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The effect of calcareous marine algae, with or without marine magnesium oxide, and sodium bicarbonate on rumen pH and milk production in mid-lactation dairy cows

E. W. Neville,[1] A. G. Fahey,[2]* V. P. Gath,[1] B. P. Molloy,[3] S. J. Taylor,[3] and F. J. Mulligan[1]
[1]School of Veterinary Medicine, University College Dublin, Belfield, Dublin 4, Ireland
[2]School of Agriculture and Food Science, University College Dublin, Belfield, Dublin 4, Ireland
[3]Celtic Sea Minerals, Currabinny, Carrigaline, Co. Cork, P43 NN62, Ireland

ABSTRACT

Two experiments were carried out to evaluate different dietary buffers and their influence on (1) rumen pH in dairy cows and (2) milk production in dairy cows. The supplements included were calcareous marine algae (CMA; *Lithothamnion calcareum*), with or without marine magnesium oxide (MM; precipitated magnesia derived from seawater), and sodium bicarbonate (SB). Dietary treatments in experiment 1 consisted of the control [32.9% starch and sugar, and 19.9% neutral detergent fiber from forage per kg of dry matter (DM)] including no dietary buffer (CON); the control plus 0.45% DM CMA (CMA); the control plus 0.45% DM CMA and 0.11% DM MM (CMA+MM); the control plus 0.9% DM SB (SB). Diets were formulated to a dry matter intake (DMI) of 18 kg per cow/d. Dietary treatments in experiment 2 also consisted of CON (28.3% starch and sugar, and 23% neutral detergent fiber from forage per kg of DM), CMA, CMA+MM, and SB and were formulated to achieve identical intakes of experimental ingredients (80 g of CMA, 80 g of CMA plus 20 g MM, and 160 g of SB per cow/d) with a DMI of 22.6 kg per cow/d. Experiment 1 used 4 rumen-cannulated dairy cows in a 4 × 4 Latin square design. Rumen pH was measured over five 2-h periods, following feeding, using rumen pH probes. In experiment 2, 52 multiparous and 4 primiparous cows (62.7 ± 3.4 d in milk) were assigned to 4 experimental treatments for 80 d. Both CMA treatments maintained a greater mean rumen pH than the CON during 4 of the 5 periods following feeding and the CON had a greater number of hours below rumen pH 5.5 compared with all other treatments. Dry matter intakes tended to be higher on the SB compared with CON. The CMA treatment increased the production of milk fat and protein yield (kg/d) compared with all other treatments. Both CMA and CMA+MM increased milk fat yield compared with CON but were similar to each other and SB. Protein yield was highest in the CMA treatment compared with CON, CMA+MM, and SB. All 3 buffer treatments increased milk fat concentration compared with CON but did not differ from each other. The SB treatment reduced milk protein concentration and milk production efficiency, energy-corrected milk per kilogram of DMI. Results indicate that the addition of CMA can benefit milk fat and protein production when included in diets based on typical feedstuffs of the northern European region. The use of CMA when compared with SB, in such diets, can increase milk protein production and milk production efficiency.

Key words: calcareous marine algae, marine magnesium oxide, rumen buffer, lactating dairy cow, rumen pH

INTRODUCTION

Improved genetics for milk production in dairy cows has resulted in the requirement for more nutrient-dense diets containing highly fermentable carbohydrates (Plaizier et al., 2008). Formulating diets to provide adequate energy levels while also supplementing sufficient effective fiber to prevent digestive upsets is a difficult task. Physically effective fiber encourages rumination and the production of saliva, which acts as a natural rumen buffer for the dairy cow (Beauchemin and Yang, 2005). However, fiber sources are often poor suppliers of energy (Mertens, 1997). The production of VFA are necessary for the energy supply of the dairy cow but can reduce rumen pH (Whelan et al., 2013) if allowed to accumulate. Prolonged periods of rumen pH depression can lead to reduced fiber digestion (Mulligan et al., 2002), negative alterations to the rumen microbial population (Allen, 1997), negatively altered milk composition (Plaizier et al., 2014), and health issues such

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*Corresponding author: alan.fahey@ucd.ie

as diarrhea, laminitis, liver abscesses, and inflammation (Plaizier et al., 2008). A decline in rumen pH for more than 3 consecutive h/d below pH 5.6 is defined as SARA (Gozho et al., 2005). Up to 20% of cows in typical northern European confinement dairy herds may be suffering from prolonged periods of rumen pH depression (Kleen et al., 2013). According to O'Grady et al. (2008), cows in pasture-based dairy herds can also succumb to SARA.

Rumen buffers [mostly mineral salts but also including calcareous marine algae (**CMA**) products] are commonly added to lactating cow diets to avoid prolonged episodes of low rumen pH and the associated production losses (Enemark, 2008). The addition of sodium bicarbonate (**SB**) to the diets of high-producing dairy cows, as a rumen buffer, has become a regular practice in most parts of the world (Rauch et al., 2012). In recent years, CMA (*Lithothamnion calcareum*) has been used to stabilize rumen pH and improve the rumen environment. Cruywagen et al. (2015) demonstrated increasing daily milk yield, 4% FCM, ECM, and milk efficiency (kg of milk per kg of DMI) in a low forage: concentrate (35:65), low NDF (26% DM) TMR fed to mid-lactation dairy cows when using CMA compared with SB and a control diet.

No previous work has been carried out on marine magnesium oxide (**MM**; precipitated magnesia derived from seawater), supplemented in combination with other rumen buffers, looking at its effects on rumen fermentation and milk production in dairy cows. However, conventional magnesium oxide (MgO) has been used to alleviate pH depressions in dairy cow diets. Both Erdman et al. (1982) and Teh et al. (1985) observed beneficial effects on rumen pH and milk fat concentration when conventional MgO, either on its own or in combination with SB, was supplemented to lactating dairy cows. Marine MgO has, however, not been studied as a rumen buffer. Furthermore, no documented studies could be found regarding CMA, MM, or SB in dairy cow diets containing low DM perennial ryegrass silages, typical of the northern European region.

The objectives of the experiments were to examine the effects of CMA, with or without MM, in comparison with SB on rumen pH, milk production, and feed efficiency parameters in mid-lactation dairy cows fed a high (25–27% DM) starch TMR based on low DM ryegrass silage, corn silage, and ingredients typical of a confined northern European feeding system.

MATERIALS AND METHODS

The research consisted of 2 studies: a rumen pH experiment (experiment 1) and a milk production experiment (experiment 2). All procedures described in both experiments were approved by the Animal Research Ethics Committee at University College Dublin and conducted under experimental license from the Health Products Regulatory Authority under the European directive 2010/63/EU and S.I. No. 543 of 2012. Each person who carried out procedures on experimental animals during the course of this experiment was authorized to do so by means of individual authorization from the Health Products Regulatory Authority.

Animal Management and Experimental Design

Experiment 1 (Rumen pH). An experiment was carried out in advance of the milk production experiment to validate the buffering capacity of the different rumen buffers. Four ruminally cannulated lactating cows were used in the rumen pH trial in a 4 × 4 Latin square design with 4 dietary treatments. This is an alternative method to measuring the reactivity of buffers using in vitro techniques, as has been published previously (Bach et al., 2018). Cows were housed individually in 5 × 7 m pens in a well-ventilated barn with a solid concrete floor for the duration of this experiment. Each pen contained a lying area with woodchip, a feeding trough, and a water trough. Cows had free access to feed and water throughout the day and both water and feed troughs were emptied and cleaned daily before adding fresh feed and water. The total duration of the trial was 100 d consisting of 4 periods of 25 d each. Each experimental period consisted of a 13-d dietary adjustment period whereby cows were acclimatized to their respective treatments followed by a 12-d sampling and data collection period.

