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Influence of nutritional management prior to adaptation to a feedlot diet on ruminal microbiota of Nellore cattle

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ABSTRACT - The objective of this study was to evaluate the effect of either a limited forage intake or concentrate supplementation prior to the adaptation to high-concentrate diets on dry matter intake, ruminal pH, bacteria, and protozoa of Nellore cattle. The experiment was designed as a two 3×3 Latin square, and six cannulated Nellore steers were used. Each experimental period was composed by three feeding phases: pre-adaptation (14 days), adaptation (12 days), and finishing (seven days) diet, in a total of 33 days per period. The steers were assigned to one of three pre-adaptation dietary treatments: control (Tifton hay fed *ad libitum* + mineral supplement), restriction (Tifton hay fed at 1.4% of BW + mineral supplement), and concentrate (Tifton hay fed *ad libitum* + 0.5% of BW of a mix of concentrate feedstuffs and mineral supplement). The adaptation period consisted of two adaptation diets, which contained 72 and 79% concentrate for six days each. The finishing diet contained 86% concentrate. During the pre-adaptation phase, restricted cattle had higher pH than concentrate-fed cattle. There was a reduction in *M. elsdenii* relative population in cattle from either restriction or concentrate groups. During adaptation and finishing phases, cattle from concentrate group had smaller *F. succinogenes* populations compared with the control group. The previous nutritional backgrounds impact ruminal microbiota during adaptation and finishing phases without causing any negative effect on ruminal pH. Feeding concentrate prior to the adaptation positively impacted the transition to high-concentrate diets and promoted increased dry matter intake.

Keywords: microorganism, Nellore, performance, rumen

1. Introduction

The Brazilian cattle industry is characterized by animals finished in pastures, but to attend the consumer market demand in recent years, the beef industry has changed by increasing the number of cattle finished in feedlots. However, transitioning cattle from pasture to a feedlot requires adjustments, because the diets consumed before feedlot arrival are typically forage-based. Therefore, some processes of adapting ruminal microorganisms to effective use of readily fermentable carbohydrate

are necessary to avoid metabolic disorders (Millen et al., 2016; Rigueiro et al., 2021). Furthermore, this transition results in ruminal fermentation and microbial changes, as well as the enlargement of the ruminal epithelium to accommodate the increase of short-chain fatty acid production (Bevans et al., 2005).

Several studies were conducted previously to determine the most appropriate adaptation period for Nellore cattle receiving high-concentrate diets in Brazilian feedlots (Estevam et al., 2020; Watanabe et al., 2022), and authors reported that Nellore cattle should not be adapted in less than 14 days. However, in all of these studies, the cattle went through a ten-day receiving period, where they consumed a forage-based diet *ad libitum* to standardize the ruminal microbiota. However, Brazilian feedlots receive cattle whose previous nutritional history is unknown. Feedlot operations in Brazil commonly receive cattle from either grazing system supplemented with concentrate feedstuffs or grazing systems that typically are unable to support maintenance requirements because of the poor quality of tropical grasses during the dry season (Pereira et al., 2020). Besides, Silvestre and Millen (2021), in a survey with Brazilian feedlot nutritionists, reported that 2.78% of the interviewed nutritionists did not adopt any reception program, and that cattle start on adaptation diet without a period to suppress possible carryover effects of a previous nutritional background.

Thus, it was hypothesized that cattle maintained under nutritional restriction or grazing with concentrate feedstuffs during the pre-adaptation period would have different ruminal microbiota, affecting the rumen fermentation patterns and animal performance. The present study was conducted to evaluate the effect of either a limited forage intake or concentrate supplementation prior to the adaptation to high-concentrate diets on dry matter intake (DMI), ruminal microbiota, and pH.

2. Material and Methods

All procedures involving the use of animals in this study were in agreement with the guidelines of Nacional Council of Animal Control and Experimentation (CONCEA) and were approved by the local Ethical Committee for Animal Research (protocol number 20/2016 - 06/15/2016). The experiment was carried out in Dracena, São Paulo, Brazil (21°29' S, 51°52' W, 421 m).

