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# Comparison of marine algae-based rumen buffer with sodium bicarbonate buffer and its role in increasing milk value

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## Research Article

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**Abstract**

This study aimed to evaluate the effect of marine-based rumen buffer (*Lithothamnium calcareum*) supplementation on rumen health as well as milk yield and composition and also behavioural and metabolic parameters of dairy cows. Thirty-six lactating multiparous Holstein cows were used with a milk yield average of 39 kg/d and 64 d in milk. The experiment was conducted over 60 d using two groups: control (CON; $n = 18$) was supplemented with sodium bicarbonate at 1.1% dry matter and a treatment group that received *Lithothamnium calcareum* at 0.5% of dry matter (LITHO; $n = 18$). Each group was fed daily with the buffers mixed to the total mixed ration containing 29.28% starch. Ruminal fluid collections were performed weekly to evaluate pH and volatile fatty acids. Feeding behaviour data were obtained through automatic feeders, while overall behavioural data were obtained using monitoring collars. Milk yield was recorded daily and adjusted for fat and energy. Milk samples were retrieved once weekly for analysis of fat, protein, lactose and total solids. Blood samples were collected weekly for metabolic analysis and faecal samples were collected weekly to evaluate pH and starch concentrations. LITHO produced more fat- and energy-corrected milk ($P \leq 0.01$) as well as the highest percentage of fat and solids ($P < 0.05$) when compared to the CON group. Data on feeding behaviour showed an increased eating time ($P \leq 0.01$) in the LITHO group but a higher eating rate ($P < 0.01$) in the CON group. Animals from the LITHO group had lower faecal pH ($P < 0.05$). The treatment did not affect dry matter intake, animal behaviour, ruminal acid–base balance, or faecal starch. In summary, *Lithothamnium calcareum* supplementation at 0.5% of dry matter improved milk yield, milk composition and, presumably, feed conversion efficiency.

To meet the high nutritional demands, increase production and improve profitability, cattle feeding programs recommend starch-rich diets, especially in the beginning and middle of lactation (Mu *et al*., 2023). Starch is an important energy source for dairy cows to meet microbial protein production goals in the rumen and milk yield demands (Srivastava *et al*., 2021). However, the supply of rapidly fermentable starch in the rumen can result in the accumulation of volatile fatty acids (VFA), harming ruminal health (Mawdae *et al*., 2022). Increased VFA production, which exceeds the buffering supply, disrupts the intraruminal acid–base balance, reducing ruminal pH and leading to an acidotic environment (Golder *et al*., 2023). This change in the rumen significantly decreases the activity of fibrolytic bacteria, reducing the acetate concentration, which plays an essential role in stimulating the synthesis of milk fat (Neville *et al*., 2019; Martins *et al*., 2021).

Considering this, maintenance of the ruminal conditions is vital to ensure healthy and efficient milk yield. Thus, ruminal buffers are commonly added to the diets of lactating cows to avoid prolonged episodes of low ruminal pH and the associated production losses (Bach *et al*., 2018; Srivastava *et al*., 2021). Sodium bicarbonate is the most popular dietary buffer used in ruminant nutrition, to neutralize acidity and stabilize ruminal pH (Chalupa *et al*., 1996; Cruywagen *et al*., 2015). However, it is a soluble buffer with a short duration in the rumen that may not effectively buffer the ruminal environment (Van Soest, 1994). Consequently, marine algae have recently been studied as a potential supplement to stabilize ruminal pH due to their polymorphic nature (honeycomb structure). These organisms provide a slow release of minerals (calcium and magnesium) in an acidic environment, prolonging their neutralizing action on ruminal pH and avoiding prolonged episodes of low pH (Cruywagen *et al*., 2015; Neville *et al.,* 2019). LithoNutri is a natural and renewable food additive produced from the marine algae *Lithothamnion calcareum* which is rich in calcium (Ca) and magnesium (Mg) carbonate (Guerreiro *et al*., 2019). Its release of Ca and Mg is slower in the ruminal

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environment, a result of its polymorphic nature (77.7% magnesium calcite, 3.8% calcite, 16.6% aragonite, 2% silica), prolonging its neutralizing action in the ruminal pH due to the varying solubility of the constituents. Aragonite is more soluble than calcite, which is more soluble than magnesium calcite.