Experiment 2 (Milk Production). Fifty-six (4 primiparous and 52 multiparous) Holstein Friesian spring-calving dairy cows were selected from the main dairy herd at University College Dublin, Lyons Farm, Co. Kildare, Ireland. Cows were assigned to treatment according to a randomized complete block design with repeated measures based on parity, calving BCS (3.1 ± 0.2; ±SD), pre-experimental milk yield (34.7 ± 5.9 kg/d), previous 305-d milk yield (7,073 ± 1,431 kg) for multiparous cows, and DIM at start of trial (62.7 ± 25.5 d). Trial duration was 80 d, which included 7 d of acclimatization to their respective diets followed by 73 d of sampling and data collection.

The 54 cows were housed in a freestall barn that had 60 individual stalls available. Cows had ad libitum access to TMR for 22 h every day through specific computerized feeding boxes (RIC System, Insentec B.V., Marknesse, the Netherlands). Cows received their complete diet through the TMR once daily at 0900 h. Each treatment was mixed separately with a Keenan Feeder (Keenan Feeding Systems, Borris, Carlow, Ireland) and

each treatment received the same total mixing time, 10 min after all the ingredients were added to the mixer wagon. Water was available ad libitum, and water troughs were cleaned daily. For both experiments, cows were milked twice daily, at 0800 and 1600 h, in a rotary milking parlor (Dairymaster, Causeway, Kerry, Ireland) and stalls were also cleaned, with new sawdust bedding added, twice daily.

Feeding and Dietary Treatments

Experiment 1 (Rumen pH). Diets were formulated using the INRAtion 4.07 computer program (www.inration.educagri.fr/en) and designed to supply 100% of the energy requirements of a 650-kg lactating dairy cow yielding 23 kg of milk/d containing 4.0% of fat and 3.3% of protein. Feed was allocated to cows, at a rate of 19 kg DM per cow per day, to allow for 5% refusals. The 4 dietary treatments consisted of the control (**CON**; 55% concentrate, 32.9% starch and sugar, and 19.9% NDF from forage per kg of DM) including no dietary buffer; CON plus 0.45% DM CMA (CMA); CON plus 0.45% DM CMA and 0.11% DM MM (CMA+MM); and CON plus 0.9% DM SB (SB). The inclusion rate of the treatment supplements was based on a DMI of 18 kg of DM per cow per day to ensure that both CMA and CMA+MM treatments provided a daily intake of 80 g of CMA per cow, the CMA+MM treatment provided 20 g of MM per cow, and the SB treatment provided a daily intake 160 g of SB per cow. All treatments were balanced for calcium and sodium (except the SB treatment) as the 3 supplements contributed to the calcium, and sodium balance of their subsequent diet. The control diet included 54% DM concentrate, 22.5% DM grass silage, and 22.5% DM corn silage. The forage:concentrate ratio was 45:55.

Experiment 2 (Milk Production). The cows were trained to use the computerized feeding stations (RIC System, Insentec B.V.) for 1 wk before the trial started. Diets were formulated using the INRAtion 4.07 computer program and designed to supply 100% of the energy requirements of a 650-kg lactating dairy cow yielding 33 kg of milk/d containing 3.9% of fat and 3.2% of protein. Feed was allocated to cows, at a rate of 23.6 kg DM per cow per day, to allow for 5% refusals. The 4 dietary treatments consisted of CON (54.3% concentrate, 28.3% starch and sugar, and 23% NDF from forage per kg of DM) including no dietary buffer, CON plus 0.35% DM CMA (CMA), CON plus 0.35% DM CMA and 0.09% DM MM (CMA+MM), and CON plus 0.7% DM SB (SB). The inclusion rate of the treatment supplements was based on a DMI of 22.6 kg of DM per cow per day to ensure that both CMA and CMA+MM treatments provided a daily intake of 80 g of CMA per cow, the CMA+MM treatment provided 20 g of MM per cow, and the SB treatment provided a daily intake 160 g of SB per cow. All treatments were balanced for calcium and sodium (except the SB treatment) as the 3 supplements contributed to the calcium and sodium balance of their subsequent diet. The control diet included 54.3% DM concentrate, 22% DM grass silage, 22% DM corn silage, and 1.7% DM wheat straw. The forage:concentrate ratio was 46:54.

The difference between diets fed in experiment 1 and 2 was due to different concentrate composition; see Tables 1 and 2. For experiment 1, wheat was included as the primary ingredient in the concentrate portion of the diet to increase the fermentability of the diet used for rumen pH determination and to ensure the dietary buffers were adequately challenged. The experiments were designed in this way to ensure that the buffers used in this experiment were evaluated in scenarios where the diet provided a significant challenge to rumen pH.

The grass silage used in both experiments consisted primarily of perennial ryegrass (*Lolium perenne*). The crop was cut using a mower-conditioner during the early boot stage of vegetation (growth stage 41; Zadoks et al., 1974), wilted for 16 h, and harvested with a Claas Jaguar 860 (Claas GmbH & Co. Harsewinkel, Germany) precision chop forage harvester (mean particle length 50 mm). The crop was then ensiled under a black polythene cover without the use of an additive. Corn silage (*Zea maiz*, variety Tekni) was grown with the aid of plastic film (Samco Agricultural Manufacturing Ltd., Limerick, Ireland). The crop was harvested at the dough stage (growth stage 85; Lancashire et al., 1991) using a Claas Jaguar 860 (Claas GmbH & Co.) precision chop forage harvester (mean particle length 25mm). The harvester was equipped with a kernel processor to improve starch digestibility. The harvested corn silage was ensiled under a black polythene cover without the use of an additive. Fresh samples of both grass and corn silage were sent to a forage laboratory (AFBI-Hillsborough, Northern Ireland) for near-infrared analysis (FOSS NIR systems 5000, Foss UK, Warrington, Cheshire, UK). Ingredients included in the concentrate used are presented in Table 2. The concentrate portion of the diet was fed in the form of a pellet to prevent unwanted dietary separation and aid in mixing accuracy. The pellet was manufactured by Gain Feeds, Clonroche, Wexford, Ireland. Ingredients were added to the TMR in the following order: wheat straw, concentrate, grass silage, and corn silage.

Data and Sample Collection

Experiment 1 (Rumen pH). Rumen pH measurements were collected over 3 d, starting on d 2 of each

Table 1. Ingredient composition of the control, calcareous marine algae, with or without marine magnesium oxide, and sodium bicarbonate diets used in experiment 1 (rumen pH)

	Dietary treatment[1]			
Ingredient, % of DM	CON	CMA	CMA + MM	SB
Wheat grain (finely ground)	34.7	34.7	34.7	34.7
Grass silage	22.5	22.5	22.5	22.5
Corn silage	22.5	22.5	22.5	22.5
Soybean meal (48% CP)	10.9	10.9	10.9	10.9
Citrus pulp	2.80	2.80	2.80	2.50
Soy hulls	2.80	2.80	2.80	2.50
Sugar cane molasses	1.40	1.40	1.40	1.40
Limestone	0.78	0.45	0.45	0.78
White salt	0.45	0.45	0.45	0.18
Mono-dicalcium phosphate	0.36	0.36	0.36	0.36
PFAD (spray) blend[2]	0.34	0.34	0.34	0.34
Trace element and vitamin premix[3]	0.30	0.30	0.30	0.30
Calcined magnesite	0.16	0.10	0.00	0.16
Calcareous marine algae[4]	0.00	0.45	0.45	0.00
Sodium bicarbonate	0.00	0.00	0.00	0.90
Marine magnesium oxide[5]	0.00	0.00	0.11	0.00

[1]Treatments: CON = control; CMA = calcareous marine algae; CMA + MM = calcareous marine algae and marine magnesium oxide; SB = sodium bicarbonate.

[2]PFAD = palm fatty acid distillate.

[3]Lactating cow trace element and vitamin premix supplied by Nutribio Ltd., Cork, Ireland. Formulated to contain (per kg of DM) 340 g of Ca, 0.04 g of Co, 7.4 g of Cu, 0.3 g of I, 0.1 g of Se, 16.6 g of Mn, 25 g of Zn, 8,000 IU of vitamin A, 2,000 IU of vitamin D_3, and 10 IU of vitamin E.