2.1. Animals, treatments, and management

Six 20-month-old yearling cannulated Nellore bulls (236±20 kg) were randomly assigned to a replicated 3×3 Latin square design. Each experimental period was composed by three feeding phases: pre-adaptation (14 days), adaptation (12 days), and finishing (seven days) diet, in a total of 33 days per period. Animals were randomly distributed into Latin squares according to the type of diet (Table 1) provided in the pre-adaptation period, which represented the treatments: control (Tifton hay fed *ad libitum* plus a supplement), restriction (Tifton hay fed at 1.4% of body weight plus a supplement), and concentrate (Tifton hay fed *ad libitum* plus 0.5% of body weight of a mix of concentrate feedstuffs and supplement). After the pre-adaptation phase, the diets were the same for all animals (adaptation and finishing diets). Likewise, cattle were submitted to a seven-day washout between periods.

The adaptation phase consisted of two adaptation diets, which contained 72 and 79% concentrate offered *ad libitum* for six days each. The finishing diet, contained 86% concentrate and was offered for seven days. All the diets were composed of sugarcane bagasse, Tifton hay, cracked corn grain, cottonseed meal, urea, limestone, and mineral supplement (Table 2). The diets were formulated according to the Large Ruminant Nutrition System (Fox et al., 2004) (Tables 1 and 2).

Table 1 - Feed ingredients and nutrient content of pre-adaptation diets given to canullated Nellore cattle

Treatment	Control	Restriction	Concentrate
Ingredients (g kg^{-1} dry matter (DM))			
Tifton hay	973.8	973.8	794.4
Finely ground corn grain	-	-	166.7
Cottonseed meal	-	-	15.6
Urea	7.5	7.5	6.7
Supplement[1]	18.8	18.8	16.7
Nutrient content (g kg^{-1} DM)			
Total digestible nutrient	460.0	460.0	530.0
Net energy for maintenance (Mcal kg^{-1} of DM)	1.31	1.31	1.59
Net energy for gain (Mcal kg^{-1} of DM)	0.73	0.73	0.99
Crude protein	124.0	124.0	125.0
Neutral detergent fiber (NDF)	735.0	735.0	629.0
Ether extract	22.0	22.0	24.0
Physically effective NDF	700.0	700.0	580.0
Ca	5.8	5.8	5.3
P	2.4	2.4	2.7

[1] Ca, 9.80%; P, 4.50%; Mg, 6.15%; Na, 11.45%; Cl, 6.60%; S, 4.00%; Co, 48.50 ppm; Cu, 516 ppm; Fe, 30 ppm; Mn, 760 ppm; Se, 9 ppm; Zn, 2516.50 ppm; sodium monensin, 2000 ppm.

Table 2 - Feed ingredients and nutrient content of adaptation and finishing diets given to canullated Nellore cattle

Diet	Adaptation 1	Adaptation 2	Finishing
Concentrate level (%)	72	79	86
Ingredients (g kg^{-1} dry matter (DM))			
Sugarcane bagasse	140.0	105.0	70.0
Tifton hay	140.0	105.0	70.0
Finely ground corn grain	510.0	605.0	735.0
Cottonseed meal	187.0	157.0	90.0
Urea	8.0	10.0	12.0
Supplement[1]	10.0	12.0	15.0
Nutrient content (g kg^{-1} DM)			
Total digestible nutrient	690.0	720.0	750.0
Crude protein	150.0	150.0	141.0
Neutral detergent fiber (NDF)	368.0	313.0	247.0
Physically effective NDF	270.0	230.0	190.0
Ca	6.1	6.5	7.2
P	4.6	4.6	4.2

[1] Ca, 9.80%; P, 4.50%; Mg, 6.15%; Na, 11.45%; Cl, 6.60%; S, 4.00%; Co, 48.50 ppm; Cu, 516 ppm; Fe, 30 ppm; Mn, 760 ppm; Se, 9 ppm; Zn, 2516.50 ppm; sodium monensin, 2000 ppm.

