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Supplementation frequency of Nellore steers finished on deferred pastures

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Abstract

The study evaluates the effects of different supplementation frequencies on nutrient intake, digestibility, productive performance, carcass characteristics, ingestive behavior, ruminal pH, and nitrogen metabolism of cattle grazing deferred pastures. In Experiment 1, thirty-five Nellore steers, 24 months old and weighing 427 ± 25 kg, were used in a completely randomized design for 70 days. In Experiment 2, five rumen-fistulated Girolando cattle, averaging 440 ± 92 kg, were used in a 5 × 5 Latin square design with 17-day periods. The animals were kept on deferred *Brachiaria brizantha* pasture and received a supplement corresponding to 0.8% of BW, provided at different frequencies: once daily in the morning; twice daily (morning and afternoon); three times daily (morning, early afternoon, and late afternoon); once daily in the afternoon; or once daily in the morning with 2.5% sodium bicarbonate. Supplementation frequency did not affect intake, digestibility, or performance ($P > 0.05$). However, hot carcass weight was greater with once-daily supplementation ($P = 0.008$), whereas supplying the supplement three times a day increased final pH ($P = 0.004$) and resulted in firmer meat texture ($P = 0.017$). Higher supplementation frequency increased the number of daily grazing ($P = 0.022$) and bunk-feeding periods ($P = 0.001$), reducing the average duration per period. Ruminal pH fluctuated throughout the day ($P < 0.05$), showing greater stability with bicarbonate inclusion. Once-daily supplementation in the morning promoted greater microbial protein synthesis ($P = 0.020$), whereas twice-daily supplementation increased urinary nitrogen excretion ($P = 0.001$). Once-daily supplementation, preferably in the morning, enhances metabolic efficiency and carcass quality, representing a practical and effective strategy for finishing cattle on deferred pastures.

1. Introduction

Finishing beef cattle on deferred pastures is a widely adopted practice in tropical production systems to ensure continuity of production during the dry season, when forage availability and nutritive value are limited. During this period, advancing grass maturity results in an increased fiber fraction and reduced crude protein (CP) content, which restrict voluntary intake and compromise dry matter (DM) digestibility (Leal et al. 2021). Concentrate supplementation, when provided strategically, is an essential tool to correct nutritional deficiencies, improve forage utilization, and sustain animal performance during the dry season (Sousa et al. 2022).

However, beyond the amount of supplement offered, supplementation frequency plays a key role in ingestive behavior, the ruminal environment, and nutrient use efficiency. Reductions in supplementation frequency can decrease operational costs and minimize interference with grazing, but they may also intensify fluctuations in ruminal fermentation, with variations in pH and ammonia concentration that affect microbial activity and fiber digestibility (Palma et al. 2023). In contrast, higher frequencies tend to promote greater ruminal stability and more efficient nitrogen use, although they require increased management and labor costs (Silva et al. 2024).

Maintaining ruminal homeostasis may be enhanced by the use of buffering additives, especially in concentrate supplementation strategies with reduced frequency. Sodium bicarbonate ($NaHCO_3$) is the buffer most commonly used in ruminant diets, acting to neutralize acids produced during fermentation and helping to maintain conditions suitable for the development of cellulolytic microorganisms (Mahdavirad et al. 2021). Farghaly et al. (2019) demonstrated that the inclusion of $NaHCO_3$ increases ruminal pH and improves the fermentative environment, highlighting its potential to prevent marked pH fluctuations associated with greater intake of rapidly fermentable carbohydrates.

In addition to metabolic effects, supplementation frequency can modify ingestive behavior, altering the time allocated to grazing, rumination, idleness, and the pattern of feeder visits. These changes reflect adaptive mechanisms to the feeding regimen and are directly associated with the efficiency of use of feed resources and final productive performance (Mirzaei-Alamouti et al. 2020; Saldanha et al. 2021).

Given this context, the hypothesis of the present study is that adjusting supplementation frequency, while maintaining a constant daily amount of supplement, may optimize the ruminal environment and nutrient utilization, resulting in improved productive efficiency. Furthermore, including 2.5% $NaHCO_3$ in the concentrate provided once daily may compensate for potential ruminal fluctuations resulting from reduced supplementation frequency.

Thus, the objective was to evaluate the effects of different supplementation frequencies on nutrient intake, total-tract digestibility, productive performance, carcass characteristics, ingestive behavior, ruminal pH, nitrogen balance, and microbial protein synthesis of Nellore steers maintained on deferred pastures.

2. Material and methods

2.1. Ethical considerations and location

The study followed the ethical guidelines for animal use established by the Ethics Committee on Animal Use of the State University of Southwest Bahia (UESB), under approval protocol 05/013.

The field trial was conducted on a commercial farm in the municipality of Macarani, Bahia, located at 15°33'46" S, 40°25'39" W and at an altitude of approximately 315 meters.

2.2. Animals, experimental design, and treatments

In Experiment 1, 35 castrated Nellore steers were used, with an average age of 24 months and a body weight (BW) of 427 ± 25 kg (mean ± SD). The animals were allocated in a completely randomized design, with a total duration of 70 days, subdivided into two 35-day periods. Performance, ingestive behavior, and carcass traits were evaluated.

In Experiment 2, five castrated Girolando cattle, fitted with ruminal fistulas (4" cannulas, Kehl®) and with an average body weight of 440 ± 92 kg (mean ± SD), were used. The experimental design was a 5 × 5

Latin square with 21-day periods, consisting of 14 days of adaptation and 7 days of sampling. In this group, intake, digestibility, microbial protein synthesis, nitrogen balance, and ruminal pH were evaluated. Concentrating these procedures in this specific group was essential because such measurements require frequent handling and invasive sampling that could alter ingestive behavior, increase physiological stress, and directly compromise the performance of the animals in the productive experiment. Thus, separating the two groups ensured methodological quality, precision of ruminal measurements, and, above all, the integrity of the performance results.

The animals were maintained on *Brachiaria brizantha* pasture in a total area of 30 hectares, subdivided into 10 paddocks of equivalent size (approximately 3 hectares each).

Daily supplement allowance was 0.8% of BW, according to the following treatments:

1× day[1] – Supplement provided once daily in the morning (07:30);

2× day[2] – Supplement provided twice daily, in the morning (07:30) and in the afternoon (16:30);

3× day[3] – Supplement provided three times daily, in the morning (07:30), early afternoon (12:30), and late afternoon (16:30);

1× day[4] – Supplement provided once daily in the afternoon (16:30);

1× day[5] – Supplement provided once daily in the morning (07:30), with 2.5% sodium bicarbonate ($NaHCO_3$) added to the concentrate.

