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Ruminal metabolism, blood parameters and animal behavior of bulls submitted to sub-acute ruminal acidosis (SARA) receiving different buffers in high-concentrate diets

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ABSTRACT. This study aimed to evaluate the supplementation of four different buffers into a high-grain diet on ruminal fermentation, blood metabolites, and feeding behavior of feedlot cattle. Five rumen-cannulated bulls (492 ± 24 kg) were distributed in a 5 x 5 Latin square design, through the treatments: CONT (no buffer), RUMO, RUMF, BICA and ALGA. The buffers did not alter ($p > 0.05$) the apparent digestibility of nutrients, ruminal fluid pH, volatile fatty acid profile, and acetic/propionic ratio compared to the CONT. Except for propionic and isovaleric acids, which were different ($p < 0.05$) among treatments at 4 and zero hours after feeding, respectively. Ruminal lactic acid accumulation was greater ($p < 0.05$) in BICA, while ammoniacal nitrogen concentrations were highest ($p < 0.05$) in CONT and lowest in RUMF. Blood glucose and creatinine were unaffected ($p > 0.05$), whereas urea and lactate concentrations were reduced ($p < 0.05$) in RUMO. Among the enzymes, only gamma-glutamyl transferase and creatine kinase showed treatment effects ($p < 0.05$). Furthermore, feeding and drinking times were unaffected by treatments; however, rumination increased ($p < 0.05$) in BICA, and idleness was higher in CONT. Overall, buffer inclusion modified ruminal and metabolic responses, indicating a possible modulation of ruminal acidosis.

Keywords: animal production; *Lithothamniun calcareun*; nutritional disorder; rumen modulation; sub-acute acidosis.

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Introduction

To support their high genetic potential for meat and milk production, beef cattle and dairy cows need to receive energy-rich diets with high-carbohydrate and low fiber levels (Ornaghi et al., 2017; Rivaroli et al., 2020; Torrecilhas et al., 2021; Matos et al. 2023). These diets alter the modulation of microbiota in the rumen by reducing the pH and changing the volatile fatty acid composition towards increasing propionate, butyrate and lactate, and reducing the acetate to propionate ratio (Carvalho et al., 2021; Khalouei et al., 2021). The decrease in the rumen's pH may lead to sub-acute ruminal acidosis (Plaizier et al., 2017). This disorder affects the production and health of beef cattle and dairy cows by decreasing production, nutrient utilization, rumen epithelium functionality and feed intake, as well as by causing inflammation, laminitis, and diarrhea (Callaway & Martin 1997; Plaizier et al., 2018).

SARA is considered a nutritional disorder, which can occur through the intake of fermentable carbohydrates in sufficient amounts to cause non-physiological accumulation of acids in the rumen, and there is considerable reduction in pH than more three hours (Nagaraja & Titgemeyer, 2007; Han et al., 2021). Ruminal pH is a critical factor in normal rumen function due to its effect on microbial populations, fermentation products, and physiological functions of the rumen, particularly motility and absorption. Therefore, the non-physiological accumulation of organic acids and the consequent reduction of pH below normal (less than 5.6) have a significant impact on microbial activity, rumen function, productivity and animal health. In beef cattle fed with high-grain diets, ruminal pH can range from 5.6 to 6.5, with an average pH typically around 5.8 to 6.2, and may drop below 5.6 during the feeding cycle (Nagaraja & Titgemeyer, 2007). Thus, the acid-base balance in the rumen requires time synchronization between acid production, its neutralization by the saliva and absorption via diffusion by the ruminal epithelium (Jaramillo-López et al., 2017). To prevent this process, there is systemic buffering with basically

three systems: the body's buffer system, renal regulation and pulmonary regulation. In addition to these buffers, ruminants have buffering through saliva. A review by Herod et al. (1978) analyzed in vitro 35 different compounds with rumen fluid from cattle with high-grain diets regarding the buffering power of these compounds. The authors found that the addition of betonite and calcium bicarbonate (NaHCO3) had no effect on changing the pH, but the inclusion of calcium hydroxide (Ca(OH)), magnesium oxide (MgO), potassium bicarbonate (K2CO3), sodium bicarbonate (Na2CO3), potassium disodium (K3PO4) and sodium hydroxide (NaOH) showed a good capacity to neutralize acidity and significantly increased the ruminal pH. Furthermore, the compounds Al(OH)3, dolomite, MgSO4, NaH2PO4 and ZnSO4 had no effect on rumen pH.

The supplementation of buffer substances can be a way to limit the adverse effects of rumen acidosis. These substances have a direct effect on the pH of rumen fluids, such as neutralizing acidity through the sequestration of hydrogen (H^+) and increasing the buffering capacity of the rumen fluid (Hernández et al., 2014) and can be added to complete rations at a dosage of 0.5 to 2.5% based on dry matter (Gastaldello Júnior et al., 2013). Sodium bicarbonate, disodium carbonate, magnesium oxide, potassium carbonate and anhydrous limestone are the compounds most commonly used as buffers (Valente et al., 2017). However, studies have been carried out with the objective of testing the possible effects of using a combination of these compounds and seaweed in the diet of cattle with nutritional disorders (Almeida et al., 2012; Carvalho et al., 2016; Neville et al., 2019).

Lithothamniun calcareun is a marine coralline alga belonging to the calcareous algae group, characterized by the deposition of calcium and magnesium carbonate in its cell walls in the form of calcite crystals (Boeckaert et al., 2008), which can present positive effects on the control of ruminal pH in animals receiving high-grain diets (Cruywagen et al., 2015).

This study was carried out to evaluate the effects of four different buffers in feedlot cattle fed with a high-concentrate diet, evaluating ruminal modulation, blood and behavioral ingestive parameters.

Material and methods

The development of this study was approved by the State University of Maringá (UEM) ethics committee for animal use with protocol CEUA Nº: 1583251120.

Location, facilities and animals

This study was carried out at the Rosa and Prado feedlot facility, located at the UEM Iguatemi Experimental Farm.

Five bulls with an average body weight of 492 ± 24 Kg and average age of 30 ± 6 months, adapted with rumen cannula were utilized, randomized across individual pens with a concrete floor measuring 10 m^2, partially covered, with automatic drinkers and concrete feeders (2.0 m x 0.4 m x 0.5 m).

Experimental design and treatments

The study was carried out in a 5 x 5 Latin square, where four different buffers and a control treatment were tested in five animals and five data collection periods. The animals were randomized across treatments. The experimental period had 14 days of rest adaptation for the bulls (non-sub-acute ruminal acidosis) when they received a high-forage (corn silage) diet, and 3 days of SARA induction for data collection. During the three days of sub-acute acidosis induction, the animals received a high-concentrate diet in the proportion of 20% corn silage and 80% concentrate (finely ground corn, ground black oat, soybean meal and mineral salt). The experimental periods followed the chronology as shown in Table 1.

Table 1. Experimental days schedule.

Days	Activities
- 13 to 0	Non-sub-acute acidosis (high-forage diet)
0	pH measuring
1 to 3	Sub-acute acidosis induction (high-concentrate diet)
1	pH measuring
2	pH measuring; ruminal fluid, blood and feces samples collecting
3	Animal behavior measuring

The bulls were fed once a day (at 8:00 a.m.) throughout the study. The amount of feed was adjusted to 2.0% of the animal's body weight and both the amount of feed provided and leftovers were recorded. The leftovers were collected only with three days of sub-acute acidosis induction and 12 hours after the morning feeding.

The animals were randomly assigned to five treatments: CONT (diet without the addition of buffering compounds), RUMO (diet containing 10 g Kg^{-1} of dry matter with the inclusion of RUMOX®, based on sodium bicarbonate, calcium carbonate, magnesium oxide, magnesium, sodium carbonate, calcareous seaweed, honey aroma, neotame, sodium saccharin, maltodextrin and silicon dioxide), RUMF (diet containing 10 g Kg^{-1} of dry matter with the inclusion of RUMOXF®, based on sodium bicarbonate, calcium carbonate, magnesium oxide, calcareous seaweed, honey aroma, neotame, sodium saccharin, maltodextrin and silicon dioxide); BICA (diet containing 10 g Kg^{-1} of dry matter with the inclusion of sodium bicarbonate) and ALGA (diet containing 10 g Kg^{-1} of dry matter with the inclusion of seaweed – *Lithothamnium calcareum*) (Table 2).

Table 2. Ingredients and chemical composition of experimental diets.

Ingredients, g Kg^{-1} of DM	Treatments				
	CONT[1]	RUMO[2]	RUMF[3]	BICA[4]	ALGA[5]
Corn silage	200.00	198.00	198.00	198.00	198.00
Finely ground corn	448.00	443.20	43.20	443.20	443.20
Black oat (*Avena strigosa*)	240.00	237.60	237.60	237.60	237.60
Soybean meal	84.00	83.10	83.10	83.10	83.10
Mineral salt	28.00	27.70	27.70	27.70	27.70
Rumox®	-	10.00	-	-	-
RumoxF®	-	-	10.00	-	-
Sodium bicarbonate	-	-	-	10.00	-
Marine algae (*Lithothamnium calcareum*)	-	-	-	-	10.00
Composition, g Kg^{-1} of feed					
Dry matter	791.32	793.05	793.05	793.05	793.05
Crude protein	137.02	135.60	135.61	135.60	135.62
Organic matter	959.98	951.79	952.16	954.08	951.00
Ash	40.02	48.21	47.84	45.92	49.00
Ether extract	32.89	32.55	32.55	32.55	32.61
Neutral detergent fiber	257.76	256.08	256.04	255.33	256.43
Acid detergent fiber	108.50	107.83	107.74	107.44	107.71

[1]CONT (without addition of buffers); [2]RUMO (sodium bicarbonate, calcium carbonate, magnesium oxide, magnesium carbonate, sodium carbonate, calcareous seaweed, honey aroma, neotame, sodium saccharin, maltodextrin and silicon dioxide); [3]RUMF (sodium bicarbonate, calcium carbonate, magnesium oxide, calcareous seaweed, honey aroma, neotame, sodium saccharin, maltodextrin and silicon dioxide); [4]BICA (Calcium Bicarbonate); [5]ALGA (Marine algae – *Lithothamnium calcareum*); [6]Mineral salt (Calcium: 30g Kg^{-1}; Phosphorus: 18 g Kg^{-1}; Sodium: 95 g Kg^{-1}; Sulfur: 5,000 mg Kg^{-1}; Magnesium: 5,000 mg Kg^{-1}; Cobalt: 30 mg Kg^{-1}; Copper: 350 mg Kg^{-1}; Iodine: 12 mg Kg^{-1}; Manganese: 260 mg Kg^{-1}; Selenium: 6 mg Kg^{-1}; Zinc: 1,000 mg Kg^{-1}; Fluorine: 180 mg Kg^{-1}; CP: 200 g Kg^{-1}; NNP: 70 g Kg^{-1}; TND: 450 g Kg^{-1}; Beta Glucans: 5,250 mg Kg^{-1}; Mannanoligosaccharides: 3,000 mg Kg^{-1}.