On the first and last day of each period, cattle were weighed for body weight assessment. The Nellore cattle were housed in individual pens (6 m of linear bunk space and 72 m^2 of pen space per animal) with free access to water. Cattle were fed *ad libitum* once a day at 08:00 h, and leftovers were weighed in the next day at 07:00 h. For the restriction treatment, the animals were fed Tifton hay at 1.4% of BW plus supplement. The amount of feed offered was adjusted daily based on orts left before morning feed delivery (target leftover rate of 5% relative to the quantity of feed offered).

Dry matter intake was calculated every day by weighing and determining the dry matter (DM) of feed and the leftover feed.

2.2. Ruminal pH measurements

Ruminal pH was continuously measured using a pH data logger (Model T7-1 LRCpH, Dascor, Escondido, CA, USA; Penner et al., 2006) on days 5 (pre-adaptation phase), 16 (adaptation phase), and 27 (finishing phase). The data logger was inserted before feeding each day and was removed 24 hours later. The systems were initialized to record data at ten-minute intervals. Before the insertion in the rumen and after removal from the rumen, each electrode was standardized at pH 7.0 and 4.0. The pH data were recorded at 0, 4, 8, and 12 h after feeding (8, 12, 16, and 20 h).

2.3. Ruminal protozoa counting

For ruminal ciliated protozoa counting, 10 mL of ruminal contents were collected through the ruminal cannula with a vacuum pump. Samples were stored in vials containing 20 mL of 50% formaldehyde. The sampling was carried out on days 8 (pre-adaptation), 18 (adaptation), and 30 (finishing) at 4, 8, and 12 h after feeding for each period. Protozoa were identified (genera *Isotricha*, *Dasytricha, Entodinium*, and *Diplodiniinae* subfamily) and counted using a Neubauer Improved Bright-Line counting chamber (Hausser Scientific Partnership, Horsham, PA, United States) by optical microscopy (Olympus CH-2 R, Japan; Dehority, 1993). Samples for protozoa counting were not collected at 0 h to avoid opening the rumen canula before collecting samples for ruminal bacteria 4 h after feeding.

2.4. qPCR of ruminal bacteria

One cellulolytic bacterium (*Fibrobacter succinogenes*), one lactate producer bacteriium (*Streptococcus bovis*), and one lactate-utilizing microorganism (*Megasphaera elsdenii*) were quantified by the qPCR technique on days 8 (pre-adaptation phase), 18 (adaptation phase), and 30 (finishing phase) of each period.

The ruminal samples (solid + liquid) were collected by manually evacuating the rumen through the cannula 4 h after feeding. The ruminal content was weighed (solid and liquid phase, separately), and the proportion of solid and liquid in the rumen of each animal was calculated. For each sample 50 g of ruminal content were used according to the proportion calculated in the rumen evacuation for each animal (e.g., 30% liquid and 70% solid, so 15 g of liquid and 35 g of solid composed a 50 g of ruminal content sample). Samples were processed immediately after collection as described by Yu and Morrison (2004) and stored at −80 °C until DNA extraction.

DNA extraction was performed for each sample of rumen content, with QIAamp DNA Stool Kit (Qiagen, Valencia, CA) and used according to the manufacturer's instructions. The real-time qPCR reactions were on a 7500 Real Time PCR System (Applied Biosystems®, Life Technologies, Foster City, CA) in plate, for each ruminal sample individually, containing 10 µL of 2 × SYBR Green Master Mix (Applied Biosystems®, Life Technologies, Foster City, CA), 1.2 µL of primer Forward, and 1.2 µL of primer Reverse for respective bacteria (Table 3), 6.6 µL of MilliQ water, and 1 µL of DNA template

Table 3 - Real-time PCR primers used in the relative quantification of ruminal microorganisms from canullated Nellore cattle