Previous studies with dairy cows demonstrated that supplementation with *Lithothamnium calcareum* increased DMI and milk fat yield (Neville *et al.*, 2022) and improved feed efficiency (Bernard *et al.*, 2014), hormone regulation, pH and ruminal fermentation (Cruywagen *et al.*, 2015). Nonetheless, research using this buffer in dairy cow herds remains scarce. Particularly, its effects on feeding and overall behaviour as well as metabolic parameters have not been compared with those of sodium bicarbonate.

Thus, the present study aimed to measure the impact of *Lithothamnium calcareum* on rumen health, feeding and activity behaviour, milk yield and composition and metabolic status in Holstein dairy cows fed a diet containing 29.28% DM of starch in total mixed ration (TMR).

## Materials and methods

### Animal ethics

This study was approved by the Animal Ethics and Experimentation Committee of the Federal University of Pelotas, registered under number 13784-2021.

### Animals handling and facilities

Thirty-six multiparous Holstein cows were divided into two groups of eighteen cows (Control, CON; *Lithothamnim calcareum*, LITHO) blocked according to the average milk yield (38.46 ± 2.16 vs. 39.06 ± 2.16 kg/d; $P > 0.05$), days in milk (74.61 ± 4.05 vs. 75.88 ± 4.05 d, $P > 0.05$) and parity (3.22 ± 0.18 vs. 3.00 ± 0.18, $P > 0.05$) at the beginning of the experiment. The cows were kept in a commercial dairy property in the city of Rio Grande, Rio Grande do Sul, Brazil at geographic coordinates 32°16′S, 52°32′E and maintained in an intensive compost barn system bedded with wood shaving and rice husk, with individual access to automated feeders and freshwater.

### Experimental design and treatments

All animals were concomitantly treated over a 60 d period. Both treatments received the same basal diet as a TMR, which was formulated to be potentially acidotic (online Supplementary Table S1) and contain 29.28% starch. The diet was calculated to guarantee 24 kg/d dry matter intake (DMI), provided in two meals, each one with 50% of the total diet at 9 am and 6 pm, allowing for approximately 5% of refusals. A 0.5% dry matter (DM) level of the product (LithoNutri, Oceana, Jundiaí, Brazil) was included in the diet for the LITHO group to ensure 102 g/cow/day. The CON group received 1.1% of the diet DM of sodium bicarbonate to provide a daily intake of 280 g/cow/day (Raudi, Totalmix, São Carlos do Ivaí, Brazil).

The buffer doses were divided so that the animals received 50% of the total dose added on top of the diet during each feeding.

### DMI, feeding behaviour and animal behaviour

DMI and feeding behaviour data were obtained daily (24 h/d) through individual automatic feeders, (Intergado, Minas Gerais, Brazil). The parameters recorded were feed intake, eating time (min/day), eating frequency, number of visits to intake troughs, eating rate, kg of DM/min, meal size (kg/meal) and eating duration (min/meal). The latter was calculated by the eating time divided by the number of meals. The eating rate was calculated by dividing the amount of individual intake by the eating time.

Individual animal behaviour was monitored daily using monitoring collars (ChipInside, Santa Maria, Brazil) that indicated the time of activity, rumination, and idleness.

### Milk production and composition

The cows were milked three times a day at 8 am, 4 pm and 11 pm. Daily milk yield was recorded individually during each milking period using the electronic milking system (DeLaval, Botkyrka, Sweden). Milk samples were collected on d 0, before the supplementation period, and on d 7, 14, 21, 28, 35, 42, 49, 56 and 60 of the experiment at each of the three milkings using bottles containing Bronopol preservative (2-bromine-2-nitropropane-1,3-diol and natamycin). Samples were kept refrigerated and sent to the milk analysis laboratory (PARLEITE, Curitiba, Paraná, Brazil) for analysis of fat, protein, lactose and total solids.