Data collection and sampling

The feed and leftover samples were collected per animal in each experimental period and, the fecal grab samples were collected on the second day of sub-acute acidosis induction at zero, 2, 4, 8 and 12 hours after the morning feeding. The samples were dried in an oven with forced air circulation at 55ºC for 72h and were processed in a Wiley mill using 1 mm sieves for the chemical composition assay and 2 mm sieves for apparent digestibility.

The rumen fluid was collected from the ventral sac. Ruminal pH was measured at the time of collection with the aid of a portable pH meter (AK103 – Akso; Porto Alegre, Rio Grande do Sul, Brazil) previously calibrated with pH 4.0 and 7.0 standards, as recommended by the manufacturer, and the samples were collected on days zero, 1 and 2 of each experimental period at zero, 2, 4, 6, 8, 10 and 12 hours after the morning feeding. For the analysis of lactic acid, ammoniacal nitrogen and volatile fatty acids, samples were collected on the second day of sub-acute acidosis induction of each experimental period at zero, 2, 4, 8 and 12 hours after the morning feeding, using 50 mL falcon tubes, and the samples were abruptly frozen with the aid of liquid nitrogen and stored at -20° C for further analysis.

Blood from each head of cattle was collected on the second day of sub-acute acidosis induction at two hours after feeding the animal of each experimental period via jugular vein puncture, in vacuum tubes (Vacutainer®) with EDTA K_2 to obtain plasma and with a clot activator to obtain serum. In sequence, the samples were centrifuged at 2,500 rpm for 15 min and the supernatant was collected, stored in an *Eppendorf* tube and frozen in a freezer at -20°C for further analysis.

Animal feeding behavior data was obtained on the third day of acidosis induction of each experimental period. Records of the time spent in different activities were obtained by visually observing the animals every five minutes, performed by a trained team for 12 uninterrupted hours. Data were collected to estimate the

duration of periods spent feeding, drinking, ruminating and idling (Bürger et al., 2000) with modifications (Silva et al., 2006). The total time spent on each activity was determined by summing the repetitions. Moreover, the number of visits to the water drinker and feeder were recorded.

Laboratorial analysis

Feed, leftovers and feces samples were analyzed for dry matter (DM – method 930.15), crude protein (CP – method 984.13), ash (method 924.05) and ether extract (EE – method 920.29) according to the (Association of Officiating Analytical Chemists, 2005). Organic matter (OM) was calculated as 1000 – ash. The cell wall components such as neutral detergent fibers (NDF) were assayed using filter bags (F57 – Ankom) and a neutral detergent solution including thermostable amylase and sodium sulphite. In sequence, acid detergent fibers (ADF) were determined by the Mertens (2002), as shown in Table 2. Indigestible neutral detergent fibers (iNDF) were determined by 288 h of *in situ* ruminal incubation (Huhtanen et al., 1994).

Lactic acid concentrations present in the rumen fluid were determined using the method described by Pryce (1969) with adaptations, where lactic acid was converted to acetaldehyde by heating with sulfuric acid, then acetaldehyde was reacted with p-hydroxybiphenyl to produce a coloured complex. The ammoniacal nitrogen concentrations in the rumen fluid were determined by the colorimetric method (Chaney & Marbach, 1962). The readings were performed by spectrophotometry using the VersaMax™ ELISA Microplate Reader equipment with the SoftMax® Pro software.

The concentrations of acetic, propanoic, butyric, valeric and isovaleric acids in the ruminal fluid samples were determined by gas chromatography using a Shimadzu© GC-2010 Plus chromatograph equipped with an AOC-20i automatic injector, Stabilwax-DA™ capillary column (30 m, 0.25 mm ID, 0.25 µm df, Restek®) and flame ionization detector (FID), after acidification with 1 M o-phosphoric acid p.a. (Ref. 100573, Merck©) and fortification with a mixture of free volatile acids (Ref. 46975, Supelco®). An aliquot of 1 µL of each sample was injected with a split ratio of 40:1, using helium as a carrier gas with a linear velocity of 42 cm s^{-1}, obtaining the separation of the analytes in a chromatographic run of 11.5 minutes. The inlet and detector temperatures were, respectively, 250 and 300°C, with initial column temperature of 40°C. The column temperature ramp started with a gradient from 40 to 120°C at a rate of 40°C $min.^{-1}$, followed by a gradient from 120 to 180°C at a rate of 10°C $min.^{-1}$, and from 180 to 240°C at a rate of 120°C $min.^{-1}$, keeping the temperature at 240°C for another 3 minutes at the end. For the quantification of analytes, a calibration of the method was performed with dilutions of WSFA-2 standard (Ref. 47056, Supelco®) and glacial acetic acid (Ref. 33209, Sigma-Aldrich®) analyzed under the conditions described above. Peak determination and integration were performed using GC solution v. 2.42.00 (Shimadzu®), according to the methodology described by Del Valle et al. (2018).

In the blood plasma, the glucose, urea, lactate and creatinine concentrations were determined. In the blood serum, total proteins and fractions, gamma-glutamyl transferase (GGT), aspartate aminotransferase (AST) and creatine kinase (CK) concentrations were measured. The analyses were performed using commercial kits (GoldAnalisa®, Belo Horizonte, Minas Gerais, Brazil) according to the manufacturer's instructions and the readings were performed in a spectrophotometer (Bioplus, 2000®, São Paulo, SP).

Statistical analysis

All the data were evaluated for normality of residuals by Shapiro-Wilk test, and homogeneity of variances by Bartlett test. In the sequence, data were analyzed using the Mixed procedure of SAS (v. 9.4, SAS Inst. Inc., Cary, NC) with the following model:

$Y_{ijkl} = \mu + S_i + C(S)_{ij} + P_k + E_l + e_{ijkl}$

Where:

Y_{ijkl} = dependent variable;

μ = overall mean;

S_i = fixed effect of square;

$C(S)_{ij}$ = random effect of bull nested within square;

P_k = fixed effect of period;

E_l = fixed effect of treatments;

e_{ijkl} = residual error.

For the analyses of pH, lactic acid, ammoniacal nitrogen and volatile fatty acids, data were considering the following model:

$Y_{ijklm} = \mu + S_i + C(S)_{ij} + P_k + E_l + T_m + E \times T_{lm} + e_{ijklm}$

Where:
Y_{ijkl} = dependent variable;
μ = overall mean;
S_i = fixed effect of square;
$C(S)_{ij}$ = random effect of bull nested within square;
P_k = fixed effect of period;
E_l = fixed effect of treatments;
T_m = repeated measures in time;
E x T_{lm} = interaction between treatment and time;
e_{ijklm} = residual error.

Finally, significance was declared at $p \leqslant 0.05$ and trends at $0.05 < p \leqslant 0.10$. Tukey test was used to compare means when differences were observed.

Results

Apparent digestibility

The inclusion of different single compounds (sodium bicarbonate [BICA] or seaweed [ALGA]) or various combinations of other compounds (sodium bicarbonate, calcium carbonate, magnesium oxide, magnesium carbonate, sodium carbonate, calcareous seaweed, honey aroma, neotame, sodium saccharin, maltodextrin and silicon dioxide [RUMO] and [RUMF]) did not change ($p > 0.05$) the apparent digestibility of dry matter, organic matter, crude protein, ether extract, neutral detergent fiber and non-fibrous carbohydrates of SARA-induced bulls (Table 3).

Table 3. Apparent nutrient digestibility of bulls fed with high-grain diet and subjected to sub-acute ruminal acidosis (SARA) induction.

Nutrients, %	Treatments					SEM[6]	p <
	CONT[1]	RUMO[2]	RUMF[3]	BICA[4]	ALGA[5]		
Dry matter	71.82	68.47	70.63	71.06	69.77	2.823	0.931
Organic matter	73.08	69.51	72.04	71.99	71.00	2.744	0.912
Crude protein	85.63	82.46	84.48	83.16	83.12	1.081	0.275
Ether extract	74.07	72.60	78.54	72.44	80.12	4.607	0.671
Neutral detergent fiber	46.24	42.33	41.13	44.14	40.03	3.232	0.680
Non-fibrous carbohydrate	85.85	79.49	83.84	83.03	82.76	3.351	0.755

[1]CONT (without addition of buffers); [2]RUMO (sodium bicarbonate, calcium carbonate, magnesium oxide, magnesium carbonate, sodium carbonate, calcareous seaweed, honey aroma, neotame, sodium saccharin, maltodextrin and silicon dioxide); [3]RUMF (sodium bicarbonate, calcium carbonate, magnesium oxide, calcareous seaweed, honey aroma, neotame, sodium saccharin, maltodextrin and silicon dioxide); [4]BICA (Calcium Bicarbonate); [5]ALGA (Marine algae – *Lithothamnium calcareum*); [6]SEM (Standard error of the mean).

Rumen pH

The ruminal pH results are presented in graphical form in Figure 1. The ruminal pH of bulls in non-sub-acute acidosis had no difference ($p > 0.05$), where the means varied from 6.64 to 6.74 (Figure 1a). This was similar to the ruminal pH of animals on sub-acute acidosis induction, where the means varied between 5.95 for BICA, the lowest treatment value, and 6.09 for RUMO, the highest treatment value. However, when observing the pH values according to the hours after feeding (Figure 1b) and considering that animals with a pH lower than 5.80 are in sub-acute acidosis, it can be observed that animals that received the RUMO treatment showed a pH mean greater than 5.80 over the 12 hours of observation, while the animals that received CONT, RUMF and ALGA treatments showed pH means close to 5.70 over the 12 hours of observation, and the animals that received the BICA treatment showed an average pH of 5.60 over the 12 hours.

