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Changes in ruminal fermentation and rumen bacteria population in feedlot cattle during a high lipid diet adaptation*

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This study aimed to investigate changes in feed intake and ruminal environmental parameters during a high-lipid diet transition in cattle. Eight Nellore steers were fed a control diet composed of 30% hay and 70% concentrate for 21 days, followed by the inclusion of 60 g/kg dry matter of soybean oil for 21 days. The DM intake expressed as a percentage of BW 21 days after lipid inclusion was lower (1.75% BW) than that observed during the control diet feeding (1.81% BW) (P<0.01). Steers fed the control diet had a lower pH than the ruminal pH recorded on days 7, 14, and 21 after lipid inclusion (P=0.034). Lower total short-chain fatty acid production in the rumen and lower microbial nitrogen synthesis were observed on day 7 after lipid inclusion compared to values found when steers were fed the control diet and on days 14 and 21 after lipid inclusion (P=0.041). Lipid inclusion in the diet decreased the population of protozoa on days 7, 14, and 21 (P<0.001). The abundances of *R. albus* and *F. succinogenes* were higher when steers were fed the control diet than the abundance observed on days 7, 14, and 21 after lipid inclusion in the diet (P<0.05). The first

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seven days of lipid diet inclusion are considered the most critical for ruminal adaptation, involving reductions in fibrolytic bacteria and changes in fermentation parameters. After 14 days the rumen showed signs of recovery and adaptation.

KEY WORDS: fibrolytic bacteria / lipolytic bacteria / rumen protozoa / soybean oil

Lipids have been incorporated into ruminant diets to enhance energy density and improve overall efficiency of livestock systems. They play a crucial role in facilitating the absorption of fat-soluble vitamins and influencing the composition of essential fatty acids (FA) in animal tissue membranes [Carvalho *et al*. 2017]. However, it is important to note that high lipid content in the diet can have negative effects on ruminal fermentation. The presence of unsaturated fatty acids (UFA) in the diet can be toxic to ruminal microbiota, particularly cellulolytic bacteria, and protozoa. This can result in reduced fiber digestion and decreased dry matter intake (DMI) [Yang *et al*. 2009]. Nevertheless, the inclusion of vegetable oils in diets with added lipids has shown promising results in improving beef cattle performance, increasing the healthy UFA available in the duodenum to meat deposition (e.g. conjugated linoleic acid and vaccenic acid) [Granja-Salcedo *et al*. 2017a].

On the other hand, the inclusion of vegetable oils in beef cattle diets has been associated with significant changes in the populations of ruminal bacteria, archaea, and protozoa [Granja-Salcedo *et al*. 2017b]. When transitioning to a new diet, the adaptation of ruminants depends on various factors such as the animal itself, diet characteristics, environment, and the interactions of rumen microbes. It is crucial to provide sufficient time for adaptation to eliminate potential residues from the previous diet, particularly in the case of fibrous feeds that have a longer ruminal retention time compared to grains. The duration of the adaptation period in cattle nutrition studies varies, typically ranging from 7 to 27 days [Yang *et al*. 2009]. However, when dealing with diets containing a high lipid content (>5 g/kg of ether extract), a longer adaptation period may be necessary due to the potential toxicity of UFA on the ruminal microbiota [Maia *et al*. 2007]. An extended adaptation period could allow the rumen microbial community to adjust to the presence of high levels of lipids and minimize the negative effects on ruminal fermentation and nutrient utilization.

The importance of the adaptation period in avoiding the influence of previous treatments (diets, additives, environments, etc.) and controlling their effects is well recognized [Jóźwik *et al*. 2010, Strzałkowska *et al*. 2010, Machado *et al*. 2016]. Several studies have investigated changes in rumen bacterial populations when animals adapt to high-concentrate diets and new forage sources [Abubakr *et al*. 2013, Maia *et al*. 2010]. However, the optimal duration of the adaptation period for high lipid inclusion diets in beef cattle remains poorly understood. Several aspects of the adaptation process to lipid inclusion, such as intake and ruminal environment characteristics, have not been fully elucidated. Therefore, the objective of this study was to investigate changes in rumen fermentation parameters, as well as populations of rumen bacteria and protozoa, during transition from a diet without lipids to a high-lipid diet in feedlot cattle.

Material and methods

The experimental procedures used in this study followed the Ethical Principles for Animal Experimentation adopted by the Brazilian College of Animal Experimentation and approved by the Ethics Committee of Animal Experimentation of the School of Agricultural and Veterinary Sciences (FCAV) (protocol #07784/14).