Milk yield was corrected for 4% fat (FCM4%) and for energy (ECM) using the following formulae:

FCM4% = (0.4 × milk yield kg) + (15 × fat production kg)

ECM = (0.3273 × milk yield kg) + (7.65 × milk protein kg) + (12.97 × milk fat kg)

The feed efficiency (FE) for milk yield was obtained using the following formulas:

(FE) = Milk yield (kg/day) / DMI (kg)

(FE) = FCM4% (kg) / DMI (kg)

(FE) = ECM (kg) / DMI (kg)

### pH and VFA ruminal fluid

On the same milk collection days, ruminal fluid was collected *via* ruminocentesis in three animals per treatment 4 h after the morning feeding. The ruminal pH was measured immediately after collection with a benchtop pH meter. An aliquot of ruminal fluid was stored at −80 °C and subsequently used to determine the concentration of VFA. These were quantified using the nuclear magnetic resonance hydrogen technique tested by Wang *et al.* (2021).

### Blood collection and analysis

Blood samples were collected from all cows on d 0, before the supplementation with buffer, and on d 21, 42 and 60 in three tubes: one containing sodium fluoride to obtain plasma and evaluate glucose concentrations, a second tube with silica for serum collection for analyses of urea, albumin, total plasma protein (PPT), paraoxonase 1 (PON1), non-esterified fatty acids (NEFA), and beta-hydroxybutyrate (BHB) and a third tube containing sodium heparin for analyses of pH, bicarbonate ($HCO_3^-$), carbon dioxide pressure ($pCO_2$), and blood oxygen pressure ($pO_2$). After collection, the samples were centrifuged at 2000 ***g*** for 15 min at room temperature and serum or plasma were saved into microtubes and then stored at −80 °C for posterior analyses.

The evaluation of protein metabolism (albumin, PPT and urea) was determined using a colorimetric kit (Labtest, Belo Horizonte, Brazil). For energy metabolism, glucose (Labtest, Belo Horizonte, Brazil), NEFA and BHB (Diasys, Germany)

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concentrations were analysed through colorimetric assay using commercial kits. The protein and energy profiles of the samples were determined using the automatic biochemical analyser (Labmax pleno, Labtest, MG, Brazil). PON1 analysis was performed using a spectrophotometer (Femto, 700 Plus). These parameters were evaluated in the metabolism laboratory of NUPEEC/UFPel. pH, $HCO_3^-$ and blood gas ($pCO_2$, $pO_2$) were evaluated using a haemogasometry (i-STAT) device immediately after collection, as recommended by the manufacturer.

### Faecal pH and starch levels

To assess faecal pH and starch levels, faeces samples were collected from all animals on d 0, before the supplementation with buffer, and on d 7, 14, 21, 28, 35, 42, 49, 56 and 60 of the experiment. Faecal pH was evaluated immediately, and samples were stored at −80 °C for starch analyses. The starch concentration was assessed using a EsalqLAB/USP Laboratory and the AACC Method 76-13.01, AOAC Method 996.11, ICC Standard Method No. 168, and RACI Standard Method from the Total Starch Kit (Catalogue Number: AA/AMG – Megazyme International Ireland).

### Feed samples

Diet samples were collected twice a week for DM evaluation using the air fryer method (Nogueira *et al.*, 2021). TMR samples were frozen for subsequent chemical analyses. Samples were analysed for concentrations of ash according to AOAC (1995). The crude protein analysis was performed based on the Kjeldahl methodology (AOAC, 1995). The neutral detergent fibre (NDF) analysis and ash-corrected acid detergent fibre (ADF) were determined according to Van Soest and Robertson (1985).

