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Fermentative profile and nutritional composition of tropical grass silages from Brazilian beef farms

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ABSTRACT - This survey was conducted to assess the fermentation profile and nutritional composition of tropical grass silages on beef farms. Grass silage samples were collected from 51 beef cattle operations located in four Brazilian states (MT, MS, SP, and PR). Additionally, a questionnaire about silage production and utilization was completed. The lack of correlation between the concentration of n-butyric acid and the IVDMD ($P = 0.368$) indicated that fermentation pattern and nutritional value were independent and therefore required separate grouping analyses. A cluster analysis based on the IVDMD data identified two groups (high and low IVDMD). Of the total, 43% and 57% of the samples were clustered into the high (508 to 709 g/kg DM) and low IVDMD (314 to 499 g/kg DM) groups, respectively. The IVDMD was negatively correlated with the indigestible neutral detergent fiber content ($r = -0.875$; $P<0.001$). A second grouping was performed based on an a priori n-butyric acid threshold: ≤ 3 g/kg DM (low) or > 3 g/kg DM (high). Most samples (86%) had n-butyric acid concentration > 3 g/kg DM, and the database revealed that silages with DM > 370 g/kg had a lower risk of clostridial fermentation (risk difference = −0.956). Silage samples with a low concentration of n-butyric acid had less particles retained on the 19-mm sieve. Collectively, the results from this survey indicate that most tropical grass silage produced in beef operations had low digestibility and poor fermentation quality. These findings suggest the need to develop, transfer, and apply techniques that contribute to improving tropical grass silage conservation and quality, particularly when greater forage inclusion in the diet can enhance production efficiency (e.g., for growing cattle and breeding cows).

Keywords: *Clostridium*, digestibility, *Megathyrsus*, *Urochloa*, survey

1. Introduction

The lower agronomic risk associated with tropical grasses compared with annual forage crops, particularly their lower vulnerability to pest and disease pressures and climatic adversities (e.g., drought, irregular rainfall, and heat stress), and the inclusion of tropical grasses in integrated crop-livestock systems for no-tillage crop rotation have led to renewed interest in tropical grass silages as a complementary forage to annual crops (e.g., corn silage). This trend has been further supported by the increased adoption of self-propelled forage harvesters, which enable more precise forage chopping and improved harvest efficiency. Recently, Monsalve and Millen (2025) reported an increase in the use of tropical grass silage utilization in Brazilian feedlots from 8.3% in 2019 to 24% in 2024.

In finishing diets, tropical grass silage is a source of physically effective fiber, with low inclusion rates (<20% DM). However, in rations for growing cattle, tropical grass silage enables nutritionists to formulate diets that balance energy intake, preventing cattle from gaining weight too quickly, which may negatively impact carcass weight as animals approach their mature size (Owens et al., 1995; Blom et al., 2022). Also, beef cows may be supplemented with tropical grass silage during periods of pasture shortage (Nascimento et al., 2025). However, tropical grasses are considered difficult to ensile (Nussio, 2005). A low dry matter (DM) content (often <280 g/kg) in combination with a usually low ratio (<1) of soluble carbohydrate concentration (SC) to buffering capacity (BC) creates a high risk of butyric acid-producing clostridia development, leading to high nutrient losses during storage (Carvalho et al., 2024).

Field observations suggest that directly ensiled tropical grass silages are often associated with high storage losses and low feeding value. However, there are no reports on the fermentation profile and nutritional composition of tropical grass silages produced on commercial farms. A survey characterizing tropical grass silage production and utilization may reveal opportunities for improving production efficiency and guiding further research. We therefore hypothesized that (1) appropriate management practices are associated with improved nutritional composition and fermentation quality, (2) silage digestibility is primarily determined by fiber concentration and digestibility, and (3) fermentation pattern is mainly influenced by DM content. The objective of this study was to characterize the fermentation profile and nutritional composition of tropical grass silages and to identify their relationships with production practices on beef cattle farms.

2. Material and methods

2.1. Data collection and survey design

Beef operations in four Brazilian states [Mato Grosso (35), Mato Grosso do Sul (5), São Paulo (2), and Paraná (9)] were visited from September to November 2023. The farms were identified through consultant networks (i.e., snowball sampling). The greater representation of farms from MT, currently the Brazilian state with the largest cattle herd, might reflect its strong tradition of tropical grass silage production and utilization. One silo was sampled at each farm (n = 51), as sampling was restricted to silos that were being used for feeding at the time of the visit.

For bunkers and piles, silage samples were collected at 12 points distributed across four columns and three rows on the silo panel, avoiding the first 50 cm from the top, base, and sides. For bags, 12 subsamples were collected across the exposed silage surface. For bale silages, sampling was performed after the bales were unpacked. When present, visibly spoiled areas were discarded, and 12 subsamples were collected from different locations within the remaining silage mass. The 12 subsamples from each silo were homogenized to form one composite sample of approximately 1 kg. The samples were then identified and frozen for at least 24 h before being transported to the laboratory in a cooler with ice. In the laboratory, the samples were thawed and analyzed for chemical composition, *in vitro* digestibility, fermentation end products, and particle size distribution.

During farm visits, a questionnaire was applied to document practices associated with the production and utilization of tropical grass silage. Confidentiality was assured to all participants. The questionnaire included items addressing grass production, conservation, and silage feeding practices, encompassing information on the following: technical consultancy for silage production, grass genus, fertilization, sward management (semi-perennial, crop-livestock integration, or pasture surplus), sward age, number of cuts/yr, grass maturity at harvest, type of forage harvester, grass management at ensiling (direct ensiling or wilting), use of silage additives, type of silo, animal category fed with grass silage, and proportion of grass silage in the diet (Supplementary material S1).

2.2. Laboratory analyses

Immediately after thawing, an aqueous extract was obtained by mixing 25 g of silage with 225 mL of distilled water in a blender for 1 min. The extract was filtered through a funnel with two layers of gauze, and the pH was measured with a digital pH meter (Tec5, Tecnal®, Piracicaba, Brazil). A portion of the aqueous extract was centrifuged at 12,000 × *g* for 20 min, and the supernatant was used to analyze the fermentation products. The concentrations of lactic acid (Pryce, 1969) and ammonia (Chaney and Marbach, 1962) were determined by colorimetric methods. Other fermentation products, including volatile fatty acids (VFA), alcohols, esters, and acetone, were analyzed by gas chromatography (Nexis GC-2030 with AOC-20i Plus autoinjector, Shimadzu, Kyoto, Japan) using a capillary column (Stabilwax 60 m, 0.25 mm Ø, 0.25 µm; Restek, Bellefonte, PA). Compounds were identified based on retention times and quantified using external standards. The sum of n-butyric, i-butyric, i-valeric, and n-valeric acids (sumBVA), the sum of methanol, ethanol, isopropanol, 2-butanodiol, 1-propanodiol, acetic acid, propionic acid, 2,3-butanediol, i-butyric acid, 1,2-propanediol, n-butyric, i-valeric, and n-valeric acids (sumVFAal), and the sum of total non-nitrogenous fermentation products (sumFP) were calculated.

Another silage subsample was dried for 72 h in a forced ventilation oven at 60 °C. The DM concentration of the silage was corrected for the loss of volatiles during oven drying (Weissbach and Strubelt, 2008). Subsequently, dried samples were ground in a Willey mill (Marconi MA340, Piracicaba, Brazil) through a 1-mm sieve to determine the DM at 105 °C (method 930.15; AOAC, 1990), ash (method 942.05; AOAC, 1990) and crude protein (CP; method 984.13; AOAC, 1990). Organic matter (OM) was calculated as 1000 – ash. Neutral detergent fiber (NDF) was determined using filter bags (F57, Ankom Technology, Macedon, USA) with a neutral detergent solution containing thermostable amylase and sodium sulfite (Mertens, 2002; method 2002.04). Acid detergent fiber (ADF) and acid detergent lignin were analyzed sequentially according to Van Soest (1973; method 973.18). Neutral detergent soluble (NDS) was calculated as OM – NDF. *In vitro* digestibility of DM (IVDMD), NDF (IVNDFD), and potentially digestible NDF (IVpdNDFD) were determined using a Daisy II incubator (Ankom Technology, Macedon, USA) for 48 h with buffer solutions prepared as described by Holden (1999). Ruminal fluid was obtained from two cannulated Holstein cows grazing stargrass, 1 h after supplementation with 2 kg/day of concentrate based on ground corn grain, soybean meal, and a mineral-vitamin mixture. A dried sample ground in a Willey mill (Marconi MA340, Piracicaba, Brazil) through a 2-mm sieve was used to determine the concentration of indigestible NDF (iNDF) concentration by *in situ* incubation for 288 h in the same rumen-cannulated cows (Valente et al., 2011). Potentially digestible NDF (pdNDF) was computed as NDF – iNDF. Animal care and handling procedures were approved by the Ethics Committee for Animal Use of the Universidade Estadual de Maringá (protocol number 8208090218).