The experiment involved eight Nellore steers with an average body weight of 501±18 kg and 24±1 months old, fitted with a ruminal silicone cannula and kept in individual stables (12 m^2) with a concrete floor, 50% of the covered area, equipped with individual feed troughs and automatic drinkers. Steers were fed a control diet composed of 30% Tifton 85 hay and 70% concentrate composed by ground corn, soybean meal, and urea (Tab. 1) for 21 days, followed by the inclusion of 60 g/kg of dry matter (DM) of soybean oil substituting partially ground corn for 21 days. Diets were formulated according to the recommendations of the Agricultural and Food Research Council [AFRC, 1993] and were isonitrogenous. Soybean oil used

Table 1. Experimental diets: proportion of ingredients, bromatologic composition, and fatty acid profiles

Item	Experimental diets	
	control	lipid[1]
Ingredients (%)		
tifton hay	30.00	30.00
ground corn	56.00	48.50
soybean meal	13.00	14.50
urea	1.00	1.00
soybean oil[1]	0.00	6.00
Bromatologic composition		
dry matter (%)	83.99	89.64
organic matter (% DM)	94.60	87.26
crude protein (% DM)	15.12	15.24
ANDFom[2] (% DM)	44.60	43.05
ether extract (DM %)	3.30	8.92
metabolizable energy (Mcal/kg)	2.55	2.48
Fatty acid profile (g/kg of DM)		
stearic acid (C18:0)	0.55	7.84
oleic acid (C18:1)	3.29	20.93
linoleic acid (C18:2)	7.47	27.55
linolenic acid (C18:3)	0.09	0.21
total SFA[3]	5.69	25.00
total UFA[4]	20.65	58.25

[1] Concentrate with 6% of soybean oil.
[2] Neutral detergent fiber assayed with a heat stable amylase and expressed exclusive of residual ash.
[3] SFA, saturated fatty acids (sum of C8:0, C10:0, C12:0, C13:0, C14:0, C15:0, C16:0, C17:0, C18:0; C20:0 and C22:0).
[4] UFA, unsaturated fatty acids (sum of C14:1 *cis* 9, C16:1 *cis* 9, C18:1 *cis* 9, C18:2 *cis* 9 *cis* 12, C18:3 *cis* 9 cis 12 *cis* 15, C20:1 *cis* 11 and C22:1 *cis* 13).

contained 121.6 g/kg stearic acid; 301.6 g/kg oleic acid; 347.83 g/kg linoleic acid; and 2.29 g/kg linolenic acid.

The animals were fed experimental diets twice per day at 06:00 h and 16:00 as the total mixed ration, and a mineral supplement was offered (containing per kg: calcium, 146 g; phosphorus, 40 g; sulfur, 40 g; sodium, 130 g; copper, 1.35 g; manganese, 1.04 g; zinc, 5 g; iodine, 100 mg; cobalt, 80 mg; selenium, 26 mg; fluorine, ≤800 mg). The duration of the experiment was 42 d, with daily intake evaluation and ruminal environmental parameter sampling on days 0, 7, 14, and 21 after soybean oil inclusion. Throughout the experimental period the allowance was adjusted to allow refusal of approximately 5% of the total amount consumed on the previous day.

Before the morning feeding diets and feed refusals were collected and weighed daily; samples of ∼100 g/kg were obtained, dried in a forced-air-circulation oven at 55°C for 72 h, and ground in a Wiley mill (Thomas Scientific, Swedesboro, NJ) to pass a 1-mm screen. The DM (DM; 934.01) and ether extract (EE; 920.39) contents were analyzed following the methods prescribed by AOAC [1990]. Nitrogen was determined using a LECO FP-528 nitrogen analyser (LECO Corp., St. Joseph, MI). Gross energy was obtained by combustion of the samples in an adiabatic bomb calorimeter (IKA model 2000 Basic). Acid detergent fiber (ADF) and Neutral detergent fiber (NDF) determination was conducted in an ANKOM 200 Fiber Analyzer (ANKOM Technology Corporation, Fairport, NY, USA), using α-amylase without the addition of sodium sulfite in NDF determination [Van Soest *et al*. 1991].