Species	Sequence (5´ - 3´)	Amplicon size (bp)	Reference
F. succinogenes	F: GGTATGGGATGAGCTTGC R: GCCTGCCCCTGAACTATC	445	Tajima et al. (2001)
S. bovis	F: CTAATACCGCATAACAGCAT R: AGAAACTTCCTATCTCTAGG	127	Stevenson and Weimer (2007)
M. elsdenii	F: GACCGAAACTGCGATGCTAGA R: CGCCTCAGCGTCAGTTGTC	129	Ouwerkerk et al. (2002)
Eubacteria	F: CCTACGGGAGGCAGCAG R: ATTACCGCGGCTGCTGG	193	Muyzer et al. (1993)

F - forward; R - reverse.

in a final volume of 20 µL per reaction. The extracted DNA was used as a template in a real-time PCR reaction using specific primers for the desired rumen bacteria (Table 3), herewith a universal primer for eubacteria (universal).

The PCR amplification protocol was as follows: an initial denaturation step at 95 °C for 10 min, then 44 cycles of heating and cooling at 95 °C for 15 s, followed by annealing step at 60 °C for 30 s, and extension at 72 °C for 30 s. The samples were run in duplicate, and a negative control was included in each assay to assess the specificity of PCR reaction. The melting curves were analyzed at the end of the reactions to verify the specificity of each amplification.

2.5. Statistical analysis

Data were analyzed in a replicated Latin square design by SAS software (Statistical Analysis System, version 9.1), and tests for normality (Shapiro–Wilk's and Kolmogorov–Smirnov's) and heterogeneity of treatment variances (GROUP option of SAS) were performed before analyzing the data. The effects of period, square, period × square, square × treatments, animal nested within square, and period animal nested within square were considered random factors. The qPCR of ruminal bacteria was analyzed by Mixed procedure of SAS. The model accounted for the same effects as described above. Results were considered significant at P≤0.05 level. All means presented are least squares means, and effects were separated by PDIFF option of SAS. The mathematical model used was:

$$y_{ijkl} = \mu + \tau_i + \rho_j + \sigma_k + a_l(\sigma_k) + e_{ijkl}, \quad (1)$$

in which y_{ijkl} = observed value of the dependent variable, μ = overall mean, τ_i = treatment effect, ρ_j = period effect, σ_k = Latin square repetition effect, $a_l(\sigma_k)$ = animal within Latin square repetition, and e_{ijkl} = random residual error.

The variables involving DMI, rumen protozoa, and ruminal pH were analyzed by MIXED procedure of SAS with repeated measures (Littell et al., 1998). The model accounted for the same effects as described above plus time and its interactions with treatments. Results were considered significant at P≤0.05 level. All means presented are least squares means, and effects were separated by PDIFF option of SAS. The mathematical model used was:

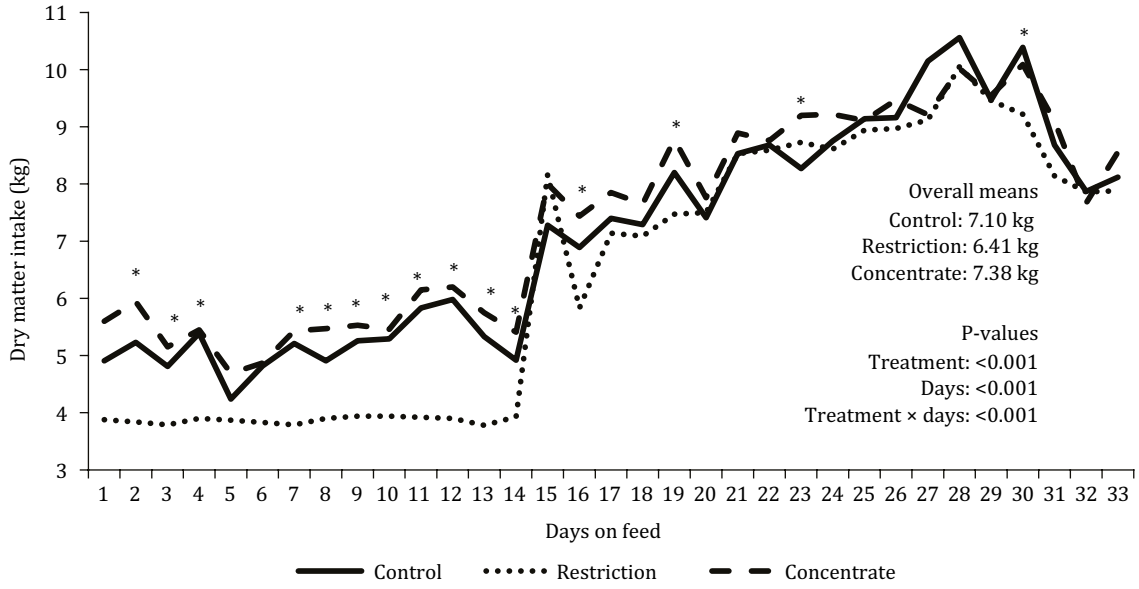
$$y_{ijklm} = \mu + \tau_i + \rho_j + \sigma_k + o_l + \Theta_{il} + a_m(\sigma_k) + e_{ijklm}, \quad (2)$$

