8 kg/d in cows receiving normal tap water (196 mg/kg dissolved salts) to 32.9 kg/d in cows receiving saline water (tap water plus 2500 mg/L NaCl). Subclinical Na toxicity, resulting in diarrhea and reduced milk production, may occur in beef cattle consuming water or a diet with a Na concentration above 1 g/kg (i.e., 0.393 Na/kg; Van Leeuwen, 1999), but caution must be used when extrapolating from beef to dairy cows due to differences

in their physiology. Nevertheless, our C and SB diets contained 3.0 and 5.0 g Na/kg DM, respectively, compared to the NRC (2001) recommended 2.2 g Na/kg DM for cows with a milk yield of 45 kg/d. A reduction in milk yield may have occurred due to increased water loss in urine without an equivalent increase in water intake. Urine volume is partly a function of Na intake (Bannink et al., 1999), and therefore SB cows likely excreted more urine than C cows. Using the equation proposed by Bannink et al. (1999), urine volumes of C and SB cows were 37.2 and 43.7 kg/d, respectively. If cows were not consuming enough water to compensate for this additional loss, or if there was a physiological limitation in absorption and metabolism of additional water required (e.g., limitations in renal capacity), there may have been a physiological shortage of fluid in the body resulting in the reduction in milk yield.

#### 4.3. Effects of CMC supplementation

##### 4.3.1. Effects on the gastrointestinal tract and dairy cow productivity

The elevated fecal pH of CMC supplemented cows without a change of *in vitro* ruminal fluid pH suggests that its buffering effect occurred post ruminally. The low pH of the abomasum is most conducive to CMC dissociation, and therefore it is likely that gastrointestinal tract buffering occurred in the abomasum as well as the small and large intestines. However, buffering may also have occurred indirectly from the Ca in CMC, which would be consistent with Noel et al. (1981), who reported that elevated dietary Ca increased pancreatic bicarbonate secretion. However, despite a buffering effect of CMC in the gastrointestinal tract, there were no changes in productivity or efficiency of the cows as assessed by similar NE output and NE concentration of the C and CMC diets. Overall, this indicates that the buffering effect of CMC probably was not physiologically required, which may have been due to the low dietary starch level. Limestone supplementation to diets with high starch levels (i.e., 518 g/kg DM) has resulted in lower fecal starch levels (89 versus 221 g/kg DM) and a substantially higher fecal pH (8.21 versus 5.67; Rogers et al., 1982). In this context, the high fecal pH of 6.60 in our C cows indicates that hindgut fermentation and acid production was limited, which is supported by the virtually complete starch digestion *in vivo*. This lack of difference in animal productivity is consistent with Crawford et al. (2008), who reported no differences in average daily gain, DM intake, gain to feed ratio, water intake or rumen pH of beef steers supplemented with 75 or 150 g/kg DM of CMC. However, if the smaller loss in BCS with CMC supplementation was real, this could be positive since a key management objective during early lactation is to minimize BCS loss in order to reduce associated depressions in productivity and incidences of related diseases such as ketosis.

##### 4.3.2. Effects on mineral metabolism and dairy FS discharge

Control and CMC cows had similar intakes of digestible Na without differences in milk Na concentration, and therefore CMC feeding did not increase Na discharge of the dairy farm and its use did not contribute to water and soil sodicity. However, considering that productivity and efficiency of CMC supplemented cows was not improved, use of CMC in similar diets and conditions is not supported. Due to limited CMC research with lactating cows, future studies should examine effects of CMC supplementation on dairy cows under other conditions, especially those known to lower rumen pH (e.g., corn silage based diets) to determine if rumen solubility of CMC is increased and, if so, whether responses in productivity occur.