Silage particle size distribution was determined using the Penn State Particle Separator (PSPS; 3-sieve model with a 4-mm sieve), adapted by adding a 40-mm sieve to the top of the assembly (total of four sieves and a bottom pan). This additional sieve was built in 20-mm thick plastic and contained thirty-three 40-mm holes, with the perforated area representing 32.1% of the sieve surface. The rationale for adapting the PSPS by including the top sieve for particle size analysis of tropical grass silages was described by Mari (2003).

2.3. Statistical analysis of data

Descriptive statistics (mean, median, minimum, maximum, standard deviation, kurtosis, and asymmetry) were used to characterize the database. The independence of the fermentation profile and nutritional value of the silages was confirmed by a Pearson correlation test between n-butyric acid concentration and IVDMD using the PROC CORR of SAS (Figure 1).

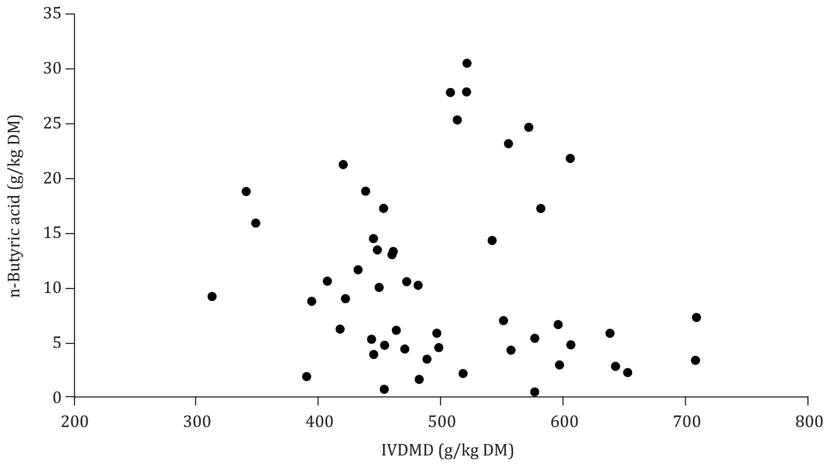
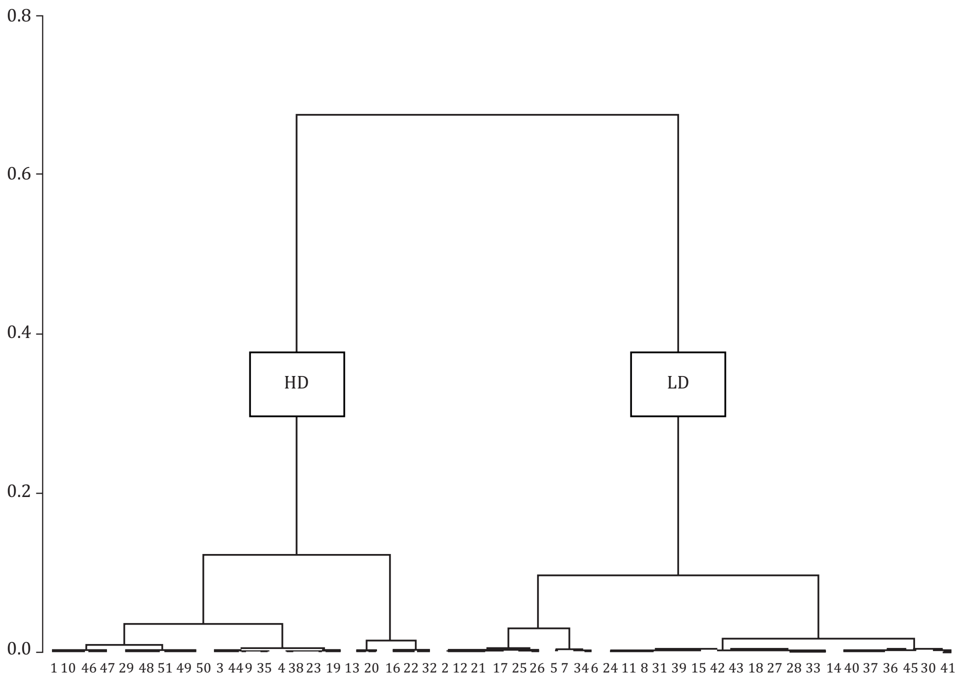


Figure 1 - Relationship between n-butyric acid content and *in vitro* dry matter digestibility in tropical grass silage samples (P = 0.368 for Pearson correlation).

A first grouping analysis based on the IVDMD was performed with PROC CLUSTER of SAS using Ward's minimum-variance method. Visual inspection of the dendrogram, considering the relative increases in branch heights between successive fusion levels, indicated that the silages were separated into two distinct groups: high IVDMD (HD) and low IVDMD (LD) (Figure 2). The separation between groups was evaluated with PROC GLM of SAS using Wilks' Lambda test ($\alpha = 0.01$). Subsequently, the group means were compared. Continuously distributed variables were compared using the PROC MIXED of SAS with a model that included the fixed effect of group, and the Kenward-Roger method used to calculate the degrees of freedom of the denominator for the F test. Discrete distribution variables were compared using Fisher's exact test, and the group means were compared using the Chi-square test (χ^2) adjusted for Bonferroni, using the PROC FREQ of SAS.



HD - high IVDMD; LD - low IVDMD.

Figure 2 - Dendrogram with grouping of tropical grass silages based on *in vitro* dry matter digestibility (IVDMD).

A second grouping was defined *a priori* based on n-butyric acid concentration (Kaiser et al., 2002), with concentrations ≤3 g/kg DM classified as low (LB) and those >3 g/kg DM classified as high (HB). Continuously distributed variables were compared using the PROC MIXED of SAS, whereas discrete variables were compared using the PROC FREQ of SAS, as described above.

Regression analyses were performed using the PROC REG of SAS to explore relationships between characteristics associated with IVDMD or n-butyric acid production. When a quadratic trend was observed, the response was modeled with segmented regression with PROC NLMIXED in SAS. Root mean square error (RMSE) and adjusted R^2 were reported as indicators of goodness of fit. As all samples with DM greater than 370 g/kg had an n-butyric acid concentration ≤ 3 g/kg DM, a risk difference (RD) analysis was performed using the PROC FREQ of SAS.

3. Results

3.1. Description of variables with continuous distribution

Among farms that used fertilizers, the average application rates were 156 kg/ha of N, 38.1 kg/ha of P_2O_5, and 64.6 kg/ha of K_2O. (Table 1) Sward age averaged 2.83 yr with 2.16 cuts/yr (Table 1). Grass maturity at harvest averaged 98.9 d, with a range of 50 to 365 d. The reported DM productivity per harvest averaged 7.06 t DM/ha (Table 1).

The reported theoretical length of cut was on average 13.1 mm. Farmers using moisture absorbent applied on average 151 g/kg as fed of chopped forage mass. Average dietary inclusion of grass silage was 553 g/kg DM for breeding cows, 530 g/kg DM for growing cattle, and 162 g/kg DM in finishing rations (Table 1).

Analysis of silage composition indicated the following ranges: DM from 166 to 466 g/kg as-fed, CP from 29.4 to 141 g/kg DM, NDF from 563 to 851 g/kg DM, ADF from 295 to 561 g/kg DM, lignin from 38.2 to 119 g/kg DM, and iNDF from 150 to 460 g/kg DM (Table 1). The iNDF:lignin ratio averaged 4.96. The IVDMD averaged 501 g/kg DM, whereas the IVNDFD and IVpdNDFD averaged 435 g/kg NDF and 768 g/kg pdNDF, respectively (Table 1).