### Statistical analysis

The results were analysed using the JMP Pro 14 program (SAS Institute Inc., 2018) by PROC MIXED, considering treatment, time and interaction as fixed effects and cow as random effects. DMI, feeding behaviour, animal behaviour, milk production and composition, pH and volatile fatty acids in ruminal fluid, faecal pH and starch levels as well as blood sample data were analysed as repeated measures using the MIXED procedure (JMP Pro 14, SAS). Statistical significance was considered at $P < 0.05$ *P*-values are presented as Tukey adjusted values. The best covariance structure was defined by the Akaike information criterion (AIC). Data were analysed according to the following statistical model:

$$Yijk = \mu + Ti + Aj + Pk + TPik + \varepsilon ijk,$$

where Yijk represents the continuous variable; μ represents the general average; Ti is the fixed effect of the treatment ($i = CON \times LITHO$); Aj is the random effect of the animal ($j = 1$ to 36); *P* is the fixed effect of time ($k = 1$ to 60); Tpik is the fixed effect of the interaction between treatment *i* and time *k*, and εijk is the random error associated with each observation.

## Results

### Feeding and animal behaviour

The results of DMI, eating time and eating rate are presented in Table 1. There was no difference in DMI between the treatments ($P > 0.05$) suggesting that the different ruminal buffer did not affect the diet's palatability. The CON and LITHO cows consumed respectively 27.19 ± 0.54 and 26.73 ± 0.53 kg DM (mean ± SEM). However, the eating rate was significantly higher for CON than LITHO (0.24 ± 0.01 *vs.* 0.20 ± 0.01 kgDM/min, $P < 0.01$) whereas

**Table 1.** Intake, eating time, eating rate, milk yield and composition of Holstein cows fed diets supplemented with sodium bicarbonate (CON) or Lithothamnium calcareum (LITHO)

| | LSMEANS ± SE | | | *P*-value | |
|---|---|---|---|---|---|
| Parameters | CON | LITHO | Treatment | Period | T × P |
| DMI (kg/day) | 27.19 ± 0.54 | 26.73 ± 0.53 | 0.49 | <0.01 | 0.14 |
| Eating time (min/day) | 121.76 ± 5.07$^b$ | 137.34 ± 4.94$^a$ | 0.01 | <0.01 | <0.01 |
| Eating rate (kgDM/min) | 0.24 ± 0.01$^a$ | 0.20 ± 0.01$^b$ | <0.01 | <0.01 | <0.01 |
| Milk yield (kg/day) | 38.59 ± 0.71 | 39.06 ± 0.69 | 0.56 | <0.01 | 0.37 |
| FCM (kg) | 37.78 ± 0.82$^b$ | 40.11 ± 0.82$^a$ | 0.01 | <0.01 | 0.97 |
| ECM (kg) | 41.01 ± 0.85$^b$ | 43.25 ± 0.85$^a$ | <0.01 | <0.01 | 0.81 |
| Efficiency – FCM/DMI | 1.48 ± 0.03$^b$ | 1.59 ± 1.3$^a$ | <0.01 | <0.01 | 0.23 |
| Efficiency – ECM/DMI | 1.62 ± 0.03$^b$ | 1.71 ± 0.03$^a$ | <0.01 | <0.01 | 0.28 |
| Fat, kg | 1.46 ± 0.03$^b$ | 1.63 ± 0.03$^a$ | <0.01 | <0.01 | 0.99 |
| Protein, kg | 1.19 ± 0.02 | 1.23 ± 0.02 | 0.50 | <0.01 | 0.24 |
| Fat, % | 3.80 ± 0.08$^b$ | 4.17 ± 0.08$^a$ | 0.01 | 0.02 | 0.65 |
| Protein, % | 3.10 ± 0.04 | 3.14 ± 0.04 | 0.50 | <0.01 | 0.10 |
| Total Solids, % | 12.27 ± 0.13$^b$ | 12.67 ± 0.12$^a$ | 0.03 | 0.05 | 0.95 |
| Lactose, % | 4.43 ± 0.04 | 4.42 ± 0.04 | 0.92 | 0.46 | 0.99 |

T × P, Treatment interaction with period; DMI, dry matter intake; FCM, fat-corrected milk; ECM, energy-corrected milk.
Different superscripts $^{ab}$ denote significant differences ($P < 0.05$) within a row for the variables/parameter.