## 5. Conclusions

Sodium bicarbonate supplementation increased milk fat concentration, but reduced milk yield. As a result, there were no differences in milk fat yield or efficiency of energy use of diets between C and SB supplemented cows. Changes in milk fat concentration and milk yield were likely due to an increase in DCAD and/or rumen buffering. While there were no productive benefits of SB use, it substantially increased Na discharge, which is known to increase water and soil sodicity. As the diet and animal characteristics, such as level of milk yield, in our study are typical of many modern dairy farms, it is likely that a large proportion of current SB supplementation occurs without benefits to productivity, while increasing Na discharge from the farm.

While CMC supplementation did not improve productivity or efficiency of dietary energy use of our cows, Na discharge from the dairy was not increased. Thus the use of CMC did not contribute to water and soil sodicity. For conditions comparable to those of this study, including many modern dairy farms, dietary use of SB or CMC is not supported due to a lack of improvement in animal performance.

## Acknowledgements

The authors wish to thank William Van Die and his farm crew for their assistance and cooperation in completion of the study. Many thanks to all the UC Davis student researchers who assisted in sample collection.

## References

- AOAC Official Method 973.18, 1997. Fiber (acid detergent) and lignin in animal feed. In: Official Methods of Analysis of AOAC International, 16th edition. AOAC International, Arlington, VA, USA, pp. 28–29 (Chapter 4).
- AOAC Official Method 990.03, 2005a. Protein (crude) in animal feed, combustion method. In: Official Methods of Analysis of AOAC International, 18th edition. AOAC International, Arlington, VA, USA, pp. 30–31 (Chapter 4).