Table 1 - Description of the database with variables with continuous distribution

Item	Mean	Med	Min	Max	SD	Kurtosis	Skewness
Applied fertilizer[1]							
N rate (kg/ha)	156	109	30.0	280	104	−1.81	0.254
P_2O_5 rate (kg/ha)	38.1	30.0	10.0	104	25.1	3.13	1.81
K_2O rate (kg/ha)	64.6	63.2	30.0	126	24.7	2.11	1.21
Plot management							
Sward age (yr)	2.83	2.00	1.00	5.00	1.65	−1.58	0.331
Harvest frequency (cuts/yr)	2.16	2.00	1.00	4.00	0.928	−0.014	0.761
Grass maturity at harvest (d)	98.9	90.0	50.0	365	46.9	24.7	4.40
Reported yield							
Fresh matter yield (t as fed/ha/cut)	26.4	27.0	4.00	85.1	12.9	8.32	1.83
Dry matter yield (t DM/ha/cut)	7.06	6.47	0.831	24.0	3.59	9.84	2.12
Reported ensiling practices							
Theoretical length of cut (mm)	13.1	12.0	10.0	18.0	2.40	−0.712	0.730
Dose of moisture absorbent (g/kg as fed)	151	100	39.0	300	112	−1.66	0.676
Inclusion of grass silage in diets							
Breeding cows (g/kg DM)	553	500	460	700	129	1.52	1.55
Growing cattle (g/kg DM)	530	500	250	750	190	−1.65	−0.041
Finishing cattle (g/kg DM)	162	140	80.0	460	69.1	10.0	2.83

Continues...

Table 1 (Continued)

Item	Mean	Med	Min	Max	SD	Kurtosis	Skewness
Chemical composition of grass silages							
DM[2] (g/kg as fed)	287	276	168	466	78.1	0.169	0.725
Ash (g/kg DM)	82.8	83.1	54.3	125	17.8	−0.759	0.330
Crude protein (g/kg DM)	64.3	59.5	29.4	141	23.9	1.44	1.18
Neutral detergent fiber (g/kg DM)	727	733	563	851	54.8	1.61	−0.756
Acid detergent fiber (g/kg DM)	449	452	295	561	53.7	0.210	−0.348
Lignin(sa) (g/kg DM)	68.6	68.5	38.2	119	22.3	−0.173	0.683
iNDF (g/kg DM)	318	324	150	460	73.4	−0.474	−0.319
pdNDF (g/kg DM)	409	420	216	596	66.2	1.09	−0.284
ADF (g/kg NDF)	616	621	516	709	38.4	0.450	−0.233
iNDF (g/kg NDF)	436	425	228	663	92.0	0.066	−0.003
iNDF:Lignin ratio	4.96	4.75	2.59	9.25	1.56	0.600	0.929
Neutral detergent solubles (g/kg DM)	190	182	92.5	373	55.8	1.64	0.97
In vitro digestibility of DM 48 h (g/kg DM)	501	483	314	709	89.0	−0.107	0.357
In vitro digestibility of NDF 48 h (g/kg NDF)	435	417	214	672	111	−0.297	0.212
In vitro digestibility of pdNDF 48 h (g/kg pdNDF)	768	792	462	1083	126	0.568	−0.105
Fermentation profile of grass silages							
pH	4.93	4.87	4.17	8.05	0.625	12.1	2.85
NH_3-N (g/kg N)	299	260	23.5	762	211	−0.745	0.651
Lactic acid (g/kg DM)	9.9	4.15	<0.001	46.1	12.2	1.34	1.47
Acetic acid (g/kg DM)	20.1	19.4	2.24	55.1	9.28	2.90	0.95
n-Butyric acid (g/kg DM)	10.7	8.83	0.57	30.5	8.03	−0.229	0.870
Ethanol (g/kg DM)	3.39	1.50	0.02	26.6	5.61	8.81	3.01
Propionic acid (g/kg DM)	2.27	1.93	<0.001	6.27	1.59	−0.425	0.671
i-Valeric acid (g/kg DM)	1.39	0.97	0.10	4.36	1.09	−0.396	0.799
n-Valeric acid (g/kg DM)	1.25	1.06	0.11	3.53	0.91	−0.390	0.635
i-Butyric acid (g/kg DM)	1.04	0.85	0.09	2.83	0.72	0.010	0.868
2,3-Butanediol (g/kg DM)	0.52	0.11	<0.001	5.53	1.13	9.44	3.06
1-Propanol (mg/kg DM)	594	226	<1	3753	838	3.88	2.00
1,2-Propanediol (mg/kg DM)	550	89.1	<1	6995	1228	15.9	3.72
Methanol (mg/kg DM)	208	106	6.02	1665	345	8.50	3.00
Isopropanol (mg/kg DM)	84.3	31.6	<1	702	154	9.52	3.12
2-Butanol (mg/kg DM)	45.5	31.0	<1	181	45.3	1.36	1.40
Acetone (mg/kg DM)	26.8	<1	<1	301	64.6	10.9	3.35
Propyl acetate (mg/kg DM)	2.09	<1	<1	37.9	6.56	18.7	4.08
Ethyl lactate (mg/kg DM)	1.52	<1	<1	22.7	5.38	10.5	3.42
Ethyl acetate (mg/kg DM)	1.49	<1	<1	24.5	4.72	13.6	3.63
SumBVA[3] (g/kg DM)	14.4	11.7	0.94	36.7	10.2	−0.831	0.629
SumVFAal[4] (g/kg DM)	42.1	37.6	5.43	102	20.7	0.123	0.635
SumFP[5] (g/kg DM)	52.0	50.6	10.1	124	22.4	1.32	0.762
Particle distribution							
40 mm (g/kg as fed)	90.6	63.1	3.26	290	73.6	2.26	1.74
19-40 mm (g/kg as fed)	369	334	92.4	668	177	−1.09	0.191
8-19 mm (g/kg as fed)	443	475	28.3	699	182	0.029	−0.751
4-8 mm (g/kg as fed)	62.5	58.8	8.50	161	40.5	−0.250	0.657
Pan (g/kg as fed)	34.7	16.0	0.000	120	35.4	−0.311	1.05
> 8 mm (g/kg as fed)	903	927	723	986	71.3	−0.232	−0.851
> 4 mm (g/kg as fed)	965	984	880	1000	35.4	−0.311	−1.05

Med - median; Min - minimum; Max - maximum; SD - standard deviation; iNDF - indigestible neutral detergent fiber; pdNDF - potentially digestible neutral detergent fiber.
[1] Application rate among farms that reported fertilizer use.
[2] DM - dry matter corrected for volatile compounds.
[3] SumBVA - sum of i-butyric, n-butyric, i-valeric and n-valeric acids.
[4] SumVFAal - sum of volatile fatty acids and alcohols (methanol, isopropanol, ethanol, 2-butanol, 1-propanol, acetic acid, propionic acid, 2,3-butanediol, 1,2-propanediol, i-butyric acid, n-butyric acid, i-valeric acid, n-valeric acid).
[5] SumFP - sum of all non-nitrogenous fermentation products.

The fermentation profile of the silages revealed the following average values: pH 4.93, NH_3-N 299 g/kg N, lactic acid 9.90 g/kg DM, and acetic acid 20.1 g/kg DM (Table 1). The n-butyric acid concentration ranged from 0.57 to 30.5 g/kg, with an average of 10.7 g/kg DM. The average concentration of propionic acid was 2.27 g/kg DM, i-valeric acid was 1.39 g/kg DM, n-valeric acid was 1.25 g/kg DM, and i-butyric acid was 1.04 g/kg DM. The average concentration of ethanol was 3.39 g/kg DM, and 2,3-butanediol was 0.52 g/kg DM. Other fermentation products were found at average concentrations below 600 mg/kg DM (Table 1).

Silage particle size distribution (as-fed basis) ranged from 3.26 to 290 g/kg on the 40-mm sieve, 92.4 to 668 g/kg on the 19-mm sieve, 28.3 to 699 g/kg on the 8-mm sieve, 8.50 to 161 g/kg on the 4-mm sieve, and 0 to 120 g/kg in the pan.

3.2. Description of variables with discrete distribution

Most farmers hired technical consultancy (67%) for grass silage production. Two-thirds (67%) of the silage was produced from grasses that had received fertilization (Table 2). *Megathyrsus* genus predominated (71%), followed by *Urochloa* (25%), with a small proportion of silage produced from a mixture of genera *Megathyrsus* + *Urochloa* (4%; Table 2). The grass used for silage production mostly came from specific semi-perennial areas (71%), followed by crop-livestock integration systems (24%) and a small portion of silage produced from pasture surplus (6%; Table 2).