in which y_{ijklm} = observed value of the dependent variable, μ = overall mean, τ_i = treatment effect, ρ_j = period effect, σ_k = Latin square repetition effect, o_l = time effect, Θ_{il} = interaction between treatments and time, $a_m(\sigma_k)$ = animal within Latin square repetition, and e_{ijklm} = random residual error.

3. Results

For DMI, an interaction was observed between treatments and day on feed, in which cattle from concentrate group had greater intakes during most part of the feeding period (Figure 1).

During the pre-adaptation phase, restricted cattle had a higher pH than concentrate-fed cattle (P = 0.05). However, there were no differences between cattle from control group and those from other treatments (Table 4). Moreover, a quadratic response was observed between time and ruminal pH (P = 0.01), in which the lowest pH was measured 12 h after feeding (0 h: 6.59, 4 h: 6.66, 8 h: 6.52, 12 h: 6.39; SE = 0.07; data not shown). Regarding the adaptation and finishing phases, no effect of treatments was observed (P>0.05). Nevertheless, a quadratic relationship was observed (P<0.01) between rumen pH and time for the adaptation phase (0 h: 6.23, 4 h: 6.22, 8 h: 5.93, 12 h: 5.78; SE = 0.04; data not shown). During finishing phase, rumen pH decreased linearly (P<0.01) from 0 to 12 hours after feeding (0 h: 6.08, 4 h: 6.00, 8 h: 5.77, 12 h: 5.56; SE = 0.09; data not shown).



* Within day, at least one mean differ from the others (P≤0.05; SE = 0.56).

Figure 1 - Interaction between treatment and days on feed for dry matter intake of Nellore cattle during the study period (P<0.001).

Table 4 - Ruminal pH measuraments of canulated Nellore cattle previously subjected to nutritional restriction or intake of concentrate ingredients

Item	Treatment (TRT)			SE	P-value		
	Control	Restriction	Concentrate		TRT	Time	TRT×Time
Pre-adaptation	6.56ab	6.61a	6.45b	0.07	0.05	0.01^{Q}	0.42
Adaptation	6.10	5.99	6.03	0.04	0.13	<0.01^{Q}	0.22
Finishing	5.85	5.93	5.79	0.09	0.10	<0.01^{L}	0.47

SE - standard error; L - linear; Q - quadratic.
a,b - Within a row, means with differnt letters differ (P≤0.05).

In the pre-adaptation phase, cattle fed concentrate had a larger *Entodinium* population than cattle from other treatments (P = 0.02), resulting in a larger total protozoa counting for steers fed concentrate as well (P<0.01, Table 5). In addition, there was an interaction (P = 0.04) between treatments and time after feeding for *Isotricha* counts, in which only at 8 h after feeding cattle either fed concentrate or restricted presented smaller *Isotricha* populations (Figure 2). No further differences for protozoal *Dasytricha* and *Diplodinium* populations were observed during the pre-adaptation phase (P>0.05).