The diets were formulated to meet the nutritional requirements of the animals according to the BR-CORTE system (BR-CORTE 2023), aiming at an estimated average daily gain (ADG) of 0.800 kg/day (Table 1).

Table 1
Ingredients and chemical composition of the supplement and pasture

Item (g/kg DM)	***Brachiaria brizantha***	**Supplement**	
		Without $NaHCO_3$	**With $NaHCO_3$**
Ground corn	-	790.0	770.0
Soybean meal	-	180.0	175.0
Urea	-	17.0	17.0
Salt[a]	-	13.0	13.0
Sodium Bicarbonate	-	-	25.0
Chemical composition (g/kg DM)			
Dry matter (g/kg as feed)	598.2	909.4	908.4
Crude protein	58.2	179.3	179.9
Ethereal extract	23.9	54.4	53.1
aNDFom [b]	662.9	151.1	146.7
ADF [c]	408.4	44.3	41.4
NFC [d]	89.1	651.5	650.5
Hemicellulose	332.5	143.8	148.3
Cellulose	89.3	19.3	12.9
Lignin	50.6	19.0	17.0
$_{i}$NDF [e]	268.8	39.4	33.7
Mineral matter	63.6	45.3	33.6
Forage Production			
Dry Matter availability (kg/ha)	3690.78	-	-
Leaf Percentage (% DM)	36.86	-	-
Stem Percentage (% DM)	27.89	-	-

[a]Mineral mixture containing 233 g Ca/kg, 80 g P/kg, 5 g Mg/kg, 48 g Na/kg, 25 mg Co/kg, 380 mg Cu/kg, 25 mg I/kg, 1080 mg Mn/kg, 3.75 mg Se/kg, 1722 mg Zn/kg. [b] aNDFom, Neutral detergent fiber corrected for ash and protein. [c]ADF, Acid detergent fiber. [d]NFC, Non-fibrous carbohydrates. [e]$_{i}$NDF, Indigestible neutral detergent fiber. [f] AU,Animal unit – 450 kg.

Item (g/kg DM)	*Brachiaria brizantha*	Supplement	
		Without $NaHCO_3$	With $NaHCO_3$
Dead Material Percentage (% DM)	35.25	-	-
Stocking Rate (AU/ha)[f]	5.25	-	-
Forage Supply (kg DM per 100 kg BW)	5.60	-	-

[a]Mineral mixture containing 233 g Ca/kg, 80 g P/kg, 5 g Mg/kg, 48 g Na/kg, 25 mg Co/kg, 380 mg Cu/kg, 25 mg I/kg, 1080 mg Mn/kg, 3.75 mg Se/kg, 1722 mg Zn/kg. [b] aNDFom, Neutral detergent fiber corrected for ash and protein. [c]ADF, Acid detergent fiber. [d]NFC, Non-fibrous carbohydrates. [e]$_i$NDF, Indigestible neutral detergent fiber. [f] AU,Animal unit – 450 kg.

The entire area designated for the experimental trial was enclosed with an electric fence to restrict access by other animals on the property, ensuring homogeneous vegetative growth of the forage across the paddocks used in the study. The experimental area remained enclosed until the beginning of the trial, corresponding to a minimum resting period of 90 days.

The animals remained on pasture throughout the entire experiment. They were only moved to covered feed bunks installed in the pasture at supplementation times. These bunks were arranged in collective pens for each treatment, delimited by movable fences to ensure proper supplement administration. As soon as all supplement was consumed, the animals were immediately released back to pasture.

Experimental procedures requiring greater animal restraint and safety were carried out in a squeeze chute, after which the animals were promptly returned to the pasture. The chute was located close to the grazing area, minimizing potential alterations in animal behavior and ensuring the maintenance of experimental conditions.

2.3. Forage evaluation

Dry matter (DM) production and forage nutritional value were determined using direct and indirect sampling methods, according to Cunha (2002). Initially, the pasture was classified into three height scores (20–30, 30–40, and 40–50 cm), characterizing the indirect method for estimating production. Subsequently, representative samples were collected using a 0.49 m² metal quadrat (0.7 × 0.7 m), tossed 40 times in a zigzag pattern in each paddock to estimate the frequency of each score and the DM production per hectare.

The samples were collected 5 cm above the soil using gardening shears, following randomization principles (three replicates per score). The collected material was weighed on a precision scale, homogenized, and divided into two portions: one composite sample and another intended for separation

of plant components (leaves, stems, and dead material), following McMeniman (1997). All samples were placed in labeled plastic bags and stored at − 15°C until bromatological analyses were performed.

The combination of direct and indirect methods allowed visual estimates of forage availability to be associated with actual measurements of production per area, providing a more accurate assessment of pasture productivity and nutritional value.

During the experiment, three forage collections were performed at distinct periods (initial, intermediate, and final). The first two were used for adjusting stocking rate and calculating supplement allowance, whereas the final collection was used to determine the residual DM production after grazing, following the same sampling, separation, and analysis procedures described above.

Stocking rate was calculated considering one animal unit equivalent to 450 kg of body weight, and forage allowance was determined based on DM availability, stocking rate, and the duration of each experimental period.

2.4. Nutrient intake and total-tract apparent digestibility

To estimate individual daily DM intake of the supplement, titanium dioxide ($TiO_{2)}$ was used as an external marker. The marker was administered directly to the animals in the collective feed bunk during supplementation times, at a dose equivalent to 10 g per animal per day for 10 consecutive days. The first 7 days were used to stabilize the marker flow in the digesta, and the last 3 days were used for fecal collection.

Fecal output was evaluated through the oral administration of a 500 mg capsule of purified and enriched lignin (LIPE) at 08:30, following the methodology described by Rodriguez et al. (2006). The first 7 days were used to stabilize digesta flow for the LIPE marker, and during the last 3 days fecal samples (approximately 200 g) were collected directly from the rectal ampulla.

The fecal material collected was stored in labeled plastic bags and frozen at − 15°C for subsequent chemical analyses.

During the same period in which the digestibility assay was conducted, through the administration of external markers and fecal collection, pasture samples were collected using the simulated grazing technique. This procedure consists of collecting the forage fraction consumed by the animals in each paddock, simulating the actual composition of the forage portion of the diet.

To estimate voluntary forage DM intake, indigestible neutral detergent fiber ($_{i}$NDF) was used as an internal marker. LIPE concentration was determined using a Fourier-transform infrared spectrophotometer (FTIR), model Varian 099-2243. TiO_2 and $_{i}$NDF concentrations in the samples were determined following the procedures described by Oliveira et al. (2012) and Detmann et al. (2012), respectively.

