New Zealand Journal of Agricultural Research

RESEARCH ARTICLE OPEN ACCESS

Inclusion of *Tithonia diversifolia* in Silages of Elephant Grass cv. BRS Capiaçu

Queila Gouveia Tavares[1] | Denise Volpi[1] | Aline Silva Oliveira[1] | Karise Fernanda Nogara[1] | Luis Augusto Calsavara[2] | Wellyngton Tadeu Vilela Carvalho[3] | Adibe Luiz Abdalla[4] | Maity Zopollatto[1] | Sanín Ortiz Grizales[5]

[1]Department of Animal Science, Federal University of Paraná, Curitiba, Brazil | [2]Department of Entomology and Acarology, University of São Paulo, Piracicaba, Brazil | [3]Department of Animal Science, Federal Institute of Southeast Minas Gerais, Barbacena, Brazil | [4]Center for Nuclear Energy in Agriculture, University of São Paulo, Piracicaba, Brazil | [5]Department of Animal Science, Universidad Nacional de Colombia Sede Palmira, Palmira, Colombia

Correspondence: Queila Gouveia Tavares (queila.tavares13@hotmail.com)

Received: 26 January 2026 | **Revised:** 23 April 2026 | **Accepted:** 21 May 2026

Keywords: aerobic stability | alternative forage | ensiling | golden button | mixed silage

ABSTRACT

This study evaluated the effects of including *Tithonia diversifolia* in the ensiling process of elephant grass cv. BRS Capiaçu, with and without wilting, on fermentation losses, microbiology, aerobic stability, and the chemical composition of the silages. A randomized complete block design was used, with six treatments and five replicates, totaling 30 experimental units. The treatments were: (i) fresh Capiaçu (FC); (ii) fresh Capiaçu + 10% *T. diversifolia* (FC + 10% TD); (iii) fresh Capiaçu + 20% *T. diversifolia* (FC + 20% TD); (iv) wilted Capiaçu (WC); (v) wilted Capiaçu + 10% *T. diversifolia* (WC + 10% TD); and (vi) wilted Capiaçu + 20% *T. diversifolia* (WC + 20% TD). The forages were chopped to a theoretical particle size of 10 mm, weighed individually, ensiled, and stored for 117 days at room temperature (±24.5°C). The inclusion of *Tithonia diversifolia* increased dry matter (up to 17.5%), crude protein (up to 8.4%), acetic acid content (up to 3.58%), and aerobic stability (average of 116 h). Silages containing 20% *Tithonia* stood out, as they remained stable for 240 h. However, treatments with *Tithonia* combined with wilted Capiaçu resulted in total dry matter losses of nearly 30%. The lactic acid bacteria population was higher in fresh silage containing 20% *Tithonia*, due to greater substrate availability. The inclusion of 20% *Tithonia diversifolia* in the ensiling process of wilted BRS Capiaçu grass is recommended to obtain a protein-rich and stable silage, despite the increase in fermentation losses.

1 | Introduction

The intensification of animal production requires nutritional strategies that ensure the availability of high-quality forage throughout the year, minimizing the effects of pasture seasonality and optimizing animal performance. In this context, ensiling is one of the main conservation techniques, enabling the preservation of plant nutrients and providing greater security for feed planning. Moreover, this technique enables the incorporation of different forage species, reducing competition with food crops and contributing to greater sustainability and competitiveness in agriculture (Okoye et al. 2023).

Among tropical grasses used for ensiling, elephant grass (*Cenchrus purpureus* Schum.) cv. BRS Capiaçu stands out for its high production. Its productivity can exceed 50 tons of dry matter (DM) per hectare per year, surpassing traditionally ensiled grasses such as corn and sorghum (Pereira et al. 2016). In addition, it is tolerant to water stress, which reduces the risk of forage shortages and ensures feed availability during critical periods.

However, it generally has low crude protein (CP) content (5.3 to 6.5% CP), which may compromise animal performance and increase the need for protein supplementation (Pereira et al. 2016). Thus, strategies that enhance the nutritional value of this

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2026 The Author(s). *New Zealand Journal of Agricultural Research* published by John Wiley & Sons Australia, Ltd on behalf of Royal Society of New Zealand Te Apārangi.

forage are essential for improving silage quality. The inclusion of protein species such as *Tithonia diversifolia* during ensiling may represent a viable alternative to increase CP while maintaining adequate fermentation characteristics.

T. diversifolia, commonly known as Mexican sunflower or wild sunflower, is a shrubby species that has gained prominence as animal feed in Colombia due to its high CP (9 to 29.8%), production potential (up to 55 t DM/ha/year), and low agricultural input requirements (Tendonkeng et al. 2014). This species is highly adaptable to different soil and climate conditions and exhibits high efficiency in nutrient absorption, making it an attractive option for low-cost production systems (Medina et al. 2009; Zapata and Silva 2010). However, it can also directly influence fermentation and microbial dynamics, affecting aerobic stability and storage losses, due to its high moisture content (>70%) and buffering capacity (Holguín et al. 2017).