- AOAC Official Method 942.05, 2005b. Ash of animal feed. In: Official Methods of Analysis of AOAC International, 18th edition. AOAC International, Gaithersburg, MD, USA, p. 8 (Chapter 4).
- AOAC Official Method 2002–04, 2006a. Amylase-treated neutral detergent fiber in feeds, using refluxing in beakers or crucibles. In: Official Methods of Analysis of AOAC International. AOAC International, Arlington, VA, USA, pp. 48–55 (Chapter 4).
- AOAC Official Method 2003.05, 2005. Crude fat in feeds, cereal grains, and forages. In: Official Methods of Analysis of AOAC International, 18th edition. AOAC International, Arlington, VA, USA, pp. 40–42 (Chapter 4).
- Bach, A., Calsamiglia, S., Stern, M.D., 2005a. Nitrogen metabolism in the rumen. *J. Dairy Sci.* 88 (E Suppl.), E9–E21.
- Bach, S.J., Selinger, L.J., Stanford, K., McAllister, T.A., 2005b. Effect of supplementing corn- or barley-based feedlot diets with canola oil on faecal shedding of *Escherichia coli* O157:H7 by steers. *J. Appl. Microb.* 98, 464–475.
- Bannink, A., Valk, H., Van Vuuren, A.M., 1999. Intake and excretion of sodium, potassium, and nitrogen and the effects on urine production by lactating dairy cows. *J. Dairy Sci.* 82, 1008–1018.
- Berg, J., Price, P., Westcott, D., Meyer, D., 2010. The industry wide salt study for existing milk cow dairies. Report prepared by the UC Davis Dairy Science Dept for Central Valley Regional Water Quality Control Board Order No. R5-2007-0035. Waste Discharge Requirements General Order For Existing Milk Cow Dairies. Central Valley Regional Water Quality Control Board, Sacramento, CA, USA.
- Blümmel, M., Ørskov, E.R., 1993. Comparison of in vitro gas production and nylon bag degradability of roughages in predicting feed intake in cattle. *Anim. Feed Sci. Technol.* 40, 109–119.
- California Regional Water Quality Control Board Central Valley Region Order No. R5-2007-0035, 2007. Waste Discharge Requirements General Order For Existing Milk Cow Dairies. [www.waterboards.ca.gov/centralvalley/board/decisions/adopted/orders/general/orders/r5-2007-0035.pdf](http://www.waterboards.ca.gov/centralvalley/board/decisions/adopted/orders/general/orders/r5-2007-0035.pdf).
- Cochran, R.C., Adams, D.C., Wallace, J.D., Galylean, M.L., 1986. Predicting digestibility of different diets with internal markers: evaluation of four potential markers. *J. Anim. Sci.* 63, 1476–1483.
- Crawford, G.L., Keeler, C.D., Wagner, J.J., Krehbiel, C.R., Erickson, G.E., Crombie, M.B., Nunnery, G.A., 2008. Effects of calcium magnesium carbonate and roughage level on feedlot performance, ruminal metabolism, and site and extent of digestion in steers fed high-grain diets. *J. Anim. Sci.* 86, 2998–3013.
- Dirksen, G., 1994. Control of metabolic disturbances in dairy cows by milk parameters. In: Proc. XVIII World Buiatrics Congr., Bologna, Italy, pp. 35–45.
- Edmonson, A.J., Lean, I.J., Weaver, L.D., Farver, T., Webster, G., 1989. A body condition scoring chart for Holstein dairy cows. *J. Dairy Sci.* 72, 68–78.
- Enemark, J.M.D., 2009. The monitoring, prevention and treatment of sub-acute ruminal acidosis (SARA): a review. *Vet. J.* 176, 32–43.
- Erdman, R.A., Botts, R.L., Hemken, R.W., Bull, L.S., 1980. Effect of dietary sodium bicarbonate and magnesium oxide on production and physiology in early lactation. *J. Dairy Sci.* 63, 923–930.
- Fuentes, M.C., Calsamiglia, S., Cardozo, P.W., Vlaeminck, B., 2009. Effect of pH and level of concentrate in the diet on the production of biohydrogenation intermediates in a dual-flow continuous culture. *J. Dairy Sci.* 92, 4456–4466.
- Galbraith, H., Miller, T.B., Paton, A.M., Thompson, J.K., 1971. Antibacterial activity of long chain fatty acids and the reversal with calcium, magnesium, ergocalciferol and cholesterol. *J. Appl. Bacteriol.* 34, 803–813.
- Goering, H.K., Van Soest, P.J., 1970. Forage fiber analyses (apparatus, reagents, procedures, and some applications). *Agric. Handbook* 379. ARS, USDA, Washington, DC, USA.
- Greene, J., Kleeman, C.R., 1991. Role of bone in regulation of systemic acid–base balance. *Kidney Int.* 39, 9–26.
- Groot, J.C.J., Cone, J.W., Williams, B.A., Debersaques, F.M.A., Lantinga, E.A., 1996. Multiphasic analysis of gas production kinetics for in vitro fermentation of ruminant feeds. *Anim. Feed Sci. Technol.* 64, 77–89.
- Hagert, C., 1991. Continuous control of the energy and protein balance in dairy cows during peak lactation concerning acetone, urea, protein and fat in milk. Thesis. University of Munich, Munich, Germany.