Samples of the ingredients used in the supplement were collected at the beginning of the experimental period, as well as samples of the pasture fraction consumed by the animals and fecal samples during the digestibility assays.

2.5. Chemical analyses

Pasture, ingredient, and fecal samples were dried in a forced-air oven (55°C for 72 h) and ground in a Wiley mill using 1 mm and 2 mm sieves.

The 1-mm samples were analyzed for DM (ID 920.15), ash (ID 942.05), CP (ID 984.13), EE (ID 920.39), acid detergent fiber and lignin (ID 973.18), according to the methods proposed by AOAC (2000). NDF was analyzed using a fiber analyzer (Ankom Tech Corp., Fairport, New York, USA) with thermostable α-amylase and without sodium sulfite in the detergent solution (Mertens 2002). The non-fibrous carbohydrate content of the diet was calculated using the equation proposed by Hall (2000).

The samples ground to 2 mm were analyzed for ¡NDF according to the methodology proposed by Detmann et al. (2012).

2.6. Productive performance

To determine performance, the cattle in Experiment 1 were weighed on days 1, 35, and 70 of the experiment after a 16-hour solid-feed fasting period. Weighing began at 07:00 using a scale with a 1,500-kg capacity. Total weight gain (TWG) was calculated from the difference between final and initial body weight; ADG was calculated by dividing TWG by the number of experimental days; and feed conversion (FC) was calculated considering dry matter intake and weight gain.

2.7. Nitrogen balance and microbial protein synthesis

On the 18th day of each experimental period, blood material samples were collected from the animals in Experiment 2. Sampling took place four hours after supplementation, by jugular vein puncture using Vacutainer® tubes. The samples were then centrifuged, and the resulting plasma was collected and analyzed for urea concentration using Bioclin® commercial kits.

On the same day, urine samples were obtained during spontaneous urination, following the same sampling interval, according to the methodology described by Barbosa et al. (2006). The collected urine was filtered and subsequently diluted (10 mL of urine with 40 mL of 0.036 N sulfuric acid solution) as described by Valadares et al. (1999). The diluted samples were then stored at − 20°C for later determination of urinary concentrations of urea, nitrogen, creatinine, allantoin, and uric acid.

Urinary concentrations of creatinine, uric acid, urea, and plasma urea were determined using Bioclin® commercial kits. Urinary allantoin and uric acid concentrations were analyzed following the procedures described by Chen and Gomes (1992), and total urinary nitrogen was determined according to AOAC (1995).

Nitrogen balance was calculated as the difference between nitrogen intake (N intake) and the amounts of nitrogen excreted in feces and urine.

Creatinine excretion (mg/kg body weight), used to estimate urinary volume from spot samples, was obtained for each animal according to the equation proposed by Chizzotti (2006). Urinary volume was estimated by dividing creatinine excretion (mg/kg body weight per day), obtained using the aforementioned equation, by its concentration in the urine samples (mg/dL), and multiplying the result by the body weight of each animal.

Total purine excretion (TP) was calculated as the sum of urinary allantoin and uric acid. The amount of absorbed microbial purines (mmol/day) was estimated from the total excretion of purine derivatives (mmol/day), using the equation proposed by Verbic et al. (1990).

2.8.Ruminal pH

To evaluate ruminal pH, data were obtained from the animals used in Experiment 2. Ruminal content samples (250 mL) were collected through a ruminal cannula in five rumen regions (cranio-dorsal, cranio-ventral, ventral, caudo-ventral, and caudo-dorsal) on days 19, 20, and 21 of each experimental period. Collections occurred at 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, and 22 hours after the first daily supplementation. On day 19, samples were collected at 7:30 am, 11:30 am, 7:30 pm, and 1:30 am; on day 20, at 9:30 am, 3:30 pm, 11:30 pm, and 5:30 am; On the 21st, samples were collected at 1:30 PM, 5:30 PM, 9:30 PM, and 3:30 AM, following the procedures described by Leite et al. (2022). The sampling sequence was organized to limit the time the animals were out of the pasture, thus reducing potential interference with their natural behavioral patterns. Time zero corresponded to the collection performed immediately before the concentrate distribution, at 7:30 AM.

Immediately after collection, the ruminal fluid was filtered and the pH determined using a digital pH meter (ORP 8651, AZ Instrument Corp., Tanzi District, Taichung City, Taiwan).

2.9. Ingestive behavior

In both experimental periods, the ingestive behavior of the animals from experiment 1 was monitored through direct observation, following the methodology described by Santana Júnior et al. (2014). Evaluations were conducted continuously for 24 hours, with records taken every 10 minutes. The time spent grazing, ruminating, idling, and at the feed bunk was quantified.

To facilitate rapid individual identification, the animals were marked in anatomically strategic regions (dorsum, right and left rib arcs, and the frontal region of the head) with Arabic numerals, ensuring that each animal maintained the same number across all marked regions. During the daytime, monitoring was carried out using binoculars to avoid close approach and minimize any influence on the animals' behavior. At night, night-vision binoculars were used to allow continuous observation without disrupting the cattle's routine.

The estimation of the average number of meric (chewing) movements per ruminal bolus (MBR) and the time spent ruminating each bolus (TBR) followed the methodology proposed by Santana Júnior et al. (2014). The time spent ruminating each bolus was recorded using a digital stopwatch. In each observation period (morning, afternoon, and night), three measurements were taken per animal, totaling nine observations for both the number of chews and the rumination time per bolus within the 24-hour cycle.

2.10. Carcass traits and classification

At the end of the 70-day experiment, the animals from Experiment 1 were transported to a commercial slaughterhouse. After a 16-hour solid-feed fasting period, they were weighed to obtain slaughter body weight (SBW). Slaughter was performed in accordance with the guidelines of the Brazilian Ministry of Agriculture, Livestock, and Supply (Normative Instruction No. 03/2000), under the Federal Inspection Service (SIF) for humane slaughter.

The animals were stunned using a pneumatic captive bolt pistol, followed by bleeding through the severing of the jugular veins and carotid arteries, and subsequently skinned and eviscerated. The head and feet were removed. The carcasses were then split lengthwise and transferred to a cold room (4°C) for 24 h.

pH was measured in triplicate in the longissimus dorsi muscle after 24 h of chilling at 4°C. A HANNA spear-type digital pH meter (model HI 99163) equipped with a penetration electrode was used, previously calibrated with pH 4.01 and 7.01 buffer solutions.