Most producers (63%) used outsourced services to harvest the grass with self-propelled harvesters (76%). Only one farm produced wilted silage. The use of microbial inoculants was reported in 82% of the farms surveyed, with 81% of the inoculants containing combinations of homo- and heterolactic bacteria. Moisture absorbents were applied at ensiling by 27% of the farmers. Among these farmers, 46% used soybean hulls, 38% used soybean residue, and 15% used dried distillers grains as moisture

Table 2 - Description of the database with variables with discrete distribution

Item	Description and frequency
Sample origin	
Brazilian state	MT (69%), MS (10%), SP (4%) and PR (18%)
Grass silage production	
Consultancy	Yes (67%) and No (33%)
Grass genera	*Megathyrsus* (71%), *Urochloa* (25%) and Mixed (4%)
Fertilization	Yes (67%) and No (33%)
Grass production area	Semi-perennial (71%), Crop-livestock integration (24%) and Pasture surplus (6%)
Harvester	Own (37%) and Outsourced (63%)
Harvester type	Pull-type (24%) and Self-propelled (76%)
Forage wilting	Yes (2%) and No (98%)
Silage additives	
Microbial inoculant	Yes (82%) and No (18%)
Inoculant type	Homolactic (19%) and Combo[1] (81%)
Moisture absorbent use	Yes (27%) and No (73%)
Moisture absorbent type	SR (38%), SH (46%) and DDG (15%)
Silo type	Bunker (49%), Drive-over (45%), Bag (2%) and Bale (4%)
Grass silage usage	
Breeding cows	Yes (12%) and No (88%)
Growing cattle	Yes (52%) and No (48%)
Finishing cattle	Yes (86%) and No (14%)
Fermentation course	
Butyric fermentation[2]	Negative (14%) and Positive (86%)

SR - soybean residue; SH - soybean hulls; DDG - dried distillers grains.
[1] Combination of homo and heterolactic bacteria.
[2] Silages with > 3 g/kg DM of n-butyric acid.

absorbent. Furthermore, 49% of the farmers reported storing silage in bunker silos, 45% in drive-over piles, 4% in bales, and 2% in bags. A small proportion of farmers fed tropical grass silage for breeding cows (12%), while 52% fed it to growing cattle and the majority fed tropical grass silage for finishing cattle (86%).

3.3. Comparison between silage groups (HD and LD) for IVDMD

In this survey, 43% (n = 22) and 57% (n = 29) of the samples were clustered into the high and low IVDMD groups, respectively. The mean values of IVDMD were 584 and 438 g/kg DM for the HD (508 to 709 g/kg DM) and LD (314 to 499 g/kg DM) groups, respectively. The nitrogen dose was higher in HD than in LD (224 and 106 kg/ha; P = 0.010; Table 3). A greater harvest frequency was observed in HD than in LD (3.00 and 1.85 cuts/yr; P<0.001). Grass maturity at harvest was higher in LD than in HD (112 and 83.8 d; P = 0.050). Forage yield at the current cut was similar between HD and LD (23.2 and 28.8 t fresh matter/ha, P = 0.146; 6.15 and 7.73 t DM/ha, P = 0.138, respectively; Table 3).

The HD presented higher CP content (74.0 g/kg CP) than LD group (56.8 g/kg; P<0.009; Table 3). The LD presented higher contents of NDF, ADF, lignin, and iNDF (751, 472, 77.8, and 365 g/kg DM) than HD (696, 419, 56.5, and 256 g/kg DM), respectively (Table 3). A higher content of pdNDF was observed in HD (439 g/kg DM) than in LD (385 g/kg DM; P = 0.003). The concentration of NDS was higher in HD than in LD (219 and 169 g/kg DM). The HD showed greater (P<0.001) IVDMD (584 and 438 g/kg DM), IVNDFD (532 and 362 g/kg NDF), and IVpdNDFD (846 and 709 g/kg pdNDF) than LD, respectively (Table 3).

The acetic acid concentration was higher (P = 0.006) in HD (24.1 g/kg DM) than in LD (17.0 g/kg DM; Table 3). A higher concentration of ethyl lactate was observed in HD (35.3 g/kg DM) than in LD (<1 g/kg DM; P = 0.019). The sumVFAal was higher (P = 0.017) in HD (49.9 g/kg DM) compared to LD (36.2 g/kg DM), as well as the sumFP (63.3 and 43.5 g/kg DM, respectively; Table 3).

Table 3 - Comparison between silage groups with high (HD, 508 – 709 g/kg DM) and low *in vitro* dry matter digestibility (LD, 314 – 499 g/kg DM), variables with continuous distribution

Item	Group		SEM	P-value
	HD	LD		
Applied fertilizer				
N rate (kg/ha)	224	106	28.7	0.010
P_2O_5 rate (kg/ha)	26.3	40.4	9.41	0.306
K_2O rate (kg/ha)	59.1	69.5	8.57	0.404
Plot management				
Sward age (yr)	2.30	3.04	0.424	0.237
Harvest frequency (cuts/yr)	3.00	1.85	0.199	<0.001
Grass maturity at harvest (d)	83.8	112	9.69	0.050
Reported yield				
Fresh matter yield (t as fed/ha/cut)	23.2	28.8	2.65	0.146
Dry matter yield (t DM/ha/cut)	6.15	7.73	0.738	0.138
Reported ensiling practices				
Theoretical length of cut (mm)	12.5	13.6	0.61	0.252
Dose of moisture absorbent (g/kg as fed)	126	169	42.7	0.540
Inclusion of grass silage in diets				
Breeding cows (g/kg DM)	460	600	121	0.567
Growing cattle (g/kg DM)	585	479	55.1	0.187
Finishing cattle (g/kg DM)	169	155	16.7	0.569

Continues...

Table 3 (Continued)

Item	Group		SEM	P-value
	HD	LD		
Chemical composition of grass silages				
DM[1] (g/kg as fed)	285	288	15.7	0.915
Ash (g/kg DM)	85.8	80.5	0.36	0.294
Crude protein (g/kg DM)	74.0	56.8	4.49	0.009
Neutral detergent fiber (g/kg DM)	696	751	9.5	<0.001
Acid detergent fiber (g/kg DM)	419	472	9.4	<0.001
Lignin(sa) (g/kg DM)	56.5	77.8	3.65	<0.001
iNDF (g/kg DM)	256	365	9.9	<0.001
pdNDF (g/kg DM)	439	385	12.2	0.003
ADF (g/kg NDF)	601	627	7.35	0.015
iNDF (g/kg NDF)	368	488	14.0	<0.001
iNDF:Lignin(sa) ratio	4.87	5.02	0.314	0.731
Neutral detergent solubles (g/kg DM)	219	169	10.1	0.001
In vitro digestibility of DM 48 h (g/kg DM)	584	438	10.2	<0.001
In vitro digestibility of NDF 48 h (g/kg NDF)	532	362	14.3	<0.001
In vitro digestibility of pdNDF 48 h (g/kg pdNDF)	846	709	21.3	<0.001
Fermentation profile of grass silages				
pH	4.75	5.07	0.122	0.071
NH_3-N (g/kg N)	285	310	4.2	0.678
Lactic acid (g/kg DM)	13.3	7.3	2.39	0.083
Acetic acid (g/kg DM)	24.1	17.0	1.73	0.006
n-Butyric acid (g/kg DM)	12.2	9.55	1.60	0.240
Ethanol (g/kg DM)	4.61	2.46	1.109	0.176
Propionic acid (g/kg DM)	2.65	1.97	0.314	0.135
i-Valeric acid (g/kg DM)	1.54	1.27	0.219	0.395
n-Valeric acid (g/kg DM)	1.06	1.40	0.180	0.196
i-Butyric acid (g/kg DM)	0.963	1.09	0.145	0.528
2,3-Butanediol (g/kg DM)	0.711	0.371	0.541	0.292
1-Propanol (mg/kg DM)	709	507	167.7	0.401
1,2-Propanediol (mg/kg DM)	894	289	239.8	0.082
Methanol (mg/kg DM)	262	167	68.9	0.337
Isopropanol (mg/kg DM)	130	49.9	29.99	0.066
2-Butanol (mg/kg DM)	56.8	36.9	8.89	0.121
Acetone (mg/kg DM)	30.5	24.1	12.99	0.731
Propyl acetate (mg/kg DM)	1.52	2.53	1.318	0.593
Ethyl lactate (mg/kg DM)	3.53	<1	1.0234	0.019
Ethyl acetate (mg/kg DM)	2.08	1.03	0.943	0.437
Sum BVA[2] (g/kg DM)	15.8	13.3	2.05	0.395
Sum VFAal[3] (g/kg DM)	49.9	36.2	3.93	0.017
Sum FP[4] (g/kg DM)	63.3	43.5	4.04	0.001
Particle distribution (g/kg as fed)				
40 mm	111	75.3	14.39	0.088
19-40 mm	384	357	35.5	0.602
8-19 mm	403	474	36.1	0.174
4-8 mm	67.1	59.0	8.12	0.486
Pan	35.0	34.5	7.14	0.966
> 8 mm	898	906	14.3	0.677
> 4 mm	965	965	7.1	0.966

iNDF - indigestible neutral detergent fiber; pdNDF - potentially digestible neutral detergent fiber; SEM - standard error of the mean.
[1] DM - dry matter corrected for volatile compounds.
[2] Sum BVA - sum of i-butyric, n-butyric, i-valeric and n-valeric acids.
[3] SumVFAal - sum of volatile fatty acids and alcohols (methanol, isopropanol, ethanol, 2-butanol, 1-propanol, acetic acid, propionic acid, 2,3-butanediol, 1,2-propanediol, i-butyric acid, n-butyric acid, i-valeric acid, n-valeric acid).
[4] Sum FP - sum of all non-nitrogenous fermentation products.