- Hu, W., Murphy, M.R., 2004. Dietary cation–anion difference effects on performance and acid–base status of lactating dairy cows: a meta-analysis. *J. Dairy Sci.* 87, 2222–2229.
- Hu, W., Murphy, M.R., 2005. Statistical evaluation of early- and mid-lactation dairy cow responses to dietary sodium bicarbonate addition. *Anim. Feed Sci. Technol.* 119, 43–54.
- Hubbert Jr., F., Cheng, E., Burroughs, W., 1958. Mineral requirements of rumen microorganisms for cellulose digestion in vitro. *J. Anim. Sci.* 17, 559.
- Jackson, J.A., Akay, V., Franklin, S.T., Aaron, D.K., 2001. The effect of cation–anion difference on calcium requirement, feed intake, body weight gain, and blood gases and mineral concentrations of dairy calves. *J. Dairy Sci.* 84, 147–153.
- Jaster, E.H., Schuh, J.D., Wegner, T.N., 1978. Physiological effects of saline drinking water on high producing dairy cows. *J. Dairy Sci.* 61, 66–71.
- Johansen, H.N., Glitso, V., Knudsen, K.E.B., 1996. Influence of extraction solvent and temperature on the quantitative determination of oligosaccharides from plant materials by high-performance liquid chromatography. *J. Agric. Food Chem.* 44, 1470–1474.
- Jones, J.B., 2001. Dionex Application Note 154. Determination of inorganic anions in environmental waters using a hydroxide-selective column. In: Laboratory Guide for Conducting Soil Tests and Plant Analysis. CRC Press, Boca Raton, FL, USA, pp. 227–228.
- Kellogg, D.W., Pennington, J.A., Johnson, Z.B., Panivivat, R., 2001. Survey of management practices used for the highest producing DHI herds in the United States. *J. Dairy Sci.* 84 (E Suppl.), E120–E127.
- Leheska, J.M., Thompson, L.D., Howe, J.C., Hentges, E., Boyce, J., Brooks, J.C., Shriver, B., Hoover, L., Miller, M.F., 2008. Effects of conventional and grass-feeding systems on the nutrient composition of beef. *J. Anim. Sci.* 86, 3575–3585.
- Lomba, F., Paquay, R., Bienfet, V., Lousse, A., 1968. Statistical research on the fate of dietary mineral elements in dry and lactating cows: II. Magnesium. *J. Agric. Sci.* 71, 181–188.
- Lucey, J.A., Horne, D.S., 2009. Milk salts: technological significance. In: McSweeney, P.L.H., Fox, P.F. (Eds.), *Advanced Dairy Chemistry. Lactose, Water, Salts and Minor Constituents*, vol. 3, 3rd ed. Springer Science + Business Media, LLC, New York, NY, USA.
- McDonald, P., Edwards, R.A., Greenhagh, J.F.D., Morgan, C.A., 2002. *Animal Nutrition*, 6th ed. Pearson Prentice Hall, Harlow, Essex, UK.
- Meyer, G.A., Keliher, P.N., 1992. An overview of analysis by inductively coupled plasma–atomic emission spectrometry. In: Montaser, A., Golightly, D.W. (Eds.), *Inductively Coupled Plasmas in Analytical Atomic Spectrometry*. VCH Publishers, New York, NY, USA, pp. 473–516.
- Mengel, K., Kirkby, E.A., 2001. Further elements of importance. In: *Principles of Plant Nutrition*, 5th ed. Kluwer Academic Publication, Dordrecht, The Netherlands.
- Menke, K.H., Steingass, H., 1988. Estimation of the energetic feed value obtained from chemical analysis and gas production using rumen fluid. *Anim. Res. Dev.* 28, 7–55.
- Mould, F.L., Ørskov, E.R., Mann, S.O., 1983. Associative effects of mixed feeds. I. Effects of type and level of supplementation and the influence of the rumen fluid pH on cellulolytic in vivo and dry matter digestion of various roughages. *Anim. Feed Sci. Technol.* 10, 15–30.
- National Research Council, 2001. *Nutrient Requirements of Dairy Cattle*, 7th revised edition. National Academy Press, Washington, DC, USA.
- NFTA, 2001. Laboratory Dry Matter by Oven Drying for 3 hours at 105 °C. Moisture Task Force Report, 2.2.2.5. National Forage Testing Association, Omaha, NE, USA.
- Noel, J., Veniene, M.C., Sarles, H.J., 1981. Dose dependant, and long lasting effects of repeated intravenous injections of calcium on the canine secretion-stimulated pancreatic juice secretion. *Eur. J. Clin. Invest.* 11, 25–31.
- Robinson, P.H., Givens, D.I., Getachew, G., 2004. Evaluation of NRC, UC Davis and ADAS approaches to estimate the metabolizable energy values of feeds at maintenance energy intake from equations utilizing chemical assays and in vitro determinations. *Anim. Feed Sci. Technol.* 114, 75–90.
- Robinson, P.H., McQueen, R.E., 1992. Influence of rumen fermentable neutral detergent fiber levels on feed intake and milk production of dairy cows. *J. Dairy Sci.* 75, 520–532.
- Robinson, P.H., Meyer, D., 2010. Total Mixed Ration Sampling Protocol. University of California Agricultural and Natural Resources, Oakland, CA, USA (Publication 8413).