The prevalence time of pH lower than 5.80 and lower than or equal to 5.6 (Figure 1c) showed no difference ($p > 0.05$) across treatments, but RUMO had the lowest prevalence time at 240 minutes with pH lower than 5.80, and 48 minutes with pH lower or equal to 5.60. ALGA and BICA were the treatments that showed greater prevalence time with pH lower than 5.80 (348 and 336 minutes, respectively), followed by RUMF and CONT (264 and 252 minutes, respectively). For prevalence time with pH lower or equal to 5.60, BICA showed the greatest time (216 min.), followed by RUMF, ALGA and CONT, at 180, 156 and 144 minutes, respectively. When the maximum and minimum pH is analyzed (Figure 1d) no differences were observed ($p > 0.05$) across treatments. However, the CONT treatments showed the lowest pH, followed by BICA, RUMF, ALGA and RUMO (4.81, 4.85, 4.97, 5.23 and 5.53, respectively).

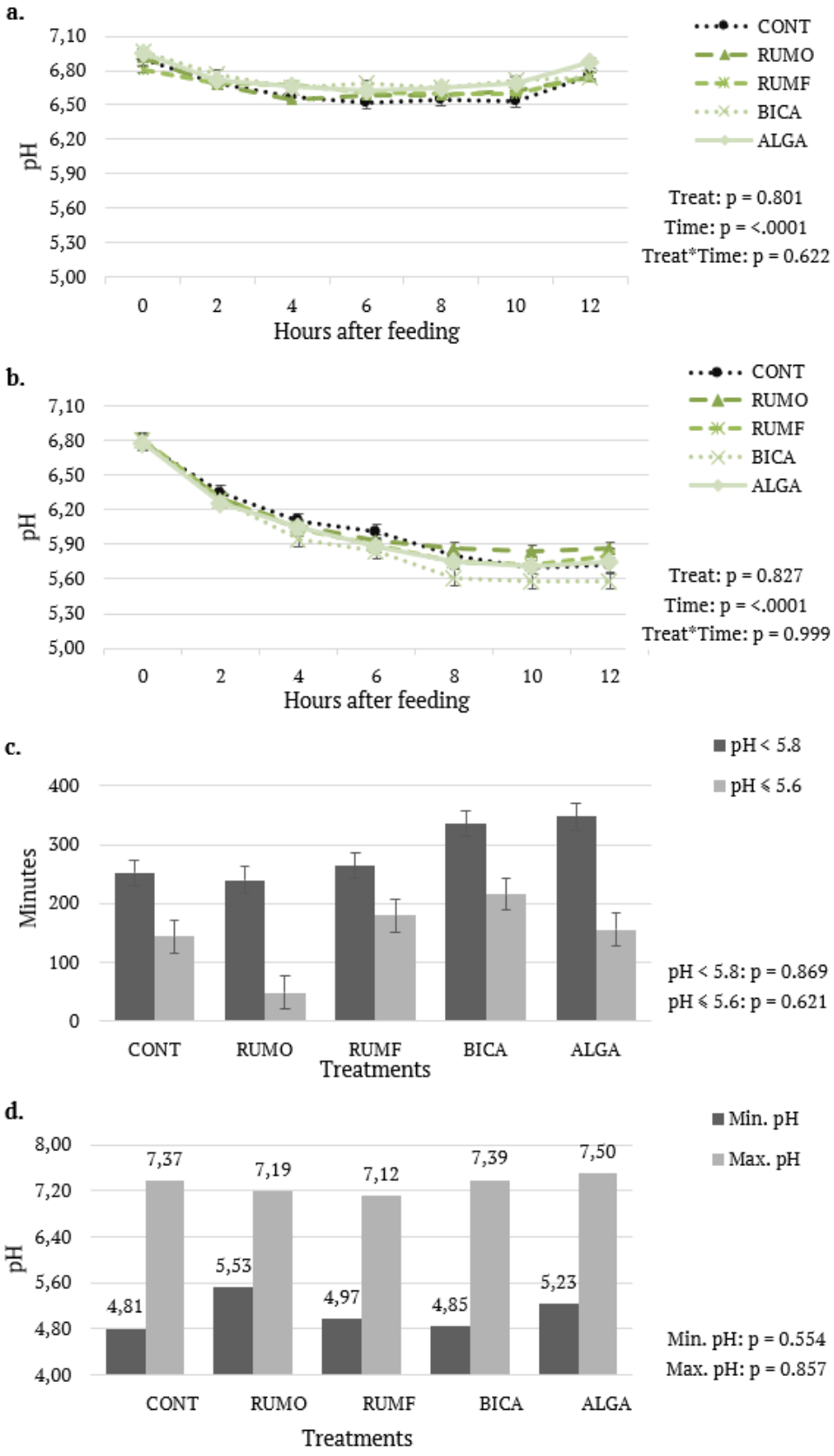

Figure 1. Ruminal pH. a. Ruminal pH of animal in non-sub-acute ruminal acidosis. b. Ruminal pH of animal in sub-acute ruminal acidosis induction. c. Prevalence time of ruminal pH below 5.8 and below or equal to 5.6 of animal in sub-acute ruminal acidosis induction. d. Minimum and maximum pH of animal in sub-acute ruminal acidosis induction. CONT (without addition of buffers); RUMO (sodium bicarbonate, calcium carbonate, magnesium oxide, magnesium carbonate, sodium carbonate, calcareous seaweed, honey aroma, neotame, sodium saccharin, maltodextrin and silicon dioxide); RUMF (sodium bicarbonate, calcium carbonate, magnesium oxide, calcareous seaweed, honey aroma, neotame, sodium saccharin, maltodextrin and silicon dioxide); BICA (calcium bicarbonate); ALGA (marine algae – *Lithothamnium calcareum*).

Volatile fatty acid

Diet effect

The treatments had no effect ($p > 0.05$) on total concentration of volatile fatty acids (VFA) (Table 4). Thus, the various buffers used, as well as their combinations, did not alter ($p > 0.05$) the ruminal metabolism of total VFA.

Table 4. Volatile fatty acid concentrations (mML^{-1}) in the rumen fluid of bulls fed with a high-grain diet and subjected to sub-acute ruminal acidosis (SARA) induction.

Time, hours	Treatments					SEM[6]	p <
	CONT[1]	RUMO[2]	RUMF[3]	BICA[4]	ALGA[5]		
Total volatile fatty acid							
0	105.88	94.32	97.07	64.46B	76.22B	18.465	0.168
2	105.77	103.64	105.48	100.98A	102.46AB	10.568	0.993
4	102.61	106.84	110.33	104.85A	106.87A	8.205	0.910
8	103.12	108.08	102.00	102.81A	109.35A	7.354	0.790
12	107.07	106.22	109.50	107.34A	112.08A	13.579	0.993
SEM	11.072	12.164	11.115	10.313	9.925		
p <	0.984	0.794	0.732	0.004	0.015		
Acetic acid							
0	59.63	53.80	49.69	38.49B	44.58B	10.097	0.273
2	55.26	57.49	51.69	56.21A	56.87A	4.523	0.746
4	53.31	59.31	54.82	61.99A	58.99A	7.006	0.728
8	51.60	59.04	50.22	60.79A	58.22A	5.986	0.328
12	52.74	57.49	53.63	60.28A	58.35A	5.451	0.609
SEM	6.292	6.629	4.907	6.087	5.520		
p <	0.636	0.924	0.802	0.007	0.049		
Propionic acid							
0	24.84	22.36	24.24B	14.74B	18.17B	4.198	0.084
2	27.09	28.13	29.40AB	25.11A	26.19A	3.007	0.798
4	26.94ab	29.80a	31.46Aa	23.62Ab	27.69Aa	1.679	0.003
8	26.85	30.18	29.62AB	23.68A	28.72A	4.014	0.510
12	26.53	28.55	31.25A	25.22A	28.05A	5.044	0.801
SEM	3.415	3.118	2.244	3.106	2.399		
p <	0.969	0.135	0.034	0.019	0.003		
Acetic:propionic ratio							
0	2.40B	2.48A	2.11A	2.70	2.47A	0.301	0.384
2	2.09A	2.06B	1.76AB	2.32	2.20B	0.213	0.316
4	2.00A	2.01B	1.76B	3.03	2.14BC	0.601	0.297
8	1.97A	1.98B	1.71B	2.01	2.04C	0.185	0.404
12	2.03A	2.04B	1.76B	2.02	2.10BC	0.201	0.479
SEM	0.082	0.111	0.095	0.577	0.050		
p <	0.004	0.002	0.006	0.662	0.001		
Butyric acid							
0	16.61	13.80	18.71	8.34	9.80B	5.132	0.211
2	19.48	13.49	20.11	16.00	15.47A	5.491	0.761
4	18.82	13.63	20.04	16.06	16.38A	5.367	0.780
8	21.37	14.66	18.49	15.53	18.64A	5.333	0.731
12	24.42	15.80	20.27	18.78	21.33A	7.948	0.860
SEM	2.771	2.647	4.333	3.732	3.554		
p <	0.129	0.898	0.989	0.116	0.049		
Isobutyric acid							
0	1.14A	0.98	0.99	0.80	0.99	0.145	0.219
2	0.89AB	1.01	0.87	0.86	0.90	0.123	0.744
4	0.80AB	0.92	0.84	0.74	0.84	0.126	0.693
8	0.67B	0.89	0.77	0.63	0.78	0.179	0.626
12	0.65B	0.92	0.93	0.74	0.85	0.200	0.587
SEM	0.097	0.087	0.076	0.105	0.101		
p<	0.002	0.631	0.075	0.287	0.318		
Valeric acid							
0	1.35	1.32	1.43	0.75	0.93	0.361	0.218
2	1.230	1.58	1.70	1.19	1.29	0.336	0.467
4	1.19	1.45	1.58	1.07	1.26	0.318	0.535
8	1.17	1.46	1.42	0.95	1.26	0.310	0.492
12	1.22	1.44	1.65	1.16	1.35	0.392	0.753

SEM	0.138	0.223	0.166	0.190	0.175		
p <	0.806	0.861	0.612	0.169	0.180		
Isovaleric acid							
0	1.91Aa	1.59ab	1.36Aab	1.14b	1.48ab	0.244	0.046
2	1.34B	1.41	1.03AB	1.12	1.21	0.249	0.564
4	1.18B	1.24	0.95AB	0.93	1.13	0.270	0.710
8	1.09B	1.35	0.91B	0.80	1.10	0.335	0.553
12	1.15B	1.55	1.16AB	1.02	1.37	0.413	0.730
SEM	0.143	0.234	0.132	0.185	0.150		
p <	0.001	0.573	0.025	0.370	0.096		
Hexanoic acid							
0	0.44	0.46	0.64	0.40	0.26B	0.247	0.366
2	0.42	0.53	0.69	0.47	0.54A	0.226	0.858
4	0.37	0.49	0.64	0.45	0.57A	0.224	0.786
8	0.37	0.51	0.57	0.42	0.63A	0.198	0.759
12	0.36	0.49	0.62	0.53	0.78A	0.260	0.581
SEM	0.087	0.118	0.169	0.144	0.143		
p <	0.765	0.978	0.993	0.243	0.027		

[1]CONT (without addition of buffers); [2]RUMO (sodium bicarbonate, calcium carbonate, magnesium oxide, magnesium carbonate, sodium carbonate, calcareous seaweed, honey aroma, neotame, sodium saccharin, maltodextrin and silicon dioxide); [3]RUMF (sodium bicarbonate, calcium carbonate, magnesium oxide, calcareous seaweed, honey aroma, neotame, sodium saccharin, maltodextrin and silicon dioxide); [4]BICA (calcium bicarbonate); [5]ALGA (marine algae – *Lithothamnium calcareum*); [6]SEM (stander error of the mean); [a, b, c]Means followed by different lowercase in the same line were different (Tukey's test ($p < 0.05$). [A, B, C]Means followed by different uppercase letter in the same column were different (Tukey's test, $p < 0.05$).