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the reverse was true for eating time (121.76 ± 5.07 *vs.* 137.34 ± 4.94 min/d). This indicates that the animals in the LITHO group consumed feed at a slower rate but for a longer period.

Other results of feeding behaviour and of animal behaviour are described in online Supplementary Table S2. There were no differences between the groups in frequency of access to feeders, daily number of meals, meal size (kg/meal) and eating duration (min/meal). However, an interaction was noted between TRT×Period in frequency of access to feeders ($P = 0.02$) and number of meals ($P = 0.01$) for the animals in the LITHO group, as well as meal size ($P = 0.01$) and eating duration for cows in the CON group. Treatment did not affect the time of rumination, activity or idleness.

### *Production, composition and feed efficiency for milk yield*

The effects of treatments, milk yield, milk composition and feed efficiency for milk yield are presented in Table 1. No differences were observed between LITHO and CON treatments for milk yield. However, LITHO exhibited greater ($P \leq 0.01$) yield of 4% FCM and ECM (+2.3 and 2.2 kg/d, respectively) than CON. Milk fat yield and concentration ($P \leq 0.01$) as well as per cent total solids ($P = 0.03$) were also higher for LITHO than for CON. No differences were observed in the yield or percentage of either protein or lactose in the milk.

The feed efficiency (milk/DMI) was not different between treatments ($P > 0.05$). However, the efficiency of FCM and ECM production was greater ($P < 0.01$) for LITHO than for CON.

### *Metabolic parameters and acid–base blood balance*

The results of metabolic parameters are presented in online Supplementary Table S3. Treatment did not affect the glucose or BHB concentrations. Values for NEFA and PON concentrations were numerically (non-significantly) lower in the LITHO group compared with the values in the CON group. There was no difference in albumin, PPT, urea, Ca and Mg levels between treatments ($P > 0.05$). The results of acid–base blood balance presented in online Supplementary Table S4, showed no difference in blood pH, $HCO_3$-, $pCO_2$, or $pO_2$ between the groups.

### *Faecal starch and pH*

The results of faecal pH and starch are shown Table 2. Faecal starch concentrations were not different among treatments, whereas faecal pH was higher ($P = 0.02$) for LITHO (6.18 ± 0.02) than for CON (6.11 ± 0.02).

## Ruminal pH and VFA

Ruminal pH and VFA results are presented in Table 3. The treatments had no significant impact on ruminal pH values 4 h after the morning feeding. In addition, the total proportions of acetate, propionate and butyrate were not different between treatments.

## Discussion

Monitoring feeding behaviour is an important tool for dairy production systems, providing important information regarding the DMI and possible changes in animal health. Additionally, it can be very helpful for predicting disease, especially when monitoring animal behaviour using collars (Beauchemin, 2018; Cecim, 2018). According to González *et al.* (2012), feeding behaviour associated with a lower ruminal pH due to high dietary starch concentrations is characterized by a greater feeding rate, less frequent meals due to increased acid production, lower feed insalivation as well as shorter chewing and rumination time.

In the present study, the lack of difference in DMI between groups is consistent with results of Rauch *et al.* (2012) and Neville *et al.* (2019), in which the effect of supplementation with sodium bicarbonate and *Lithothamnium calcareum* did not affect the DMI of dairy cows. Bernard *et al.* (2014) showed that sodium bicarbonate increased the DMI in the last weeks of the study compared to that obtained using *Lithothamnium calcareum* in cows at the beginning of lactation. According to Russell and Chow (1993) and Perez-Ruchel *et al.* (2014), the increase in DMI with supplemental sodium bicarbonate is related to increased ruminal osmotic pressure which increases the water intake and passage rate. However, in our work feeding time was greater in the LITHO group, and the consumption rate was higher for the CON group. Despite this latter difference, rumination times were within physiological parameters (Beauchemin, 2018; Cecim, 2018) and not different between treatments. The mean rumination time of LITHO and CON were 601.67 ± 5.02 and 593.93 ± 4.81 min/d, respectively.