Carcasses were weighed immediately after slaughter, evisceration, and skinning to obtain hot carcass weight (HCW), and again after chilling to obtain cold carcass weight (CCW). Cold carcass yield (CCY) was calculated as: CCY = (CCW × 100 / SBW).

Carcass measurements were taken after 24 hours of chilling. Carcass length, leg length, round width, round length, conformation, and fat cover were recorded. On the left half-carcass, a transverse cut was made between the 12th and 13th ribs to expose the longissimus dorsi muscle. A 5-cm thick steak was removed from this muscle.

The muscle sample was divided into two steaks, each 2.5 cm thick, which were used to determine subcutaneous fat thickness, ribeye area, ribeye height, ribeye width, color, intramuscular fat (marbling), and texture. The ribeye area was determined by tracing the muscle contour onto parchment paper using a planimeter.

Meat color and texture were evaluated by a trained panel of 10 assessors, previously instructed and calibrated for the criteria of tenderness and color, according to the structured scale of Müller (1987). Each attribute was scored on a scale of 1 to 5, with the following definitions:

1 – Very coarse texture and dark color;

2 – Coarse texture and dark-red color;

3 – Slightly coarse texture and slightly dark-red color;

4 – Fine texture and red color;

5 – Very fine texture and bright-red color.

Before the formal evaluation, panelists participated in training sessions with anchor samples to standardize perception of texture and color.

During evaluation, each sample was coded and randomized in a balanced order to minimize sequence effects or influence from other samples. Each assessor assigned a score independently, and each sample was evaluated in duplicate in separate sessions.

To ensure inter-assessor consistency, sample means were calculated and divergences among evaluators were monitored; significant discrepancies were discussed and resolved by consensus. Final scores, representing the mean of individual evaluations and replicates, were used for statistical analysis.

2.11. Statistical analysis

The data were analyzed using the MIXED procedure of the SAS OnDemand for Academics© 2014 (SAS Institute Inc., Cary, NC, EUA). For the performance variables, carcass traits, and ingestive behavior, a completely randomized design was used with five treatments and seven replicates, according to the following model:

$$Y_i = \mu + T_i + e_i$$

where Y_i is the dependent variable, μ is the overall mean, T_i is the fixed effect of treatment (i = 1–5; corresponding to the supplementation frequencies and the addition of sodium bicarbonate), and e_i is the random error term, assumed to be normally distributed with zero mean and constant variance.

For the meat color and texture variables evaluated by a trained panel of 10 assessors, the model was adjusted to include the random effect of assessor, in order to control for inter-individual variability:

$$Y_{ij} = \mu + T_i + A_j + e_{ij}$$

where A_j is the random effect of assessor (j = 1–10), and the remaining terms were described previously.

The variables related to intake, digestibility, microbial protein synthesis, and nitrogen balance were obtained from five Girolando cattle in a 5 × 5 Latin square experiment, using the following model:

$$Y_{ijk} = \mu + T_i + B_j + P_k + e_{ijk}$$

where Y_{ijk} is the dependent variable, μ is the overall mean, T_i is the fixed effect of treatment (i = 1–5), B_j is the random effect of animal (j = 1–5), P_k = is the random effect of period (k = 1–5), and e_{ijk}= is the residual error, assumed to be normally distributed with zero mean and constant variance.

For the evaluation of ruminal pH, five rumen-fistulated Girolando cattle were used, and the data were analyzed as repeated measures over time according to the following model:

$$Y_{ijkl}=\mu + T_i+B_j+P_k++H_l+(T_i \times H_l) + e_{ijkl}$$

where H_l= is the fixed effect of sampling time (l = 1–12; corresponding to 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, and 22 h after supplement provision), $T_i \times H_l$ is the interaction between treatment and sampling time, and the remaining terms were described previously.

Means were compared using Tukey's test, adopting a significance level of P ≤ 0.05.

3. Results

The daily supplementation frequency did not affect nutrient intake in steers finished on deferred pasture during the dry season ($P > 0.05$; Table 2). On average, the animals consumed 4.94 kg/day of pasture dry matter and 3.70 kg/day of supplement dry matter. Likewise, total apparent digestibility of nutrients was not influenced by the different supplementation frequencies ($P > 0.05$).

Table 2
Effect of daily supplementation frequency during the dry season on nutrient intake and total apparent digestibility of the digestive tract in cattle.

Items	Supplementation schedules [a]					SEM[b]	P-value*
	1 x day[1]	2 x day[2]	3 x day[3]	1 x day[4]	1 x day[5]		
Intake (kg/day)							
DM of pasture[c]	5.08	5.09	4.97	4.76	4.81	0.216	0.756
DM of the supplement[c]	3.94	3.76	3.76	3.59	3.46	0.124	0.059
Total DM[c]	9.02	8.85	8.74	8.35	8.27	0.279	0.261
Crude protein	0.98	0.95	0.95	0.90	0.88	0.028	0.100
Neutral detergent fiber	4.00	3.98	3.89	3.73	3.74	0.145	0.583
NFC[d]	3.02	2.90	2.90	2.76	2.68	0.095	0.107
Total digestible nutrients	5.38	5.01	5.08	4.79	4.80	0.219	0.311
Ether Extract	0.34	0.33	0.33	0.32	0.31	0.011	0.131
Intake (%BW)							
Total DM[c]	1.87	1.78	1.78	1.71	1.74	0.067	0.551
Neutral detergent fiber	0.83	0.80	0.79	0.76	0.79	0.032	0.764
Total-tract digestibility							
Dry matter	52.81	51.07	50.14	48.15	49.34	1.450	0.204
Crude protein	65.80	64.32	64.11	62.64	63.22	1.609	0.750
Neutral detergent fiber	39.15	32.21	34.83	33.58	35.64	2.630	0.426
NFC[d]	75.90	74.27	74.42	71.76	72.94	1.209	0.207
Ether Extract	71.48	68.49	67.97	65.97	65.30	1.666	0.063
Total digestible nutrients	59.48	56.67	57.97	57.11	58.00	1.112	0.470

* Statistical differences were considered significant at $P \leq 0.05$ (Means followed by different letters in the rows differ from each other by Tukey's test ($P \leq 0.05$)).

Items	Supplementation schedules [a]					SEM[b]	P-value*
	1 x day[1]	2 x day[2]	3 x day[3]	1 x day[4]	1 x day[5]		
Intake (kg/day)							

[a]1x day [1] - Supplement provided once a day, in the morning (7:30 AM); 2 x day [2] - Provided twice a day, in the morning (7:30 AM) and afternoon (4:30 PM); 3 x day [3] - Provided three times a day, in the morning (7:30 AM), early afternoon (12:30 PM) and late afternoon (4:30 PM); 1 x day [4] - Provided once a day, in the afternoon (4:30 PM); 1 x day [5] - Provided once a day, in the morning (7:30 AM), with the addition of 2.5% sodium bicarbonate ($NaHCO_3$) to the concentrate. [b]Standard error of the mean. [c] Dry matter. [d] Non-fibrous carbohydrates.