Total fatty acids were extracted from the feed ingredients using the methylation method described by Palmquist and Conrad [1971], and 1 mL of nonadecanoic acid C19:0 (2.0%) was added to each sample, which was used as the internal standard for FA quantification. The FA profile was quantified by gas chromatography (GC Shimatzu model 20-10, with automatic injection; Shimatzu Corporation, Kyoto, Japan) using an SP-2560 capillary column (100 m × 0.25 mm diameter, 0.02 mm thick; Supelco, Bellefonte, PA, USA), and hexane was used as the carrier gas. To identify each FA, external standards (Supelco, Bellefonte, PA, USA; Nu-Chek Prep, Elysian, MN, USA) were used.

Ruminal fluid was sampled on days 0, 7, 14, and 21 after soybean oil inclusion 3 h after morning feeding. Approximately 50 mL of rumen fluid were collected through the rumen cannula after filtering through cotton double fabric, and pH measurements were performed using an electric pH meter (Nova Tecnica, PHM, Piracicaba, SP, Brazil). Then, two aliquots of 25 ml were stored at -20°C and later used to determine ammonia N and short-chain fatty acid (SCFA) concentrations. NH3-N was assayed in duplicate in Kjeldahl distillation [Fenner 1965]. The SCFA concentration was quantified by gas chromatography (GC Shimatzu model 20-10, with automatic injection; Shimatzu Corporation, Kyoto, Japan) using an SP-2560 capillary column (30 m × 0.25 mm diameter, 0.02 mm thick; Supelco, Bellefonte, PA, USA) according to Palmquist and Conrad [1971].

Spot urine samples were collected 4 h after morning feeding on days 0, 7, 14, and 21 of each experimental period. Urine was collected while the animals were in a pen after spontaneous urination using collector funnels coupled to tubes that collected urine in plastic bottles. The samples were filtered through two layers of gauze, and 10 mL urine aliquots were diluted in 40 mL 0.036 N sulfuric acid and stored at -20°C for subsequent analysis. Concentrations of uric acid were determined using commercial kits (Acid Uric Liquiform, cat. no. 140), whereas allantoin was determined using the colorimetric method described by Chen and Gomes [1992]. The total urinary volume was estimated using the proportion of creatinine concentration in the urine with daily creatinine excretion (CE) estimated using the equation for Nellore steers proposed by Costa e Silva *et al*. [2012] as follows: CE (g/d) = 0.0345 (FBW) 0.9491, in which CE is the daily creatinine excretion (g/day) and FBW = fasted body weight, obtained by the equation 0.96 BW. Microbial protein synthesis was calculated via urinary total excretion of purine derivatives (allantoin + uric acid) according to Fujihara *et al.* [1987] and Pina *et al.* [2009].

Quantitative polymerase chain reaction (qPCR) was used to identify and quantify the main fibrolytic bacterial species (*Ruminococcus albus, Ruminococcus flaviefaciens*, and *Fibrobacter succinogenes*), amylolytic (*Selenomonas spp*. and *Prevotella spp*.), and lipolytic species (*Butyrivibrio fibrisolvens* and *Anaerovibrio lipolytica*). For this purpose on days 0, 7, 14, and 21 after soybean oil inclusion, before the morning feeding, 50g samples of solid and liquid fractions of ruminal content were collected from the medial region of the rumen through a ruminal cannula. The samples were immediately frozen at -80°C until DNA extraction. A microbial pellet was obtained as described by Granja-Salcedo *et al*. [2017c]. Metagenomic DNA extraction for 150 mg of the pellet was performed using the Fast DNA SPIN Kit for Soil (MP Bio, Biomedicals, Illkirch, France) following the manufacturer's recommendations. The integrity and quantity of DNA were verified on a 0.8% agarose gel stained with ethidium bromide (5 mg/mL), while the yield and quality of DNA were evaluated by spectrophotometry (NanoDrop 1000, Thermo Fisher Scientific, Waltham, MA, USA) and fluorometry (Qubit 3.0, Life Technology, Waltham, MA, USA).

All forward and reverse primers used in this study (Tab. 2) were tested at five concentrations (100, 200, 400, 600, and 800 nM) to determine the minimum concentration of the primer with the lowest threshold cycle (Ct) and to reduce nonspecific amplification. The slope value was determined and efficiency was calculated. The selected primer concentrations were validated using different concentrations of DNA (150, 125, 100, 50, 25, and 12.5 ng). The qPCRs (final reaction volume of 12.5 µL) contained 6.25 µL of SYBR Green PCR Master Mix (Bio-Rad, Hercules, CA, USA), 400 nM of each primer pair, ultrapure water (Milli-Q, Millipore Corporation, Merck, Billerica, MA, USA), and 100 ng of the metagenomic DNA. The reactions were performed using an Applied Biosystems 7500 Real-time PCR System (Thermo Fisher Scientific, Waltham, MA, USA). Negative controls were included in the analysis by omitting metagenomic DNA. ROX was used as the passive reference