The genus *Megathyrsus* occurred in 86% of the samples in LD against 50% in HD, whereas the genus *Urochloa* occurred in 46% of the samples in HD against 10% in LD (P = 0.006; Table 4). Higher frequency of fertilizer application (P = 0.007) was observed in LD (83%) than in HD (46%). The use of a specific area for grass production occurred in 90% of farms in LD, but 45% in HD (P<0.001). Grass production in crop-livestock integration systems occurred in 50% of farms in HD and 3% in LD (Table 4). A small proportion of farms produced silage from pasture surplus (7% in LD and 5% in HD).

Table 4 - Comparison between silage groups with high (HD, 508 – 709 g/kg DM) and low *in vitro* dry matter digestibility (LD, 314 – 499 g/kg DM), variables with discrete distribution

Item	Class	Group		Fisher test[1]
		HD	LD	
Consultancy	Yes	59%	72%	0.377
	No	41%	28%	
Grass genera	*Megathyrsus*	50%b	86%a	0.006
	Urochloa	46%a	10%b	
	Mixed	4%a	4%a	
Fertilization	Yes	46%b	83%a	0.007
	No	54%a	17%b	
Grass production area	Semi-perennial	45%b	90%a	<0.001
	Crop-livestock integration	50%a	3%b	
	Pasture surplus	5%a	7%a	
Harvester	Own	36%	38%	0.999
	Outsourced	64%	62%	
Harvester type	Pull-type	32%	17%	0.320
	Self-propelled	68%	83%	
Inoculant	Yes	73%	90%	0.150
	No	27%	10%	
Inoculant type[2]	Bal Homo	19%	19%	0.999
	Combo	81%	81%	
Moisture absorbent	Yes	27%	28%	0.999
	No	73%	72%	
Moisture absorbent type	SR	20%	50%	0.246
	SH	80%	25%	
	DDG	0%	25%	
Silo type	Bunker	50%	48%	0.999
	Pile	45%	45%	
	Bag	0%	3%	
	Bale	5%	3%	
Breeding cows feeding	Yes	6%	16%	0.632
	No	94%	84%	
Growing cattle feeding	Yes	50%	54%	0.999
	No	50%	46%	
Finishing cattle feeding	Yes	100%	77%	0.067
	No	0%	23%	

SR - soybean residue; SH - soybean hulls; DDG - dried distillers grains.
[1] Fisher's exact test.
[2] Combination of homo- and hetero-lactic bacteria.

3.4. Comparison between silage groups (LB and HB) for n-butyric acid concentration

Upon grouping silages by butyric fermentation, 86% (n = 44) of the samples were classified as HB (>3 g n-butyric acid/kg DM), and only 14% (n = 7) were classified as having low n-butyric acid concentration (LB, ≤ 3 g/kg DM). The LB group showed greater values for grass maturity at harvest (P<0.001), DM (P<0.001), NDS (P = 0.023), particles retained on the 4-mm sieve (P<0.001), and particles retained on the pan of the PSPS (P<0.001), and lower values of harvest frequency (P = 0.036), NDF (P = 0.035), NH_3-N (P<0.001), acetic acid (P = 0.012), n-butyric acid (P<0.001), propionic acid (P = 0.020), i-valeric acid (P<0.001), n-valeric acid (P<0.001), i-butyric acid (P<0.001), 2-butanol (P = 0.024), sumBVA (P<0.001), sumVFAal (P<0.001), sumFP (P = 0.023), particles retained on the 19-mm sieve (P = 0.013), particles >8 mm (P< 0.001), and particles >4 mm (P<0.001) compared with the group HB. Other variables did not show differences between groups (Table 5).

Table 5 - Comparison between silage groups with low (LB, ≤ 3 g/kg DM) and high n-butyric concentration (HB, > 3 g/kg DM), variables with continuous distribution

Item	Group		SEM	P-value
	LB	HB		
Applied fertilizer				
N rate (kg/ha)	30.0	163	63.14	0.224
P_2O_5 rate (kg/ha)	30.0	33.5	17.39	0.903
K_2O rate (kg/ha)	30.0	66.7	14.90	0.156
Plot management				
Sward age (yr)	1.33	2.97	0.607	0.102
Harvest frequency (cuts/yr)	1.25	2.27	0.298	0.036
Grass maturity at harvest (d)	152	90.5	12.07	<0.001
Reported yield				
Fresh matter yield (t as fed/ha/cut)	18.5	27.8	3.38	0.078
Dry matter yield (t DM/ha/cut)	7.10	7.05	0.98	0.971
Reported ensiling practices				
Theoretical length of cut (mm)	12.8	13.2	0.79	0.745
Dose of moisture absorbent (g/kg as fed)	59.0	159	74.1	0.419
Inclusion of grass silage in diets (g/kg DM)				
Breeding cows	700	480	24.1	0.099
Growing cattle	440	539	89.1	0.497
Finishing cattle	186	157	20.6	0.355
Chemical composition of grass silages				
DM[1] (g/kg as fed)	410	267	16.1	<0.001
Ash (g/kg DM)	79.1	83.4	4.80	0.557
Crude protein (g/kg DM)	71.9	63.0	36.04	0.365
Neutral detergent fiber (g/kg DM)	687	733	14.0	0.035
Acid detergent fiber (g/kg DM)	420	454	14.0	0.118
Lignin(sa) (g/kg DM)	74.9	67.6	5.90	0.425
iNDF (g/kg DM)	274	325	19.0	0.084
pdNDF (g/kg DM)	413	408	17.7	0.854
ADF (g/kg NDF)	610	617	10.2	0.644
iNDF (g/kg NDF)	399	442	24.2	0.254
iNDF:Lignin(sa) ratio	3.94	5.12	0.40	0.061
Neutral detergent solubles (g/kg DM)	234	183	14.1	0.023
In vitro DM digestibility 48 h (g/kg DM)	531	496	23.5	0.341
In vitro NDF digestibility 48 h (g/kg NDF)	442	434	29.7	0.863
In vitro pdNDF digestibility 48 h (g/kg pdNDF)	731	774	33.4	0.417

Continues...

Table 5 (Continued)

Item	Group		SEM	P-value
	LB	HB		
Fermentation profile of grass silages				
pH	5.06	4.91	0.166	0.565
NH_3-N (g/kg N)	89.4	332	51.7	<0.001
Lactic acid (g/kg DM)	15.3	9.03	3.22	0.212
Acetic acid (g/kg DM)	12.1	21.3	2.32	0.012
n-Butyric acid (g/kg DM)	1.79	12.1	1.92	<0.001
Ethanol (g/kg DM)	1.00	3.67	1.470	0.229
Propionic acid (g/kg DM)	0.98	2.47	0.402	0.020
i-Valeric acid (g/kg DM)	0.250	1.57	0.270	<0.001
n-Valeric acid (g/kg DM)	0.254	1.41	0.220	<0.001
i-Butyric acid (g/kg DM)	0.022	0.117	0.0172	<0.001
2,3-Butanediol (g/kg DM)	0.70	0.49	0.301	0.646
1-Propanol (mg/kg DM)	510	608	224	0.777
1,2-Propanediol (mg/kg DM)	1131	458	321.9	0.180
Methanol (mg/kg DM)	68.2	231	90.99	0.252
Isopropanol (mg/kg DM)	114	79.6	40.98	0.588
2-Butanol (mg/kg DM)	10.1	51.1	11.47	0.024
Acetone (mg/kg DM)	2.34	30.7	17.04	0.284
Propyl acetate (mg/kg DM)	1.16	2.24	1.749	0.688
Ethyl lactate (mg/kg DM)	1.90	1.46	1.436	0.844
Ethyl acetate (mg/kg DM)	0.00	1.72	1.248	0.374
SumBVA[2] (g/kg DM)	2.52	16.3	2.42	<0.001
SumVFAal[3] (g/kg DM)	19.1	45.8	4.94	<0.001
SumFP[4] (g/kg DM)	34.4	54.8	5.66	0.023
Particle size distribution (g/kg DM)				
>40 mm	78.5	92.5	19.61	0.646
19-40 mm	221	392	44.5	0.016
8-19 mm	522	431	48.0	0.222
4-8 mm	104	55.9	9.85	<0.001
Pan	74.2	28.4	8.46	<0.001
> 8 mm	822	916	16.9	<0.001
> 4 mm	926	972	8.5	<0.001