Productive performance also did not vary as a function of the number of daily supplement offerings ($P > 0.05$) (Table 3). However, some carcass traits were affected (Table 4). Hot carcass weight (HCW) was greater in animals supplemented once daily, either in the morning or afternoon, compared with those supplemented three times daily ($P = 0.008$). Meat texture scores were lower in the treatment with three daily supplementations compared with the animals supplemented once daily, regardless of schedule (morning or afternoon) ($P = 0.017$). Final carcass pH was higher in the animals supplemented three times per day compared with those supplemented once (morning or afternoon) or twice daily ($P = 0.004$). The remaining carcass variables did not differ among frequencies evaluated ($P > 0.05$).

Table 3
Effect of daily supplementation frequency during the dry season on productive performance.

Items	Supplementation schedules[a]					SEM[b]	P-value[*]
	1 x day[1]	2 x day[2]	3 x day[3]	1 x day[4]	1 x day[5]		
IBW (kg)[c]	425.00	432.14	428.57	421.67	425.14	8.709	0.960
FBW (kg)[d]	471.83	476.00	466.86	469.17	467.86	8.800	0.969
ADG (kg/day)[e]	0.66	0.63	0.55	0.68	0.61	0.071	0.793
FC[f]	16.05	14.87	18.22	13.90	15.72	1.970	0.711

[*] Statistical differences were considered significant at P ≤ 0.05 (Means followed by different letters in the rows differ from each other by Tukey's test (P ≤ 0.05)).

[a]1x day ¹ - Supplement provided once a day, in the morning (7:30 AM); 2 x day ² - Provided twice a day, in the morning (7:30 AM) and afternoon (4:30 PM); 3 x day [3] - Provided three times a day, in the morning (7:30 AM), early afternoon (12:30 PM) and late afternoon (4:30 PM); 1 x day [4] - Provided once a day, in the afternoon (4:30 PM); 1 x day [5] - Provided once a day, in the morning (7:30 AM), with the addition of 2.5% sodium bicarbonate ($NaHCO_3$) to the concentrate. [b] Standard error of the mean. [c] Initial body weight. [d] Final body weight. [e]Average daily gain. [f] Feed conversion.

Table 4
Effect of daily supplementation frequency during the dry season on the physical and chemical characteristics of cattle carcasses.

Items	Supplementation schedules[a]					SEM[b]	P-value*
	1 x day [1]	2 x day [2]	3 x day [3]	1 x day [4]	1 x day [5]		
HCW (kg) [c]	267.24a	254.36ab	243.68b	269.47a	259.26ab	5.409	0.008
Carcass yield (%)	56.83	53.48	52.36	57.54	55.52	1.390	0.068
Car.Length (cm) [d]	135.93	136.43	136.28	138.00	137.57	1.117	0.777
Thigh width (cm)	27.43	26.78	27.78	27.56	27.43	0.401	0.564
Thigh length (cm)	78.64	78.00	79.07	80.50	80.28	1.000	0.448
Carc. conf [e]	11.66	11.00	10.80	11.70	11.28	0.140	0.610
SFT (mm) [f]	4.28	3.11	4.07	3.84	3.77	0.503	0.677
LEA (cm^2) [g]	64.14	62.23	61.71	66.83	61.76	2.600	0.728
H LEA (cm^2) [h]	5.64	5.86	5.80	5.90	5.73	0.222	0.954
W LEA (cm) [i]	12.42	12.23	11.89	12.31	11.90	0.302	0.719
Color	4.00	3.85	4.00	4.00	4.00	0.085	0.756
Marbling	2.42	2.50	2.50	2.58	2.71	0.340	0.987
Texture	3.64a	3.36ab	3.07b	3.67a	3.50ab	0.131	0.017
pH	5.59b	5.57b	5.68a	5.57b	5.63ab	0.018	0.004

* Statistical differences were considered significant at $P \leq 0.05$ (Means followed by different letters in the rows differ from each other by Tukey's test ($P \leq 0.05$)).

[a]1x day ¹ - Supplement provided once a day, in the morning (7:30 AM); 2 x day ² - Provided twice a day, in the morning (7:30 AM) and afternoon (4:30 PM); 3 x day [3] - Provided three times a day, in the morning (7:30 AM), early afternoon (12:30 PM) and late afternoon (4:30 PM); 1 x day [4] - Provided once a day, in the afternoon (4:30 PM); 1 x day [5] - Provided once a day, in the morning (7:30 AM), with the addition of 2.5% sodium bicarbonate ($NaHCO_3$) to the concentrate. [b] Standard error of the mean.[c] Hot carcass weight. [d] Carcass length. [e] Carcass conformation. [f] Subcutaneous fat thickness. [g] Loin eye area. [h] Height of the rib eye. [i] Width of the rib eye.

Regarding ingestive behavior (Table 5), time spent at the feed bunk was greater in animals supplemented once daily with $NaHCO_3$ compared with those supplemented once in the afternoon and twice daily ($P = 0.001$). The number of daily periods differed for grazing ($P = 0.022$), idling ($P = 0.008$), and feed bunk activity ($P = 0.001$). Animals supplemented once daily (morning and afternoon) had fewer grazing periods than those supplemented three times daily ($P = 0.022$). The highest numbers of idling and feed bunk periods were observed in animals supplemented two and three times daily compared with the other treatments ($P = 0.008$ and $P = 0.001$, respectively).

Table 5
Effect of daily supplementation frequency during the dry season on the ingestive behavior of cattle.

