

Evaluación de dos buffers ruminales comerciales a base de algas calcáreas para vacas lecheras a pastoreo: Ensayo piloto en granjas comerciales

Evaluation of two commercially available rumen buffers derived from calcified seaweed for grazing dairy cows: Pilot trial in commercial farms

Rivero, J.M.,¹ Merino, V.M.,^{2,*} Plaza, J.E.,³ Pena, J.⁴

¹ Rothamsted Research, North Wyke, Okehampton, Devon EX20 2SB, UK

² Department of Animal Production, Faculty of Agronomy, Universidad de Concepción, PO Box 160-C, Concepción 4030000, Chile

³ Anasac Chile S.A., Trébol Sur, Ruta U-55 s/n, Osorno 5290000, Chile

⁴ Devenish Nutrition Limited, 96 Duncrue Street. Belfast, BT3 9AR, UK.

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*Corresponding author:

Merino, V.M.

E-mail address:

veronicamerino@udec.cl

ABSTRACT

Calcified seaweed (CS) based products have been proposed as an alternative ruminal pH buffer to sodium bicarbonate. It has been reported that magnesium can aid in ruminal pH control too, especially those natural marine forms. We assessed the rumen buffer capacity and milk production and milk composition of two CS: Acid Buf (AB) compared with M12 (a natural marine magnesium enriched CS) on grazing dairy cows in a replicated Latin Square design involving 2 farms with the dairy herd in good sanitary and health status, 2 supplements and 4 measurements periods of one month each. Holstein Friesian dairy cows fed with a partial mixed ration (PMR) were supplemented for 30-d periods with either AB or M12. Total milk production and milk composition were measured in each farm, and five cows in each herd had rumen boluses for the monitoring of ruminal pH, allowing us to identify and monitor total subacute ruminal acidosis (SARA) events (i.e., = 2 h with pH < 5.8). The average pH during the experimental period was 2% greater ($p < 0.05$) and the number of SARA events per cow was lower ($p = 0.007$) for cows supplemented with M12 compared to AB. If only the cows that developed SARA events were considered, M12 showed a trend ($p = 0.068$) to reduce the number of SARA events and their duration compared to those cows supplemented with AB. Milk production and composition were similar between both rumen buffers at all periods.

RESUMEN

Los productos a base de algas marinas calcificadas (CS) se han propuesto como un tampón de pH ruminal alternativo al bicarbonato de sodio. Se ha reportado que el magnesio puede ayudar en el control del pH ruminal, especialmente las formas naturales marinas. Evaluamos la capacidad amortiguadora del pH del rumen y la producción y composición de la leche de dos CS: Acid Buf (AB) y M12 (un CS natural enriquecido con magnesio marino), en vacas lecheras en pastoreo en un diseño de cuadrado latino replicado que involucró 2 granjas con rebaños lecheros en buen estado sanitario y de salud, 2 suplementos y 4 periodos de evaluación de un mes cada uno. Vacas lecheras Holstein Friesian se alimentaron con una ración parcial mixta (PMR) y se suplementaron durante cuatro períodos de 30 días cada uno con AB o M12. Se midió la producción total y la composición de la leche en cada granja y cinco vacas en cada rebaño recibieron bolos ruminales para monitorear el pH ruminal, permitiéndonos identificar y monitorear eventos de acidosis ruminal subaguda total (SARA) (es decir = 2 h con pH < 5.8). El pH promedio durante el período experimental fue 2% mayor ($p < 0,05$) y el número de eventos SARA por vaca fue menor ($p = 0,007$) para vacas suplementadas con M12 en comparación con AB. Considerando solo las vacas que desarrollaron eventos SARA, M12 tendió ($p = 0,068$) a reducir el número de eventos de SARA y su duración en comparación con aquellas vacas suplementadas con AB. La producción y composición de la leche fueron similares entre ambos tampones ruminales en todos los períodos.