[4]Calcareous marine algae = *Lithothamnion calcareum*.

[5]Marine magnesium oxide = precipitated magnesia derived from seawater.

Table 2. Ingredient composition of the control, calcareous marine algae, with or without marine magnesium oxide, and sodium bicarbonate diets used in experiment 2 (milk production)

	Dietary treatment[1]			
Ingredient, % of DM	CON	CMA	CMA + MM	SB
Grass silage	22.0	22.0	22.0	22.0
Corn silage	22.0	22.0	22.0	22.0
Wheat grain (finely ground)	16.6	16.7	16.7	16.6
Barley grain (finely ground)	8.70	8.70	8.70	8.70
Soybean meal (48% CP)	8.70	8.70	8.70	8.70
Corn grain (finely ground)	6.50	6.50	6.50	6.50
Unmolassed beet pulp	4.30	4.30	4.30	4.30
Soy hulls	4.32	4.30	4.39	3.98
Sugar cane molasses	2.40	2.40	2.40	2.40
Straw (wheat)	1.70	1.70	1.70	1.70
Limestone	0.84	0.48	0.48	0.84
White salt	0.60	0.60	0.60	0.24
Mono-dicalcium phosphate	0.45	0.45	0.45	0.45
PFAD (spray) blend[2]	0.34	0.34	0.34	0.34
Trace element and vitamin premix[3]	0.30	0.30	0.30	0.30
Calcined magnesite	0.25	0.18	0.00	0.25
Calcareous marine algae[4]	0.00	0.35	0.35	0.00
Sodium bicarbonate	0.00	0.00	0.00	0.70
Marine magnesium oxide[5]	0.00	0.00	0.09	0.00

[1]Treatments: CON = control; CMA = calcareous marine algae; CMA + MM = calcareous marine algae and marine magnesium oxide; SB = sodium bicarbonate.

[2]PFAD = palm fatty acid distillate.

[3]Lactating cow trace element and vitamin premix supplied by Nutribio Ltd., Cork, Ireland. Formulated to contain (per kg of DM) 340 g of Ca, 0.04 g of Co, 7.4 g of Cu, 0.3 g of I, 0.1 g of Se, 16.6 g of Mn, 25 g of Zn, 8,000 IU of vitamin A, 2,000 IU of vitamin D_3, and 10 IU of vitamin E.

[4]Calcareous marine algae = *Lithothamnion calcareum*.

[5]Marine magnesium oxide = precipitated magnesia derived from seawater.

sampling period. The feeding times and milking routines were the same as in the production experiment. Rumen pH was measured using internal pH probes linked to a data logger (Intech Instruments Ltd., Lincoln, New Zealand). The pH data loggers were connected to straps that were securely fastened around the shoulder of the cow to prevent damage to the data logger while also avoiding irritation of the cow. The electrodes were housed in specially designed stainless-steel capsules and joined to the cannulas via water-tight hoses and fittings. This specially designed rumen cannula, holding the pH probe, allowed the pH probe to reside in the center of the rumen.

On d 2 of each data collection period, the pH loggers and probes were introduced at 1100 h, 2 h after feeding. The pH probes were cleaned, checked for accuracy, and re-calibrated, with pH 4.0 and 7.0 standards every 24 h. This process took approximately 15 min to complete. Continuous pH measurements from the indwelling probe were sent to the data logger every 10 min. After the device had been removed each day, pH measurements were retrieved from the data logger. Measurements taken over the 3 d were combined to create a daily mean pH (24 h) and also for the first 12 h after morning feeding for each cow/period, which was then divided into 5 separate 2-h periods.

Experiment 2 (Milk Production). Experimental wk 2 to 12 were used for sampling and data collection. Milk samples were collected on 2 separate days each week during the data collection period. Each day consisted of an a.m. and p.m. sample, pooled in proportion to the specific a.m. and p.m. yields to create 1 milk sample per day and 2 milk samples per cow for each week. Samples were preserved (Broad Spectrum Microtabs II, D&F Control Systems Inc., Norwood, MA) and stored at 4°C until analyzed. Milk samples were analyzed within 48 h of collection. Daily milk yields were automatically recorded using the Weighall milk meter system (Dairymaster, Causeway, Kerry, Ireland). Samples of TMR were collected daily and dried at 104°C in a forced-air oven for 16 h to establish the DM content of the TMR. Individual daily feed intakes were recorded on a computerized feeding system (RIC System, Insentec B.V.) and used, in combination with daily TMR DM content, to calculate DMI. Concentrations of milk fat, protein, lactose, urea, and casein were determined in a commercial milk laboratory (Progressive Genetics, Dublin, Ireland) using infrared analysis (CombiFoss 5000, Foss Analytical, A/S, Hillerod, Denmark). Body condition score of each cow was graded once per week, by the same trained individual adopting a 5-point scale method, as described by Edmondson et al. (1989). Samples of TMR were taken from each treatment during feed out 3 times per week, pooled into weekly samples by treatment, and stored at −20°C until they were analyzed. Concentrate, grass silage, corn silage, and straw samples were collected weekly and stored at −20°C before analysis.

Sample Analyses

Subsamples of the composite TMR from both experiments, corn silage, grass silage, and concentrate samples were dried at 55°C for 72 h. The subsequent dried samples were ground using a Norris hammer mill fitted with a 1-mm screen (Lab Mill, Christy Turner, Suffolk, UK). Ash content was determined by incineration of a 5-g sample in a muffle furnace (Nabertherm GmbH, Lilienthal, Germany) at 550°C for 5.5 h. The N content of the feed was determined by combustion using a Leco 528 instrument (Leco Instruments UK, Cheshire, UK). Crude Protein was then calculated using N × 6.25. Neutral detergent fiber and ADF were determined according to the method of Van Soest et al. (1991) using the Ankom 220 Fiber Analyzer (Ankom Technology, Fairport, NY). As part of the NDF procedure, both sodium sulfite (Na_2SO_3; FSS, Ankom Technology) and heat stable α-amylase (FAA, Ankom Technology) were used for the analysis of TMR and corn silage subsamples whereas only sodium sulfite (Na_2SO_3) was used for grass silage subsamples. Starch content was determined using the Megazyme Total Starch Assay Procedure (product no. K-TSTA, Megazyme International Ltd., Wicklow, Ireland). Water-soluble carbohydrate was analyzed according to the method used by Birch and Mwangelwa (1974). Ether extract was measured using a Soxtec instrument (Tecator) according to the method of AOAC 107 (AOAC, 1970).

Experiment 2 (Milk Production). Values for ECM yield and 4% FCM were calculated using the following formulae:

$$\text{ECM} = (0.3273 \times \text{milk yield kg}) + (7.65 \times \text{milk protein kg}) + (12.97 \times \text{milk fat kg})$$

(Tyrrell and Reid, 1965);

$$4\%\ \text{FCM} = (0.4 \times \text{milk yield kg}) + (15 \times \text{fat yield kg})$$

(Gaines, 1928).

Data Screening and Statistical Analyses

Data residuals from both experiments were examined for normality using the UNIVARIATE procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC). Following assessment of normality, outliers were removed (±3 SD from the mean).

Table 3. The analyzed and predicted chemical and nutrient profile of the control, calcareous marine algae, with or without marine magnesium oxide, and sodium bicarbonate diets used in experiment 1 (rumen pH)

	Dietary treatment[1]			
Item	CON	CMA	CMA + MM	SB
Chemical composition, % of DM				
DM	40.1	40.1	40.1	40.1
CP[2]	15.7	15.7	15.7	15.7
PDIN[2]	10.5	10.5	10.5	10.5
PDIE[2]	10.5	10.5	10.5	10.5
NDF	27.2	27.2	27.2	27.2
f-NDF[3]	19.9	19.9	19.9	19.9
ADF	12.9	12.9	12.9	12.9
Ash	6.80	6.80	6.80	7.00
Starch	27.5	27.5	27.5	27.5
Sugar	5.40	5.40	5.40	5.40
Ca[2]	0.79	0.79	0.79	0.79
Mg[2]	0.20	0.20	0.15	0.20
Na[2]	0.21	0.21	0.21	0.26
UFL[2]	0.88	0.88	0.96	0.96
NE_L,[4] Mcal/kg of DM	1.50	1.50	1.50	1.50

[1]Treatments: CON = control; CMA = calcareous marine algae; CMA + MM = calcareous marine algae and marine magnesium oxide; SB = sodium bicarbonate.