Items	Supplementation schedules[a]					SEM[b]	P-value*
	1 x day[1]	2 x day[2]	3 x day[3]	1 x day[4]	1 x day[5]		
Time spent (min/day)							
Grazing	491.25	440.00	469.37	447.50	428.75	28.52	0.567
Rumination	298.75	346.25	294.37	303.12	292.50	26.27	0.597
Idleness	581.25	601.25	618.12	643.75	641.25	33.08	0.649
Trough	68.75ab	52.50bc	58.14abc	45.63c	77.50a	6.480	0.001
Feeding efficiency (kg/h day)							
Dry matter of the pasture	0.64	0.70	0.65	0.64	0.67	0.042	0.816
Neutral detergent fiber of the pasture	0.42	0.47	0.43	0.42	0.45	0.028	0.834
Total digestible nutrients of diet	0.59	0.64	0.60	0.61	0.60	0.035	0.895
Rumination efficiency (kg/h day)							
Dry matter of the pasture	1.07	0.92	1.11	0.94	1.04	0.103	0.678
Neutral detergent fiber of the pasture	0.71	0.61	0.73	0.63	0.69	0.067	0.677
Chewing							
TCT (minutes/day)[c]	858.75	838.75	821.75	796.25	798.75	33.08	0.649
NBR (number/day)[d]	320.71	341.96	319.38	330.77	327.43	3.210	0.284
TBR (second/bolus)[e]	55.76	56.75	55.37	56.50	54.17	0.507	0.301
NCB (number/bolus)[f]	50.12	50.12	49.00	49.00	53.12	1.560	0.389
Feeding behavior (number of periods/day)							
Grazing	8.25b	8.75ab	11.30a	8.25b	8.62ab	0.850	0.022
Rumination	8.00	9.62	7.87	8.00	7.50	0.820	0.117

Items	Supplementation schedules[a]					SEM[b]	P-value*
	1 x day[1]	2 x day[2]	3 x day[3]	1 x day[4]	1 x day[5]		
Time spent (min/day)							
Idleness	12.45b	15.62a	15.55a	12.24b	12.45b	0.900	0.008
Trough	2.00b	3.50a	3.49a	2.50b	2.62b	0.310	0.001
Time per period of feeding behavior (minutes)							
Grazing	54.81a	44.81b	40.90b	52.24a	52.89a	3.480	0.021
Rumination	42.79	35.79	35.49	38.60	44.79	2.950	0.176
Idleness	47.72	40.16	40.83	52.00	46.10	4.602	0.075
Trough	25.60ab	21.87b	18.28b	25.60ab	28.31a	2.140	0.001

* Statistical differences were considered significant at P ≤ 0.05 (Means followed by different letters in the rows differ from each other by Tukey's test (P ≤ 0.05)).

[a]1x day ¹ - Supplement provided once a day, in the morning (7:30 AM); 2 x day ² - Provided twice a day, in the morning (7:30 AM) and afternoon (4:30 PM); 3 x day [3] - Provided three times a day, in the morning (7:30 AM), early afternoon (12:30 PM) and late afternoon (4:30 PM); 1 x day [4] - Provided once a day, in the afternoon (4:30 PM); 1 x day [5] - Provided once a day, in the morning (7:30 AM), with the addition of 2.5% sodium bicarbonate ($NaHCO_3$) to the concentrate.[b] Standard error of the mean. [c] Total chewing time. [d] Number of boluses ruminated per day. [e]Time spent per boluse ruminated. [f] Number of chews per boluse.

Mean time per grazing period and feed bunk period was shorter in animals supplemented two and three times daily ($P = 0.021$ and $P = 0.001$, respectively).

Ruminal pH fluctuated throughout the day ($P < 0.05$; Fig. 1). The treatment with once-daily supplementation plus sodium bicarbonate showed greater pH stability, whereas the lowest values occurred with supplementation three times daily. The lowest pH value was observed four hours after supplementation in animals fed once in the morning.

Nitrogen balance and microbial synthesis showed important differences among strategies (Table 6). Urinary nitrogen excretion was highest in animals supplemented twice daily, followed by those supplemented once in the afternoon and once with sodium bicarbonate, and lowest in those supplemented once in the morning and three times daily ($P = 0.001$). Urinary urea concentrations and excretion also differed among treatments ($P = 0.001$), with the lowest values observed in the sodium bicarbonate treatment. Microbial nitrogen production ($P = 0.020$) and microbial protein production ($P =$

0.020) were greater when supplementation occurred once daily in the morning and lower when animals were supplemented twice daily, although microbial synthesis efficiency was not affected (P = 0.850).

Table 6
Effect of daily supplementation frequency during the dry season on nitrogen balance and microbial protein synthesis and microbial efficiency in cattle.

Items	Supplementation schedules[a]					SEM[b]	P-value*
	1 x day[1]	2 x day [2]	3 x day [3]	1 x day [4]	1 x day [5]		
Nitrogen balance (g/day)							
Ingested N	155.30	158.24	156.68	153.73	150.44	2.421	0.191
Fecal N	53.62	54.04	54.13	53.32	51.71	1.970	0.920
Digested N	101.68	104.20	102.55	100.41	98.74	3.033	0.776
Urinary N	10.52c	15.26a	11.55c	13.65b	13.44b	0.680	0.001
Retained N	91.16	88.94	91.00	86.76	85.30	3.040	0.595
Concentrations							
Urine urea nitrogen	309.54a	177.48c	227.31b	224.34b	125.94d	23.63	0.001
Plasma urea nitrogen	14.34	14.91	13.57	13.87	14.04	0.410	0.178
Excretions (g/day)							
Urine urea nitrogen	19.35a	17.06b	17.47ab	19.30a	10.47c	1.277	0.001
Urine urea/liter	70.35a	40.34c	51.66b	50.99b	28.62d	5.347	0.001
Microbial synthesis (g/day)							
Microbial N	106.57a	92.43b	95.10ab	98.08ab	96.64ab	3.265	0.020
Microbial CP	666.06a	577.68b	594.38ab	613.00ab	604.00ab	30.39	0.020
Microbial efficiency							
g CP/kg TDN intake	125.78	121.90	119.88	130.84	121.41	7.345	0.850

* Statistical differences were considered significant at $P \leq 0.05$ (Means followed by different letters in the rows differ from each other by Tukey's test ($P \leq 0.05$)).

[a]1x day ¹ - Supplement provided once a day, in the morning (7:30 AM); 2 x day ² - Provided twice a day, in the morning (7:30 AM) and afternoon (4:30 PM); 3 x day [3] - Provided three times a day, in the morning (7:30 AM), early afternoon (12:30 PM) and late afternoon (4:30 PM); 1 x day [4] - Provided once a day, in the afternoon (4:30 PM); 1 x day [5] - Provided once a day, in the morning (7:30 AM), with the addition of 2.5% sodium bicarbonate ($NaHCO_3$) to the concentrate.[b] Standard error of the mean.