iNDF - indigestible neutral detergent fiber; pdNDF - potentially digestible neutral detergent fiber; SEM - standard error of the mean.
[1] DM - dry matter corrected for volatile compounds.
[2] SumBVA - sum of i-butyric, butyric, i-valeric and n-valeric acids.
[3] SumVFAal - sum of volatile fatty acids and alcohols sum of volatile fatty acids and alcohols (methanol, isopropanol, ethanol, 2-butanol, 1-propanol, acetic acid, propionic acid, 2,3-butanediol, 1,2-propanediol, i-butyric acid, n-butyric acid, i-valeric acid, n-valeric acid).
[4] SumFP - sum of all non-nitrogenous fermentation products.

The Chi-square test (χ^2) applied to variables with discrete distribution revealed that outsourced harvest service was used in 100% of cases in group LB. Other discrete variables showed no difference between groups (Table 6).

3.5. Relationships between silage characteristics

The iNDF concentration had a negative correlation with IVDMD (P<0.001; R^2 = 0.766; Figure 3). There was a linear and positive correlation between the concentrations of n-butyric acid and propionic acid (P<0.001; R^2 = 0.693; Figure 4A). The concentration of n-butyric acid showed a linear and positive correlation with NH_3-N (P<0.001; R^2 = 0.523; Figure 4B). The concentration of lactic acid was inversely correlated with n-butyric acid, and a broken-line regression model indicated a negative linear segment, followed by a plateau, after the break point (P<0.001; R^2 = 0.456; Figure 4C). Silages with DM content higher than 370 g/kg present a lower risk of clostridial fermentation (risk difference = −0.956; Figure 4D).

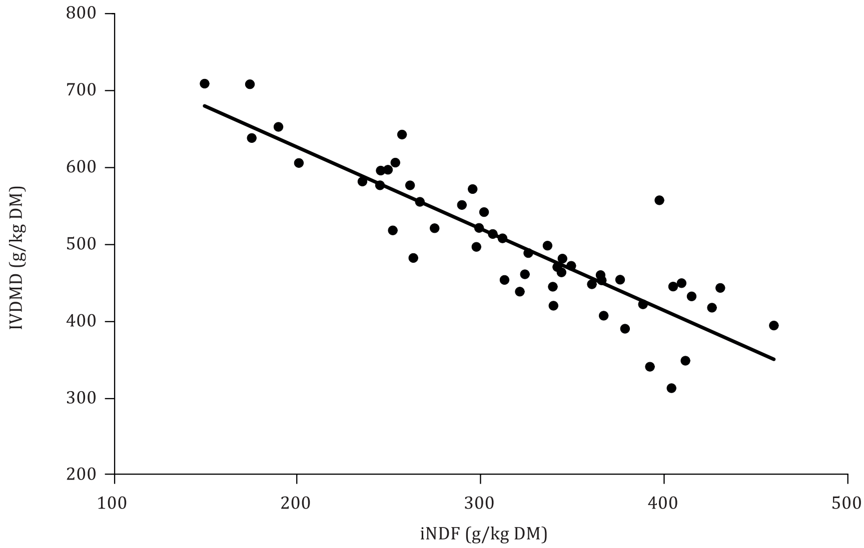
Table 6 - Comparison between silage groups with low (LB, ≤ 3 g/kg DM) and high n-butyric concentration (HB, > 3 g/kg DM), variables with discrete distribution

Item	Class	Group		Fisher test[1]
		LB	HB	
Consultancy	Yes	86%	64%	0.401
	No	14%	36%	
Grass genera	*Megathyrsus*	57%	73%	0.527
	Urochloa	43%	23%	
	Mixed	0%	5%	
Fertilization	Yes	57%	68%	0.673
	No	43%	32%	
Grass production area	Semi-perennial	57%	73%	0.354
	Crop-livestock integration	29%	23%	
	Pasture surplus	14%	5%	
Harvester	Own	0%b	43%a	0.037
	Outsourced	100%a	57%b	
Harvester type	Pull-type	0%	27%	0.177
	Self-propelled	100%	73%	
Inoculant	Yes	100%	80%	0.328
	No	0%	20%	
Inoculant type[2]	BAL homo	14%	20%	0.999
	Combo	86%	80%	
Moisture absorbent	Yes	14%	30%	0.657
	No	86%	70%	
Moisture absorbent type	SR	100%	33%	0.539
	SH	0%	50%	
	DDG	0%	17%	
Silo type	Bunker	29%	52%	0.515
	Drive-over	71%	41%	
	Bag	0%	2%	
	Bale	0%	5%	
Breeding cows feeding	Yes	14%	12%	0.999
	No	86%	88%	
Growing cattle feeding	Yes	29%	56%	0.237
	No	71%	44%	
Finishing cattle feeding	Yes	86%	86%	0.999
	No	14%	14%	

SR - soybean residue; SH - soybean hulls; DDG - dried distillers grains.
[1] Fisher's exact test.
[2] Combination of homo- and hetero-lactic bacteria.

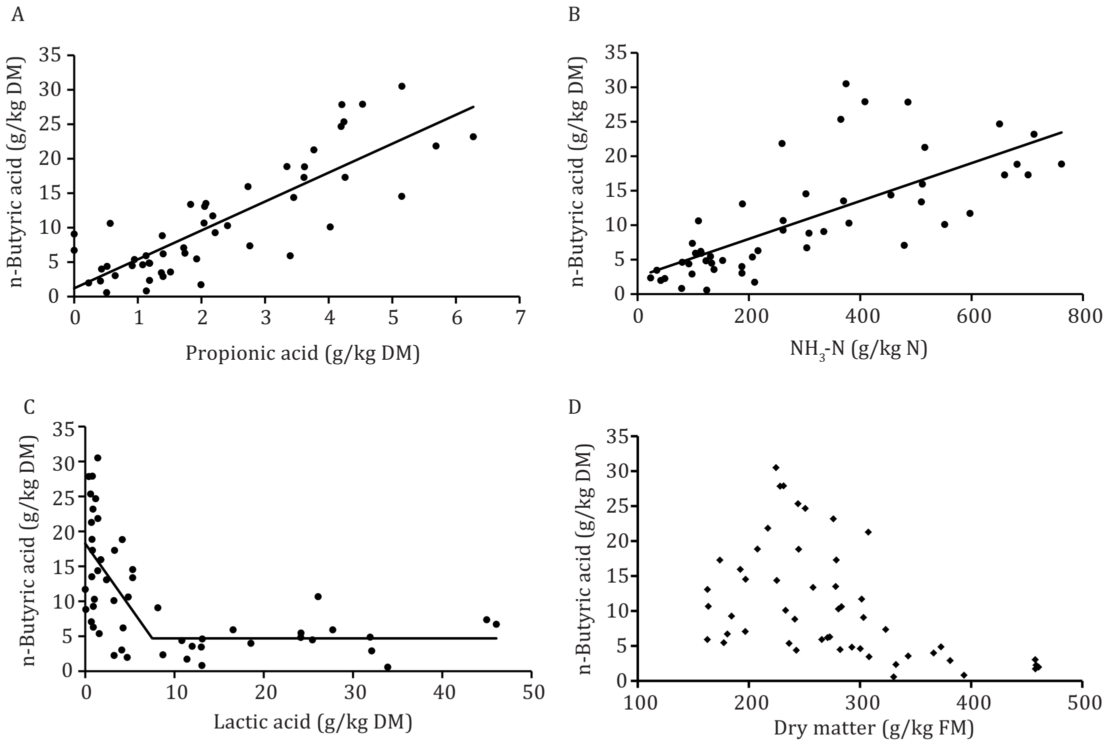
4. Discussion

While the use of tropical grass silage in beef production systems is expanding (Monsalve and Millen, 2025), field observations suggest high storage losses and low feeding value. However, the fermentation pattern and nutritional composition of tropical grass silages produced on commercial farms have not been characterized. In this survey, we demonstrated that most tropical grass silage produced in



IVDMD (g/kg DM) = 839 – 1.06 × iNDF (g/kg DM); RMSE = 43.5; R^2 = 0.766; P<0.001.