Palabras clave: M12; Acid Buf; acidosis ruminal subaguda; algas calcáreas marinas; óxido de magnesio marino.

INTRODUCTION

Diets high in grain or other rapidly fermentable (non-fibre) carbohydrates are offered to deliver adequate energy supply to increase milk production and microbial protein synthesis in the rumen, and therefo-

re increase milk protein production of high producing dairy cows (Bargo et al., 2003). Providing such diets with limited amounts of effective fibre frequently reduces ruminal pH below 5.6, increasing the risk of ruminal acidosis in two forms, i.e., acute or subacute ruminal acidosis (SARA) (Krause and Oetzel, 2006; Zhao et al.,

2018), depending on factors such as ruminal pH threshold, predominant acid (volatile fatty acids or lactic acid), and ruminal population bacteria (Hernández et al., 2014). SARA is described as a disorder of high prevalence in commercial dairy cows (Dohme et al., 2008) which is defined by a moderate rumen pH depression (between 5.2–5.6) for more than 3 consecutive hours per day and variable clinical signs (Gozho et al., 2005). Ruminal acidosis has significant impacts on microbial activity, rumen function, fibre digestion (Mulligan et al., 2002), animal production and health, being associated with several metabolic diseases including laminitis and liver abscesses that eventually may increase culling rates on a herd-level (Nagaraja and Lechtenberg, 2007; Rossi et al., 2019).

A wide variety of dietary buffers mostly mineral salts such as sodium bicarbonate, sodium bentonite and calcium carbonate are commonly added to stabilize the rumen pH of lactating dairy cows fed diets with high-energy and low-fibre content. These buffers improve the productivity and the animal health status (Enemark, 2008; Krause and Oetzel, 2006), with different responses between animals depending upon the type and dose of the buffer used (Sen et al., 2006). Calcium is considered an important mineral component in buffering supplements, with sodium bicarbonate as the most common calcium source used to buffer the rumen pH of ruminants (Hu and Murphy, 2005; Jallow and Hsia, 2014). Sodium bicarbonate has been successful in alleviating the symptoms of SARA, especially the drop in dry matter intake (DMI) and milk fat depression (Rauch et al., 2012). However, it is rapidly solubilized in the rumen and, therefore, it cannot effectively buffer a continuous production of acids in the rumen (Krause, 2008).

Calcified seaweed (CS, i.e. dead remains of the red algae *Lithothamnium corraloides* (P.Crouan & H.Crouan) or *Phytomalithion calcareum* (Pallas)) based products consist mainly of calcium carbonate have been proposed as an alternative to sodium bicarbonate, characterized by a singular molecular composition and structures, which increases their buffering capacity (Cruywagen et al., 2015). It is composed of multiple minerals including calcium (300 g kg^{-1}), magnesium (55 g kg^{-1}), potassium (7 g kg^{-1}) and other trace minerals (Cruywagen et al., 2015). Calcium in the form of calcium carbonate is a more effective rumen buffer than bicarbonate because carbonate ion (CO_3^{2-}) can react with two protons (H^+) while bicarbonate ion (HCO_3^-) can react with one H^+ (Sgoifo Rossi and Compiani, 2016). The addition of seaweed-derived calcium supplements in the diet of mid-lactation dairy cows have shown to increase milk production and milk production efficiency (kg of milk per kg of DMI) (Cruywagen et al., 2015), and improve milk fat and protein production in a low forage and low fibre con-