4. Discussion

The absence of an effect of daily supplementation frequency on nutrient intake and total apparent digestibility is associated with the fact that all animals grazed the same deferred pasture, consuming forage with identical nutritional characteristics across treatments. The pasture showed a high NDF content (662.9 g/kg DM) and, notably, a large ¡NDF fraction (268.8 g/kg DM), as well as low CP concentration (58.2 g/kg DM). Deferred pastures often accumulate lignified material, increasing ¡NDF and reducing both the degradation and passage rates of digesta (Leal et al. 2021). Under these conditions, rumen fill becomes the main physiological constraint on intake, since the elevated ¡NDF increases particle retention time in the rumen, limiting ruminal emptying and preventing greater voluntary intake (Harper and McNeill 2015; Allen et al. 2019).

Thus, because all animals ingested forage of the same quality and structure, they faced the same physical limitation, reducing the likelihood of differences in intake among supplementation strategies. In addition, all treatments received the same daily amount of supplement (0.8% of BW), ensuring identical total supply of fermentable energy and rumen-degradable nitrogen. Therefore, although the timing and frequency of supplementation varied, the daily nutrient supply remained constant, contributing to the absence of differences in intake and digestibility among the evaluated frequencies.

Similarly, supplementation frequency did not alter steer performance, as indicated by the similar ADG across treatments (0.55–0.68 kg/day). This response is consistent with the principle that, in forage-based systems, weight gain is determined primarily by the total daily supply of digestible nutrients rather than the temporal distribution of supplementation, as long as total intake is equivalent (Sousa et al. 2022). Studies show that changes in feeding frequency may modify ingestive behavior and ruminal dynamics but do not produce consistent performance differences when total nutrient supply is maintained (Mirzaei-Alamouti et al. 2020; Saldanha et al. 2021).

Although the diet was formulated to support a gain of 0.800 kg/day, the observed performance was lower than expected due to limitations imposed by the deferred pasture, whose reduced digestibility compromised the effective supply of metabolizable energy (Públio et al. 2023). As plant maturity increases, the cell wall fraction increases and ruminal degradability decreases, reducing the flow of available nutrients for growth and thus limiting productive response even when supplementation is provided (Paula et al. 2024). Therefore, in the present study, supplementation was not able to fully compensate for the forage limitation, resulting in moderate and similar gains across the different feeding frequencies.

Despite the maintenance of intake, digestibility, and performance, carcass traits responded to the supplementation pattern. The greater HCW recorded in the once-daily treatments (morning or afternoon) suggests greater metabolic efficiency, possibly due to the concentration of fermentable carbohydrates at a single time, which increases propionate production in the rumen (Silva et al. 2024). Higher propionate enhances hepatic gluconeogenesis and improves the energy supply for tissue synthesis, favoring both

muscle and lipid deposition (Cappellozza et al. 2021). In addition, once-daily supplementation reduces movement and competitive behavior at the feed bunk, lowering the energy expenditure associated with competition for supplement access and allowing more net energy to be directed toward tissue accretion (Bica et al. 2020).

In contrast, supplementation three times daily reduced HCW and increased ultimate carcass pH, resulting in firmer meat. This effect is related to lower muscle glycogen deposition, a consequence of increased behavioral energy expenditure, as animals had to walk to the feed bunk at three different times during the day, reducing the immediate availability of energy for tissue deposition (Clinquart et al. 2022). Moreover, increased daily movement may have induced adjustments in muscle fiber profile, favoring relatively greater proportions of oxidative fibers (type I), which have lower glycogen content and lower glycolytic potential compared with type II fibers (Ferguson and Gerrard 2014). Because muscles with a predominance of oxidative fibers naturally show higher ultimate pH due to the smaller availability of substrate for post-mortem glycolysis (Clinquart et al. 2022), this physiological adjustment may have further contributed to the smaller pH decline observed in the more frequent supplementation treatment. Although moderate changes in muscle fiber type alone are not sufficient to markedly alter the rate of post-mortem glycolysis, the combination of reduced glycogen availability and increased muscular oxidative activity promoted by higher physical activity tends to limit the pH decline after slaughter, resulting in firmer meat in animals supplemented three times daily.

Supplementation frequency also modulated ingestive behavior. The bicarbonate treatment resulted in longer feeding-bunk time and more prolonged meals, a response consistent with the buffering effect of $NaHCO_3$, which attenuates postprandial acidification and promotes a more stable ruminal environment during ingestion (Bach et al. 2018). This improved ruminal stability reduces discomfort associated with pH decline, allowing animals to remain longer at the feed bunk and maintain less-interrupted meals.

Providing supplement three times daily increased the number of grazing bouts, whereas animals supplemented once daily showed less fragmented grazing activity. This pattern occurs because each supplementation event acts as a stimulus that interrupts and restarts the feeding cycle, intensifying fragmentation of ingestive activities, a behavior previously observed when feeding schedules are distributed across multiple times of the day (Mirzaei-Alamouti et al. 2020). In the twice- and thrice-daily treatments, more idle and bunk-time events were also recorded, resulting from additional time spent approaching, waiting, and moving associated with supplement delivery. This increased alternation between grazing and bunk activities is consistent with studies showing that higher feeding frequency leads to more interruptions in continuous ingestive behavior (Saldanha et al. 2021).

Despite the greater number of behavioral events, these animals showed shorter average duration per grazing and feeding-bunk period, characterizing shorter and more frequent meals, a pattern commonly reported when feed is offered at shorter intervals without altering total nutrient supply (Mirzaei-Alamouti et al. 2020). Thus, although supplementation fragmentation reorganized the temporal distribution of activities, the total time allocated to grazing, rumination, and idling remained stable because it is

determined by constant requirements for effective fiber intake, mastication, and ruminal processing (Saldanha et al. 2021).

These adjustments in ingestive pattern help explain the observed ruminal pH dynamics. The $NaHCO_3$ treatment showed a smaller daily amplitude in pH variation, resulting from the combination of longer meals and the additional buffering action of bicarbonate, which enhances the rumen's ability to neutralize acids produced during fermentation (Bach et al. 2018). In the three-times-daily treatment, the lower pH values recorded at specific times reflect the cumulative effect of successive fermentation peaks generated by short intervals between supplement meals, reducing the time available for pH recovery through salivary buffering and absorption of volatile fatty acids. Similar behavior was reported by Hamdon et al. (2024), who observed progressive pH decline throughout the day with more frequent feeding.

In the once-daily supplementation, the sharper drop following the morning feeding corresponds to the classic pattern of rapid fermentation associated with the concentrated intake of NFC (DelCurto-Wyffels et al. 2021). Thus, the feeding pattern throughout the day directly modulated the rate and intensity of acid production in the rumen, determining the shape of the pH curves. Even so, oscillations remained within a physiologically acceptable range for normal rumen function and forage utilization (5.8–6.6) (Cappellozza et al. 2021).