These results show that animals that received diets rich in starch, which generally decrease rumination time, responded positively to supplementation with buffers (DeVries *et al.*, 2009; Beauchemin, 2018). The lower consumption rate and longer feeding time of our LITHO cows was beneficial for the digestibility of the diet, as can be demonstrated by the intestinal buffering, represented by the higher pH of the faeces in these animals. There was no difference in faecal starch concentrations between treatments. Starch escaping digestion is excreted in the faeces and may result in performance impairment and economic losses. For both treatments, values were within the normal range of faecal starch concentration (< 3%, Neiderfer *et al.*, 2020).

According to Erdman (1988), calcium carbonate, magnesium oxide and limestone have low rumen solubility and can pass to the lower tract and modify intestinal fermentation resulting in increased intestinal and faecal pH. Our results are consistent with those of Neiderfer *et al.* (2020), who also reported no difference in faecal starch. Nevertheless, faecal pH was higher for cows

**Table 2.** Faecal pH and starch concentrations of Holstein cows fed diets supplemented with sodium bicarbonate (CON) or Lithothamnium calcareum (LITHO)

| | LSMEANS ± SE | | *P*-value | | |
|---|---|---|---|---|---|
| Parameters | CON | LITHO | Treatment | Period | T × P |
| Faecal pH | 6.11 ± 0.02[b] | 6.18 ± 0.02[a] | 0.02 | 0.21 | 0.66 |
| Faecal starch % | 2.98 ± 0.38 | 2.79 ± 0.39 | 0.71 | 0.05 | 0.61 |

T × P, Treatment interaction with period.
Different superscripts [ab] denote significant differences ($P < 0.05$) within a row for the variables/parameter

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**Table 3.** Ruminal pH and molar proportions (mol/100 mol) of individual volatile fatty acids 4 h after the morning feeding from Holstein cows fed diets supplemented with sodium bicarbonate (CON) or *Lithothamnium calcareum* (LITHO)

| | LSMEANS ± SE | | *P*-value | | |
|---|---|---|---|---|---|
| Parameters | CON | LITHO | Treatment | Period | T × P |
| Ruminal pH | 6.80 ± 0.11 | 6.70 ± 0.11 | 0.57 | 0.48 | 0.56 |
| Acetic acid | 62.60 ± 1.21 | 62.13 ± 1.33 | 0.56 | 0.26 | 0.44 |
| Propionic acid | 22.98 ± 1.13 | 23.71 ± 1.19 | 0.67 | 0.43 | 0.27 |
| Butyric acid | 14.42 ± 0.59 | 14.16 ± 0.74 | 0.59 | 0.81 | 0.99 |

T × P, Treatment interaction and period.

that received LITHO. Wheeler and Noller (1977) reported increased pH of the lower gastrointestinal tract and faeces using limestone supplementation. Clark *et al.* (1989) showed that the increase in faecal pH observed with calcium carbonate supplementation is accompanied by a decrease in faecal starch. This inverse correlation can be explained by improved buffering of the small intestine, increasing the efficiency of enzymatic starch digestion (Neiderfer *et al.*, 2020). In the current experiment, faecal starch remained within acceptable levels. It was not affected by treatment, although differences in intestinal acid neutralization were evidenced by increased faecal pH in LITHO animals. However, the low number of faeces samples analysed for starch may have limited our ability to observe this correlation.

Milk yield was similar between treatments. However, the yield of FCM and ECM was higher in the LITHO group compared with the CON group, which may be due to greater VFA production, as suggested by studies conducted by Cruywagen *et al.* (2015) and Neville *et al.* (2022), who attributed the greater milk production and parameters (4% FCM and ECM) in cows supplemented with *Lithothamnium calcareum* to an increase in VFAs.