[2]As calculated by using INRAtion 4.07 feed formulations program (www.inration.educagri.fr/en), based on ingredient analyses; similar for all treatments. PDIN = protein truly digestible in the small intestine where nitrogen is limiting microbial protein synthesis; PDIE = protein digested in the small intestine where energy is limiting microbial protein synthesis; UFL = unit of energy for lactation.

[3]f-NDF = contribution of the forage component of the diet to NDF.

[4]NE_L at production level, NRC (2001).

Experiment 1 (Rumen pH). Rumen pH data were analyzed as a 4 × 4 Latin square design using the MIXED procedure of SAS (SAS, version 9.4). The model included fixed effects of treatment and period, with cow considered as the random effect. All data presented in Table 5 are expressed as least squares means ± standard error of the mean. Statistical significance was declared at $P \leq 0.05$ and a tendency was assumed at $0.05 < P \leq 0.10$. *P*-values are presented as Tukey adjusted values.

Experiment 2 (Milk Production). Milk production data were analyzed using the MIXED procedure (SAS, version 9.4). The model included fixed effects of treatment, week, and parity as well as treatment by week interaction with cow considered as the random effect. Before analysis, SCC was transformed to SCS [$SCS = \log_2(SCC/100{,}000) + 3$] (Schutz, 1994). Pre-experimental milk yield and calving BCS were included in the final model as covariates. The covariance structure for repeated measures of week was chosen using the lowest Bayesian information criterion value. Compound symmetry heterogeneous covariance structure was used for FCM, ECM efficiency, protein composition, and urea composition. Toeplitz covariance structure was used for FCM efficiency. Compound symmetry covariance structure was used for the remaining parameters. All data presented in Table 6 are expressed as least squares means ± standard error of the mean. Statistical significance was declared at $P < 0.05$ and a tendency was assumed at $P > 0.05$ and <0.10. *P*-values are presented as Tukey adjusted values.

RESULTS

Chemical Analysis of TMR

The nutrient composition of the 4 dietary treatments is presented in Table 3 for experiment 1 and Table 4 for experiment 2. Values for CP, PDIN, PDIE, UFL, and NE_L are predicted based on the INRAtion 4.07 computer program and NRC (2001).

Experiment 1 (Rumen pH)

The effects of CON, CMA, CMA+MM, and SB on mean rumen pH per 2-h period from 2 to 12 h post-feeding, the overall daily mean rumen pH, and the hours below pH 5.5 are presented in Table 5. The CON cows had a lower mean rumen pH compared with CMA ($P < 0.01$), CMA+MM ($P < 0.01$), and SB ($P < 0.05$) during the 2- to 4-h period. The CMA, CMA+MM, and SB had a similar mean rumen pH at 2 to 4 h. Cows fed CMA tended to have a greater mean rumen pH during 4 to 6 h compared with SB ($P < 0.10$) but similar to CMA+MM and CON ($P > 0.10$). Both CMA and CMA+MM had a greater mean rumen pH compared

Table 4. The analyzed and predicted chemical and nutrient profile of the control, calcareous marine algae, with or without marine magnesium oxide, and sodium bicarbonate diets used in experiment 2 (milk production)

Item	Dietary treatment[1]			
	CON	CMA	CMA + MM	SB
Chemical composition, % of DM				
DM	38.3	38.3	38.3	38.3
CP[2]	16.5	16.5	16.5	16.5
PDIN[2]	9.90	9.90	9.90	9.90
PDIE[2]	10.3	10.3	10.3	10.3
NDF	31.7	32.6	32.3	32.1
f-NDF[3]	23.0	23.0	23.0	23.0
ADF	19.5	20.3	20.5	20.4
Ash	7.50	7.40	7.20	7.30
Starch	25.0	25.5	25.5	25.8
Sugar	3.30	3.30	3.30	3.30
Ca[2]	0.82	0.80	0.80	0.82
Mg[2]	0.25	0.25	0.20	0.25
Na[2]	0.28	0.28	0.28	0.33
UFL[2]	0.96	0.96	0.96	0.96
NE_L,[4] Mcal/kg of DM	1.60	1.60	1.60	1.60

[1]Treatments: CON = control; CMA = calcareous marine algae; CMA + MM = calcareous marine algae and marine magnesium oxide; SB = sodium bicarbonate.

[2]As calculated by using INRAtion 4.07 feed formulations program (www.inration.educagri.fr/en), based on ingredient analyses; similar for all treatments. PDIN = protein truly digestible in the small intestine where nitrogen is limiting microbial protein synthesis; PDIE = protein digested in the small intestine where energy is limiting microbial protein synthesis; UFL = unit of energy for lactation.

[3]f-NDF = contribution of the forage component of the diet to NDF.

[4]NE_L at production level, NRC (2001).

with CON ($P < 0.05$) during 6 to 8 h but were not different from each other or SB ($P > 0.10$). Controls and SB were similar during 6 to 8 h. Both CMA and CMA+MM maintained a greater rumen pH at 8 to 10 h compared with CON ($P < 0.01$) and SB ($P < 0.05$) but were similar to each other. The cows fed CON and SB had similar pH values during 8 to 10 h. The CMA and CMA+MM cows had a greater mean rumen pH during 10 to 12 h compared with CON ($P < 0.01$) but had similar mean rumen pH values to each other and SB ($P > 0.10$). Controls tended to have lower daily (24 h) mean rumen pH values to SB ($P < 0.10$). Both CMA and CMA+MM maintained a greater daily (24 h) mean rumen pH compared with CON ($P < 0.01$) but were similar to each other and SB ($P > 0.10$). The control cows had a greater number of hours below pH 5.5 compared with CMA, CMA+MM, and SB ($P < 0.01$), but CMA was not different from CMA+MM ($P > 0.10$). The SB had a greater number of hours below rumen pH 5.5 compared with CMA and CMA+MM ($P < 0.01$) and fewer hours below rumen pH 5.5 compared with CON ($P < 0.01$).

Table 5. The effect of calcareous marine algae, with or without marine magnesium oxide, and sodium bicarbonate on mean rumen pH over a 2-h period relative to feeding time in experiment 1 (rumen pH)

Hours relative to feeding	Dietary treatment[1]				SEM	Trt[2] *P*-value
	CON	CMA	CMA + MM	SB		
2 to 4 h	5.78^{a}	6.34^{b}	6.32^{b}	6.14^{b}	0.136	<0.01
4 to 6 h	5.78^{xy}	6.04^{x}	6.01^{xy}	5.74^{y}	0.136	<0.01
6 to 8 h	5.75^{a}	6.12^{b}	6.10^{b}	5.84^{ab}	0.136	<0.01
8 to 10 h	5.34^{a}	5.90^{b}	6.00^{b}	5.53^{a}	0.136	<0.01
10 to 12 h	5.12^{a}	5.66^{b}	5.70^{b}	5.40^{ab}	0.136	<0.01
Overall mean (24 h)	$5.92^{a,x}$	$6.23^{b,xy}$	$6.32^{b,xy}$	$6.15^{ab,y}$	0.079	<0.01
Hours ≤5.5	6.10^{a}	0.77^{b}	0.67^{b}	3.17^{c}		<0.01

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

[x,y]Means within a row with different superscripts differ ($P < 0.10$).