This study was based on the hypothesis that the inclusion of *T. diversifolia* in elephant grass cv. BRS Capiaçu silages could improve the nutritional composition, while maintaining a good fermentative profile, microbial population, and aerobic stability of these silages. Therefore, the objective was to evaluate the chemical composition, fermentation losses, microbiology, and aerobic stability of wilted and unwilted elephant grass cv. BRS Capiaçu silages with different levels of *T. diversifolia* inclusion.

2 | Material and Methods

2.1 | Location Description

The research was conducted at the Animal Science Department of the Federal Institute of Education, Science and Technology of Southeast Minas Gerais, in Barbacena—MG (latitude 21° 13′ 33″ S, longitude 43° 46′ 25″ W, and altitude 1,164 m). The climate, according to the Köppen classification, is tropical highland, with average temperatures between 17 and 27°C during the period in which the study was conducted. Figure 1 presents the meteorological data obtained from the National Institute of Meteorology (INMET), from the Barbacena Meteorological Station, corresponding to the ensiling period (April 4th to 5th, 2022).

2.2 | Crop Management and Harvesting

The *T. diversifolia* (TD) and elephant grass cv. BRS Capiaçu areas were not irrigated, relying on natural rainfall for soil water replenishment. Soil samples for analysis were also not collected, as no chemical fertilization was applied. Both cultures have already been established and managed in accordance with the practices of the remaining experimental area, receiving only bovine manure in nature, with an estimated average taxa of 15 t ha^{-1} $year^{-1}$, applied periodically throughout the production cycle. The specific procedures carried out for each foragem are described below.

The TD was established in 2018 in a 100 m^2 area, with 0.75 × 0.75 m spacing, and was manually managed. Elephant grass cv. BRS Capiaçu was also planted in 2018 and managed by hand, but in an area of approximately 5,000 m^2. Both crops were cut uniformly at 30 cm above the ground to standardize the regrowth time for ensiling (80 and 50 days for Capiaçu and TD, respectively). No pesticides were used throughout the crop cycle.

The wilted Capiaçu was harvested 1 day before the TD and the fresh ensiled, all manually cut approximately 10 cm above the ground. The material was spread on plastic tarps in a covered warehouse in 15 cm layers without compaction. It was turned at 3-h intervals and manually leveled with a rake and fork until it reached 20% DM, which occurred after 24 h of drying.

On the following day, the whole TD plant and fresh Capiaçu were harvested at approximately 15 and 17% DM, respectively. The DM contents were monitored using the microwave oven method (Oliveira et al. 2015), in which approximately 100 g of fresh matter (FM) was subjected to successive heating cycles until constant weight was achieved. All materials were processed in a stationary forage harvester (JF 60 MAXXIUM,

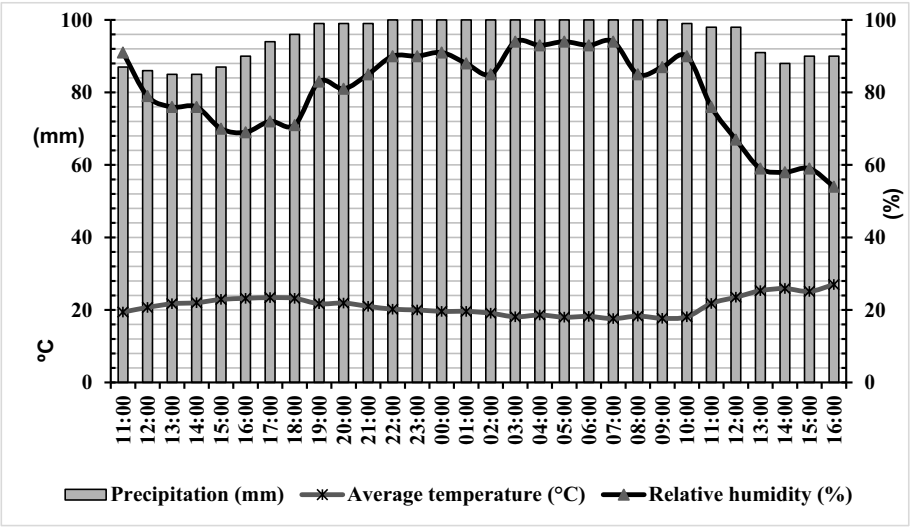


FIGURE 1 | Precipitation, relative humidity and average temperature during the ensiling stages. *Source:* Adapted from (INMET 2022).

JF Máquinas, Guararapes, SP, Brazil) set to cut to an average particle size of 10 mm.

2.3 | Treatments and Ensiling

On a floor covered with previously sanitized tarps, the forage materials were divided into six piles of 35 kg each, corresponding to the following treatments: (i) fresh Capiaçu (FC); (ii) fresh Capiaçu + 10% *T. diversifolia* (FC + 10% TD); (iii) fresh Capiaçu + 20% *T. diversifolia* (FC + 20% TD); (iv) wilted Capiaçu (WC); (v) wilted Capiaçu + 10% *T. diversifolia* (WC + 10% TD); and (vi) wilted Capiaçu + 20% *T. diversifolia* (WC + 20% TD). The inclusion levels were calculated on a FM basis. The characterization of the ensiled plants is presented in Table 1.