The treatment also had a significant effect on feed utilization efficiency for milk production. Considering FCM/DMI and ECM/DMI, efficiency was higher in the LITHO than in the CON group. This improvement can be attributed to an improvement in milk components. Cruywagen *et al.* (2015) and Bernard *et al.* (2014) reported an improved efficiency of 4% FCM/DMI and ECM/DMI, respectively, for cows fed with *Lithothamnium calcareum* compared to cows fed sodium bicarbonate, and our results are consistent with these data.

All metabolic parameters were within the physiological reference range for bovine species (Beigh *et al.*, 2018; Quiroz-Rocha *et al.*, 2009). Serum concentrations of NEFA are used as indicators of body fat mobilization and poor metabolic status. *Lithothamnium calcareum* supplementation did not affect the serum glucose and BHB levels in this study. However, serum NEFA showed a numercial (non-significant) decrease in the LITHO group compared to that in the CON group, indicating an improvement in energy utilization.

Serum protein and mineral (Ca and Mg) concentrations were similar in both groups, which is consistent with the results of Wu *et al.* (2015) and Neville *et al.* (2022). An increase in Ca and Mg concentrations in the LITHO group could be expected due to the increased bioavailability of this supplement. However, Cruywagen *et al.* (2015) demonstrated that *Lithothamnium calcareum* is sparingly soluble in water due to its chemical and physical properties, releasing cations slowly and thereby maintaining the buffer effect for longer in the ruminal environment. Due to this slower release, there was no change in the absorption rate, keeping these minerals at physiological rates in the blood.

The blood acid–base balance was not affected by the treatments. Blood pH is mainly regulated by the respiratory system, which efficiently provides sufficient $CO_2$ to stabilize the pH. An increased $CO_2$ concentration causes increased $H^+$ ions and acidity in the bloodstream. Conversely, a decrease in $CO_2$ levels causes blood alkalinity (Carlson, 1997; Kozloski, 2011). Only a few studies have evaluated the effects of seaweed on blood characteristics. Wheeler *et al.* (1981) reported no difference in blood pH or $HCO^{3-}$ when assessing the effect of calcium and sodium bicarbonate levels in high-concentrate diets for beef cattle. Bigner *et al.* (1997) found that sodium bicarbonate and sodium propionate effectively increased the concentration of $HCO^{3-}$ and maintained the blood pH at 7.4 in acidotic dairy cows. Carvalho *et al.* (2016) observed an increase in blood pH of cows supplemented with *Lithothamnium calcareum* compared to that of control cows. Our values for blood pH, $HCO^{3-}$ and $pO_2$ were slightly above the normal range in both groups. $pO_2$ is linked to blood pH, which means as pH increases, the pressure also increases. Conversely, $pCO_2$ decreases as the blood pH increases (Carlson, 1997; Kozloski, 2011), which did not occur in the present study. However, based on the blood and ruminal characteristics we observed, there was no ruminal and metabolic acidosis in the animals in both groups.

Our results show that the two treatments with buffers did not alter ruminal pH and were effective in maintaining the ruminal health. VFA production was also not altered, which possibly contributed to the unaltered glucose concentration. These results are consistent with the study by Neville *et al.* (2019), in which cows supplemented with seaweed (*Lithothamnium calcareum*) maintained an average ruminal pH (over 24 h) similar to that obtained with sodium bicarbonate.

A difference could be expected in variations in ruminal pH and VFA production over time, contributing to improvements in ruminal fermentation and digestibility. The difference observed in the milk constituents in the LITHO group may have resulted from this, since because it is more stable and has a slower release of its constituents (calcite, vaterite, aragonite and dolomite) in the rumen, *Lithothamnium calcareum* buffers effectively for a longer period of time than sodium bicarbonate (Carvalho *et al.*, 2016).

In conclusion, supplementation with *Lithothamnium calcareum* algae provides an effective alternative to sodium bicarbonate as a means of preventing rumen acidosis in lactating dairy cows fed a high starch diet. The results also showed greater feed efficiency when assessed relative to fat- or energy-corrected production.

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