[1]Treatments: CON = control; CMA = calcareous marine algae; CMA + MM = calcareous marine algae and marine magnesium oxide; SB = sodium bicarbonate.

[2]Trt = treatment effect.

Table 6. The effect of calcareous marine algae, with or without marine magnesium oxide, and sodium bicarbonate on intake, milk production, milk composition and milk efficiency in experiment 2 (milk production)

Item	Dietary treatment[1]				SEM	Trt[2] *P*-value
	CON	CMA	CMA + MM	SB		
Intake, kg/d						
DMI[3]	20.7^{x}	21.7^{xy}	20.9^{xy}	22.6^{y}	0.75	<0.10
Output, kg/d						
Milk yield	32.2^{ab,x}	33.0^{a,xy}	31.2^{b,y}	32.4^{a,xy}	0.43	<0.01
FCM[3]	30.4	32.4	31.1	31.3	1.13	0.39
ECM[3]	33.7	35.4	34.0	34.5	1.21	0.51
Milk fat and protein	2.19^{a}	2.35^{b}	2.26^{a}	2.25^{a}	0.033	<0.01
Milk fat	1.18^{a}	1.27^{b}	1.24^{b}	1.23^{ab}	0.023	<0.01
Milk protein	1.03^{a}	1.07^{b}	1.01^{a}	1.03^{a}	0.013	<0.01
Milk efficiency, kg/d						
ECM/DMI[4]	1.61^{a}	1.62^{a}	1.63^{a}	1.50^{b}	0.028	<0.01
FCM/DMI[4]	1.44^{a}	1.47^{a}	1.47^{a}	1.36^{b}	0.024	<0.01
Milk composition, %						
Protein	3.21^{ac}	3.26^{ab}	3.27^{b}	3.18^{c}	0.021	<0.01
Fat	3.68^{a}	3.87^{b}	3.93^{b}	3.87^{b}	0.037	<0.01
Lactose	4.37	4.37	4.40	4.40	0.014	0.04
Urea, mg/dL	15.8	15.3	16.1	16.5	0.75	0.73
Casein	2.48^{a}	2.50^{ab}	2.53^{b}	2.47^{a}	0.013	<0.01

$^{a–c}$Means within a row with different superscripts differ ($P < 0.05$).

^{x,y}Means within a row with different superscripts differ ($P < 0.10$).

[1]Treatments: CON = control; CMA = calcareous marine algae; CMA+ MM = calcareous marine algae and marine magnesium oxide; SB = sodium bicarbonate.

[2]Trt = treatment effect.

[3]FCM = 4% FCM.

[4]ECM/DMI = kg of ECM produced per kg of DM consumed; FCM/DMI = kg of FCM produced per kg of DM consumed.

Experiment 2 (Milk Production)

Table 6 shows the effect of CON, CMA, CMA+MM, and SB on milk production, milk composition, DMI, and milk efficiency. No significant treatment × week interactions were detected for any of the parameters presented in Table 6. Cows fed the SB treatment tended to have a greater DMI than that of cows consuming the control diet ($P < 0.10$) but were similar to the CMA and CMA+MM treatments. No difference was observed in DMI between CON, CMA, and CMA+MM.

Cows fed CON tended to have greater milk yields than cows fed CMA+MM ($P < 0.10$), and cows fed CMA+MM had lower yields than CMA ($P < 0.01$) and SB ($P < 0.01$). No differences were observed among treatments CON, CMA, and SB for milk yield. No treatment effect was found during this trial for 4% FCM or ECM. Milk fat and protein (kg/d) production increased for cows fed the CMA treatment compared with CON ($P < 0.01$), CMA+MM ($P < 0.05$), and SB ($P < 0.05$). Cows fed CMA ($P < 0.01$) and CMA+MM ($P < 0.05$) had greater milk fat yield compared with the CON treatment but were similar to each other. Cows fed SB were similar to cows fed CON, CMA, or CMA+MM, for total fat yield. Protein yield was greater in the CMA treatment group compared with CON, CMA+MM, and SB ($P < 0.01$). No difference was observed in protein yield between CON, CMA+MM, and SB treatments.

The 2 milk efficiency measurements displayed similar trends. Energy-corrected milk efficiency (kg of ECM/kg of DMI) was less in the SB treatment compared with all other treatments ($P < 0.01$). The CON, CMA, and CMA+MM were similar to each other. Fat-corrected milk efficiency (kg of 4% FCM/kg of DMI) was reduced in the SB treatment ($P < 0.01$) with all other treatments being equal.

The control cows had similar milk protein concentration to CMA and SB. However, CON had lower protein concentration than CMA+MM ($P < 0.05$). Both CMA and CMA+MM had greater milk protein concentration than SB ($P < 0.01$). Milk fat concentration of cows on the CMA, CMA+MM, and SB treatments were greater than CON ($P < 0.01$) but similar to each other.

No treatment effect ($P > 0.10$) was evident for milk lactose or urea concentration throughout the trial. Cows supplemented with CMA+MM had a greater concentration of milk casein than cows supplemented with SB ($P < 0.01$) and CON ($P < 0.05$) but similar to CMA. No difference was observed in milk casein concentration between the CON, CMA, and SB.

The average BCS across all experimental groups was 2.67 ± 0.23 (SD) at the start of trial and 2.78 ± 0.29

(SD) at the end of the trial. Because no differences were observed among treatments during the trial, BCS data are not reported.

DISCUSSION

According to the NRC (2001), lactating dairy cow diets should contain a minimum of 25% NDF (DM) with 19% coming from forage (**f-NDF**). Furthermore, a review from Mertens (1997) suggested 22% as the minimum level of peNDF in dairy cow diets to maintain a rumen pH of 6.0 and avoid SARA. The NRC (2001) also recommends a maximum of 40% NSC (DM) to maintain normal rumen function. The main components of NSC are starch and sugars; therefore, a combined starch and sugar content of less than 35% should remain within these dietary guidelines. Dietary treatments fed during experiment 1 (27.2% NDF, 19.9% f-NDF, and 32.9% starch and sugars per kg of DM) and experiment 2 (31.7–32.6% NDF, 23% f-NDF, and 28.3–29.1% starch and sugars per kg of DM) were within NRC (2001) guidelines and are representative of dairy cow diets in confined herds in northern Europe. However, other factors must also be considered, such as the rumen fermentability of the diets. Diets used in both experiments were based on rapidly degradable grain sources (>25% wheat and barley; Herrera-Saldana et al., 1990). Experiment 1 (rumen pH study) was designed to challenge the rumen buffers over a short period of time (<25 d), whereas experiment 2 (milk production study) was designed to be slightly less of a challenge to rumen pH as it was being fed over a longer period (80 d). Feeding the diets used in experiment 1 for a long period of time to a large group of cows might conceivably cause health problems and failure to complete the research. Hence the decision was made that in the production experiment (experiment 2), cows would be exposed to less dietary risk, while keeping the diets as similar as possible. There were also many similarities between the diets such as basal forage type and inclusion level. The differences in f-NDF (3%) and starch content (2%) between the 2 diets are not large, and therefore the results of experiment 1 can be extrapolated to help explain possible mechanisms for the outcomes of experiment 2. Based on the rumen pH results from experiment 1, it was then hypothesized that the diets fed during experiment 2 could lead to a reduced rumen pH, especially in the control treatment.

Rumen buffers have been used for many years to stabilize rumen pH in dairy cow diets and SB is the most commonly used (Pérez-Ruchel et al., 2014). Calcareous marine algae has also been added to lactating cow diets to prevent rumen pH depression (Bernard et al., 2014; Cruywagen et al., 2015). No previous work has been carried out on MM, but effects of conventional magnesium oxide in dairy cow diets have been examined by numerous authors (Thomas and Emery, 1969; Teh et al., 1985; Kaplan et al., 2010). According to a review conducted by Hu and Murphy (2005), SB had no effect on milk production or milk composition in diets that were based on alfalfa silage, alfalfa hay, barley silage, and wheat silage, but milk composition was improved in corn silage-based diets.