Time effect

For treatments CONT, RUMO and RUMF, the collection times of rumen fluid (before and 12 hours after feeding) had no effect ($p > 0.05$) in the concentration of total volatile fatty acids (Table 4). However, before the morning feeding, the concentration of total volatile fatty acids was lower ($p < 0.05$) in the BICA and ALGA treatments. Nevertheless, two hours after the morning feeding, the concentration of VFA increased and remained close to 105.3 mmL^{-1} (Table 4).

Acetic acid concentrations were similar and unaffected ($p > 0.05$) by the CONT, RUMO and RUMF treatments. On the other hand, before the morning feeding, acetic acid concentrations were low, then increased ($p < 0.05$) two hours after feeding and remained high until the end of the collection period (12 hours).

The time of collection had no effect on propionic acid concentrations in the CONT and RUMO treatments. Concentrations were low before the morning feeding, then increased in the first two hours and remained high until the last collection time (12 hours) in the RUMF, BICA and ALGA treatments (Table 4).

Before the morning feeding, the acetic/propionic ratio was at 2.43, then was reduced ($p < 0.05$) to 2.05 two hours after feeding and remained at these levels until the end of the day (Table 4).

For the other fatty acids (butyric, isobutyric, valeric, isovaleric and hexanoic), the collection time of the ruminal fluid was not important, with the exception of butyric and hexanoic fatty acids, with an increase in concentration two hours after the morning feeding (Table 4).

Ruminal lactic acid

Before the morning feeding, lactic acid concentrations in the ruminal fluid were similar ($p > 0.05$) in bulls of all treatments (Table 5). Lactic acid concentrations in the ruminal fluid varied from 1.43 mML^{-1} in bulls fed with the BICA diet, to 1.83 mML^{-1} in bulls fed with the RUMO diet. However, from the second to the twelfth postprandial hours, lactic acid concentrations were higher ($p < 0.01$) in the ruminal fluid of bulls fed with BICA, compared to the concentrations observed in the ruminal fluids of bulls fed with other diets (Table 5). Ruminal fluid lactic acid concentrations in bulls fed with the CONT, RUMO, RUMF and ALGA diets were similar ($p > 0.05$) at all times studied. For these four diets, lactic acid concentrations ranged from 1.43 to 3.02 mMol L^{-1}, while the concentrations observed in bulls fed with the BICA diet were above 5.0 mMol L^{-1} two hours after SARA induction, up until 12 hours from collection time.

Collection times, before and after the morning feeding had no effect ($p > 0.05$) on lactic acid concentrations in the ruminal fluid of bulls fed with the CONT, RUMO, RUMF and ALGA diets, with the exception of an increase observed at 12 hours in bulls fed with ALGA (Table 5). However, in BICA bulls, there was a linear increase ($p < 0.05$) in lactic acid concentrations as a function of collection time, from 1.66 before the morning feeding to 8.83 mMol L^{-1} 12 hours after feeding (Table 5).

Table 5. Lactic acid concentrations (mML^{-1}) in rumen liquid of bulls fed with a high-grain diet and subjected to sub-acute ruminal acidosis (SARA) induction.

Time, hours	Treatments					SEM[6]	p <
	CONT[1]	RUMO[2]	RUMF[3]	BICA[4]	ALGA[5]		
0	1.72	1.81	1.65	1.66A	1.43A	0.179	0.611
2	2.02a	2.13a	1.75a	5.17bB	1.88aA	1.517	0.048
4	1.83a	1.45a	1.92a	7.44bC	1.87aA	2.514	0.041
8	2.32a	1.85a	2.14a	8.09bC	1.92aA	2.775	0.046
12	2.09a	1.89a	1.96a	8.83bC	3.02aB	3.164	0.048
SEM[6]	0.118	0.114	0.097	2.285	0.026		
P <	0.248	0.903	0.262	0.035	0.050		

[1]CONT (without addition of buffers); [2]RUMO (sodium bicarbonate, calcium carbonate, magnesium oxide, magnesium carbonate, sodium carbonate, calcareous seaweed, honey aroma, neotame, sodium saccharin, maltodextrin and silicon dioxide); [3]RUMF (sodium bicarbonate, calcium carbonate, magnesium oxide, calcareous seaweed, honey aroma, neotame, sodium saccharin, maltodextrin and silicon dioxide); [4]BICA (calcium bicarbonate); [5]ALGA (marine algae – *Lithothamnium calcareum*); [6]SEM (stander error of the mean); [a, b, c]Means followed by different lowercase in the same line were different (Tukey's test, $p < 0.05$). [A, B, C]Means followed by different uppercase in the same column were different (Tukey's test, $p < 0.05$).

Ruminal N-NH_3

Before the morning feeding (0 hour) and two hours after, ammoniacal nitrogen (N-NH_3) concentrations were higher ($p < 0.05$) in the ruminal fluid of bulls fed with the CONT (14.6 and 16.7 mg dL^{-1}) and RUMO (14.2 and 15.4 mg dL^{-1}) diets, lower in bulls fed with the RUMF diet (8.11 and 8.77 mg dL^{-1}) and intermediate in bulls fed with the BICA (10.2 and 12.86 mg dL^{-1}) and ALGA (11.7 and 15.9 mg dL^{-1}) diets (Table 6). Four hours post-feeding, N-NH_3 concentrations were higher ($p < 0.05$) in the ruminal fluid of bulls fed with CONT (12.5 mg dL^{-1}), lower for those fed with RUMO (7.04 mg dL^{-1}) and RUMF (4.79 mg dL^{-1}) and intermediate for those fed with BICA (8.57 mg dL^{-1}) and ALGA (8.21 mg dL^{-1}) (Table 6). Eight hours post-feeding, N-NH_3 concentrations in the ruminal fluid of bulls fed with the CONT diet (9.43 mg/dL) were higher for cattle fed with the RUMF diet (5.20 mg dL^{-1}). The concentration of N-NH_3 was 7.79, 7.42 and 7.44 mg dL^{-1} for bulls fed with RUMO, BICA and ALGA, respectively (Table 6). In the last collection of the day (12 hours), the concentrations of N-NH_3 in the ruminal fluid of bulls were higher ($p < 0.05$) for CONT (11.8 mg dL^{-1}), lower for RUMO (6.29 mg dL^{-1}) and ALGA (7.02 mg dL^{-1}), and intermediate for RUMF (9.96 mg dL^{-1}) and BICA (8.95 mg dL^{-1}) (Table 6). In general, regardless of the ruminal fluid collection time, the highest N-NH_3 concentrations were observed in the ruminal fluid of bulls fed with CONT (13.0 mg dL^{-1}), lowest for RUMF (7.3 mg dL^{-1}) and intermediate for RUMO (10.2 mg dL^{-1}), BICA (9.6 mg dL^{-1}) and ALGA (10.0 mg dL^{-1}) (Table 6).

Table 6. N-NH_3 concentrations (mg dL^{-1}) in the rumen liquid of bulls fed with a high-grain diet and subjected to sub-acute ruminal acidosis (SARA) induction.

Times, hours	Treatments					SEM[6]	p <
	CONT[1]	RUMO[2]	RUMF[3]	BICA[4]	ALGA[5]		
0	14.59a	14.16aA	8.11cA	10.18bA	11.68bA	2.287	0.026
2	16.67a	15.40aA	8.77cA	12.79bA	15.86aA	3.991	0.063
4	12.50a	7.04bB	4.79cB	8.57bB	8.21bB	2.125	0.017
8	9.61a	7.79aB	5.21bB	7.42aB	7.44aB	1.763	0.007
12	11.81a	6.29bB	9.96aA	8.95aB	7.02bB	2.231	0.043
SEM	1.462	1.194	0.889	1.135	1.312		
P <	0.056	0.022	0.043	0.014	0.100		

[1]CONT (without addition of buffers); [2]RUMO (sodium bicarbonate, calcium carbonate, magnesium oxide, magnesium carbonate, sodium carbonate, calcareous seaweed, honey aroma, neotame, sodium saccharin, maltodextrin and silicon dioxide); [3]RUMF (sodium bicarbonate, calcium carbonate, magnesium oxide, calcareous seaweed, honey aroma, neotame, sodium saccharin, maltodextrin and silicon dioxide); [4]BICA (calcium bicarbonate); [5]ALGA (marine algae – *Lithothamnium calcareum*); [6]SEM (stander error of the mean); [a, b, c]Means followed by different lowercase in the same line were different (Tukey's test, $p < 0.05$). [A, B, C]Means followed by different uppercase in the same column were different (Tukey's test, $p < 0.05$).

Parameters in blood plasma

Parameters were measured in blood plasma before the morning feeding and two hours after, on the second day of ruminal acidosis induction (Table 7). Thus, these parameters could be altered due to the induction of acidosis on the previous day.

Before the morning feeding and two hours after, glucose concentrations were similar ($p < 0.05$) in the blood plasma of bulls from all diets (Table 7). Similarly, collection times had no effect ($p > 0.05$) on blood plasma glucose concentrations (Table 7).

Before the morning feeding, blood plasma urea concentrations were higher ($p < 0.05$) in bulls fed with CONT (16.5 mg dL^{-1}) and BICA (15.2 mg dL^{-1}), lower for bulls fed with RUMO (12.1 mg dL^{-1}) and RUMF (11.6 mg dL^{-1}), and intermediate for the animals fed with ALGA (13.1 mg dL^{-1}). However, after 2 postprandial hours, blood plasma urea concentrations of bulls showed no differences ($p < 0.05$) across diets. Likewise, observed concentrations before and 2 hours later were similar ($p > 0.05$).