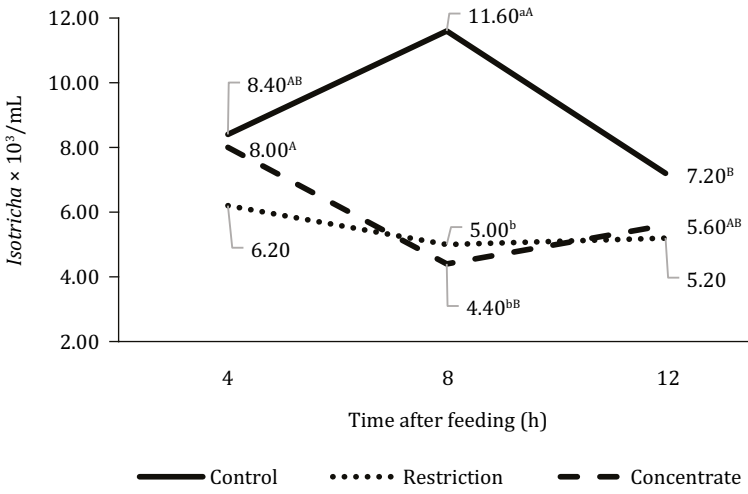
During the adaptation phase, cattle fed concentrate had the lowest counting of *Dasytricha* (P<0.01) and *Isotricha* (P = 0.05) when compared with cattle from control or restricted groups. Furthermore, cattle from restricted group showed higher number of *Dasytricha* (P<0.01) and a smaller number of *Isotricha* (P = 0.05) than the control animals. No effect of treatments was observed (P>0.05) on *Entodinium* and *Diplodinium* populations and total protozoa.

Regarding the finishing phase, steers either restricted or fed concentrate had larger *Diplodinium* (P<0.01) and *Dasytricha* (P<0.01) relative populations than control animals. Furthermore, restricted cattle had lower (P = 0.02) numbers of *Isotricha* when compared with either control or concentrate-fed steers. Finally, a time effect was observed (P = 0.03) for *Isotricha*, in which its counts decreased linearly (4 h: 2.33; 8 h: 1.20; 12 h: 2.20). No significant effect (P>0.05) was detected for *Entodinium* relative populations and total protozoa.

Table 5 - Ruminal protozoa counting of canulated Nellore cattle previously subjected to nutritional restriction or intake of concentrate ingredients

Protozoa (× 10^3/mL)	Treatment (TRT)			SE	P-value		
	Control	Restriction	Concentrate		TRT	Time	TRT×Time
Pre-adaptation							
Entodinium	56.33b	44.47b	101.67a	13.21	0.02	0.37	0.20
Diplodinium	18.13	28.53	19.00	6.70	0.49	0.40	0.36
Isotricha	9.07	5.47	6.00	2.70	0.29	0.21	0.04
Dasytricha	5.27	3.33	3.40	1.08	0.24	0.13	0.71
Total protozoa	89.13b	82.20b	130.80a	14.95	<0.01	0.87	0.35
Adaptation							
Entodinium	153.60	163.80	148.20	21.21	0.26	0.56	0.27
Diplodinium	18.00	19.13	12.13	6.26	0.54	0.27	0.42
Isotricha	7.13a	3.73b	1.73c	1.71	0.05	0.32	0.12
Dasytricha	2.00b	2.67a	1.27c	0.49	<0.01	0.13	0.87
Total protozoa	181.13	189.53	163.40	18.01	0.19	0.53	0.58
Finishing							
Entodinium	192.80	186.53	198.60	16.64	0.25	0.22	0.08
Diplodinium	12.20b	25.53a	25.73a	9.91	<0.01	0.99	0.14
Isotricha	2.00a	0.87b	1.87a	1.18	0.02	0.03^{L}	0.23
Dasytricha	0.06b	1.13a	1.40a	0.24	<0.01	0.16	0.31
Total protozoa	207.80	215.00	228.07	22.91	0.16	0.23	0.50

SE - standard error; L - linear.
a,b,c - Within a row, means with different letters differ (P≤0.05).



a,b - Within time, means with different letters differ (P≤0.05; SE = 2.70).
A,B - Within treatment, means with different letters differ (P≤0.05; SE = 2.40).