Figure 3 - Relationship between indigestible neutral detergent fiber (iNDF) content and *in vitro* dry matter digestibility (IVDMD) in tropical grass silage samples.



A: ButAc (g/kg DM) = 1.20 + 4.19 × PropAc (g/kg DM); RMSE = 4.49; R^2 = 0.693; P<0.001. B: ButAc (g/kg DM) = 2.50 + 0.028 × Ammonia (g/kg N); RMSE = 5.60; R^2 = 0.523; P<0.001. C: If LatAc < 7.51 g/kg DM, ButAc = 4.69 + 1.81 × (7.51 – LatAc); If LatAc ≥ 7.51, then ButAc = 4.69 g/kg DM. RMSE = 5.86; R^2 = 0.456; P<0.001. D: Silages with DM > 370 g/kg had a lower risk of butyric fermentation (Risk difference = −0.956; exact 95% confidence interval −0.996 to −0.526).

Figure 4 - Relationship between n-butyric acid (ButAc) and propionic acid (PropAc; A), NH_3-N (Ammonia; B), lactic acid (LatAc; C), and dry matter (DM; D) in tropical grass silage samples.

commercial beef farms in central Brazil has low feeding value and high variability in forage yield. The wide range of DM yield (0.8 to 24 t DM/ha/cut) highlights the substantial variability in production conditions across these operations.

The composition of tropical grass silages also showed substantial variability, indicating the broad range of conditions represented in the data set. For example, DM content ranged from extremely low (168 g/kg) to high (466 g/kg), while IVDMD (314 to 709 g/kg DM) and n-butyric acid concentration from 0.6 to 31 g/kg DM, likely reflecting differences in agronomic and ensiling conditions among fields. The absence of correlation between n-butyric acid concentration and IVDMD indicated that fermentation pattern and nutritional value were not directly associated. These findings indicate that complementary strategies are needed to produce highly digestible, well-fermented silage.

4.1. Nutritional value of silages

In this study, silages with the highest digestibility were obtained from production systems that adopted greater doses of nitrogen fertilization, greater cutting frequency, and lower regrowth age at harvest, which together may contribute to greater annual productivity. More frequent cutting usually allows forage production with a higher leaf:stem ratio and, consequently, better nutritional value at harvest. This intensive management, when soil nutrient availability is adequate, promotes faster growth and efficient plant recovery, maximizing the biomass produced. In addition, this practice helps to maintain plants in the vegetative phase, with greater nutritional quality (Mari, 2003). On the other hand, the application of greater doses of N allows faster plant growth while maintaining desirable leaf:stem ratio (Pinto et al., 1994; Oliveira et al., 2020).

An intriguing finding was the dichotomy in fertilization practices. While a higher proportion of farmers in the low in LD applied fertilizer, the actual nitrogen dose was significantly lower than that in HD group. This may suggest that in the LD group, fertilization is done as a standard practice but at suboptimal rates, which might be insufficient to maintain high proportion of leaves and delay stem elongation. Lower application rates of fertilizers combined with a longer regrowth interval observed in LD likely resulted in poorer forage quality. This underscores that the dose of fertilizer is critical for affecting grass productivity and nutritive value.

As the plant matures, the proportion of highly digestible components (i.e., NDS) tends to decrease. On the other hand, the proportions of fibrous fractions increase, resulting in lower digestibility (Moser, 1995; Jung and Allen, 1995). This pattern was evidenced in the present study by the lower concentrations of NDS and higher concentrations of fibrous fractions in the group with lower IVDMD, as well as by the negative correlation between iNDF and IVDMD. Therefore, forage harvested at a more advanced stage of maturity was associated with higher iNDF and lower IVDMD of the silages, as cell wall lignification hinders fiber digestibility by rumen microorganisms (Van Soest, 1994). In this survey, the average grass maturity at harvest was nearly 100 days, consistent with the high iNDF (318 g/kg DM) and low IVDMD (501 g/kg DM) observed. A high proportion of concentrate was reported even in diets for breeding cows and growing cattle, likely to compensate for the low feeding value of the silage.

In grasses from *Urochloa* and *Megathyrsus* genus, the IVDMD decreases with maturity, with a more pronounced decline after approximately two months of regrowth (Mari, 2003; Tomaz et al., 2018). The high iNDF:lignin(sa) ratio (averaging 4.96) corroborates that lignification has a greater negative effect on fiber digestibility in tropical grasses than in corn silage and temperate grasses (Daniel et al., 2017; Raffrenato et al., 2019; Moraes et al., 2023). The strong negative correlation between iNDF and IVDMD confirms that, for tropical grass silages, the indigestible fiber fraction is a major driver of overall DM digestibility. Therefore, iNDF may be more informative than total NDF for benchmarking the nutritive value of tropical grass silages.

The association between the *Megathyrsus* genus and the low IVDMD group (LD) is noteworthy. While both genera are tropical, their morphological and agronomic traits differ. It is plausible that *Megathyrsus* grasses on these farms were managed more extensively (e.g., lower N fertilization, longer regrowth intervals), leading to more lignified fiber at harvest. This aligns with the lower N application and numerically greater maturity at harvest observed in LD, where *Megathyrsus* predominated. Conversely, the higher prevalence of *Urochloa* in HD suggests that, under the management practices adopted on these farms, it may have been harvested at a less mature stage, preserving its nutritive value. This highlights the importance of on-farm management in determining the expression of the genetic potential of these grasses. The HD had 3.00 cuts/yr compared with 1.85 cuts/yr in LD, while regrowth age was lower in HD (83.8 vs 112 d). Such differences reflect not only botanical differences but also potentially distinct forage production systems.

Crop-livestock integration areas accounted for 50% of HD but only 3% of LD, whereas semi-perennial systems predominated in LD (90%). These results suggest that integrated systems may substantially enhance soil fertility, nitrogen cycling, and overall crop productivity (Lemaire et al., 2014), facilitate harvesting at earlier phenological stages, thereby indirectly contributing to higher digestibility.

The concentration of several fermentation products, either considered individually (e.g., acetic acid) or as the sum of compounds (sumFP) were higher for the group with greater IVDMD, indicating a greater extent of fermentation, which must have occurred due to the greater availability of soluble substrates for microorganisms in the younger better-fertilized plants. Despite the difference in maturity, the DM content was similar between silage groups. Previous studies have shown that maturity advancement is not an exclusive factor determining the moisture content of tropical grasses (Wilkinson, 1983; Santos et al., 2014; Tomaz et al., 2018; Moraes et al., 2023).

Although no differences were observed for the use of moisture absorbent (27 and 28% in HD and LD, respectively), the dose of absorbent (126 and 169 g/kg in HD and LD, respectively), and the type of absorbent between digestibility groups, the results from this clustering based on digestibility should be interpreted with caution as the mixture of those ingredients with the grass can modify the silage digestibility.

4.2. Silage fermentation pattern

In this study, most tropical grass silage samples (86%) were considered poorly fermented, with n-butyric acid concentrations > 3 g/kg DM (Kaiser et al., 2002). The relationship between DM content and n-butyric acid concentration indicated that silages with DM contents below 370 g/kg DM were at a high risk of clostridial development, whereas silages with more than 370 g/kg DM had lower risk of butyric fermentation. Recently, Carvalho et al. (2024) confirmed that the FC model proposed by Weissbach et al. (1974) is applicable to tropical grasses, including the *Megathyrsus* and *Urochloa* genus. The authors observed that, on average, the minimum DM content necessary to reduce the risk of butyric fermentation was approximately 350 g/kg DM, which is very similar to the value found in the present study. Thus, it can be concluded that the minimum DM value of 250 g/kg mentioned by McDonald et al. (1991) or even the value of 300 g/kg commonly adopted in practice may not be sufficient to restrict butyric fermentation and minimize nutrient losses in tropical grass silages, especially those from the genera *Megathyrsus* and *Urochloa*. This finding, derived from an on-farm survey, provides field-based support for the controlled experimental data reported by Carvalho et al. (2024) and suggests a practical benchmark for farmers and consultants. For *Megathyrsus* and *Urochloa* grasses, wilting or blending moisture absorbents to achieve a DM concentration above 370 g/kg represents the single most critical factor for mitigating the risk of clostridial fermentation. As the availability of harvesting and forage conservation machinery increases, the production of wilted tropical grass silage is expected to become more widespread.