The experimental silos were 10 L plastic buckets, each fitted with a Bunsen valve on the lid and a 10 cm high plastic grate at the bottom, overlaid with plastic mesh and cotton fabric. After homogenizing the respective forage proportions, 6.0 ± 0.7 kg of forage per silo were manually compacted using a wooden tamper. This procedure aimed to achieve a target density of 600 kg FM/m^3. Finally, all silos were sealed with plastic tape and stored at room temperature (~24°C) for 117 days.

2.4 | Sampling and Laboratory Analyses

All samples were collected in duplicate from each forage to be ensiled ($n = 6$) and from each replicate ($n = 60$) after silo opening. Before sampling, the upper 5 cm of each replicate was discarded to avoid interference from potential microaeration. Subsequently, the entire silage mass from each silo was removed, placed in an individual plastic bag identified by replicate ($n = 30$), and homogenized for subsampling.

TABLE 1 | Chemical, bromatological, and microbiological characteristics of elephant grass cv. BRS Capiaçu and *Tithonia diversifolia* at the time of ensiling.

Variables[a]	Forage		
	Fresh Capiaçu	Wilted Capiaçu	*Tithonia diversifolia*
pH	6.1	6.0	6.0
DM, %	17.06	20.20	15.00
Ash, % DM	14.21	13.26	12.91
NDF, % DM	73.74	81.55	78.47
ADF, % DM	49.99	53.18	54.57
CP, % DM	9.01	8.27	15.21
EE, % DM	2.27	1.57	2.00
Lignin, % DM	9.83	7.75	12.86
LAB, log CFU/g	6.08	6.11	—
Yeasts, log CFU/g	5.60	5.80	—
Molds, log CFU/g	5.18	5.20	—

[a]pH - hydrogen potential; DM - corrected dry matter; NDF - neutral detergent fiber; ADF - acid detergent fiber; CP - crude protein; EE - ether extract; LAB - lactic acid bacteria; CFU - colony forming units.

Effluent losses (EL), gas losses (GL), and total dry matter losses (TDML) were calculated according to the following equations proposed by Jobim et al. (2007)

$$\text{EL}\,(\text{kg/t FM}) = [(\text{Wef} - \text{Wei})/\text{FM}] \times 1000$$

$$\text{GL}\,(\%\,\text{DM}) = [(\text{Wsi} - \text{Wsf})/\text{DMf}] \times 100$$

$$\text{TDML}\,(\%\,\text{DM}) = [(\text{DMi} - \text{DMf})/\text{DMi}] \times 100$$

where: Wei and Wef (kg) = initial and final weights, respectively, obtained by the collection system; FM (kg) = FM ensiled; DM (%) = dry matter; Wsi and Wsf (kg) = initial and final silo weights, respectively; DMi and DMf (%) = initial and final dry matter content of the mass ensiled.

For chemical and bromatological analyses, samples of approximately 300 g of forage were collected both during ensiling and after silo opening. Of these, 25 g were used to determine pH through silage extract (Kung et al. 1984); about 30 g were frozen for later determination of volatile fatty acids (VFA – analysis of lactic, acetic, and butyric acids) and ethanol; and the remaining fraction was used for DM determination in a forced-air oven at 65°C for 72 h (AOAC, 1990). The VFA concentrations were determined by high-performance liquid chromatography (HPLC, model LC-10Ai, Shimadzu, Tokyo, Japan) using Shimadzu ion-exclusion columns (Shim-pack SCR-101H (L17), 300 × 7.9 mm) and the analytical parameters recommended by the manufacturer (mobile phase: 100 mmol/L $HClO_4$; flow rate: 0.6 mL/min; column temperature: 50°C). Subsequently, oven-dried samples were ground in a Wiley mill with a 1 mm sieve, packed in labeled plastic bags, and sent to the laboratory for chemical composition analyses. Ash, CP, and ether extract (EE) were determined according to AOAC (1990), while neutral detergent fiber, acid detergent fiber (ADF), and lignin fractions were analyzed following Van Soest et al. (1994).

For microbiological analyses, 25 g samples were aseptically diluted in 225 mL of sterile saline solution (adapted from Kung and Ranjit 2001) and mixed for 4 min at 3850 rpm. The suspensions were filtered through three layers of cheesecloth and subjected to serial decimal dilutions. For lactic acid bacteria (LAB), dilutions were plated in MRS broth (MRS Kasvi, Kasvi, Pinhais, PR, Brazil) using Petri dishes (Qualividro, Passos, MG, Brazil). The dishes were incubated at 30°C for 48 h in a microbiological culture incubator, after which colony counts were performed. For yeast and filamentous fungi, dilutions were plated in yeast glucose chloramphenicol agar (YGC, Himedia, Mumbai, India) and incubated in a BOD chamber at an average temperature of 23.5°C. Colony counts were performed after 72 h for yeasts and after 120 h for filamentous fungi. Microbial populations were expressed as colony forming units per gram of fresh material (colony-forming units [CFU] g^{-1}).