Lactate concentrations were higher ($p < 0.05$) in the blood plasma of bulls fed with CONT (18.5 mg dL^{-1}), RUMF (20.1 mg dL^{-1}) and ALGA (17.2 mg dL^{-1}), and lower for RUMO (10.0 mg dL^{-1}) and BICA (12.7 mg dL^{-1}) (Table 7).

High blood plasma lactate values are characteristic of animals suffering from ruminal acidosis. In this way, cattle fed with the RUMO and BICA diets had better comfort in relation to ruminal acidosis related to blood lactate concentrations.

As observed for glucose concentrations, diets and blood collection times had no effect ($p > 0.05$) in blood plasma creatinine concentrations (Table 7). Creatinine values ranged from 14.9 to 20.1 mg dL^{-1} across diets and collection times. This variation is within the values observed for blood creatine concentrations in ruminants (Jain & Jain, 1993; Kramer, 2000).

Table 7. Parameters in the blood plasma of bulls fed with a high-grain diet and subjected to sub-acute ruminal acidosis (SARA) induction.

	Treatments						
Parameters	CONT[1]	RUMO[2]	RUMF[3]	BICA[4]	ALGA[5]	SEM[6]	p <
Glucose, mg dL^{-1}							
0 hour	62.5	71.1	71.2	70.1	71.4	3.57	0.067
2 hours	66.0	67.4	70.0	62.4	64.3	3.06	0.101
SEM	3.36	3.66	3.28	3.06	3.33		
p <	0.670	0.445	0.505	0.611	0.542		
Urea, mg dL^{-1}							
0 hora	16.5a	12.1b	11.6c	15.2a	13.1b	0.802	0.013
2 horas	18.4a	13.5b	15.3ab	15.2ab	16.2ab	1.020	0.034
SEM	0.778	0.665	0.771	0.772	0.567		
p <	0.677	0.675	0.564	0.541	0.556		
Lactate, mg dL^{-1}							
0 hour	18.5a	10.0b	20.1aA	12.7bB	17.2aA	0.152	0.024
2 hours	18.9a	12.1c	16.3bB	15.0bA	14.9bB	0.108	0.011
SEM	0.133	1.032	1.254	1.250	1.345		
p <	0.726	0.876	0.050	0.046	0.037		
Creatinine, mg dL^{-1}							
0 hour	2.04	1.79	1.92	2.04	2.17	0.070	0.058
2 hours	1.85	1.75	1.99	2.10	1.97	0.083	0.057
SEM	0.031	0.033	0.028	0.030	0.028		
p <	0.887	0.776	0.675	0.556	0.441		

[1]CONT (without addition of buffers); [2]RUMO (sodium bicarbonate, calcium carbonate, magnesium oxide, magnesium carbonate, sodium carbonate, calcareous seaweed, honey aroma, neotame, sodium saccharin, maltodextrin and silicon dioxide); [3]RUMF (sodium bicarbonate, calcium carbonate, magnesium oxide, calcareous seaweed, honey aroma, neotame, sodium saccharin, maltodextrin and silicon dioxide); [4]BICA (calcium bicarbonate); [5]ALGA (marine algae – *Lithothamnium calcareum*); [6]SEM (stander error of the mean); [a, b, c]Means followed by different lowercase in the same line were different (Tukey's test ($p < 0.05$). [A, B, C]Means followed by different uppercase in the same column were different (Tukey's test, $p < 0.05$).

Blood serum parameters

Diets and blood collection times (before feeding and 2 hours after) had no effect ($p < 0.05$) on total protein, albumin and globulin concentrations in the blood serum of bulls subjected to SARA induction (Table 8). The variations in the blood serum observed across diets and collection times are considered normal, according to reference data (Jain & Jain, 1993; Kramer, 2000).

Gamma-glutamyl transferase (GGT) concentrations were lower ($p < 0.05$) in the blood serum of cattle fed with RUMO (9.60 u L^{-1}) compared to the other diets (Table 6), where it had similar concentrations ($p > 0.05$). Although kidney tissue has the highest levels of GGT, this enzyme in the blood appears to originate from the hepatobiliary system. Its activity is increased in all forms of liver disease. GGT can be elevated in even small subclinical levels of liver dysfunction. Thus, in this study, the RUMO diet showed greater protection of the hepatic system in cattle. On the other hand, observed concentrations before feeding and 2 hours after were similar ($p > 0.05$). Therefore, the short time between blood collections (2 hours) did not influence the concentrations of this enzyme.

The concentrations of the enzyme aspartate aminotransferase (AST) were lower ($p < 0.05$) in the blood plasma of cattle fed with BICA (44.0 IU L^{-1}) in relation to the concentrations observed in the blood serum of animals on the other diets, and there was no difference ($p < 0.05$) between the concentrations of the animals fed with CONT, RUMO, RUMF or ALGA (Table 8). On the other hand, collection time (before and 2 hours after feeding) had no effect ($p > 0.05$) on this enzyme.

Creatinine kinase enzyme concentrations were similar ($p > 0.05$) in the blood serum of cattle fed with CONT and RUMF both before and 2 hours after feeding, but were higher in comparison to RUMO, BICA and ALGA (Table 8). On the other hand, creatine kinase enzyme concentrations were higher two hours after feeding, compared to concentrations observed before feeding (Table 8).

Table 8. Parameters in the blood serum of bulls fed with a high-grain diet and subjected to sub-acute ruminal acidosis (SARA) induction.

	Treatments						
Parameters/hours	CONT[1]	RUMO[2]	RUMF[3]	BICA[4]	ALGA[5]	SEM[6]	p <
Total protein, g dL^{-1}							
0 hour	8.06	8.64	8.28	8.41	8.69	0.881	0.448
2 hours	7.76	8.15	8.11	8.29	8.25	0.992	0.552
SEM	0.776	0.811	0.772	0.776	0.791		
p <	0.443	0.445	0.334	0.671	0.682		
Albumin, g dL^{-1}							
0 hour	4.08	4.19	4.05	4.38	4.47	0.441	0.711
2 hours	3.99	4.16	4.16	4.23	4.29	0.338	0.665
SEM	0.405	0.398	0.401	0.388	0.398		
p <	0.611	0.554	0.445	0.505	0.662		
Globulin, g dL^{-1}							
0 hour	3.98	4.45	4.22	4.03	4.22	0.385	0.722
2 hours	3.77	3.99	3.95	4.06	3.96	0.332	0.446
EPM	0.315	0.332	0.344	0.328	0.235		
p <	0.772	0.778	0.656	0.442	0.488		
Gamma-glutamyl transferase, u L^{-1}							
0 hour	12.8ab	9.60c	10.9bc	11.7ab	13.5[a]	0.667	0.013
2 hours	9.60	12.6	12.5	9.90	11.9	0.655	0.012
SEM	0.565	0.617	0.711	0.688	0.665		
p <	0.561	0.668	0.786	0.778	0.743		
Aspartate aminotransferase, UI L^{-1}							
0 hour	48.2	47.0	49.7	44.0	48.7	3.886	0.059
2 hours	45.4	45.6	53.7	46.6	47.9	3.778	6.111
SEM	4.233	4.444	3.895	3.886	4.764		
p <	0.334	0.334	0.376	0.440	0.336		
Creatine kinase, u L^{-1}							
0 hour	121.0aB	68.0bB	156.0aB	75.2bB	89.5bB	10.012	0.015
2 hours	188.1aA	110.3bA	230.3aA	130.2bA	105.0bA	12.355	0.325
SEM	10.333	9.887	8.785	6.225	5.883		
p <	0.001	0.001	0.001	0.001	0.001		

[1]CONT (without addition of buffers); [2]RUMO (sodium bicarbonate, calcium carbonate, magnesium oxide, magnesium carbonate, sodium carbonate, calcareous seaweed, honey aroma, neotame, sodium saccharin, maltodextrin and silicon dioxide); [3]RUMF (sodium bicarbonate, calcium carbonate, magnesium oxide, calcareous seaweed, honey aroma, neotame, sodium saccharin, maltodextrin and silicon dioxide); [4]BICA (calcium bicarbonate); [5]ALGA (marine algae – *Lithothamnium calcareum*); [6]SEM (stander error of the mean); [a, b, c]Means followed by different lowercase in the same line were different (Tukey's test, $p < 0.05$). [A, B, C]Means followed by different uppercase in the same column were different (Tukey's test, $p < 0.05$).

Animal behaviour

Animal feeding behaviour (Table 9) showed no difference ($p > 0.05$) in feeding and drinking time (minutes) across diets. When analyzing rumination time, a difference ($p < 0.0176$) was observed. The BICA treatment had the longest rumination time (109.00 minutes) followed by ALGA (81.00 minutes), RUMF (80.00 minutes), CONT (59.00 minutes) and RUMO (55.20 minutes), respectively. For the idleness time parameter, a difference was observed ($p < 0.0023$) among diets, as the animals that received the CONT and RUMOX diets showed an increase in their idle time. However, the RUMOX diet did not differ from other diets. In regards to the number of visits to the feeder, CONT was different from BICA ($p < 0.03$). Finally, no difference was observed ($p > 0.05$) in the number of visits to the water drinker across all diets.

Table 9. Animal ingestive behaviour of bulls fed with a high-grain diet and subjected to sub-acute ruminal acidosis (SARA) induction.

Parameters	Treatments					SEM[6]	p <
	CONT[1]	RUMO[2]	RUMF[3]	BICA[4]	ALGA[5]		
Feeding time, min	176.00	206.00	206.00	217.00	213.00	17.642	0.220
Drinking time, min	35.00	47.00	52.00	44.00	47.00	14.224	0.812
Rumination time, min	59.00b	55.20b	80.00ab	109.00a	81.00ab	14.186	0.018
Idleness time, min	450.00a	395.00ab	382.00b	340.00b	383.80b	19.863	0.002
Feeder, no. visit	11.80b	13.80ab	14.20ab	17.40a	15.8ab	1.545	0.033
Water drinker, no. visit	5.80	6.40	7.20	7.80	7.80	1.818	0.756

[1]CONT (without addition of buffers); [2]RUMO (sodium bicarbonate, calcium carbonate, magnesium oxide, magnesium carbonate, sodium carbonate, calcareous seaweed, honey aroma, neotame, sodium saccharin, maltodextrin and silicon dioxide); [3]RUMF (sodium bicarbonate, calcium carbonate, magnesium oxide, calcareous seaweed, honey aroma, neotame, sodium saccharin, maltodextrin and silicon dioxide); [4]BICA (calcium bicarbonate); [5]ALGA (marine algae – *Lithothamnium calcareum*); [6]SEM (stander error mean). [a, b, c]Means followed by different lowercase in the same line were different (Tukey's test, $p < 0.05$).