tent diet compared with sodium bicarbonate (Neville et al., 2019). Magnesium can aid in ruminal pH control due to its alkaline properties and have a vital role in animal metabolism as an enzymatic cofactor of various enzymes and its influence in energy metabolism, protein synthesis, cell growth and reproduction, DNA and RNA synthesis and stabilization of mitochondrial membranes (Schonewille and Beynen, 2005). Maintenance of normal plasma Mg concentration of cows is almost dependent on its ruminal absorption, which in turn relies on the solubility of the supplemental source of Mg (Goff, 2018). In this regard, magnesium oxide (MgO) is the most common supplemental source of Mg due to its high ruminal solubility and absorption, and another effective buffer used to alleviate pH depressions in dairy cow diets (Cruywagen et al., 2015; NRC, 2001). It can sustain better the pH fluctuations in the rumen when compared to sodium bicarbonate in dairy cows subjected to a high concentrate diet (Bach et al., 2018), and may improve milk fat content as it increases the uptake of plasma acetate and triglycerides by the mammary gland (Srivastava et al., 2021). The bioavailability and intestinal absorption of magnesium are influenced by solubility, food matrix and dose (Schuchardt and Hahn, 2017). In this sense, the seaweed-derived magnesium provides increased bioavailable magnesium levels for gut health (Crowley et al., 2018) probably because of the three-dimensional structure of calcareous marine algae (CMA) which results in a slow release of minerals in an acid environment (Rauch et al., 2012). Therefore, we hypothesized that the use of enriched marine magnesium oxide (precipitated magnesia derived from seawater; Neville et al., 2019) in combination with seaweed CS as ruminal buffer supplements would increase the buffer capacity and reduce SARA while improving milk production and composition. This pilot trial aimed to compare the rumen buffer capacity, milk production and milk composition of AB (Acid Buf, Celtic Sea Minerals, Cork, Ireland; Calitz, 2009) and M12 (Devenish Nutrition Ltd., UK) on grazing dairy cows in commercial farms.

MATERIALS AND METHODS

The methodology used in this study followed the guidelines of the Committee of Ethical, Bioethical and Biosecurity of the Universidad de Concepción, Chile. Ethical review and approval were granted under the certificate CEBB 1069-2021.

Farms and Animals

The field trials were carried out in two commercial farms (Farm 1 and Farm 2) located in the Los Lagos Region ($40^{\circ}37'S$ $73^{\circ}09'W$, south of Chile), and lasted

19 weeks (October 2019 – February 2020). The farms and dairy herds were in good sanitary and health status (i.e. tuberculosis, brucellosis and leukosis free) and were selected based on their similar annual milk yield (both herds had achieved 9,000 litres of milk in the last lactation) and milk composition (total milk solids of 7.8% as well as 4.2% of milk fat, and 3.7% of milk protein on average). In each dairy farm, five multiparous dairy cows (between second and third calving) were selected out of 700 multiparous lactating dairy cows on average. The selected animals were producing between 7,500 and 8,000 L per lactation and between 5 to 10 days in milk. The effect of the treatment on each animal and herd was measured considering that all cows will be susceptible at any stage, but the standardization of the experimental design would be able to neutralize all variables including animal body condition score, days in milking, productive and reproductive stage, number of calving and the diet itself. A smart intraruminal bolus providing real-time pH information was placed in those selected cows. The entire population of 1,400 milking cows was used to determine the effect of the two buffer ruminal supplements.

Diets and Feeds

Cows in both farms grazed a *Lolium perenne* L. pasture ("Base" variety) during the whole period, and the expected dry matter intake (DMI) at the beginning of the trial (15th October – Control period) was 12 kg cow⁻¹ day⁻¹, decreasing gradually to 8 kg cow⁻¹ day⁻¹ towards the end of the experiment (25th February). The cows were under a partial mixed ration (PMR) program which was offered at the parlour during milking time. The PMR was mainly composed of grass silage (ryegrass and triticale silages), steam flakes rolled, maize silage, wheat straw, salts with live yeast, cosetan (beet pulp dry + wheat bran, 50:50) and soybean meal (51% crude protein). Diets differed between farms in terms of the average amount offered of each feed (forage and PMR) and the average DMI throughout the experiment for each farm was 20.5 kg cow⁻¹ day⁻¹.