Aerobic stability was assessed according to Moran et al. (1996). A 2 kg sample from each replicate was placed in an open container,

without compaction, and stored in a temperature-controlled room at 25°C. A digital contact thermometer (HI145–00, Hanna Instruments, Tamboré Barueri, Brazil) was inserted into the geometric center of the silage mass, and temperature was recorded manually every hour for 10 days. Additionally, another 2 kg sample was kept under the same conditions for pH determination on days 0, 2, 4, 6, 8, and 10 of aerobic exposure.

2.5 | Statistical Analysis

A randomized complete block design was used, consisting of six treatments with five replicates (totaling 30 experimental units), according to the following statistical model

$$Yij = \mu + Ti + Bj + \varepsilon ij$$

where: YijY_{ij}Yij = observed value of the experimental unit subjected to treatment i in block j; μ\muμ = overall mean of the dependent variable; TiT_iTi = effect of the *i*th treatment (i = 1, 2, …, 6); BjB_jBj = effect of the *j*th block (j = 1, 2, …, 5); and εij\varepsilon_{ij}εij = experimental error, assumed to be independently and identically distributed as N(0,σ2)N(0, \sigma^2)N(0,σ2), that is, normally distributed with zero mean and constant variance.

Data were subjected to analysis of variance (ANOVA) at 5% significance level. Prior to ANOVA, the assumptions of normality and homogeneity of variances were verified using the Shapiro–Wilk and Levene tests, respectively. When treatment effects were significant, means were compared using Tukey's test ($p \leq 0.05$). Microbiological data, expressed as CFU, were transformed to base-10 logarithmic scale (log CFU) prior to statistical analysis. All statistical procedures were performed using SAS software (SAS Institute Inc., Cary, NC, USA).

3 | Results

3.1 | Chemical and Bromatological Composition and Fermentation Products

The chemical and bromatological composition and VFA concentration of Capiaçu silages with different levels of *T. diversifolia* inclusion after 117 days of fermentation are presented in Table 2.

The treatments had a significant effect on the dry matter (DM; $p = 0.0002$), CP ($p = 0.0131$), and ether extract ($p = 0.0306$) chemical variables. Except for the fresh Capiaçu silage (FC), which showed the lowest DM content, all other treatments presented similar DM values for this variable. On average, the FC treatment resulted in 7.72% less DM compared to the other silages evaluated (average of 17.36% DM). A similar effect was observed for CP content. Wilted Capiaçu silages with the addition of 20% *T. diversifolia* (WC + 20% TD) had the highest absolute CP value (+21.35%) compared to FC treatment. The other treatments presented intermediate values (average of 7.59% CP).

In general, the EE of the silages showed a trend opposite to that of the CP, with the FC silages containing 39.82% more energy than those made from WC + 10% TD, which had a lower absolute value. However, the FC treatment was similar to the FC + 10% TD, FC + 20% TD and WC treatments (average of 2.88% EE).

TABLE 2 | Chemical-bromatological composition and volatile fatty acid concentration of elephant grass cv. BRS Capiaçu silages exclusively or combined with *Tithonia diversifolia*.

	Treatments[b]							
Variables[a]	FC	FC + TD10%	FC + TD20%	WC + TD10%	WC + TD20%	WC	*p*-value	SEM
pH	4.23	4.26	4.42	4.20	5.12	4.36	0.487	0.437
DM, %	16.02^{b}	17.16^{a}	17.48^{a}	17.51^{a}	17.38^{a}	17.29^{a}	0.0002	0.144
Ash, % DM	13.03	12.58	12.23	12.64	13.25	13.94	0.093	1.046
CHOt, % DM	76.54	77.56	76.69	77.94	75.95	75.37	0.155	6.456
NDF, % DM	80.59	81.95	81.05	79.04	79.04	82.64	0.206	6.80
ADF, % DM	54.80	56.32	56.08	55.98	56.99	57.51	0.552	6.634
OM, % DM	86.97	87.42	87.77	87.36	86.75	86.06	0.093	7.353
CP, % DM	6.93^{b}	7.09^{ab}	8.11^{ab}	7.35^{ab}	8.41^{a}	7.80^{ab}	0.013	0.761
EE, % DM	3.16^{a}	2.79^{abc}	2.96^{ab}	2.26^{c}	2.38^{bc}	2.89^{abc}	0.002	0.284
Lignin, % DM	6.90	8.95	9.07	8.90	9.25	8.06	0.302	1.001
Acetic acid, % DM	2.11^{c}	2.03^{c}	2.85^{abc}	3.58^{ab}	3.81^{a}	2.65^{bc}	<0.000	1.015
Lactic acid, % DM	3.27^{b}	4.53^{ab}	4.28^{ab}	4.13^{ab}	4.18^{ab}	5.42^{a}	0.017	1.027
Butyric acid, % DM	0.34	0.42	0.39	0.49	0.47	0.42	0.103	0.083
Ethanol, % DM	0.45	0.59	0.44	0.67	0.68	0.67	0.102	0.082

Note: Means followed by different lowercase letters, in the same line, differ statistically by Tukey's test at 5% significance level.
[a]pH – hydrogen potential; DM – corrected dry matter; CHOt – total carbohydrates; NDF – neutral detergent fiber; ADF – acid detergent fiber; OM – organic matter; CP – crude protein; EE – ether extract.
[b]FC – fresh Capiaçu silage; FC + TD10% – fresh Capiaçu silage + 10% Tithonia; FC + TD20% – fresh Capiaçu silage + 20% Tithonia; WC + TD 10% – wilted Capiaçu silage + 10% Tithonia; WC + TD 20% - wilted Capiaçu silage + 20% Tithonia; WC – wilted Capiaçu silage.