Figure 2 - Interaction between treatment and time after feeding for *Isotricha* counts in the rumen of Nellore cattle during pre-adaptation phase (P = 0.04).

Regarding the real-time PCR of ruminal bacteria, there was no effect of treatments on *F. succinogenes* population (P>0.05, Table 6) during the pre-adaptation phase. However, there was a reduction in *M. elsdenii* relative population (P<0.01) in cattle from either restriction or concentrate groups. Likewise, restricted cattle had smaller *S. bovis* relative population when compared with animals receiving concentrate (P = 0.05).

Table 6 - Relative population of ruminal microrganisms of Nellore subjected to nutritional restriction or intake of concentrate ingredients

Item	Treatment (TRT)			SE	P-value
	Control	Restriction	Concentrate		TRT
Pre-adaptation					
F. succinogenes	0.490	0.491	0.544	0.0044	0.14
M. elsdenii	0.256a	0.238b	0.230c	0.130	<0.01
S. bovis	0.129ab	0.118b	0.136a	0.0099	0.05
Adaptation					
F. succinogenes	0.613a	0.566a	0.501b	0.0041	0.05
M. elsdenii	0.261	0.247	0.248	0.0224	0.73
S. bovis	0.135	0.141	0.147	0.62	0.08
Finishing					
F. succinogenes	0.686a	0.637ab	0.585b	0.0030	0.05
M. elsdenii	0.189	0.178	0.168	0.0107	0.16
S. bovis	0.166	0.157	0.156	0.0090	0.31

SE - standard error.
a,b,c - Within a row, means with different letters differ (P≤0.05).
The relative population size is presented as a percentage of a microbial population.

Regarding the adaptation and finishing phases, no effect (P>0.05) of treatments were observed on *M. elsdenii* and *S. bovis* relative populations. However, cattle from concentrate group had smaller *F. succinogenes* populations (P = 0.05) when compared with animals from the control group.

4. Discussion

The present study was part of a larger research performed by this research group, a compendium of studies assessing the effect of either nutritional restriction or intake of concentrate feedstuffs prior to the adaptation to high-concentrate diets on animal performance, DMI, ruminal fermentation, and microbiota. Pereira et al. (2020) aimed to test the hypothesis that cattle from pasture, coming either from nutritional restriction or from intake of concentrate feedstuffs prior to the adaptation period, require a different adaptation length and present different overall feedlot performance. The authors reported that either restriction or concentrate supplementation before beginning the adaptation period to high-concentrate diets did not impact adaptation length, and both may be used as nutritional strategies to improve performance and carcass characteristics of feedlot Nellore cattle. In this context, it was hypothesized that restriction or intake of concentrate feedstuffs prior to the adaptation could affect rumen fermentation patterns and, consequently, ruminal microbiota. Thus, Pinto et al. (2020) reported that cattle previously exposed to concentrate exhibited decreased bacterial richness during the pre-adaptation phase and increased bacterial diversity during the adaptation phase. Moreover, restricted animals had lower DMI during the adaptation phase, as well as lower DM digestibility, starch, and total digestible nutrients when compared with cattle consuming concentrate.

In the present study, greater ruminal fermentation during pre-adaptation phase decreased ruminal pH, which may have negatively affected *M. elsdenii*, a pH-sensitive microorganism (Nocek, 1997). However, we observed an increase in *Entodinium* populations, which is considered the most pH-tolerant species when compared with other genera of rumen protozoa (Mackie et al., 1978; Lyle et al., 1981). Furthermore, *Entodinium* populations present high amylase activity to digest engulfed starch granules (Nagaraja, 2016). *Entodinum* ferment cell wall carbohydrates, as well as starch and soluble sugars, but in general, these microorganisms use starch as main source to growth, which may have favored *Entodinium* population in the rumen of concentrate-fed cattle, since the

number of protozoa can be relatively low in animals receiving exclusive forage diets and higher in forage and grain mixtures (Veira, 1986).