dye. The PCR conditions were as follows: amplification at 50°C for 2 min, 95°C for 10 min; 35 denaturing cycles at 95°C for 15 s, annealing at 60°C for 60 s, and extension at 78°C for 1 min. After the amplification cycle a step was added, increasing the temperature from 60°C to 95°C to obtain a dissociation curve of the reaction products and analyze specificity of the amplification. Relative quantification was used to determine the proportion of target bacterial species. The results were expressed as a proportion of the 16S rRNA associated with total bacteria, according to the following equation: [relative quantification = 2 − [Ct target-Ct total bacteria]], where Ct is defined as the number of cycles required for the fluorescent signal to cross the threshold.

For the quantification and identification of rumen ciliate protozoa, samples of ruminal content were collected via a cannula from various sites within the rumen after 3 h of morning feeding on days 0, 7, 14, and 21 after soybean oil inclusion. The liquid and solid contents were immediately preserved in formalin (1:1 solution of water and 370 ml/l formaldehyde). Cell counts were obtained from preserved rumen content aliquots according to D'Agosto and Carneiro [1999] in a Sedgewick-Rafter counting chamber. The samples were diluted with 200 ml glycerol per l and stained using Lugol's solution before counting the cells [D'Agosto and Carneiro 1999]. Using a light microscope, 50 grids were evenly spaced over a 20 × 50 mm chamber and counted.

Statistical analysis

Initially, the assumptions of normality and homoscedasticity were verified using the Shapiro-Wilk and Bartlett's tests,

Table 2. Primers used for specific quantification of ruminal bacteria by qPCR

Item	Primer (5'-3')		Product (bp)	Efficiency (%)
	forward	reserve		
Total bacteria[1,2]	CGGCAACGACAACCC	CCATTGTAGCACCTGTGTAGCC	130	100
Ruminococcus flavefaciens[2]	CGAACGGAGATAATTTGAGTTTACTTAGG	CGGTCTCTGTATGTTATGAGGTATTACC	132	95
Ruminococcus albus[3]	CCCTAAAAGCAGTCTTAGTTCG	CCTCCTTGCGGTTAGAACA	175	94
Fibrobacter succinogenes[2]	GTTCGG AATTAC TGG GCGTAAA	CGCCTGCCCCTGAACTATC	121	97
*Butyrivibrio fibrisolvens*5	*TGAAAAACTCCGGTGGTATGAGAT*	*CCGTGTCTCAGTCCCAATGTG*	126	95
Anaerovibrio lipolytica[5]	TTGGGTGTTAGAAATGGATTCTAGTG	TCGAAATGTTGTCCCCATCTG	82	96
Selenomonas sp.[4]	GGCGGGAAGGCAAGTCAGTC	CCTCTCCTGCACTCAAGAAAGACAG	83	101
Prevotella sp.[2]	GGTTCTGAGAGGAAGGTCCCC		83	97

[1] Primers used for the normalization of qPCR.
[2] Primer designed and validated by Denman and McSweeney [2006].
[3] Primer designed and validated by Koike and Kobayashi [2001].
[4] Designed and validated by Khafipour *et al.* [2009].
[5] Designed and validated by Fuentes *et al.* [2009].

respectively. For feed intake data a repeated measures analysis of variance was conducted, considering a mixed model that included the fixed effects of diet, day, and their interaction, as well as random effects for the animal and the residuals corresponding to the model. For ruminal fermentation parameters and ruminal protozoa, a one-way repeated measures analysis of variance was used to compare measurements between days. When the analysis of variance indicated significant differences, Tukey's post-hoc test was applied, considering statistical significance at $P \leq 0.05$. Ruminal bacterial abundances were compared between sampling days using Kruskal-Wallis and post-hoc Dunn's tests, and data are showed as medians and interquartile ranges. The computations were performed using the R Software version 3.2.2 (R Core Team, 2015).