Results of the rumen pH study confirm that these diets, based on low DM perennial ryegrass silage and rapidly fermentable grains, lead to periods of low rumen pH on the CON treatment.

In experiment 1, the control diet used caused rumen pH to fall below 5.5 for significant periods each day. The results from this experiment show that the 3 dietary buffer treatments positively affected rumen pH as they reduced the time spent below pH 5.5 compared with the control. Both CMA and CMA+MM reduced the time spent below pH 5.5. This agrees with Cruywagen et al. (2015) who reported a reduction in time spent below pH 5.5 with CMA compared with SB and CON. During the 8 to 10 h and 10 to 12 h period, mean rumen pH on the CON reached levels below pH 5.5. The 3 buffer treatments proved effective at preventing a drop in rumen pH, experienced by the CON cows. Among the buffer treatments, CMA and CMA+MM were most effective in maintaining high rumen pH with a greater daily mean rumen pH, a reduction in hours below pH 5.5, and greater mean pH during the 2 to 4 h, 6 to 8 h, 8 to 10 h, and 10 to 12 h periods compared with CON and during the 8 to 10 h period compared with SB. This is in agreement with Cruywagen et al. (2015) where the authors reported a reduction in the time spent below rumen pH 5.5 in both the CMA and SB treatments compared with CON and in the CMA treatment compared with SB. The addition of MM to the CMA diet did not have any additional benefits in terms of buffering the rumen, demonstrated by the similarities in all rumen pH parameters. To the best of the authors' knowledge, this is the first experiment comparing CMA to CMA+MM and further work may be required to investigate why there was no additional buffering effect of MM over the CMA.

In the production study, the positive effect of SB on DMI agrees with Hu and Murphy (2005), for corn silage-based diets, and Kawas et al. (2007). However, studies carried out by Rauch et al. (2012) and Khorasani and Kennelly (2001) found no effect of SB on DMI in dairy cows. Bernard et al. (2014) observed that SB increased DMI in the last 2 wk of a 10-wk trial comparing CMA and SB to a control diet in early lactation dairy cows. According to Russell and Chow (1993), increased DMI observed when feeding SB is due to increased osmotic

pressure in the rumen leading to increased water intake and fluids leaving the rumen quicker, and in turn the rate of passage in the rumen is increased, leading to a larger DMI. Work by Pérez-Ruchel et al. (2014) reported that SB reduced rumen retention time, which helps to validate the theory proposed by Russell and Chow (1993).

We observed a reduction in milk yield with CMA+MM compared with CON, CMA, and SB. Previous work carried out by Cruywagen et al. (2015) demonstrated that CMA increased milk yield in lactating dairy cows by 4.2 kg/d compared with control and 2.7 kg/d compared with the SB-supplemented diet. However, Bernard et al. (2014) reported no difference in milk yield between CMA, SB, or the control diet in early lactation dairy cows. In the present study, CMA was similar to CON and SB in milk yield, which is in agreement with Bernard et al. (2014). Greater forage and NDF content of the diets used in the present study (45.7% DM forage and 31–32.6% DM NDF) may be the reason why we observed no significant difference in milk yield between CON, CMA, and SB compared with the work of Cruywagen et al. (2015) who fed CMA and SB using a much lesser forage (30% DM) and lesser NDF (25% DM) control diet. Cruywagen et al. (2015) reported a mean rumen pH, over a 24-h period, of 5.56 on the control treatment, which is lower than the overall mean rumen pH of 5.69 for the 12-h period post-feeding for the control in this experiment.

No effect was observed of CMA, CMA+MM, or SB on 4% FCM or ECM in the current experiment, which is in agreement with Bernard et al. (2014). Despite this, Cruywagen et al. (2015) reported an increase in both 4% FCM and ECM with CMA compared with SB and control. Khorasani and Kennelly (2001) also reported increased production of 4% FCM when SB was added to the diet of late-lactation dairy cows consuming a low-forage (25% DM) diet.

Supplementation of CMA resulted in greater milk fat and protein production (milk fat yield + milk protein yield) during this experiment. This increase can be attributed to an improvement in milk components while not negatively affecting milk yield. Increased milk fat and protein production with CMA may be due to improvements in rumen fermentation as indicated in the mean rumen pH differences between CMA, SB, and CON during experiment 1 of this study and previously demonstrated by Cruywagen et al. (2015) where CMA reduced the time spent below pH 5.5 in the rumen.

In the current study, SB had reduced milk protein concentration compared with CMA and CMA+MM. The CMA+MM treatment improved the milk protein concentration compared with CON, but CMA was similar to CON. When cows were fed a SARA-inducing diet, by feeding a high level of starch or by particle size deficiency in the diet, milk protein concentration was increased, and milk fat concentration decreased (Plaizier et al., 2008). However, there is no evidence of rumen buffers reducing milk protein concentration. Similar work carried out to investigate the effects of CMA and SB by Cruywagen et al. (2015) showed no difference in milk protein concentration. Furthermore, Bernard et al. (2014) reported that the control diet tended to increase milk protein concentration compared with CMA and SB. Milk protein concentration can often be improved in dairy cows when diets containing high levels of fermentable OM or starch are consumed, likely due to an increase in rumen available energy, resulting in greater microbial protein synthesis and AA supply to the mammary gland (Zhao et al., 2016). The degradation of OM in the rumen is dependent on the activity of the rumen microbial population, which, in turn, is reliant on a stable rumen pH. According to Strobel and Russell (1986), the efficiency of microbial protein synthesis is reduced as rumen pH decreases from pH 7.0 to 6.0. Hoover and Stokes (1991) also summarized work to demonstrate the importance of rumen pH for optimum microbial protein synthesis, reporting that a drop in rumen pH from pH 6.5 to pH 5.5 reduced the effectiveness of microbial protein synthesis. This effect can be further explained by Sinclair et al. (1995) who stated that consequences of low rumen pH on microbial protein synthesis is due to an increased energy reliant discharge of protons at lower pH and a diversion of energy to nongrowth activities. Therefore, differences in rumen pH, observed during experiment 1, may be one of the reasons why CMA+MM increased milk protein concentration over the control diet and why CMA and CMA+MM had greater milk protein concentration compared with SB. Kennelly et al. (1999) reported that the addition of SB to a diet containing 75% DM of concentrate fed to mid-lactation dairy cows reduced milk protein concentration compared with the nonsupplemented diet. This agrees with results of the present experiment. The reason for this reduction in milk protein concentration could also be explained by the theory of Russell and Chow (1993) who proposed that increased rate of passage caused by feeding SB reduced the amount of starch degradation in the rumen leading to lesser propionate production. However, it must also be mentioned that some authors reported no negative effects on milk protein concentration when SB was supplemented to lactating dairy cows (Khorasani and Kennelly, 2001; Rauch et al., 2012).

Results from the current production study (experiment 2) study demonstrated a positive effect of using the 3 buffer treatments, CMA, CMA+MM, and SB, on increasing milk fat concentration. Low milk fat concen-

tration is commonly associated with low rumen pH in dairy cows (Plaizier et al., 2014). Allen (1997) demonstrated the effect of NDF on increasing rumen pH and how rumen pH was positively correlated with milk fat concentration. The effect of rumen buffers on milk fat concentration in lactating dairy cows has been investigated in detail over the years. According to Erdman (1988), rumen buffers have a positive influence on milk fat concentration, but their effects are less pronounced in diets containing greater than 30% DM of forage. A meta-analysis conducted by Hu and Murphy (2005) suggested that SB induced greater levels of milk fat concentration in lactating dairy cows consuming corn silage-based diets. Studies by Rauch et al. (2012) along with Khorasani and Kennelly (2001) also reported that SB could prevent low milk fat concentration due to its positive effect on rumen pH. When Cruywagen et al. (2015) previously investigated CMA and SB, they obtained similar results to this experiment when both CMA and SB increased milk fat concentration compared with CON. Bernard et al. (2014) found no effect of CMA or SB on milk fat concentration when added to an early lactation dairy cow diet containing 49% DM forage. The mean rumen pH results of this study demonstrate an increasing effect of all buffer treatments compared with the CON during the 2 to 4 h period following feeding, following the same trend as milk fat concentration. Milk fat yield in this experiment was also influenced by the presence of a rumen buffer, with CMA and CMA+MM having better performance compared with controls and SB being intermediate as it did not differ from CON.