During the pre-adaptation, restricted steers presented higher rumen pH than concentrate-fed cattle, due to the lack of substrate available for fermentation based on the DMI (Figure 1). In addition, the lack of substrate may have played a role in reducing *M. elsdenii* and *S. bovis* populations during pre-adaptation without negative effects on protozoa. When concentrate diets were introduced during adaptation phase, bacterial populations were reestablished, and no differences were observed when restricted animals were compared with cattle from control or concentrate groups during adaptation and finishing.

The lower pH during pre-adaptation phase may have negatively affected the *F. succinogenes* relative populations in both adaptation and finishing phases, as well as *Isotricha* and *Dasytricha* populations during adaptation. *Fibrobacter succinogenes* is considered the major ruminal cellulolytic bacterium in the rumen, which is sensitive to low ruminal pH; almost none of ruminal cellulolytic bacteria grow significantly at pH values below 6.0 (Weimer, 1996). Likewise, *Isotricha* and *Dasytricha* populations were negatively affected when the level of concentrate in diets became higher (Dehority, 1995). However, since they do not ferment structural carbohydrates (Nagaraja, 2016), the lower ruminal pH during adaptation and finishing phase when compared with pre-adaptation may have had more impact on their populations. On the other hand, the lower inclusion of roughage sources in the diet in the adaptation and finishing phases may have played a more significant role in reducing *F. succinogenes* population than the ruminal pH itself, since there would be smaller amounts of structural carbohydrates available for fermentation entering the rumen. Pinto et al. (2020) reported that the lower relative abundances of *F. succinogenes* could be related to the larger area of pH below 6.2, resulting in a reduction in total tract digestibility of neutral and acid detergent fibers.

In the finishing phase, steers receiving concentrate reestablished protozoa populations that were negatively affected in previous phases, such as *Diplodinium*, which may be related to the increased DMI presented by cattle fed concentrate without negatively impacting ruminal pH. Moreover, *Dasytrichia* and *Diplodinium* populations were higher in restricted cattle than in the control cattle, which may be related to the lower intake presented by these animals most of the feeding period. So, the intake of concentrate feedstuffs during pre-adaptation phase may have promoted positive effects in the process of cattle adaptation to high-concentrate diets. In this context, early exposure to concentrate feedstuffs is thought to prepare the ruminal bacterial community for higher levels of non-fibrous carbohydrates (Pereira et al., 2020; Pinto et al., 2020). Thus, Pinto et al. (2020) reported an increase in ruminal starch degradability in animals exposed to concentrate feedstuffs prior to the adaptation phase.

5. Conclusions

The previous nutritional background impacts dry matter intake and ruminal microbiota during adaptation and finishing phases without causing any negative effect on rumen pH. Furthermore, feeding concentrate prior to the adaptation positively impacts the transition to high-concentrate diets, since cattle receiving concentrate partially reestablishes the ruminal microbiota during the finishing phase, even presenting greater dry matter intake.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: G.D. Cruz and D.D. Millen. Data curation: P.H.M. Rodrigues, G.D. Cruz and D.D. Millen. Formal analysis: A.C.J. Pinto and D.D. Millen. Funding acquisition: D.D. Millen. Investigation:

P.H.M. Rodrigues, G.D. Cruz and D.D. Millen. Methodology: A.C.J. Pinto, L.D. Felizari, B.L. Demartini, E.F.F. Dias, M.M. Squizatti, A.M. Silvestre, F. Perna Junior, L.G. Mesquita and P.H.M. Rodrigues. Project administration: G.P. Bertoldi, M.M. Squizatti, A.M. Silvestre and D.D. Millen. Supervision: A.C.J. Pinto, G.P. Bertoldi and D.D. Millen. Validation: J.M. Souza and D.D. Millen. Writing – original draft: J.M. Souza. Writing – review & editing: J.M. Souza and D.D. Millen.

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