Results and discussion

The daily DMI expressed in kg was higher when steers were fed the control diet on day 14 (9.76±0.89 vs. 9.13±0.76 kg) and day 21 (10.42±1.67 kg vs. 10.02±1.33 kg) compared to when animals were fed the high-lipid diet ($P<0.01$) - Figure 1. Additionally, DMI expressed as a percentage of BW at 21 days after lipid inclusion was lower (1.75±0.21% BW) than the intake observed during the control diet feeding (1.81±0.20% BW) ($P<0.01$). Following the inclusion of a high-lipid diet, a reduction in DMI was observed on day 1 of adaptation (9.07±2.59 kg) ($P=0.048$). The decrease in DMI continued during days 2 to 6 of adaptation, with a mean of 8.13 kg (ranging from 7.66 to 9.05). After 10 days of adaptation the DMI increased to 9.78±1.83 kg, reaching 10.02±1.33 kg after 21 days. Interestingly, DMI on day 14 was similar to that observed on day 21 days after lipid inclusion (9.39±1.37 vs. 10.17±1.45, $P=0.71$). The literature on DMI in ruminants supplemented with lipids presents variable results, which can be attributed to such factors as the type (saturated or unsaturated) and amount of supplemental fat [Fiorentini *et al*. 2015]. Different studies have reported reductions

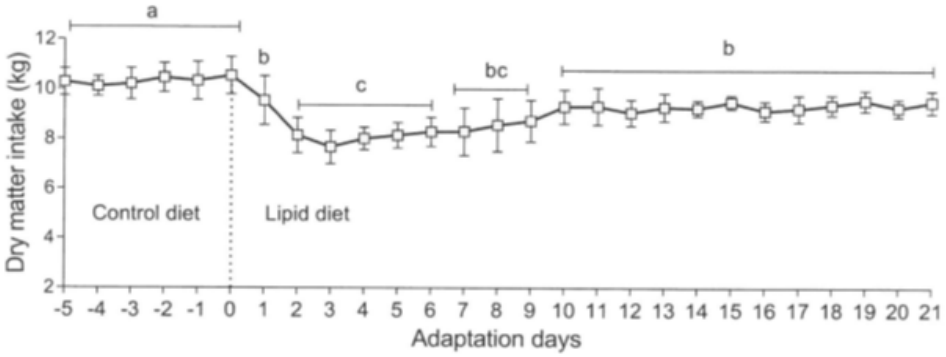

Fig. 1. Dry matter intake expressed as kg of DM per day in feedlot Nellore steers during a high lipid diet transition. Different letters represent a significant difference ($P<0.05$), as obtained using Tukey's test.

in DMI when high-lipid content diets were provided to cattle. This reduction can be attributed to factors as a decrease in the number of rumen protozoa and possibly an increase in rumen retention time due to reduced fiber digestion [Torres *et al*. 2023]. For example, Granja-Salcedo *et al*. [2017a] reported a decrease in DMI in Nellore steers with the inclusion of 6% soybean oil in combination with 10% glycerine. However, it is important to note that some studies reported no significant effect on DMI when feeding lipid diets to cattle, such as the study conducted by Santana *et al*. [2017]. On the other hand, an increase in the content of UFA in the blood serum can have an impact on reducing DMI in ruminant animals. UFA can activate the hypothalamic feedback satiety signal center, leading to a decrease in feed intake [Allen *et al.* 2009]. This mechanism can be mediated through the action of GLP-1 (peptide-like glucagon 1), which has a hypophagic effect in ruminants. The concentration of GLP-1 in the blood is known to increase with the addition of fat in the diet [Relling and Reynolds, 2007]. In addition, it has been observed that the concentration of GLP-1 is higher when diets containing fats rich in monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) are fed to cattle compared to diets with sources of saturated fatty acids (SFA) [de Paiva Ferreira *et al*. 2020]. High levels of PUFA in the diet can be toxic to rumen microbiota. PUFA can alter permeability and fluidity of microbial membranes, leading to changes in the ruminal environment and potentially affecting microbial populations [Maia *et al.* 2007].

Lower total SCFA production in the rumen and decreased microbial nitrogen synthesis were observed on day 7 after lipid inclusion compared to values observed when steers were fed the control diet and on days 14 and 21 after lipid inclusion (P=0.041, Tab. 3). The change in carbohydrate sources (relatively more hemicellulose owing to less ground corn) due to the replacement of grains with oil in the diet, along with a reduction in cell wall fermentation due to reduced bacterial activity, may partially explain the reduction in total SCFA concentrations [Yang *et al*. 2009]. The

Table 3. Rumen fermentation parameters during a high lipid diet transition in feedlot Nellore steers