Nitrogen metabolism showed consistent differences among supplementation strategies, indicating variations in ruminal nutrient utilization. The greater urinary N excretion observed in animals supplemented twice daily indicates that the nutrient supply did not favor efficient use of rumen-degradable N. Under this condition, fermentable energy availability was insufficient to support microbial incorporation of N, resulting in greater ammonia absorption, hepatic conversion into urea, and consequently higher urinary nitrogen losses (Cappellozza et al. 2021).

Conversely, once-daily supplementation in the morning resulted in greater microbial nitrogen and protein synthesis, indicating better synchrony between forage intake and the release of energy from the supplement. When the supplement is provided at this time, the availability of rapidly fermentable carbohydrates coincides with the period of greatest grazing activity (Iqbal et al. 2023), improving energy–nitrogen synchrony in the rumen (Haro et al. 2024). This synchronization creates a more stable ruminal environment conducive to microbial growth, allowing nitrogen to be used more efficiently for microbial protein synthesis, the main source of metabolizable amino acids for the animal (Millen et al. 2025).

The variation in supplementation frequency does not affect intake, digestibility, or performance of Nellore steers. However, strategies with lower frequency, particularly once-daily supplementation in the morning, improve carcass weight, increase microbial protein synthesis, and reduce urinary nitrogen losses, reflecting greater metabolic efficiency. Therefore, providing the supplement once a day represents a practical and efficient alternative for finishing cattle on deferred pastures, aligning productive performance, carcass quality, and simplified management.

Declarations

Ethics approval

The Committee of Ethics authorized all procedures involving the animals in the Use of Animals of the School of the State University of Southwest Bahia (UESB), under approval protocol 05/013.

Competing interests

The authors have no relevant financial or non-financial interests to disclose.

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Authors' contributions

All authors contributed to the study conception and design. Gonçalo Mesquita da Silva: Data curation. Fabiano Ferreira da Silva: Conceptualization, formal analysis, funding acquisition, methodology, project administration, resources and validation. Robério Rodrigues Silva: Roles/Writing - original draft and resources. José Esler de Freitas Júnior and Lara Maria Santos Brant: formal analysis and writing - review & editing. Pedro Henrique Souza Cardoso, Flávia França Cerqueira and Luan Brito Matos: Investigation. All authors read and approved the final manuscript.

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Not applicable

Data availability

The datasets generated during and/or analyzed during the current study are not publicly available due to [WHY DATA ARE NOT PUBLIC] but are available from the corresponding author on reasonable request.

Code availability

Not applicable

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Figures

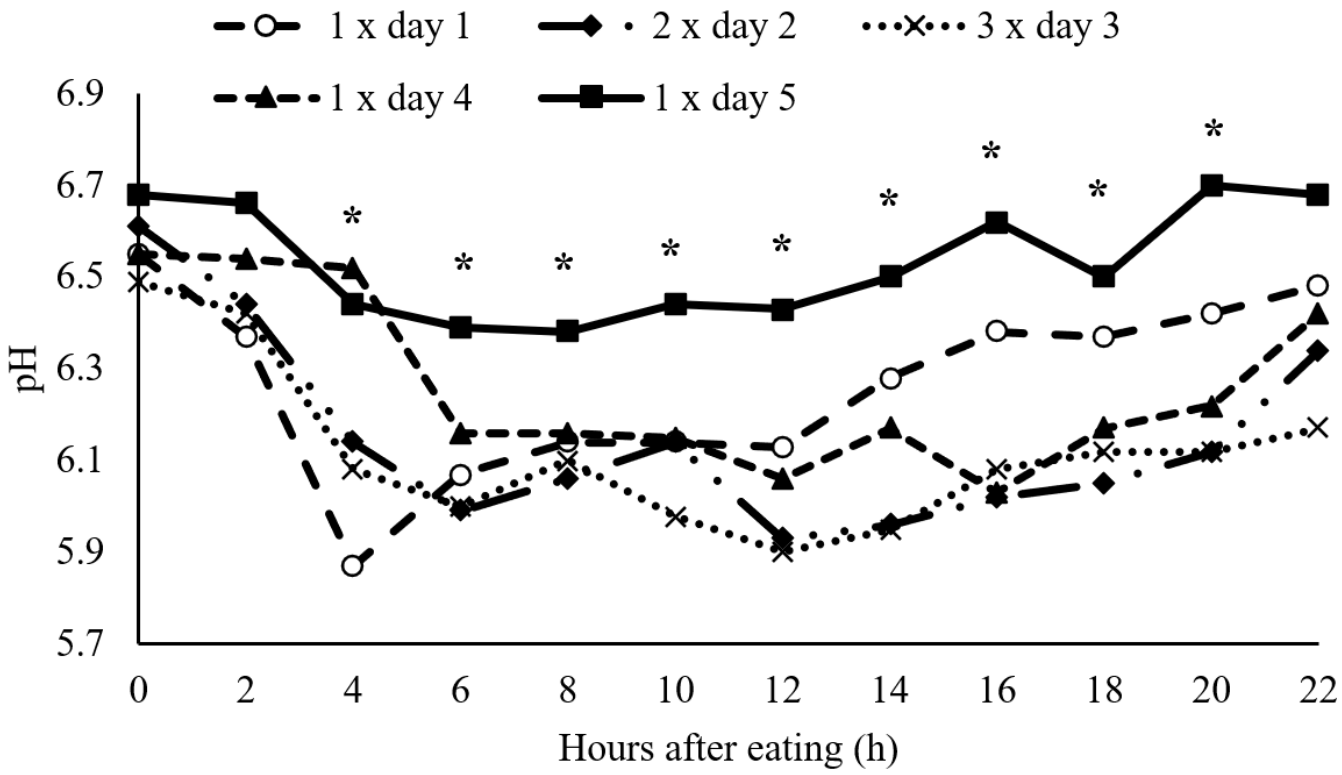


Figure 1

Effect of daily supplementation frequency during the dry season on ruminal pH in cattle. 1x day [1] - Supplement provided once a day, in the morning (7:30 AM); 2 x day [2] - Provided twice a day, in the morning (7:30 AM) and afternoon (4:30 PM); 3 x day 3 - Provided three times a day, in the morning (7:30 AM), early afternoon (12:30 PM) and late afternoon (4:30 PM); 1 x day 4 - Provided once a day, in the afternoon (4:30 PM); 1 x day 5 - Provided once a day, in the morning (7:30 AM), with the addition of 2.5% sodium bicarbonate ($NaHCO_3$) to the concentrate. *Significant values ($P < 0.05$) for times 4, 6, 8, 10, 12, 14, 16, 18 and 20 days after the first daily supplementation.