The other treatments presented lower concentrations, being 21.35% below the overall average (average of 2.95% DM). Regarding organic acids, treatments significantly affected lactic acid ($p = 0.0170$) and acetic acid ($p < 0.0001$) concentrations. Wilting of Capiaçu (WC) increased lactic acid content by 65.75% compared with fresh Capiaçu. Regardless of wilting, the addition of *T. diversifolia* also promoted lactic acid production, resulting in intermediate values (average 4.28% DM) between the lowest and highest levels observed. For acetic acid, wilted silages with *T. diversifolia* inclusion showed the highest concentrations (average 3.69% DM), which were 53.11% higher than the mean of the other treatments (2.41% DM).

3.2 | Fermentative Losses

TDML ($p < 0.0001$) and GL ($p = 0.0002$) during the fermentation process were also significantly influenced by wilting and/or Tithonia inclusion (Table 3). The lowest losses were recorded in the FC treatment, which showed a 72.09% reduction compared to the average (27.63% DM) of the treatments with the highest losses (WC + 10% TD and WC + 20% TD). The fresh treatments with Tithonia inclusion (FC + 10% TD and FC + 20% TD) also exhibited a significant reduction of 57.98% compared to the treatments with the highest losses. The treatment with wilted grass alone (WC) presented the lowest loss among the wilted treatments, but this value was 52.94% higher than in the treatments containing fresh grass.

Silages made with fresh Capiaçu (FC) showed the lowest GL also showed the lowest GL among all treatments evaluated, with the other treatments showing similar values, resulting in an average difference of approximately one percentage point (p.p.).

3.3 | Silage Microbial Population

Regarding the microbiological profile, only the LAB count differed significantly ($p = 0.0354$) between treatments (Table 4). The FC + 20% TD treatment presented the highest average, followed by WC + 20% TD and WC + 10% TD, with relatively similar values. The lowest count was observed in the FC + 10% TD treatment, representing a reduction of approximately 7.92% compared to the highest value recorded.

3.4 | Aerobic Stability

The aerobic stability of the silages is presented in Table 5. Treatments had a significant effect on aerobic stability ($p = 0.0457$) and on dry matter losses after exposure to air (PMSea; $p = 0.0009$). The longest stability time was observed in the WC + 20% TD treatment (240 h), although it was statistically similar to the other treatments (mean = 99.46 h), except for the FC + 10% TD treatment, which showed a reduction of 86.20% relative to the overall mean (125.54 h).

This same treatment (FC + 10% TD) also exhibited the highest dry matter losses after exposure to air (PMSea), with values approximately twice the average of the other treatments (9.30% DM), which did not differ statistically from each other.

TABLE 3 | Fermentation losses in elephant grass cv. BRS Capiaçu silages exclusively or combined with *Tithonia diversifolia*.

	Treatments[b]							
Variables[a]	FC	FC + 10%TD	FC + 20%TD	WC + 10%TD	WC + 20%TD	WC	*p*-value	SEM
TDML, % DM	7.71^{d}	14.15^{c}	9.08^{cd}	29.98^{a}	25.29^{ab}	21.91^{b}	<0001	1.408
GL, % DM	14.82^{b}	15.63^{a}	15.82^{a}	15.92^{a}	15.96^{a}	15.76^{a}	0.0002	1.178
EL, kg/t FM	7.77	7.86	8.51	7.24	7.32	6.12	0.5030	0.918

Note: Means followed by different lowercase letters, in the same line, differ statistically by Tukey's test at 5% significance level.
[a]TDML – total dry matter losses; GL – gas losses; EL – effluent losses.
[b]FC – fresh Capiaçu silage; FC + TD10% – fresh Capiaçu silage + 10% Tithonia; FC + TD20% – fresh Capiaçu silage + 20% Tithonia; WC + TD 10% - Wilted Capiaçu silage + 10% Tithonia; WC + TD 20% - Wilted Capiaçu silage + 20% Tithonia; WC – Wilted Capiaçu silage.

TABLE 4 | Microbial count in elephant grass cv. BRS Capiaçu silages exclusively or combined with *Tithonia diversifolia*.