Parameters	Sampling days				SEM[1]	*P value*
	control	high lipid inclusion				
	0d	7d	14d	21d		
pH	6.23[b]	6.41[a]	6.36[a]	6.37[a]	0.103	0.034
NH_3-N (mg/dL)	28.29	27.32	28.56	29.80	1.347	0.142
Total SCFA (mmol/L)	93.41[a]	88.42[b]	90.06[ab]	89.76[ab]	3.028	0.041
acetate (%)	71.30	70.98	69.33	71.11	1.140	0.237
propionate (%)	17.00[b]	19.29[a]	18.20[ab]	17.90[b]	0.935	0.022
butyrate (%)	10.60[a]	7.70[b]	9.93[a]	10.52[a]	0.104	0.003
Acetate: Propionate ratio	4.20[a]	3.67[b]	3.80[b]	3.97[b]	0.088	<0.001
Microbial N (g/d)	225.77[a]	154.32[b]	216.19[a]	223.13[a]	16.498	0.302
Protozoa (n x10^4/mL)	123.3[a]	70.8[b]	77.2[b]	76.7[b]	2.149	<0.001

[1]Standard error mean.
[abc] In the same row means bearing different superscripts differ significantly at P<0.05.

ruminal NH3-N concentration and acetate proportion in the rumen were not affected by diet or sampling duration (P>0.05).

Steers fed the control diet had a lower pH than the ruminal pH observed on days 7, 14, and 21 after lipid inclusion (P=0.034, Tab. 3). When lipid sources such as soybean oil are added to cattle feed, they increase the concentration of C18:2 (linoleic acid) in the rumen. This increase in C18:2 could promote a better use of hydrogen in the rumen environment through the process of biohydrogenation, due to utilization of hydrogen and a subsequent increase in rumen pH [Jenkins and Bridges 2007]. Therefore, it is expected that after lipid supplementation higher pH values would be observed in the rumen.

A reduction in microbial nitrogen synthesis was observed on day 7 after the inclusion of lipids in the diet, along with a decrease in the abundance of the bacteria *F. succinogenes* and *B. fibrisolvens*. This is consistent with the findings regarding the reduction in protozoan populations, indicating that the inclusion of lipids significantly affects these microorganisms. In contrast, a study conducted with heifers that evaluated the effect of different concentrations of lipid supplementation (soybean oil at 8.6%, soybean at 520 g/kg, and protected fat at 10.4%) found no influence of the diet on microbial nitrogen synthesis [Santana *et al*. 2017]. However, it was mentioned in that study that rumen passage rate and digestibility may influence microbial nitrogen. It is possible that the reduction in microbial nitrogen observed in the current study is associated with the inhibition of bacterial growth, as approximately 90% of the protein reaching the duodenum of ruminants is of bacterial origin. Microbial nitrogen is of great importance in meeting the protein requirements of ruminants, providing between 50% and 90% of their protein needs [Krysl *et al*. 1991].

High lipid inclusion in the diet reduced the population of protozoa on days 7, 14, and 21 (P<0.001), corroborating that the effect of lipid supplementation reduces ruminal protozoa, as was also evidenced in cattle with a high lipid ratio of palm oil, linseed, and protected fat [Fiorentini *et al*. 2015]. Furthermore, it has been evidenced in goats that protozoa numbers in the rumen decreased rapidly after day 4 of feeding diets containing oil palm [Abubakr *et al*. 2013].

The relative abundance of *R. flavefaciens* was lower after lipid diet inclusion when compared to the abundance observed when steers were fed the control diet, with the lowest abundance found on days 14 and 21 (P<0.05). Similar findings have been reported in studies using diets based on soybean oil and linseed, which also showed a reduction in *R. flavefaciens* [Yang *et al*. 2009]. The decline in fibrolytic bacteria, such as *R. flavefaciens*, can be attributed to the toxic effects of fatty acids, especially unsaturated acids, on these bacterial populations [Maia *et al*. 2007]. The altered ruminal environment caused by the lipid inclusion in the diet may have negatively affected the growth and activity of fibrolytic bacteria, leading to a reduction in their abundance. The relative abundance of the fibrolytic bacteria *R. albus* and *F. succinogenes* was higher when steers were fed the control diet compared to the abundance observed on days 7, 14, and 21 after lipid inclusion in the diet (P<0.05, Fig. 2). On days 14