- Rogers, J.A., Davis, C.L., Clark, J.H., 1982. Alteration of rumen fermentation, milk fat synthesis, and nutrient utilization with mineral salts in dairy cows. *J. Dairy Sci.* 65, 577–586.
- Rogers, J.A., Muller, L.D., Davis, C.L., Chalupa, W., Kronfield, D.S., Krocher, L.F., Cummings, K.R., 1985. Response of dairy cows to sodium bicarbonate and limestone in early lactation. *J. Dairy Sci.* 68, 646–660.
- Russell, J.B., Chow, J.M., 1993. Another theory for the action of ruminal buffer salts: decreased starch fermentation and propionate production. *J. Dairy Sci.* 76, 826–830.
- SAS Institute Inc., 2000. SAS/STAT® Software: changes and enhancements, Release 8.1. SAS Institute Inc., Cary, NC, USA.
- Schofield, P., Pell, A.N., 1995. Measurement and kinetic analysis of the neutral detergent soluble-carbohydrate fraction of legumes and grasses. *J. Anim. Sci.* 73, 3455–3463.
- Solomon, R., Miron, J., Ben-Ghedalia, D., Zomberg, Z., 1995. Performance of high producing dairy cows offered drinking water of high and low salinity in the Arava desert. *J. Dairy Sci.* 78, 620–624.
- Snyder, T.J., Rogers, J.A., Muller, L.D., 1983. Effects of 1.2% sodium bicarbonate with two ratios of corn silage:grain on milk production, rumen fermentation, and nutrient digestion by lactating dairy cows. *J. Dairy Sci.* 66, 1290–1297.
- Stensig, T., Robinson, P.H., 1997. Digestion and passage kinetics of forage fiber in dairy cows as affected by fiber-free concentrate in the diet. *J. Dairy Sci.* 80, 1339–1352.
- Stern, M.D., Bach, A., Calsamiglia, S., 2006. New concepts in protein nutrition of ruminants. In: 21st Annual Southwestern Nutrition and Management Conference, February 23–24, 2006, Tempe, AZ, USA.
- Stern, M.D., Hoover, W.H., 1979. Methods for determining and factors affecting rumen microbial protein synthesis: a review. *J. Anim. Sci.* 49, 1590–1603.
- Swanepoel, N., Robinson, P.H., Erasmus, L.J., 2010. Amino acid needs of lactating dairy cows: predicting limiting amino acids in contemporary rations fed to high producing dairy cattle in California using metabolic models. *Anim. Feed Sci. Technol.* 161, 103–120.
- Tracy, M.L., Moeller, G., 1990. Continuous flow vapor generation for inductively coupled argon plasma spectrometric analysis. Part 1: selenium. *J. Assoc. Off. Anal. Chem.* 73, 404–410.
- Tyrrell, H.F., Reid, J.T., 1965. Prediction of the energy value of cow's milk. *J. Dairy Sci.* 48, 1215–1223.
- Van Leeuwen, J.A., 1999. Salt poisoning in beef cattle on coastal pasture on Prince Edward Island. *Can. Vet. J.* 40, 347–348.
- Wiedmeier, R.D., Arambel, M.J., Lamb, R.C., Marcinkowski, D.P., 1987. Effect of mineral salts, carbachol, and pilocarpine on nutrient digestibility and ruminal characteristics in cattle. *J. Dairy Sci.* 70, 592–600.
- Wilson, G.F., 1980. Effects of magnesium supplements on the digestion of forages and milk production of cows with hypomagnesaemia. *Anim. Prod.* 31, 153–157.
- Wolin, M.J., 1960. A theoretical rumen fermentation balance. *J. Dairy Sci.* 43, 1452–1459.