Feed samples were collected once a month from November 2019 according to the number of trial periods,

totalizing four samples in each farm. Average nutritional values for the main diet components are shown in Table 1. A sample of the cow's drinking water was collected on 18th December from each farm and sent to the laboratory for pH measurement. The water pH values for Farms 1 and 2 were 7.37 and 6.57 (both values within the preferred range).

Treatments and experimental design

The two treatments consisted of the different rumen buffer additives: M12, a commercial natural magnesium enriched calcified seaweed (250 g kg⁻¹ Ca, 120 g kg⁻¹ Mg, 0.3 g kg⁻¹ P⁻¹) commercialised by Devenish Nutrition Ltda., United Kingdom, which in this case we will call "M12" and the other is Acid Buf (300 g kg⁻¹ Ca, 55 g kg⁻¹ Mg, 0.58 g kg⁻¹ P), commercialized by Celtic Sea Minerals, Ireland, abbreviated here as "AB" (Table 2). The farm was considered the experimental unit (n = 2). The treatments were applied to the experimental units in a replicated Latin Square design with four periods (Table 3).

A short period of 10 days, where cows were not supplemented with any ruminal buffer products, was used to measure the baseline levels (control) of the response variables before the experimental periods. Then, each experimental period was intended to last 30 days (15 days of adaptation to the additive and 15 days of

Table 2. Main chemical constituents of the feed additives M12 and AB as % of ash.

Cuadro 2. Principales constituyentes químicos de los aditivos dietarios M12 y AB como % de cenizas.

Element	M12	AB
Calcium (Ca)	25	30
Magnesium (Mg)	12	3.5
Sodium (Na)	0.4	0.8
Crude Ash	95	95
Moisture	1	<5

Table 1. Nutritional composition of feeds (% dry matter otherwise stated) in the experimental farms.

Cuadro 1. Composición nutricional de los alimentos en las granjas experimentales.

Farm	Feed	Dry matter (g kg ⁻¹)	Crude protein	NDF	Sugars	Starch	Ca	P	Mg
Farm 1	Pasture	154	22.1	40.7	10.8	2.4	0.655	0.345	0.185
	PMR	537	11.4	34.0	6.9	22.4			
Farm 2	Pasture	170	19.6	39.8	14.3	2.6	0.753	0.323	0.233
	PMR	543	14.5	32.6	5.7	22.5			

Table 3. Periods for the two treatments applied to the farms.

Cuadro 3. Períodos de los dos tratamientos aplicados a las granjas.

Period (P)	Farm 1	Farm 2
P1 (October-November)	AB	M12
P2 (November-December)	M12	AB
P3 (December-January)	AB	M12
P4 (January-February)	M12	AB

measurements per period). The rumen buffer additive was supplied to the cows in the mineral mix offered at the milking parlour: 75 g cow⁻¹ day⁻¹ of AB and 50 g cow⁻¹ day⁻¹ of M12 following the manufacturer's dose recommendation.

Three aspects of the experimental protocol were not met: i) most of the periods did not last 30 days each (range: 26-40 days), ii) diets slightly differed between farms, and iii) only the high-yielding cows (400-410 cows) received the treatment in the farm 2 (712 cows received the treatment in farm 1). Nevertheless, the statistical model applied was able to identify and isolate the effect of each additive in each farm (i.e. the difference in milk yield of the cow's diet) and each period. Regarding the duration of every period, the data used for the analysis were those collected from the 16th day after the start of the treatment, i.e., the experimental period, to allow the rumen to become accustomed to the new additive and metabolic conditions (i.e. wash-out period).

Measurements

Milk production and its composition (milk fat, milk protein and urea concentrations) were obtained daily from the bulk milk tanks (daily reports supplied by the milk processor and measured by infrared spectrophotometry (Milkoscan, System 4300 Foss Electric, Hillerød, Denmark). Average daily individual milk production was calculated by dividing the total volume produced each day by the number of cows milked. Individual milk solids production (milk fat, milk protein and their sum) was calculated by multiplying the percentage of each solid by the daily average of milk produced (volume). This procedure was applied to both farms.