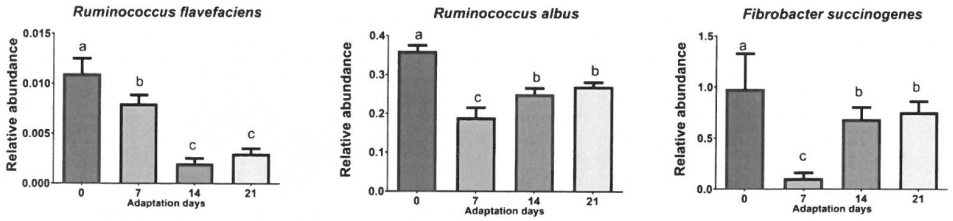

Fig. 2. Relative abundance of the main ruminal fibrolytic bacteria in feedlot Nellore steers during a high lipid diet transition. The different letters represent a significant difference (P<0.05), obtained by the Friedman test.

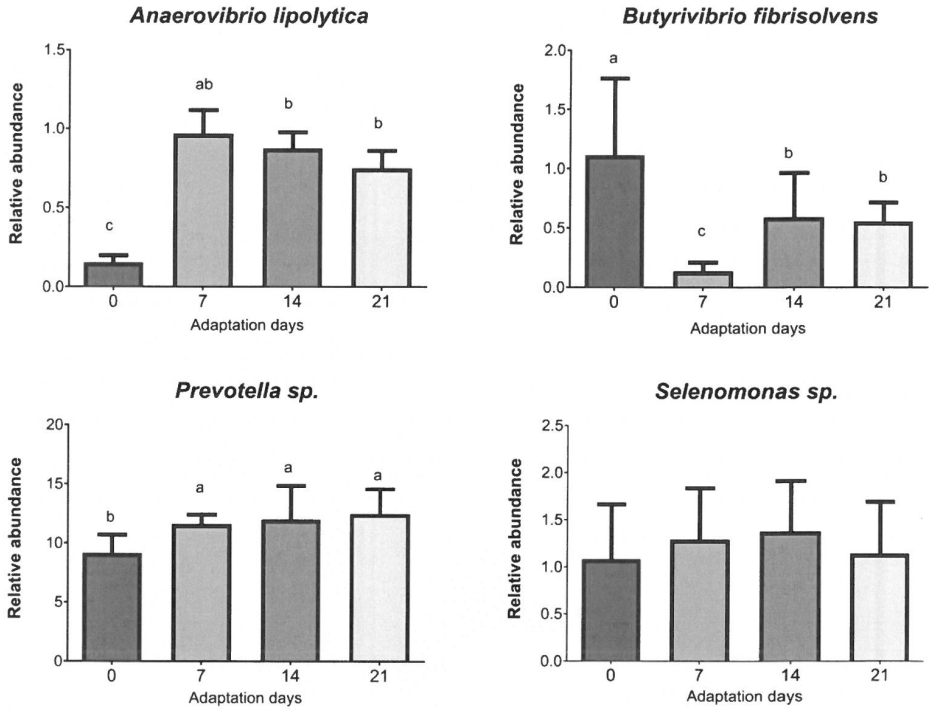

Fig. 3. Relative abundance of major lipolytic bacteria (*Anaerovibrio lipolytica* and *Butyrivibrio fibrisolvens*) and non-structural carbohydrate fermenting bacteria (*Prevotella spp. and Selenomonas spp*.) in the rumen feedlot Nellore steers during a high lipid diet transition. The different letters represent a significant difference (P <0.05), obtained by the Friedman test.

and 21 after lipid inclusion in the diet the proportion of *R. albus* and *F. succinogenes* increased compared to that on adaptation day 7.

A lower relative abundance of the *A. lipolytic* population was observed when steers were fed the control diet and it increased with lipid inclusion in the diet, with a higher relative abundance on days 7, 14, and 21 (P<0.05, Fig. 3). This enrichment may

be attributed to the presence of linoleic acid in the diet at a concentration of 27.55g/kg DM. *A. lipolytica* could hydrolyze tri- and di-glycerides, leading to an increase in its products, including propionate [Lourenço *et al*. 2010]. The increase in *A. lipolytica* population and the subsequent production of propionate can have several positive effects. Propionate is an important substrate for glucose synthesis in ruminants [Loncke *et al*. 2020]. It provides energy for the animal and can contribute to better growth and production. The enrichment of *A. lipolytica* and the increase in propionate production indicate improved energy utilization in cattle fed high-lipid diets. These findings are consistent with other studies conducted on beef cattle under feedlot conditions, where a 14-day adaptation to a high-lipid diet also resulted in increased ruminal *A. lipolytica* abundance and propionate production [Granja-Salcedo *et al*. 2017b]. A higher ruminal molar proportion of propionate was observed on day 7 after lipid inclusion compared to the molar proportion of propionate observed during the control diet and 14 and 21 days of adaptation to the lipid diet ($P=0.003$). In addition, the acetate: propionate ratio was higher in steers fed the control diet ($P<0.001$).