	Treatments[b]							
Variables, log CFU/g[a]	FC	FC + TD10%	FC + TD20%	WC + TD 10%	WC + TD 20%	WC	*p*-value	SEM
LAB	6.76^{ab}	6.51^{b}	7.07^{a}	6.86^{ab}	6.91^{ab}	6.84^{ab}	0.035	0.106
Yeasts	4.63	4.73	5.16	5.17	5.56	4.74	0.529	0.360
Molds	<3.00	<3.00	<3.00	<3.00	<3.00	<3.00	—	—

Note: Means followed by different lowercase letters, in the same line, differ statistically by Tukey's test at 5% significance level.
[a]CFU – Colony forming units; LAB – lactic acid bacteria.
[b]FC – fresh Capiaçu silage; FC+ TD10% – fresh Capiaçu silage + 10% Tithonia; FC + TD20% – fresh Capiaçu silage + 20% Tithonia; WE+ TD 10%- Wilted Capiaçu + 10% Tithonia; WE + TD 20%- Wilted Capiaçu + 20% Tithonia; WE – Wilted Capiaçu.

TABLE 5 | Aerobic stability of elephant grass silages cv. BRS Capiaçu exclusively or combined with *Tithonia diversifolia*.

Variables[a]	Treatments[b]						*p*-value	SEM
	FC	FC + TD10%	FC + TD 20%	WC + TD10%	WC + TD20%	WC		
AS, hours	83.60[ab]	17.60[b]	81.25[ab]	158.60[ab]	240[a]	74.40[ab]	0.045	38.953
MaxT, °C	27.84	28.18	29.62	29.12	26.78	29.22	0.083	0.650
DMLae, % DM	9.18[b]	19.87[a]	9.37[b]	10.5[b]	8.72[b]	8.72[b]	0.0009	1.341

Note: Means followed by different lowercase letters, in the same line, differ statistically by Tukey's test at 5% significance level.
[a]AS – aerobic stability; MaxT – maximum temperature; DMLae – dry matter losses after exposure to air.
[b]FC – fresh Capiaçu silage; FC + TD10% – fresh Capiaçu silage + 10% Tithonia; FC + TD20% – fresh Capiaçu silage + 20% Tithonia; WC + TD 10% – wilted Capiaçu silage + 10% Tithonia; WC + TD 20% – wilted Capiaçu silage + 20% Tithonia; WC – wilted Capiaçu silage.

Figure 2 shows the pH values during the aerobic stability assessment of Capiaçu silages, both with and without *T. diversifolia* supplementation.

The pH of the silages increased gradually over the ten days of air exposure, with similar trends observed among treatments during the initial days. From the fourth day onward, more pronounced differences began to emerge, particularly in the FC + 20% TD treatment, which exhibited a sharper pH increase between days 8 and 10, indicating greater instability. In contrast, the FC treatment maintained lower pH values until the fourth day. The other treatments showed intermediate variations, without abrupt changes in the curve pattern.

4 | Discussion

4.1 | Chemical and Bromatological Composition and Fermentation Products

Despite the unfavorable climatic conditions during the experiment (Figure 1), which prolonged the wilting time to 29 h, the data showed that both wilting and *T. diversifolia* addition were effective in increasing the dry matter (DM) content of the silages. The highest absolute DM value was observed in the fresh grass treatment supplemented with TD (17.61% DM), with higher values observed in treatments containing TD, even in the absence of wilting. These results suggest that, under unfavorable climatic conditions, the inclusion of TD may be a viable strategy to increase forage DM, reducing reliance on prolonged and often inefficient wilting.

The increase in DM due to *T. diversifolia* addition and the use of wilting, even if modest, indicates lower moisture, which may favor silage preservation and reduce effluent losses. Despite this beneficial effect, the observed values are still below the ideal for well-conserved tropical grass silages, which generally should present between 30% and 35% DM (McDonald, Henderson and Heron 1991), suggesting that wilting under optimal conditions could be an additional strategy to increase DM and improve the fermentative quality of silages. However, despite the nutritional advantages of *T. diversifolia*, the low dry matter content likely limited the full expression of its benefits, particularly by favoring undesirable fermentations and increasing losses.

Certainly, losses in DM are expected due to the fermentative process resulting from cellular respiration and metabolism of anaerobic microorganisms, which consume sugars and produce