Rumen boluses (smaXtec v.4.7.8, Graz, Austria) were administered to 5 randomly selected cows in each commercial farm on 1st October, totalizing 10 smart boluses in the whole experimental herd. Therefore, the rumen pH was recorded every 10 minutes during the next 5 months, providing precise information for every treatment and experimental time. Fif-

teen days after the ruminal boluses were placed, and ~8 days before the first period started (initial ruminal buffer additive supplementation), data were recorded to characterise the pre-experimental conditions of the herds. Blood samples were collected from these cows on periods 3 and 4 and analysed for phosphorus (P), calcium (Ca) and magnesium (Mg) concentration in plasma for each treatment group using commercial reagents (Phosphorus liquicolor®, Human; Ca-color arsenazo III®, Wiener; Magnesium liquicolor®, Human, respectively), according to the established and approved protocols and methodologies of the Universidad Austral de Chile.

Statistical analysis

The data collected during each experimental period (from the 16th day of each period) were averaged across the days for each farm. At the same time, the data recorded from the ruminal boluses during the experimental periods were analysed in order to identify the total number of subacute ruminal acidosis (SARA) events (i.e. = 2 h with pH < 5.8, according to Dohme et al., 2008) and their duration.

All data were analysed as a replicated Latin Square design using Genstat 20th edition with the following structure:

1. Experimental unit: commercial farm (Farm 1 and Farm 2)
2. Rows: period (r = 1 to 4)
3. Columns: farm (c = 1 to 2)
4. Treatments: rumen buffer type (AB or M12)

RESULTS

Control Period

Milk production was similar between farms before the additives were added to the diet (mean ± SD: 25.1 ± 0.43 and 25.5 ± 0.57 L, cow⁻¹ d⁻¹ for Farm 1 and 2, respectively). Milk solids concentration seemed slightly higher for Farm 1 than for Farm 2: 3.82 ± 0.144 vs. 3.61 ± 0.095 % milk fat and 3.48 ± 0.032 vs. 3.41 ± 0.033 % protein in milk. Similar average pH values were observed for the five cows with boluses in each herd (6.16 ± 0.348 for Farm 1 and 6.18 ± 0.300 for Farm 2).

Mean pH and SARA events

Average ruminal pH during the experimental period was 2% greater (p < 0.05) every time the herd was supplemented with M12, compared to AB supplemented periods (Table 4, Figure 1). On the other hand, the proportion of cows affected by SARA (at least one event) and the duration of the SARA events were not

affected by treatments (Table 4). However, wide variability was observed (10-75%) for these variables (standard error of the difference (SED) = 20% and 67 min, respectively).

Regarding the number of SARA events per cow that occurred in a period of 15 d (calculated as the number of events recorded on the 5 cows per herd and the actual duration of each experimental period), the cows supplemented with M12 had 5 events less ($p = 0.007$) than those cows supplemented with AB (Table 4). Additionally, considering only the cows that developed SARA events, the additive M12 tended ($p = 0.068$) to reduce the number of SARA events: 6.5 SARA events less than those cows supplemented with AB (Table 4).

Milk production and composition

The effect of the period and the farm was not statistically significant for any of the variables analysed.

There was no statistical evidence to support the hypotheses that milk yield and milk composition differed between rumen buffer additives (Table 5). Although some numerical differences were observed between treatments, being higher when the cows received M12 in the diet (Table 5), the reduced number of degrees of freedom (df) of the error term in the statistical test ($df = 3$) may have prevented from finding significant differences.

Nutrients concentrations in blood plasma

Plasma concentrations of phosphorus (P), calcium (Ca) and magnesium (Mg) for each treatment group did not show differences between rumen buffer additives throughout the whole trial period, averaging 1.92, 2.57 and 1.07 mmol L⁻¹, respectively (Table 6). All the values are within the reported range for dairy cows (NRC, 2001).