Although no significant differences in pH were observed between the fermentation quality groups (LB vs. HB) or between the digestibility groups (HD vs. LD), the pH value alone was not a reliable indicator of fermentation quality in this study. Silage pH is influenced by multiple factors, including the concentration of buffering compounds (either originating from the forage or formed during fermentation), the presence of other organic acids, the use of additives, and moisture level. Notably, silages with higher DM content tend to exhibit relatively high pH values even under good fermentation conditions (Muck, 1988). Therefore, relying on pH alone, without profiling fermentation products can be misleading.

The correlation between n-butyric acid and lactic acid concentrations was inverse. The segmented regression model suggests a threshold of approximately 7.5 g/kg DM of lactic acid. Below this level, butyric acid concentration increased sharply. When enough lactic acid is produced in the fermentation process, microbial activity is reduced, mainly due to the effect of the acidic environment, allowing the silage to be stored aerobically (McDonald et al., 1991; Pahlow et al., 2003; Rooke and Hatfield, 2003). On the other hand, the lactic acid produced at the beginning of fermentation may have been degraded by the activity of undesirable microorganisms in silages classified as poorly fermented, especially by bacteria of the genus *Clostridium* (McDonald et al., 1991; Pahlow et al., 2003; Rooke and Hatfield, 2003). Both mechanisms may have occurred and the real cause of the low lactic acid concentration in silages with high concentration of n-butyric acid cannot be established in this study. Nevertheless, these findings suggest that achieving and maintaining an adequate lactic acid concentration during fermentation may help inhibit clostridial activity. Management strategies, as the use of efficient silage additives, must therefore help promote adequate lactic acid production and favorable fermentation.

In addition to n-butyric acid, several fermentation products can be associated with the development of clostridia in silages, such as i-valeric acid, n-valeric acid, i-butyric acid, 2,3-butanediol, isopropanol, 2-butanol, ethanol, acetic acid, ammonia, and biogenic amines (McDonald et al., 1991; Rooke and Hatfield, 2003). Several of these compounds are also associated with DM losses and reduced feeding value. In this study, n-butyric acid content was positively correlated with propionic acid, NH_3-N, and other fermentation products typically produced by clostridia. Furthermore, silages classified as HB had higher concentrations of NH_3-N, acetic acid, propionic acid, i-butyric acid, n-valeric acid, i-valeric acid, and 2-butanol.

High concentrations of NH_3-N, i-butyric acid, i-valeric acid, and n-valeric acid indicate severe proteolysis and deamination triggered by proteolytic microorganisms (McDonald et al., 1991). The observed differences in NH_3-N concentration suggest marked degradation of true protein and a potential reduction in nitrogen-use efficiency in HB silages. Moreover, a higher NH_3-N concentration (and likely other hypophagic metabolites) in grass silage has been reported to be inversely related to feed intake in cattle (Krizsan and Randby, 2007), further reducing silage feeding value. These nutritional and economic consequences warrant further investigation in future research. Although propionic acid does not contribute significantly to pH reduction high concentrations of this acid in moist silages typically indicates poor fermentation (Kung et al., 2018).

The development of undesirable microorganisms in tropical grass silages generally results in the degradation of readily available soluble nutrients and decreased digestibility (Gomes et al., 2021; Moraes et al., 2023). In fact, in the present study, poorly fermented silages had lower levels of SDN and higher levels of NDF, but the difference of IVDMD between LB and HB was only numerical (531 to 496 g/kg DM), as delayed harvest was associated with less n-butyric acid primarily through higher DM in the LB group. However, this does not imply that delayed harvest is an appropriate management strategy (Tomaz et al., 2018), because late harvest reduces nutritive value. Therefore, plant composition and cell wall digestibility at harvest likely had a stronger effect on silage IVDMD than did fermentation pattern, supporting the distinction between these two quality attributes in tropical grass silages.

In addition to plant composition, silage management can also influence the conservation process. In our dataset, the group of well-preserved silages showed better physical processing of the forage, as indicated by the lower proportion of particles retained on the 19- and 40-mm sieves. The association between finer particle size and reduced butyric fermentation in the LB group reinforces the importance of mechanical processing. Effective chopping serves a dual purpose: (1) it releases more soluble carbohydrates needed for the growth of lactic acid bacteria (McDonald et al., 1991; Pahlow et al., 2003), promoting rapid lactic acid production and pH drop; and (2) it facilitates better compaction, reducing porosity and creating the anaerobic conditions necessary to inhibit undesirable microorganisms. Hence, particle size seems more relevant to fermentation pattern than to silage digestibility and this dataset supports the concept of independence between fermentation pattern and bromatological composition. In future surveys, silage density and its relationship with particle size and fermentation pattern warrant further characterization.

In the current survey, more than 80% of producers used bacterial inoculants, with a predominance of products containing a combination of homolactic and heterolactic bacteria (81%). Under favorable growth conditions, homolactic bacteria can accelerate the pH decline at the onset of fermentation, inhibiting undesirable microorganisms such as enterobacteria and clostridia (Pahlow et al., 2003). However, in this study, the use of inoculants was not associated with improved fermentation or nutritional value. The effects of bacterial inoculants applied to wet tropical grass silages have been inconsistent (Mühlbach, 2000; Coan, 2005; Tomaz et al., 2018). The high moisture and low soluble carbohydrate content of these forages create an environment in which even inoculated LAB may fail to dominate the epiphytic microbiota. Furthermore, the predominance of inoculants containing a combination of homo- and heterolactic bacteria may not be an optimal strategy for direct-cut wet forages, as heterofermentative bacteria prioritize acetic acid production rather than rapid acidification, potentially delaying pH decline and increasing the risk of clostridial activity (Arriola et al., 2021). This suggests that current commercial inoculant strategies, as applied by farmers, are insufficient to overcome the fundamental ensiling challenges posed by these tropical grasses, highlighting the need for more robust technological interventions.

The use of moisture-absorbing additives (e.g., soybean hulls, soybean residue, and by-products from corn ethanol production), as an alternative to wilting, was adopted by a relatively small proportion of farmers and did not differ between the LB and HB groups. Although some studies have demonstrated positive effect on the fermentation profile of grass silages (Igarasi, 2002; Ribeiro et al., 2007; Avila et al., 2022), the addition of moderate levels of moisture absorbents as the only management strategy does not necessarily ensure adequate fermentation (Tomaz et al., 2018; Gouvêa et al., 2020; Gomes et al., 2021) and can increase the complexity of the ensiling process in large operations. In this study, the lack of efficacy noted for the use of absorbents might be due to the dose, type of absorbent or either initial forage DM.

5. Conclusions

Most of the surveyed silages exhibited low IVDMD (< 500 g/kg DM) and high concentrations of n-butyric acid (> 3 g/kg DM). Moreover, the results confirmed the independence between fermentation dynamics and the bromatological composition of tropical grass silages, suggesting that complementary management strategies are required to produce highly digestible, well-fermented silage. Even on farms where grass silage is used primarily as a source of physically effective fiber, strategies such as wilting and/or the application of proven silage additives are necessary to improve preservation. Collectively, our findings highlight the need to develop, transfer, and implement techniques that enhance the conservation and quality of tropical grass silage on Brazilian beef farms, particularly in systems where greater forage inclusion in the diet can improve production efficiency (e.g., for growing cattle and breeding cows).

Supplementary material

The supplementary material of this article can be found online at: https://www.rbz.org.br/wp-content/uploads/articles_xml/1806-9290-rbz-55-e20260048/1806-9290-rbz-55-e20260048-suppl01.pdf

Data availability

The datasets supporting the results of this study is available upon request to João Daniel (jlpdaniel@uem.br).

Author contributions

Conceptualization: Daniel, J. L. P. **Formal analysis:** Bragatto, J. M.; Silva, A. F.; Silva, N. G.; Zapponi, G. H. and Daniel, J. L. P. **Funding acquisition:** Daniel, J. L. P. **Investigation:** Bragatto, J. M.; Silva, A. F.; Silva, N. G.; Zapponi, G. H. and Campanili, P. R. B. **Methodology:** Bragatto, J. M.; Bánkuti, F. I. and Daniel, J. L. P. **Project administration:** Daniel, J. L. P. **Resources:** Daniel, J. L. P. **Visualization:** Bánkuti, F. I.; Bernardes, T. F.; Nussio, L. G. and Daniel, J. L. P. **Writing – original draft:** Bragatto, J. M. **Writing – review & editing:** Bánkuti, F. I.; Bernardes, T. F.; Nussio, L. G. and Daniel, J. L. P.

Conflict of interest

The authors declare no conflict of interest.

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