The rumen biohydrogenation theory, as proposed by Bauman and Griinari (2003), suggests that low milk fat concentration is a result of dietary conditions that alter pathways of the rumen biohydrogenation of fatty acids to produce intermediates that are effective inhibitors of milk fat synthesis. Fuentes et al. (2009) established that the levels of intermediates produced from incomplete biohydrogenation in the rumen is greatly increased as the rumen pH dropped from pH 6.4 to 5.6. Furthermore, Van Nevel and Demeyer (1996) demonstrated that the process of rumen biohydrogenation was greatly reduced below pH 6.0. The *Butyrivibrio* species of ruminal bacteria are thought to be of major importance to rumen biohydrogenation (Jenkins et al., 2008). Work by Russell and Dombrowski (1980) demonstrated how the growth pattern of *Butyrivibrio fibrisolvens* was diminished at a pH less than 5.7. Thus, it is possible that this phenomenon occurred for cows fed the CON in this experiment.

In our findings, CMA+MM had greater milk casein concentration compared with CON and SB. This followed a similar trend to crude milk protein concentration and may be due to the fact that casein is the largest component of crude milk protein. Milk casein can be used to make specific dairy products such as cheese. To the best of the authors' knowledge, no reports of a rumen buffer affecting milk casein concentration are present in the literature.

In the current study, 2 measurements were used to report milk efficiency: (1) FCM as a proportion of total DMI and (2) ECM as a proportion of total DMI. We observed similar trends for both measurements. Sodium bicarbonate reduced FCM efficiency and ECM efficiency compared with CON, CMA, and CMA+MM. Feed efficiency can be defined as the fraction of feed energy captured in saleable products and allows producers to use their resources more effectively, with the possibility of increasing economic profitability while also maintaining the environment. As already mentioned in this section, Russell and Chow (1993) proposed that SB promoted an increased rate of passage, therefore reducing the time feed particles spend in the rumen. This can lead to a reduction in nutrient utilization, particularly in the rumen, when SB is supplemented. This may then have prompted the negative effect on feed efficiency as cows fed SB tended to consume more feed than CON, CMA, and CMA+MM while producing similar levels of ECM and FCM. Previous work by Cruywagen et al. (2015) agreed with the results of the present study as they also reported a negative effect of SB on feed efficiency (4% FCM/DMI) compared with CMA. Bernard et al. (2014) also observed a negative response of SB on feed efficiency (ECM/DMI) compared with CMA in the last 2 wk of a 10-wk trial using early lactation dairy cows.

CONCLUSIONS

The addition of rumen buffering products can increase rumen pH, milk fat, and protein production in lactating dairy cows fed a diet containing 45.7% DM forage, with typical forages and feeds of the northern European region. Calcareous marine algae, SB, and CMA in combination with MM were all successful at increasing milk fat concentration, although SB failed to affect total fat yield. Calcareous marine algae in combination with MM increased milk protein concentration, whereas SB had a negative effect on milk protein concentration. Supplementing diets with CMA can increase the production of total milk fat and protein in lactating dairy cows. The addition of MM to diets already containing CMA may be of limited benefit to milk production parameters, based on the outcomes of these experiments. This may be of significant importance to dairy producers as milk pricing based on components is becoming common across Europe. Our research also illustrates that the

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increased DMI reported with feeding SB negatively affected feed efficiency in lactating dairy cows.

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REFERENCES

Allen, M. S. 1997. Relationship between fermentation acid production in the rumen and the requirement for physically effective fiber. J. Dairy Sci. 80:1447–1462. https://doi.org/10.3168/jds.S0022-0302(97)76074-0.

AOAC. 1970. Official methods of analysis. 11th ed. Association of Official Analytical Chemists, Washington, DC.

Bach, A., I. Guasch, G. Elcoso, J. Duclos, and H. Khelil-Arfa. 2018. Modulation of rumen pH by sodium bicarbonate and a blend of different sources of magnesium oxide in lactating dairy cows submitted to a concentrate challenge. J. Dairy Sci. 101:9777–9788. https://doi.org/10.3168/jds.2017-14353.

Bauman, D. E., and J. M. Griinari. 2003. Nutritional regulation of milk fat synthesis. Annu. Rev. Nutr. 23:203–227. https://doi.org/10.1146/annurev.nutr.23.011702.073408.

Beauchemin, K. A., and W. Z. Yang. 2005. Effects of physically effective fiber on intake, chewing activity, and ruminal acidosis for dairy cows fed diets based on corn silage. J. Dairy Sci. 88:2117–2129. https://doi.org/10.3168/jds.S0022-0302(05)72888-5.

Bernard, J. K., J. W. West, N. Mullis, Z. Wu, and S. J. Taylor. 2014. Evaluation of calcareous marine algae supplements on production and metabolic parameters of early lactation dairy cows. Prof. Anim. Sci. 30:649–656. https://doi.org/10.15232/pas.2014-01339.

Birch, G. G., and O. M. Mwangelwa. 1974. Colorimetric determination of sugars in sweetened condensed milk products. J. Sci. Food Agric. 25:1355–1362.

Cruywagen, C. W., S. Taylor, M. M. Beya, and T. Calitz. 2015. The effect of buffering dairy cow diets with limestone, calcareous marine algae, or sodium bicarbonate on ruminal pH profiles, production responses, and rumen fermentation. J. Dairy Sci. 98:5506–5514. https://doi.org/10.3168/jds.2014-8875.

Edmondson, A. J., I. J. Lean, L. D. Weaver, T. Farver, and G. Webster. 1989. A body condition scoring chart for Holstein dairy cows. J. Dairy Sci. 72:68–78. https://doi.org/10.3168/jds.S0022-0302(89)79081-0.

Enemark, J. M. D. 2008. The monitoring, prevention and treatment of sub-acute ruminal acidosis (SARA): A review. Vet. J. 176:32–43. https://doi.org/10.1016/j.tvjl.2007.12.021.

Erdman, R. A., R. Hemken, and L. Bull. 1982. Dietary sodium bicarbonate and magnesium oxide for early postpartum lactating dairy cows: Effects of production, acid-based metabolism, and digestion. J. Dairy Sci. 65:712–731. https://doi.org/10.3168/jds.S0022-0302(82)82259-5.

Erdman, R. A. 1988. Dietary buffering requirements of the lactating dairy cow: A review. J. Dairy Sci. 71:3246–3266. https://doi.org/10.3168/jds.S0022-0302(88)79930-0.

Fuentes, M. C., S. Calsamiglia, P. Cardozo, and B. Vlaeminck. 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. https://doi.org/10.3168/jds.2008-1722.

Gaines, W. L. 1928. The energy basis of measuring milk yield in dairy cows. Ill. Agric. Exp. Sta. Bull. 308:403–436.

Gozho, G. N., J. C. Plaizier, D. O. Krause, A. D. Kennedy, and K. M. Wittenberg. 2005. Subacute ruminal acidosis induces ruminal lipopolysaccharide endotoxin release and triggers an inflammatory response. J. Dairy Sci. 88:1399–1403. https://doi.org/10.3168/jds.S0022-0302(05)72807-1.

Herrera-Saldana, R., J. Huber, and M. Poore. 1990. Dry matter, crude protein, and starch degradability of five cereal grains. J. Dairy Sci. 73:2386–2393. https://doi.org/10.3168/jds.S0022-0302(90)78922-9.