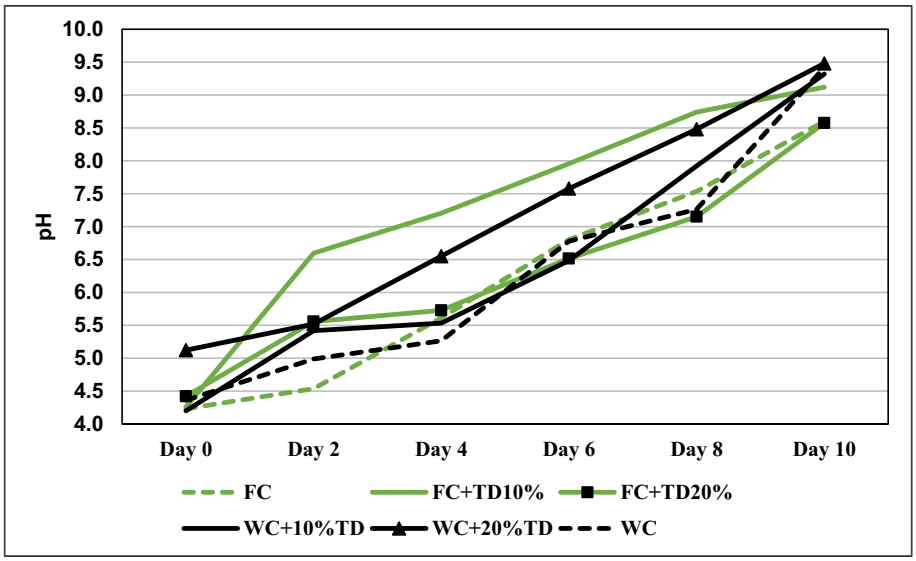


FIGURE 2 | Shows the pH curves of elephant grass silages (cv. BRS Capiaçu), alone or in combination with *Tithonia diversifolia*, during air exposure. The abbreviations are as follows: FC-fresh Capiaçu silage; FC + TD10%-fresh Capiaçu silage + 10% Tithonia; FC + TD20%-fresh Capiaçu silage + 20% Tithonia; WC + TD 10%-wilted Capiaçu silage + 10% Tithonia; WC + TD 20%-wilted Capiaçu silage + 20% Tithonia; WC-wilted Capiaçu silage.

CO_2 and water during the process (Junges et al. 2013). However, pH values were within the expected range for elephant grass and *T. diversifolia* silages, which, due to the high buffering capacity of these materials, tend to stabilize at a higher pH (McDonald, Henderson and Heron 1991).

As expected, CP content increased with higher levels of *T. diversifolia* inclusion, even when ensiled with fresh Capiaçu, which has low CP. This effect is attributed to the high protein concentration (15.21% DM) found in the leaves of *T. diversifolia* at the time of ensiling (Table 1). Proportionally, the data from the present study corroborate those reported by Morales et al. (2016), who evaluated the inclusion of *T. diversifolia* in elephant grass silages (cv. Cuba CT-169) at 40:60 and 60:40 proportions on a FM basis. The authors found that the higher proportion of TD (60%) increased CP concentration (18.86% DM) compared to the lower inclusion (10.82% DM). Similarly, values close to those observed in the present study were reported by Gutierrez et al. (2014), who analyzed silages containing 20% TD and 80% Capiaçu, finding CP values of 7.79% DM.

However, the WC treatment presented higher CP content than the WC + 10% TD treatment, both wilted. This difference, although small, contradicts the general trend observed with higher levels of TD inclusion and may be explained by the low proportion of TD (10%), which may not have been sufficient to promote a significant increase in CP, especially considering the natural variability of the harvested elephant grass. Studies by Ribeiro et al. (2020) and Sosa (2024) indicate that low inclusion levels of legumes or other protein sources do not necessarily guarantee an increase in silage CP content and may even dilute the nutritional content depending on the composition of the materials involved. Furthermore, the chemical composition of elephant grass can vary significantly due to maturity stage, environmental conditions, and wilting duration.

The lowest ether extract (EE) content was observed in wilted silages with the addition of *Tithonia diversifolia* compared to the other treatments. This reduction can be attributed to the low lipid concentration in Tithonia, which contributes to the dilution of total silage lipids, particularly due to the proportional increase in the fibrous fraction. Additionally, the wilting process may favor losses of volatile lipid compounds through oxidation or volatilization during the preensiling period. It should also be noted that, at the time of ensiling, elephant grass already had a naturally high EE content (Table 1), which may have influenced the observed results. Nevertheless, the values obtained are within the range recommended by Marques et al. (2021), which should be below 5% to avoid limiting intake.

Fermentation products (lactic, acetic, and butyric acids) were within the ranges of 3%–8%, 2%–5%, and <0.5%, respectively, as reported in the literature for wet silages, which are at higher risk of undesirable fermentations (McDonald, Henderson and Heron 1991; Kung Jr. et al. 2018). However, silages containing TD showed higher acetic acid concentrations, characteristic of wet silages (>70% moisture), due to prolonged fermentations (Kung Jr. et al. 2018). Nevertheless, butyric acid was detected in these silages, indicating clostridial fermentation, in which lactic acid is converted to butyric acid, favored by low DM content that hinders rapid pH decline during ensiling. According to Kung Jr. et al. (2018), legume and grass silages with low DM tend to have higher pH than normal; however, in the present study, no differences were observed among treatments. It is noteworthy that TD is not a legume, but it possesses similar characteristics.

4.2 | Fermentative Losses

Higher TDML were observed in wilted treatments combined with *T. diversifolia*. Although these losses were initially thought to be associated with increased gas production, the data show that GL were relatively similar among treatments, except for FC. According to Bueno et al. (2020), most gas is produced during the first days of ensiling, driven by the respiration of residual oxygen in the pores of the plant material. However, these same treatments also exhibited higher acetic acid concentrations, suggesting that heterolactic fermentation may have been more intense, resulting in greater production of volatile compounds, such as acetic acid itself, and consequently higher losses through volatilization during oven drying. This pattern was observed in the FC + 10% TD treatment, which showed high GL but low TDML, reinforcing that loss mechanisms differ among treatments and cannot be attributed solely to gas loss.