Discussion

Apparent digestibility

Apparent digestibility was not altered by the inclusion of different buffers in the diets. However, the apparent digestibility of all nutrients was high (greater than 70% for dry matter and organic matter, greater than 80% for crude protein and non-fiber carbohydrates). The high apparent digestibility for all treatments, including CONT, could be explained in part by the diet's composition (high content of grains rapidly degraded in the rumen) and the feeding system (restricted intake, 2% of body weight). In general, cattle finished in feedlot and finished with high grain diets have high apparent digestibility (Cruz et al., 2014; Ornaghi et al., 2017; Souza et al., 2019; Mottin et al., 2020). Sharma et al. (2018) carried out a long review of studies carried out using various buffers in diets for beef cattle, dairy cows and sheep, and concluded that these compounds have little significant effect on feed intake and apparent digestibility of dry matter, organic matter, crude protein, ether extract, neutral detergent fiber and carbohydrates, as observed in this study. Thus, the use of buffers would have little effect on the utilization of nutrients in the diets.

Ruminal pH

Ruminal pH is a critical factor in the normal and stable function of the rumen, because it has a profound effect on microbial populations, fermentation products, and physiological functions, mainly motility and absorptive function (Nagaraja & Titgemeyer, 2007; Abdela, 2016). Thus, low ruminal pH for prolonged periods can negatively affect dry matter intake and fiber degradation, resulting in reduced productive performance and, subsequently, large financial losses (Vyas et al., 2014). Furthermore, there may be a reduction in fiber digestibility at low ruminal pH due to the reduction of the fibrolytic population, given the reduced ability of fibrolytic bacteria to bind to feed particles (Calsamiglia et al., 2002).

In this study, no significant differences were observed in ruminal pH across diets (Table 4 and Figure 1). In animals fed with high-grain diets, according to Nagaraja & Titgemeyer (2007), ruminal pH can range from 6.50 to 5.60, with an average pH around 5.80 to 6.20, and it can drop below 5.60 for a period during the feeding cycle. Moreover, in this study, we considered that animals showing ruminal pH below 5.80 were in sub-acute acidosis (Beauchemin et al., 2003). Thus, observing the development of the ruminal pH over 12 hours, it is considered that all treatments had sub-acute acidosis except for RUMO. Furthermore, Gozho et al. (2007) suggest that a drop in ruminal pH below 5.60 for more than 180 minutes was used as the threshold to characterize sub-acute acidosis. In this way, we observed that the use of 10 g Kg^{-1} of dry matter with the inclusion of sodium bicarbonate was not effective in reducing sub-acute acidosis.

Volatile fatty acids

The mean overall concentration of volatile fatty acids (all treatments) was 102.6 mML^{-1}, with no differences among treatments. Thus, the average concentration of fatty acids is close to the concentrations observed by several authors with the use of different buffers (Erdman, 1988; Iwaniuk & Erdman, 2015; Watanabe et al., 2019; Khalouei et al., 2021; Minami et al., 2021). Likewise, the concentrations of acetic, propionic, butyric, isobutyric, valeric, isovaleric and hexanoic acids, and the ratio between acetic and propionic acids were also not altered by the buffers, remaining close to the concentrations observed in other studies (Erdman, 1988; Iwaniuk & Erdman, 2015; Watanabe et al., 2019; Khalouei et al., 2021; Minami et al., 2021).

Lactic acid

Only the BICA diet (calcium bicarbonate) was not efficient in reducing lactic acid production. According to Nagaraja & Titgemeyer, 2007), the reason the pH drops below 5.60 is the accumulation of volatile fatty acids, which is a combination of overproduction (increased substrate), possibly decreased absorption, and the fact that, when pH is near 5.0 or below for a sustained period, the growth of lactate-fermenting bacteria is inhibited. Hence, lactate begins to accumulate. Ruminal lactate acid concentrations in SARA and acute acidosis mostly range from 0.17 to 0.74 and 50 to 120 mM, respectively (Arik et al., 2019). In this study, lactate acid concentrations between 1.81 (RUMO) and 6.48 mM L^{-1} (BICA) were observed, showing accumulation of lactate acid. When pH is near 5.0 or less for a sustained period, the growth of lactate-fermenting bacteria is inhibited, and lactate begins to accumulate in the ruminal environment, which is the result of increased production and decreased acid fermentation. The increase in lactic acid production is essentially due to the establishment of an amino acid-tolerant lactobacillus population, which accumulates in two isomers: D-lactate (produced in lesser quantity) and L-lactate (Jaramillo-López et al., 2017). Thus, decreased fermentation occurs because the pH is below the range required for lactate-fermenting bacteria to be active (Nagaraja & Titgemeyer, 2007) Total VFA concentration generally increases at the onset of acidosis but, with its progression, VFA concentrations decline drastically due to the destruction of normal bacterial flora, and ruminal dilution of fluid influx, to compensate for increased osmolality (Nagaraja & Titgemeyer, 2007).

There is a change in the ruminal microbiota, and bacteria that degrade fibers, such as *Butyrivibrio fibrisolvens*, *Bacteroides succinogenes*, *Ruminococcus albus*, and *Ruminococcus flavefaciens* cease growth with a pH lower than 6.00. Starch-degrading species such as *Selemonas ruminatiun* and *Streptococcus bovis* continue to grow at a pH closer to 5.00 (Kozloski, 2011). Furthermore, one population of protozoa did not survive prolonged exposure to pH below 5.50 (Quinn et al., 1962). The less species of bacteria and protozoa present in the rumen, the less stable the microflora, and the harder to maintain the pH close to normal during periods of sudden dietary changes (Soto et al., 2013).

Ruminal N-NH_3

The evolution of the kinetics of N-NH_3 concentrations in the ruminal fluid of cattle showed a similar dynamic for the CONT, RUMO, RUMF, and BICA treatments, with significant reduction 4 hours after the morning feeding (Table 6), with the exception of BICA. In this way, all buffers used in the diets were Maeda et al. (2007) able to reduce the production of N-NH_3 in the rumen, with emphasis on the RUMF treatment. Still, the others (RUMO, BICA, and ALGA) showed a better ability to buffer the rumen when compared to CONT. In general, N-NH_3 concentrations in the rumen are strongly elevated before and in the first two hours after feeding, and decrease after 4 hours (Fregadolli et al., 2001; Maeda et al., 2007; Prado et al., 2010).

Blood plasma parameters

Blood parameters were not influenced by the inclusion of various buffers. Glucose and urea levels ranged from 62.3 to 70.0 mg dL^{-1} and from 13.5 to 18.4 mg dL^{-1}, respectively. In general, the blood levels of glucose and urea in ruminants vary from 60 to 80 mg dL^{-1} for glucose and from 15 to 30 mg dL^{-1} for urea. Thus, the levels found in this study are within the normal range for ruminants. However, Xu et al. (2016) observed a significant reduction in dairy cows with sub-acute acidosis.

Glucose concentrations in the blood plasma range from 62.5 to 71.4 mg dL-1. These values are in agreement with the values considered normal (Jain & Jain, 1993; Kramer, 2000). In ruminants, diets and postprandial time have little effect on blood glucose concentrations, since glucose is metabolized very rapidly in the rumen and transformed into VFAs.

Before the morning feeding, blood plasma urea concentrations were higher ($p < 0.05$) in bulls fed with CONT (16.5 mg dL^{-1}) and BICA (15.2 mg dL^{-1}), lower for bulls fed with RUMO (12.1 mg dL^{-1}) and RUMF (11.6 mg dL^{-1}), and intermediate for the animals fed with the ALGA diet (13.1 mg dL^{-1}). However, after 2 postprandial hours, blood plasma urea concentrations of bulls showed no differences ($p < 0.05$) across diets. Likewise, observed concentrations before and 2 hours later were similar ($p > 0.05$).

Urea concentrations observed before and after feeding in blood plasma are within ruminant standards (5 – 20 mg dL^{-1}) (Jain & Jain, 1993; Kramer, 2000). Changes in the concentrations of urea in the blood of the animals are due to the degradation of the protein in the rumen. A higher rate of degradation means higher concentrations of urea in the blood. Thus, the CONT and BICA diets had lower buffering power in the rumen after the induction of ruminal acidosis. On the other hand, the RUMO and RUMF diets were more efficient in reducing protein degradation in the rumen; ALGA was neutral.

The levels of lactate in the blood ranged from 12.1 to 18.9 mg dL^{-1}; these values being normal for cattle in normal health conditions. Although without significant differences, lactate levels were 30% higher in the CONT treatment animals at 17.9 mg dL^{-1}, versus the 14.6 mg dL^{-1} mean of the other treatments. However, Xu et al. (2016) observed higher levels of lactate in the blood of dairy cows with sub-acute acidosis.

Blood serum parameters

The levels of total proteins, albumins, and globulins were not altered by the treatments. Levels of these components were normal for animals in good health. However, Xu et al. (2016) observed lower levels of total proteins, albumins, and globulins in the blood of dairy cows with sub-acute acidosis. When the liver is injured or diseases like diarrhea cause dehydration, the levels of total proteins, albumins, and globulins will be increased.

Gamma-glutamyl transferase, aspartate aminotransferase, creatinine, and creatinine kinase are increased when the animal's muscle or liver is damaged. In our study, treatments did not change creatinine, gamma-glutamyl transferase, and aspartate aminotransferase levels. Although without significant numerical differences, in the CONT treatment animals, creatine kinase levels were 70% higher. In dairy cows, levels of gamma-glutamyl transferase, aspartate aminotransferase, creatinine, and creatinine kinase are increased when animals are in sub-acute acidosis (Xu et al., 2016).

AST is an enzyme that catalyzes the conversion of the nitrogenous portion of an amino acid to an amino acid residue. This enzyme is essential for energy production in the Krebs cycle and is found in the cytoplasm and mitochondria of many cells, primarily the liver, heart, skeletal muscle, kidney, pancreas, and red blood cells. This enzyme is released into the blood in large amounts when there is damage to the hepatocyte membrane. Thus, the absolute elevation of aspartate aminotransferases has great diagnostic significance in acute liver diseases. Therefore, the addition of sodium bicarbonate to the diet enabled better protection of the hepatic system, while the other diets were neutral.