Table 4. Mean pH and SARA events of dairy cows receiving two different rumen buffer additives (M12, and a commercial natural product produced from *L. calcareum* - AB).

Cuadro 4. Eventos promedio de pH y SARA de vacas lecheras que recibieron dos aditivos diferentes de tampón ruminal (M12, y un producto natural comercial producido a partir de *L. calcareum* - AB).

Additive	Mean pH	% Cows with SARA	SARA duration (min)	SARA events per cow in 15 d	SARA events per cow with SARA in 15 d
M12	6.25	25	197	1.2	5.1
AB	6.13	55	262	6.3	11.6
SED	0.028	20.0	67.0	0.42	1.80
P value	0.05	0.272	0.437	0.007	0.068

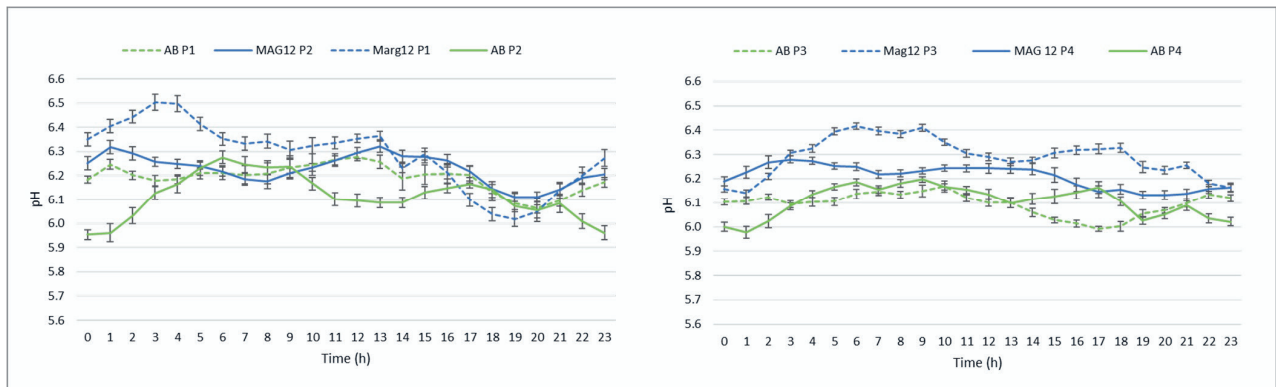


Figure 1. Hourly mean ruminal pH (average of five cows measured over the last 12 days of periods 3 (P3) and period 4 (P4); bars represent standard error of the mean) of dairy cows receiving two different rumen buffer additives (M12, and a commercial natural product elaborated from *L. calcareum* - AB).

Figura 1. Promedio de pH ruminal por hora (promedio de cinco vacas medido durante los últimos 12 días del período 3 (P3) y del período 4 (P4); las barras representan el error estándar de la media) de las vacas lecheras que reciben dos aditivos de tampón ruminal diferentes (M12, y un producto natural comercial producido a partir de *L. calcareum* - AB).

Table 5. Mean milk yield and milk components concentrations of dairy cows receiving two different rumen buffer additives (M12, and a commercial natural product produced from *L. calcareum* – AB).

Cuadro 5. Promedio de producción de leche y concentraciones de componentes de la leche de vacas lecheras que recibieron dos aditivos diferentes de tampón ruminal (M12, y un producto natural comercial producido a partir de *L. calcareum* - AB).