Lipid inclusion in the diet resulted in a lower relative abundance of *B. fibrisolvens* on day 7 compared to the other days of analysis ($P<0.05$), while the highest abundance was observed when steers were fed the control diet, indicating an alteration in response to the provision of soybean oil. This reduction in its population could be attributed to the inhibitory effects of UFA from soybean oil [Maia *et al*. 2007].

The bacterial population of *Prevotella spp*. was lower when steers were fed the control diet and increased with the inclusion of lipids in the diet, maintaining the same abundance on days 7, 14, and 21 ($P<0.05$, Fig. 3). Some studies have reported that the addition of soybean oil in the diet can increase the population of *Prevotella spp.*, but it can also affect fungi, protozoa, and methanogenic microorganisms, leading to a decrease in their relative abundance [Shang *et al*. 2020]. The increase in *Prevotella spp.* may be an opportunistic response to the addition of soybean oil, potentially hindering the growth of other bacteria [Ibrahim *et al*. 2021].

In contrast, *Selenomonas spp.* was not influenced by the inclusion of soybean oil in the diet, with similar ruminal abundance in all sampling days. The diet containing soybean oil in association with glycerine can increase in abundance on some proteolytic and amylolytic bacteria, including *Selenomonas spp.* [Granja-Salcedo *et al*. 2017b]. Additionally, Mirzaei *et al*. [2021] observed that *S. ruminantium* showed no significant effects in the case of oil supplement in small ruminants. Therefore, lipid inclusion in cattle diets can have variable effects due to such factors as lipid concentration, the type of bacterial population, and adaptation time and capacity.

The reduction in protozoan population observed on days 7, 14, and 21 compared to day 0 in response to the inclusion of a lipid diet indicates a negative relationship between lipid inclusion and protozoa abundance. This reduction in protozoa population can have several implications. One benefit of reducing the protozoa population in the rumen is a decrease in methanogenic activity. Protozoa are known to contribute to methane production in the rumen, and their reduction can lead to a decrease in energy

losses associated with enteric methane production [Patra and Yu 2013]. Methane is a potent greenhouse gas and mitigating its production in ruminants is an important aspect of reducing the environmental impact of livestock production. Furthermore, protozoa play a role in shaping the bacterial community in the rumen. They selectively prey on bacteria, including proteolytic bacteria. This selective predation can result in improved protein digestibility and microbial protein synthesis, leading to greater efficiency in nitrogen (N) utilization. By reducing protozoa population, the recycling of N in the rumen may be affected, potentially improving N utilization [Belanche *et al*. 2011]. However, it is important to consider the overall impact on rumen fermentation and microbial interactions, as the presence of protozoa also has beneficial effects on certain aspects of rumen function.

Diet changes can disrupt the microbiota and fermentation in the rumen, leading to alterations in intake behaviour, bacterial populations, and fermentation parameters. Therefore, a period of ruminal stabilization and adaptation is necessary. Previous studies have suggested an adaptation period of 14 days [Machado *et al*. 2016], while others have indicated that the response of diets on intake, ruminating, and resting behaviours can stabilize within 9-14 days [Barducci *et al*. 2019]. In the current study the results suggest that the first 7 days are crucial for ruminal adaptation to the diet change, as evident by the observed changes in rumen parameters and bacterial populations. It is important to note that the specific adaptation period may vary depending on various factors, including the composition and concentration of the lipid diet, the animal's individual response, and other environmental factors. Further research is needed to optimize the adaptation period and understand the underlying mechanisms of rumen adaptation to lipid inclusion diets in ruminants. Our findings suggest that the inclusion of a lipid diet has a significant impact on rumen fermentation parameters during the initial 7-day period of lipid inclusion in the diet. After 14 days of adaptation to the lipid diet there is a recovery in ruminal fermentation parameters, reflected in increased production of SCFA, microbial nitrogen, and the population of *R. albus, F. succinogenes, A. lipolytica*, and *Prevotella spp.* This indicates that the rumen undergoes an adaptation process during this period, leading to an improvement in ruminal environment and microbial populations.

Conflict of interest

No potential conflict of interest was reported by the authors.

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