Animal behavior

When the animals exhibit sub-acute acidosis, feed intake and performance might be reduced, even though the animal does not appear sick (Owens et al., 1998). Because of this, this study analyzed the animals' intake behaviour, and significant differences were observed across treatments in rumination time, idleness time, and number of visits to the feeder. For rumination time, BICA showed the greatest time (109.00 minutes), which may be correlated with the amount of acid in the ruminal environment, and one possibility to control the acidosis. This is because, according to Bailey (1961) saliva secretion during rumination is similar to that while eating (25- and 20-mL $min.^{-1}$, respectively) and about 2 times greater than during resting in cattle (10 mL $min.^{-1}$). Moreover, saliva production is very important for rumen function, because it has high buffering capacity as a result of high concentrations of bicarbonate (125 mEq mL^{-1}) and phosphate (20 mEq mL^{-1}). Consequently, idleness time in the BICA treatment was reduced.

Furthermore, the BICA treatment showed the highest number of visits to the feeder, and CONT showed the lowest number (17.40 and 11.80 visits). Greater meal frequency and wider distribution of daily intake throughout the day lead to better synchronization in time between bouts of acid and saliva production, and absorption and passage of organic acids from the rumen. The time periods between feeding events allow timely metabolization of acids and elimination through urine of protons absorbed from the rumen (González et al., 2012). However, some factors can influence the animal's ingestive behavior, such as the grain utilized, particle size, carbohydrate availability, low ruminal pH, and the feed intake control mechanisms. In addition, the use of sodium bicarbonate can show alterations in animal behaviour (González et al., 2012). Besides, short-term feed intake behaviour can be summarized by meal size and frequency, eating rate, and distribution of intake throughout the day. Moreover, it is important to note that the method used to calculate these short-term feeding behaviour measurements may have a great influence on the outcomes (Nielsen, 1999; González et al., 2012).

Conclusion

The present results showed all treatments were submitted in sub-acute ruminal acidosis. However, only the RUMO treatment showed less impact on the ruminal pH and ruminal lactate acid, being a mixture of sodium bicarbonate, calcium carbonate, magnesium oxide, magnesium, sodium carbonate, calcareous seaweed, honey aroma, neotame, sodium saccharin, maltodextrin, and silicon dioxide. In contrast, the animals that received the BICA treatment were more susceptible to ruminal acidosis than those with other

treatments, which had lower ruminal pH and greater time prevalence with pH < 5.80 or ⩽ 5.60. However, further studies must be carried out to define the best dose for animal supplementation and possible financial impact on cattle production.

Data availability

Not applicable.

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References

Abdela, N. (2016). Sub-acute ruminal acidosis (SARA) and its consequence in dairy cattle: a review of past and recent research at global prospective. *Achievements in the Life Sciences, 10*(2), 187-196. https://doi.org/10.1016/j.als.2016.11.006

Almeida, F., Schiavo, L. V., Vieira, A. D., Araújo, G. L., Queiroz-Junior, C. M., Teixeira, M. M., Cassali, G. D., & Tagliati, C. A. (2012). Gastroprotective and toxicological evaluation of the *Lithothamnion calcareum* algae. *Food and Chemical Toxicology, 50*(5), 1399-1404. https://doi.org/10.1016/j.fct.2012.02.028

Association of Officiating Analytical Chemists. (2005). *Official method of Analysis*. Association of Officiating Analytical Chemists.

Arik, H. D., Gulsen, N., Hayirli, A., & Alatas, M. S. (2019). Efficacy of *Megasphaera elsdenii* inoculation in subacute ruminal acidosis in cattle. *Journal of Animal Physiology and Animal Nutrition, 103*(2), 416-426. https://doi.org/10.1111/jpn.13034

Bailey, C. B. (1961). Saliva secretion and its relation to feeding in cattle. *British Journal of Nutrition, 15*(4), 489-498. https://doi.org/10.1079/bjn19610062

Beauchemin, K. A., Yang, W. Z., & Rode, L. M. (2003). Effects of particle size of alfalfa-based dairy cow diets on chewing activity, ruminal fermentation, and milk production. *Journal of Dairy Science, 86*(2), 630-643. https://doi.org/10.3168/jds.S0022-0302(03)73641-8.

Boeckaert, C., Vlaeminck, B., Dijkstra, J., Issa-Zacharia, A., Van Nespen, T., Van Straalen, W., & Fievez, V. (2008). Effect of dietary starch or micro algae supplementation on rumen fermentation and milk fatty acid composition of dairy cows. *Journal of Dairy Science, 91*(12), 4714-4727. https://doi.org/http://dx.doi.org/10.3168/jds.2008-1178

Bürger, P. J., Pereira, J. C., Queiroz, A. C., Coelho, J. F., Valadares Filho, S. C., Cecon, P. R., Casali, A. D. P. (2000). Comportamento ingestivo de bezerros holandeses alimentados com dietas contendo diferentes níveis de concentrado. *Revista Brasileira de Zootecnia, 29*(1), 236-242. https://doi.org/http://dx.doi.org/10.1590/S1516-35982000000100031

Callaway, E. S., & Martin, S. A. (1997). Effects of a Saccharomyces cerevisiae culture on ruminal bacteria that utilize lactate and digest cellulose. *Journal of Dairy Science, 80*(9), 2035-2044. https://doi.org/10.3168/jds.S0022-0302(97)76148-4

Calsamiglia, S., Ferret, A., & Devant, M. (2002). Effects of pH and pH fluctuations on microbial fermentation and nutrient flow from a dual-flow continuous culture system. *Journal of Dairy Science, 85*(3), 574-579. https://doi.org/10.3168/jds.S0022-0302(02)74111-8

Carvalho, V. M., Ávila, V. A. D., Bonin, E., Matos, A. M., Prado, R. M., Castilho, R. C., Silva, R. R., Abreu Filho, B. A., & Prado, I. N. (2021). Effect of extract from baccharis, tamarind, cashew nut shell liquid and clove on animal performance, feed efficiency, digestibility, rumen fermentation and feeding behavior of bulls finished in feedlot. *Livestock Science, 244,* 104361. https://doi.org/10.1016/j.livsci.2020.104361

Carvalho, R. F., Mazon, M. R., Silva, A. P. S., Oliveira, L. S., Zotti, C. A., Silva, S. L., & Leme, P. R. (2016). Use of calcareous algae and monensin in Nellore cattle subjected to an abrupt change in diet. *Ciência Rural, 46*(4), 713-718. https://doi.org/10.1590/0103-8478cr20150278

Chaney, A. L., & Marbach, E. P. (1962). Modified reagents for determination of urea and ammonia. *Clinical Chemistry, 8*(2), 130-132. https://doi.org/10.1093/clinchem/8.2.130

Cruywagen, C. W., Taylor, S, Beya, M. M., & Calitz, T. (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. *Journal of Dairy Science, 98*(8), 5506-5514. https://doi.org/10.3168/jds.2014-8875

Cruz, O. T. B., Valero, M. V., Zawadzki, F., Rivaroli, D. C., Prado, R. M., Lima, B. S., & Prado, I. N. (2014). Effect of glycerine and essential oils (*Anacardium occidentale* and *Ricinus communis*) on animal performance, feed efficiency and carcass characteristics of crossbred bulls finished in a feedlot system. *Italian Journal of Animal Science, 13*(4), 3492. https://doi.org/10.4081/ijas.2014.3492

Erdman, R. A. (1988). Dietary buffering requirements of the lactating dairy cow: a review. *Journal of Dairy Science, 71*(12), 3246-3266. https://doi.org/10.3168/jds.S0022-0302(88)79930-0

Fregadolli, F. L., Zeoula, L. M., Branco, A. F., Prado, I. N., Caldas Neto, S. F., Guimarães, K. C., Kassies, M. P., & Dalponte, A. O. (2001). Efeitos das fontes de amido e nitrogênio de diferentes degradabilidades ruminais. 2. pH, concentração de amônia no líquido ruminal e eficiência de síntese microbiana. *Revista Brasileira de Zootecnia, 30*(3), 870-879. https://doi.org/10.1590/S1516-35982001000300036

Gastaldello Júnior, A. L., Pires, A. V., Susin, I., Mendes, C. Q., Queiroz, M. A. A., Amaral, R. C., Gentil, R. S., Ferreira, E. M., Mourão, G B., & Eastridge, M. L. (2013). Limestone with different particle size and sodium bicarbonate to feedlot lambs fed high grain diets with or without monensin. *Small Ruminant Research, 114*(1), 80-85. https://doi.org/10.1016/j.smallrumres.2013.05.009

González, L. A., Manteca, X., Calsamiglia, S., Schwartzkopf-Genswein, K. S., & Ferret, A. (2012). Ruminal acidosis in feedlot cattle: Interplay between feed ingredients, rumen function and feeding behavior (a review). *Animal Feed Science and Technology, 172*(1-2), 66-79. https://doi.org/10.1016/j.anifeedsci.2011.12.009

Gozho, G. N., Krause, D. O., & Plaizier, J. C. (2007). Ruminal lipopolysaccharide concentration and inflammatory response during grain-induced subacute ruminal acidosis in dairy cows. *Journal of Dairy Science, 90*(2), 856-866. https://doi.org/10.3168/jds.S0022-0302(07)71569-2

Han, G., Gao, X., Duan, J., Zhang, H., Zheng, Y., He, J., Huo, N., Pei, Li, H., & Gu, S. (2021). Effects of yeasts on rumen bacterial flora, abnormal metabolites, and blood gas in sheep with induced subacute ruminal acidosis. *Animal Feed Science and Technology, 280*, 115042. https://doi.org/10.1016/j.anifeedsci.2021.115042

Hernández, J., Benedito, J. L., Abuelo, A., & Castillo, C. (2014). Ruminal acidosis in feedlot: from aetiology to prevention. *The Scientific World Journal, 2014*, 702572. https://doi.org/10.1155/2014/702572

Herod, E. L., Bechtle, R. M., Bartley, E. E., & Dayton, A. D. (1978). Buffering ability of several compounds in vitro and the effect of a selected buffer combination on ruminal acid production in vivo. *Journal of Dairy Science, 61*(8), 1114-1122. https://doi.org/10.3168/jds.S0022-0302(78)83695-9

Huhtanen, P., Kaustell, K., & Jaakkola, S. (1994). The use of internal markers to predict total digestibility and duodenal flow of nutrients in cattle given six different diets. *Animal Feed Science and Technology, 48*(3-4), 211-227. https://doi.org/10.1016/0377-8401(94)90173-2

Iwaniuk, M. E., & Erdman, R. A. (2015). Intake, milk production, ruminal, and feed efficiency responses to dietary cation-anion difference by lactating dairy cows. *Journal of Dairy Science, 98*(12), 8973-8985. https://doi.org/10.3168/jds.2015-9949

Jain, N. C., & Jain, A. H. (1993). *Essentials of veterinary hematology*. Wiley-Blackwell.