Additive	Milk yield (L cow ⁻¹ d ⁻¹)	Milk fat (%)	Milk protein (%)	Milk solids (%)	Milk urea (mg L ⁻¹)	Milk fat (kg cow ⁻¹ d ⁻¹)	Milk protein (kg cow ⁻¹ d ⁻¹)	Milk solids (kg cow ⁻¹ d ⁻¹)
M12	24.2	3.78	3.39	7.17	276	0.92	0.82	1.74
AB	23.6	3.83	3.42	7.25	313	0.90	0.81	1.71
SED	0.47	0.033	0.064	0.093	54.3	0.020	0.028	0.483
P value	0.315	0.290	0.698	0.500	0.570	0.594	0.672	0.638

Table 6. Phosphorus, calcium and magnesium concentration in blood plasma (mmol/L) of dairy cows receiving two different rumen buffer additives (M12, and a commercial natural product produced from *L. calcareum* – AB).

Cuadro 6. Concentración en plasma sanguíneo (mmol/L) de fósforo, calcio y magnesio de vacas lecheras que recibieron dos aditivos amortiguadores ruminales diferentes (M12, y un producto natural comercial producido a partir de *L. calcareum* – AB).

Additive	Ca	P	Mg
M12	2.54	1.79	1.08
AB	2.60	2.05	1.05
SED	0.100	0.120	0.235
P value	0.656	0.275	0.906

DISCUSSION

Average ruminal pH during the experimental period for all cows administered with the rumen buffer pH boluses was on average above the 5.5 and 5.8 pH thresholds considered for severe and total SARA conditions (Dohme et al., 2008), respectively, regardless of treatment. Despite the numerical difference (30 percentage units), more cows under AB supplementation developed SARA, and the percentage of cows affected by SARA (at least one event) did not show a statistical difference between additives and the variability was high for this variable. This variability can be explained by the fact that for M12 supplemented cows, the percentage was 20 for Periods 2 and 4, and the percentage was 0 for Period 3, but the percentage was 60 for Period 1: three cows presented at least one SARA event (two cows presented 2 SARA events which lasted less than 3 hours, and one cow presented 6 SARA events that lasted less than 4 hours; raw data not shown). However, for AB supplemented cows this percentage was always between 40 and 60% (between 2 and 3 cows had at least one SARA event that lasted on average

more than 4 h, raw data not shown). Therefore, if the number of cows with rumen boluses had been greater, a more accurate estimation of this parameter would have been possible and the difference in the percentage of cows affected by SARA might have reached statistical significance. Similarly, despite the numerical differences (1 hour and 5 minutes in favour of M12) between the mean duration of the SARA events, there was no evidence to support the hypothesis that the rumen buffer additive affected the duration of the SARA events.

The higher mean rumen pH and efficiency of M12 in preventing SARA events compared to AB could be due to its physical and chemical characteristics (Le Ruyet and Tucker, 1992) that could influence its solubility within the rumen and buffer capacity (Khorrami et al., 2021). Considering that calcite is the most abundant form of CaCO₃ in CMA (650 g kg⁻¹) and its solubility is increased in the presence of Mg (Cruywagen et al., 2015), the higher amount of magnesium and its availability in M12 when compared to AB (12% and 5.5%, respectively) could have improved the solubility of calcite within the rumen. Ideally, buffers should be providing a continuous release of minerals to avoid the increase in free proton (H⁺) concentration (Le Ruyet and Tucker, 1992). In this case, the natural structure of both additives could be responsible for these differences in terms of ruminal pH buffering and eventually in optimising the production of volatile fatty acids, as well as a more balanced profile in a challenged rumen.

Despite the differences in the mineral compositions found between the buffers used in this study, there were no statistical differences yet numerical between bioactive buffers in either the proportion of cows affected by metabolic acidosis or in the duration of rumen pH depression. This would suggest that using a lower dose of M12 might be similarly effective than AB used in a higher dose when it comes to regulating rumen pH under the same challenged rumen condition. Also, the low number of SARA events recorded would suggest that the amount and quality of the forage available

(mostly concentrations of rapidly fermentable carbohydrate, NDF and physical effective fibre) were appropriate to ensure an adequate flow of saliva of grazing dairy cows to maintain buffer capacity, and therefore prevent a drop in ruminal pH (Rivero and Anrique, 2015). The NDF content of the rations administered to lactating dairy cows was greater than the minimum amount of dietary NDF of 25% recommended by NRC (NRC, 2001) for optimal ruminal fermentation.