Hoover, W. H., and S. Stokes. 1991. Balancing carbohydrates and proteins for optimum rumen microbial yield. J. Dairy Sci. 74:3630–3644. https://doi.org/10.3168/jds.S0022-0302(91)78553-6.

Hu, W., and M. R. Murphy. 2005. Statistical evaluation of early- and mid-lactation dairy cow responses to dietary sodium bicarbonate addition. Anim. Feed Sci. Technol. 119:43–54. https://doi.org/10.1016/j.anifeedsci.2004.12.005.

Jenkins, T. C., R. J. Wallace, P. J. Moate, and E. E. Mosley. 2008. Recent advances in biohydrogenation of unsaturated fatty acids within the rumen microbial ecosystem: A review. J. Anim. Sci. 86:397–412. https://doi.org/10.2527/jas.2007-0588.

Kaplan, O., S. Deniz, M. A. Karsli, H. Nursoy, and M. Avci. 2010. Effects of sodium bicarbonate, magnesium oxide and dried sugar beet pulp in diets of dairy cows on milk yield, milk composition and rumen fluid and some blood parameters. J. Anim. Vet. Adv. 9:1570–1574.

Kawas, J. R., R. García-Castillo, H. Fimbres-Durazo, F. Garza-Cazares, J. F. G. Hernández-Vidal, E. Olivares-Sáenz, and C. D. Lu. 2007. Effects of sodium bicarbonate and yeast on nutrient intake, digestibility, and ruminal fermentation of light-weight lambs fed finishing diets. Small Rumin. Res. 67:149–156. https://doi.org/10.1016/j.smallrumres.2005.09.010.

Kennelly, J. J., B. Robinson, and G. R. Khorasani. 1999. Influence of carbohydrate source and buffer on rumen fermentation characteristics, milk yield, and milk composition in early-lactation Holstein cows. J. Dairy Sci. 82:2486–2496. https://doi.org/10.3168/jds.S0022-0302(99)75500-1.

Khorasani, G. R., and J. J. Kennelly. 2001. Influence of carbohydrate source and buffer on rumen fermentation characteristics, milk yield, and milk composition in late-lactation Holstein cows. J. Dairy Sci. 84:1707–1716. https://doi.org/10.3168/jds.S0022-0302(01)74606-1.

Kleen, J. L., L. Upgang, and J. Rehage. 2013. Prevalence and consequences of subacute ruminal acidosis in German dairy herds. Acta Vet. Scand. 55:48. https://doi.org/10.1186/1751-0147-55-48.

Lancashire, P. D., H. Bleiholder, T. V. D. Boom, P. Langeluddeke, R. Stauss, E. Weber, and A. Witzenberger. 1991. A uniform decimal code for growth stages of crops and weeds. Ann. Appl. Biol. 119:561–661.

Mertens, D. R. 1997. Creating a system for meeting the fiber requirements of dairy cows. J. Dairy Sci. 80:1463–1481. https://doi.org/10.3168/jds.S0022-0302(97)76075-2.

Mulligan, F. J., P. J. Caffrey, M. Rath, J. J. Callan, P. O. Brophy, and F. P. O'Mara. 2002. An investigation of feeding level effects on digestibility in cattle for diets based on grass silage and high fibre concentrates at two forage:concentrate ratios. Livest. Prod. Sci. 77:311–323. https://doi.org/10.1016/S0301-6226(02)00030-1.

NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Press, Washington, DC.

O'Grady, L., M. L. Doherty, and F. J. Mulligan. 2008. Subacute ruminal acidosis (SARA) in grazing Irish dairy cows. Vet. J. 176:44–49. https://doi.org/10.1016/j.tvjl.2007.12.017.

Pérez-Ruchel, A., J. Repetto, and C. Cajarville. 2014. Use of $NaHCO_3$ and MgO as additives for sheep fed only pasture for a restricted period of time per day: effects on intake, digestion and the rumen environment. J. Anim. Physiol. Anim. Nutr. (Berl.) 98:1068–1074. https://doi.org/10.1111/jpn.12173.

Plaizier, J. C., D. O. Krause, G. N. Gozho, and B. W. McBride. 2008. Subacute ruminal acidosis in dairy cows: The physiological causes, incidence and consequences. Vet. J. 176:21–31. https://doi.org/10.1016/j.tvjl.2007.12.016.

Plaizier, J. K., S. Li, G. Gozho, and E. Khafipour. 2014. Minimizing the risk for rumen acidosis. Pages 11–26 in Proc. 23rd Tri-State

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Dairy Nutrition Conference, Fort Wayne, IN. Ohio State University.

Rauch, R. E., P. H. Robinson, and L. J. Erasmus. 2012. Effects of sodium bicarbonate and calcium magnesium carbonate supplementation on performance of high producing dairy cows. Anim. Feed Sci. Technol. 177:180–193. https://doi.org/10.1016/j.anifeedsci.2012.08.016.

Russell, J. B., and J. M. Chow. 1993. Another theory for the action of ruminal buffer salts: Decreased starch fermentation and propionate production. J. Dairy Sci. 76:826–830. https://doi.org/10.3168/jds.S0022-0302(93)77407-X.

Russell, J. B., and D. Dombrowski. 1980. Effect of pH on the efficiency of growth by pure cultures of rumen bacteria in continuous culture. Appl. Environ. Microbiol. 39:604–610.

Schutz, M. M. 1994. Genetic evaluation of somatic cell scores for United States dairy cattle. J. Dairy Sci. 77:2113–2129. https://doi.org/10.3168/jds.S0022-0302(94)77154-X.

Sinclair, L., P. Garnsworthy, J. Newbold, and P. Buttery. 1995. Effects of synchronizing the rate of dietary energy and nitrogen release in diets with a similar carbohydrate composition on rumen fermentation and microbial protein synthesis in sheep. J. Agric. Sci. 124:463–472. https://doi.org/10.1017/S0021859600073421.

Strobel, H. J., and J. B. Russell. 1986. Effect of pH and energy spilling on bacterial protein synthesis by carbohydrate-limited cultures of mixed rumen bacteria. J. Dairy Sci. 69:2941–2947. https://doi.org/10.3168/jds.S0022-0302(86)80750-0.

Teh, T. H., R. Hemken, and R. Harmon. 1985. Dietary magnesium oxide interactions with sodium bicarbonate on cows in early lactation. J. Dairy Sci. 68:881–890. https://doi.org/10.3168/jds.S0022-0302(85)80905-X.

Thomas, J., and R. Emery. 1969. Additive nature of sodium bicarbonate and magnesium oxide on milk fat concentrations of milking cows fed restricted-roughage rations. J. Dairy Sci. 52:1762–1769. https://doi.org/10.3168/jds.S0022-0302(69)86838-4.

Tyrrell, H. F., and J. T. Reid. 1965. Prediction of the energy value of cow's milk. J. Dairy Sci. 48:1215–1223.

Van Nevel, C. J., and D. Demeyer. 1996. Influence of pH on lipolysis and biohydrogenation of soybean oil by rumen contents in vitro. Reprod. Nutr. Dev. 36:53–63.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583–3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2.

Whelan, S. J., F. J. Mulligan, J. J. Callan, B. Flynn, and K. M. Pierce. 2013. Effect of forage source and a supplementary methionine hydroxyl analogue on rumen fermentation parameters in lactating dairy cows offered a low crude protein diet. Anim. Feed Sci. Technol. 183:62–66. https://doi.org/10.1016/j.anifeedsci.2013.04.017.

Zadoks, J. C., T. T. Chang, and C. F. Konzak. 1974. A decimal code for the growth stages of cereals. Weed Res. 14:415–421.

Zhao, M., D. Bu, J. Wang, X. Zhou, D. Zhu, T. Zhang, J. Niu, and L. Ma. 2016. Milk production and composition responds to dietary neutral detergent fiber and starch ratio in dairy cows. Anim. Sci. J. 87:756–766. https://doi.org/10.1111/asj.12482.