Jaramillo-López, E., Itza-Ortiz, M. F., Peraza-Mercado, G., & Carrera-Chávez, J. M. (2017). Ruminal acidosis: strategies for its control. *Austral Journal of Veterinary Sciences, 49*(3), 139-148. https://doi.org/10.4067/S0719-81322017000300139

Khalouei, H., Seranatne, V., Fehr, K., Guo, J., Yoon, I., Khafipour, E., & Plaizier, J. C. (2021). Effects of Saccharomyces cerevisiae fermentation products and subacute ruminal acidosis on feed intake, fermentation, and nutrient digestibilities in lactating dairy cows. *Canadian Journal of Animal Science, 101*(1), 143-157. https://doi.org/10.1139/cjas-2020-0018

Kozloski, G. V. (2011). *Bioquímica dos ruminantes*. Editora Universidade Federal de Santa Maria.

Kramer, J. W. (2000). Normal hematology of cattle, sheep and goats. In B. F. Feldman, J. G. Zinkl, & N. C. Jain (Eds.), *Schalm's veterinary hematology* (pp. 1075-1084). Lippincott Williams and Wilkins.

Maeda, E. M., Zeoula, L. M., Juliano, L., Geron, V., Best, J., Prado, I. N., Martins, E. N., & Kazama, R. (2007). Digestibilidade e características ruminais de dietas com diferentes níveis de concentrado para bubalinos e bovinos Digestibility and ruminal metabolism on bubalines and bovines fed diets with different concentrate levels. *Revista Brasileira de Zootecnia, 36*(3), 716-726. https://doi.org/10.1590/S1516-35982007000300027

Matos, A. M., Duarte, V., Tagiariolli, M., Bonin, E., Vital, A. C. P., Guerrero, A., Prado, R. M., Costa e Silva, L. F., Ávila, V. D., Carvalho, V. M., & Prado, I. N. (2023). Meat acceptability of crossbred bulls fed a high-grain feedlot diet with antimicrobial compounds and a blend of organic trace minerals and yeast. *Animal Production Science, 63*(1), 85-95. https://doi.org/10.1071/AN22092

Mertens, D. R. (2002). Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: collaborative study. *Journal of AOAC International, 85*(6), 1217-1240.

Minami, N. S., Sousa, R. S., Oliveira, F. L. C., Dias, M. R. B., Cassiano, D. A., Mori, C. S., Minervino, A. H. H., & Ortolani, E. L. (2021). Subacute ruminal acidosis in Zebu cattle: Clinical and behavioral aspects. *Animals, 11*(1), 21. https://doi.org/10.3390/ani11010021

Mottin, C., Catalano, F. A. R., Chefer, D. M., Araújo, F. L., Carvalho, V. M., Guerrero, A., Ornaghi, M. G., Souza, K. A., Castilho, R. A., & Prado, I. N. (2020). Effect of essential and vegetable oil blend supplementation on animal performance, feed intake, rumen fermentation and rumen microbial populations of crossbred steers finished in a pasture system. *Research, Society and Development, 9*(9), 1-20. https://doi.org/10.33448/rsd-v9i9.8057

Nagaraja, T. G., & Titgemeyer, E. C. (2007). Ruminal acidosis in beef cattle: the current microbiological and nutritional outlook. *Journal of Dairy Science, 90*(Suppl), E17-E38. https://doi.org/10.3168/jds.2006-478

Neville, E. W., Fahey, A. G, Gath, V. P., Molloy, B. P., Taylor, S. J., & Mulligan, F. J. (2019). 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. *Journal of Dairy Science, 102*(9), 8027-8039. https://doi.org/10.3168/jds.2019-16244

Nielsen, B. L. (1999). On the interpretation of feeding behaviour measures and the use of feeding rate as an indicator of social constraint. *Applied Animal Behaviour Science, 63*(1), 79-91. https://doi.org/10.1016/S0168-1591(99)00003-9

Ornaghi, M. G., Passetti, R. A. C., Torrecilhas, J. A., Mottin, C., Vital, A. P., Guerrero, A., Sañudo, C., Campo, M. M., & Prado, I. N. (2017). Essential oils in the diet of young bulls: effect on animal performance, digestibility, temperament, feeding behaviour and carcass characteristics. *Animal Feed Science and Technology,* 234, 274-283. https://doi.org/10.1016/j.anifeedsci.2017.10.008

Owens, F. N., Secrist, D. S., Hill, W. J., & Gill, D. R. (1998). Acidosis in cattle: a review. *Journal of Animal Science, 76*(1), 275-286. https://doi.org/10.2527/1998.761275x

Plaizier, J. C., Li, S., Tun, H. M., & Khafipour, E. (2017). Nutritional models of experimentally-induced subacute ruminal acidosis (SARA) differ in their impact on rumen and hindgut bacterial communities in dairy cows. *Frontiers in Microbiology, 7*, 2128. https://doi.org/10.3389/fmicb.2016.02128

Plaizier, J. C., Mesgaran, M. D., Derakhshani, H., Golder, H., Khafipour, E., Kleen, J. L., Lean, I., Loor, J., Penner, G., & Zebeli, Q. (2018). Review: enhancing gastrointestinal health in dairy cows. *Animal, 12*(suppl 2), s399-s418. https://doi.org/10.1017/S1751731118001921

Prado, O. P. P., Zeoula, L. M., Moura, L. P. P., Franco, S. L., Prado, I. N., & Gomes, H. C. C. (2010). Digestibilidade e parâmetros ruminais de dietas à base de forragem com adição de própolis e monensina sódica para bovinos. *Revista Brasileira de Zootecnia, 39*(6), 1336-1345. https://doi.org/10.1590/S1516-35982010000600024

Pryce, J. D. (1969). A modificacion of the Barker-Summerson method for the determination of lactic acid. *Analyst, 94*(1125), 1151-1152.

Quinn, L. Y., Burroughs, W., & Christiansen, W. C. (1962). Continuous culture of ruminal microorganisms in chemically defined medium: II. Culture medium studies. *Applied Microbiology, 10*(6), 583-592. https://doi.org/10.1128/am.10.6.583-592.1962

Rivaroli, D. C., Campo, M. M., Sañudo, C., Guerrero, A., Jorge, A. M., Vital, A. C. P., Valero, M. V., Prado, R. M., & Prado, I. N. (2020). Effect of an essential oils blend on meat characteristics of crossbred heifers finished on a high-grain diet in a feedlot. *Animal Production Science, 60*(4), 595-602. https://doi.org/10.1071/AN18620

Sharma, H., Pal, R. P., Mir, S. H., Mani, V., & Ojha, L. (2018). Effect of feeding buffer on feed intake, milk production and rumen fermentation pattern in lactating animals: a review. *Journal of Entomology and Zoology Studies, 6*(4), 916-922.

Silva, R. R., Silva, F. F., Prado, I. N., Carvalho, G. G. P., Franco, I. L., Almeida, V. S., Cardoso, C. P., & Ribeiro, M. H. S. (2006). Comportamento ingestivo de bovinos. Aspectos metodológicos. *Archivos de Zootecnia, 55*(211), 293-296.

Soto, E. C., Molina-Alcaide, E., Khelil, H., & Yáñez-Ruiz, D. R. (2013). Ruminal microbiota developing in different in vitro simulation systems inoculated with goats' rumen liquor. *Animal Feed Science and Technology, 185*(1-2), 9-18. https://doi.org/http://dx.doi.org/10.1016/j.anifeedsci.2013.06.003

Souza, K., Monteschio, J. O., Mottin, C., Ramos, T., Pinto, L. A. M., Eiras, C. E., Guerrero, A., & Prado, I. N. (2019). Effects of diet supplementation with clove and rosemary essential oils and protected oils (eugenol, thymol and vanillin) on animal performance, carcass characteristics, digestibility, and ingestive behavior activities for Nellore heifers finished in feedl. *Livestock Science, 220*, 190-195. https://doi.org/10.1016/j.livsci.2018.12.026

Torrecilhas, J. A., Ornaghi, M. G., Passetti, R. A. C., Mottin, C., Guerrero, A., Ramos, T. R., Vital, A. C. P., Sañudo, C., Malheiros, E. B., & Prado, I. N. (2021). Meat quality of young bulls finished in a feedlot and supplemented with clove or cinnamon essential oils. *Meat Science, 174*, 108412. https://doi.org/10.1016/j.meatsci.2020.108412

Valente, T. N. P., Sampaio, C. B., Lima, E. D. S., Deminicis, B. B., Cezário, A. S., & Santos, W. (2017). Aspects of acidosis in ruminants with a focus on nutrition: a review. *Journal of Agricultural Science, 9*(3), 90-97.

Del Valle, T. A., Antonio, G., Zenatti, T. F., Campana, M., Zilio, E. M. C., Ghizzi, L. G., Gandra, J. R., Osório, J. A. C., & Morais, J. P. G. (2018). Effects of xylanase on the fermentation profile and chemical composition of sugarcane silage. *The Journal of Agricultural Science, 156*(9), 1123-1129. https://doi.org/10.1017/S0021859618001090.

Vyas, D., Uwizeye, A., Mohammed, R., Yang, W. Z., Walker, N. D., & Beauchemin, K. A. (2014). The effects of active dried and killed dried yeast on subacute ruminal acidosis, ruminal fermentation, and nutrient digestibility in beef heifers. *Journal of Animal Science, 92*(2), 724-732. https://doi.org/10.2527/jas.2013-7072

Watanabe, Y., Kim, Y-H., Kushibiki, S., Ikuta, K., Ichijo, T., & Sato, S. (2019). Effects of active dried Saccharomyces cerevisiae on ruminal fermentation and bacterial community during the short-term ruminal acidosis challenge model in Holstein calves. *Journal of Dairy Science, 102*(7), 6518-6531. https://doi.org/10.3168/jds.2018-15871.

Xu, C., Shen, T., Yang, W., Yu, H., Gao, S., & Huang, B. (2016). The effect of subacute ruminal acidosis of dairy cows on productivity, digestibility and greenhouse gas emission. Journal of Agricultural Science, 8(4), 92. https://doi.org/10.5539/jas.v8n4p92.