Despite the higher average ruminal pH of cows supplemented with M12, we found no differences between M12 and AB in milk yield and composition. This is contrary to the results of Neville et al. (2019), who found that the inclusion of CMA in combination with marine Mg oxide promoted a similar ruminal pH but a reduced milk yield of mid-lactation dairy cows by 1.8 kg d⁻¹ compared to CMA alone. Moreover, cows supplemented with the composed bioactive buffer showed 5.6% lower milk protein yield than CMA, using diets containing 45.7% DM forage. The greater forage proportion (61% DM) and lower nonstructural carbohydrates (21.5% DM sugars and starch) content of the diets used in the present study might explain the reason why no differences in milk yield and milk protein yield were observed between AB and M12.

Dairy cows grazing at highly digestible perennial pastures with high concentrations of rapidly fermentable carbohydrates and low concentrations of physical effective fibre are potentially at risk of developing SARA (Rivero and Anrique, 2015) even when low amounts of concentrate supplementation were offered (1.83 kg d⁻¹) (O'Grady et al., 2008). To prevent acidosis and milk fat decrease, rumen buffering products are routinely added to lactating dairy cows receiving high concentrate-diets (Enemark, 2008) where buffer flow from saliva is inadequate (Oetzel, 2003). Calcareous marine algae and more recently marine Mg oxide are used in lactating dairy cow diets as an alternative to sodium bicarbonate to stabilise rumen pH and prevent SARA (Bernard et al., 2014; Cruywagen et al., 2015).

The observed values for plasmatic Phosphorus, Calcium, and Magnesium did not differ from the recommended level for dairy cows in the NRC (NRC, 2001), also they were within the normal range of mineral concentration for mid-lactating cows, as reported by the Animal Health Laboratory at that University of Guelph (Hoff and Duffield., 2015). However, a lower dose of M12 showed similar effectiveness than AB used in a higher dose to regulate rumen pH and mineral uptake under the same challenging rumen conditions, taking into consideration that Southern Chilean milk production is characterised by pasture grazing systems with low energy and mineral imbalances associated to concerning scenarios for metabolic diseases like hypocalcemia and hypomagnesemia (Melendez et al., 2023). These metabolic diseases have an economic impact on

the herd where hypomagnesemia and hypocalcemia, especially in a subclinical form, are important due to their high prevalence, affecting grazing production systems reaching 48.9% and 64.8%, respectively. Unfortunately, like in many other countries in South America, the monitoring of magnesium concentrations in blood as a predictor of magnesium status in dairy herds is not a common practice in southern Chile.

CONCLUSIONS

Under the prevailing conditions of this pilot trial in commercial farms, cows supplemented with M12 had a greater mean pH and lesser SARA events than cows supplemented with AB. However, there was no statistical evidence to support the hypotheses that milk yield and milk composition differed between the rumen buffer additives M12 and AB. The fact that the df of the error term was low (df = 3) may have prevented finding statistical differences in some variables, where numerical differences were observed. If the number of cows with rumen boluses had been greater, the estimation of the percentage of cows affected by SARA in the herd would have been more accurate. The findings of this initial pilot study comparing the two different rumen buffers could be assessed further in controlled conditions to confirm the potential of M12 to reduce SARA incidence. Moreover, M12 could have a better dose response in terms of minimising the negative impact of SARA, as well as keeping the recommended levels of plasmatic minerals in lactating dairy cows.

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CONFLICTS OF INTEREST

The funders' product managers designed the study and collected the data. The funders did not participate in the analysis and interpretation of data, nor in the writing of the manuscript. All the authors, including the funders', decided to publish the results.

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