Thus, in the present study, the data indicate that the highest TDML was more associated with volatile acid production than with initial respiration or gas formation. However, it is important to emphasize that higher DM losses do not necessarily indicate lower fermentative quality, as factors such as aerobic stability and microbial populations also influence silage preservation (Kung Jr. et al. 2018). Nevertheless, the elevated moisture content of the material may have contributed to these losses, reinforcing that inadequate dry matter levels can compromise fermentation efficiency even when using nutritionally promising additives such as *T. diversifolia*.

4.3 | Silage Microbial Population

As shown in Table 4, most treatments presented statistically similar LAB counts. This performance may be related to the epiphytic microbial composition of *T. diversifolia*, which, when added in higher proportions, likely provided fermentable substrates and bioactive compounds such as lactones, flavonoids, tannins, and saponins. These compounds may have contributed to modulating the silage microbiota, favoring LAB growth while inhibiting other potential spoilage organisms not evaluated in the present study. Additionally, the dry matter content and the initial fermentation environment in these treatments may have been more favorable for the multiplication of this beneficial microbiota. In contrast, the FC + 10% TD treatment showed the lowest count (6.51 log CFU/g), which may be associated with the lower proportion of Tithonia and a possible limitation in soluble substrate availability, resulting in less favorable conditions for LAB colonization and growth (McDonald, Henderson and Heron 1991). These results indicate that both the inclusion level of Tithonia and the condition of the grass influence the microbial response of the silage.

The wilted treatments (WC, WC + 10% TD, and WC + 20% TD) exhibited high LAB counts, ranging from 6.86 to 6.91 log CFU/g,

suggesting that wilting the forage before ensiling may have contributed to maintaining an environment favorable for LAB growth. However, the FC + 20% TD treatment showed the lowest value, which may be related to the absence of wilting combined with the higher proportion of Tithonia, which has high moisture and buffering capacity. This could have hindered rapid pH decline and favored competition with undesirable microorganisms in the initial stages of fermentation. Consequently, LAB growth and the early establishment of the desirable microbiota may have been inhibited.

Nevertheless, LAB predominance over yeasts and filamentous fungi was observed in most treatments, which, together with the low butyric acid concentration (≤0.42% DM) and pH within the adequate range (4.10–5.12), suggests that fermentation was predominantly lactic and efficient, even under the limitations imposed by low dry matter content.

4.4 | Aerobic Stability

Although the WC + 20% TD treatment exhibited the highest aerobic stability (>240 h), it is important to consider that this result did not differ statistically from other treatments, such as FC and WC, which were neither wilted nor received the same levels of Tithonia inclusion. Although sensory characteristics were not evaluated, it is noteworthy that after 10 days of air exposure, wilted Capiaçu silages combined with TD showed slightly darker coloration, preserved odor, and lower visible fungal presence. This effect may be more directly related to the higher acetic acid concentration observed in this treatment, as acetic acid has recognized antifungal activity and may have contributed to inhibiting the growth of spoilage microorganisms during air exposure (Kung Jr. et al. 2018).

Thus, although Tithonia contains bioactive compounds with potential antifungal properties, such as sesquiterpene lactones (Ajao, Moteetee and Gray 2017), the results of this study indicate that acetic acid production appears to have played a more significant role in maintaining aerobic stability. Holguín et al. (2017) reported that higher TD inclusions (100% and 67% on FM basis) provided greater aerobic stability compared to those with a higher proportion of elephant grass, due to its buffering capacity maintaining pH after silo opening.

In more humid materials, such as elephant grass and TD, aerobic deterioration is not always accompanied by a temperature rise in the material because the heat generated is absorbed by water (Woolford 1990). Therefore, for wetter materials, it is recommended to evaluate pH concurrently during air exposure. Considering dry matter losses and pH increase as a result of degradation, the control silages showed higher resistance to deterioration (Figure 2). From the first day, a considerable pH increase was noted in all treatments, indicating material degradation and corroborating the data in Table 5, where the stability of control silages lasted about 3 days (83.6 h), approximately 156.4 h less than the WC + 20% treatment, which maintained its stability (240 h). As mentioned by Heinritz et al. (2012), tropical grasses present nonstructural carbohydrate concentrations ranging from 31 to 77 g/kg DM; thus, acidification is more effective when the mixture has a higher proportion of grasses, providing greater substrate availability for LAB and favoring silage acidification.

The pH reduction observed in silages with higher proportions of Capiaçu may be associated with its lower buffering capacity compared to Tithonia, which is naturally more buffered due to its mineral and protein composition. However, when evaluating aerobic stability, it is evident that wilting had a more pronounced effect than TD inclusion levels on pH during air exposure. Although Holguín et al. (2017) reported that TD can contribute to greater aerobic stability by delaying abrupt pH increases after silo opening, in the present study, wilting proved more decisive in this regard, possibly by reducing moisture and limiting the growth of spoilage microorganisms during the postopening period.

Dry matter losses after aerobic stability (DMLae) were higher in the FC + 10% TD silages (19.87%), while the opposite was observed in the other treatments (average of 9.30%). At first glance, this result could be interpreted as indicative of lower aerobic stability in this treatment. However, considering the complete dataset, particularly the low TDML and the higher lactic acid concentration observed in this same treatment, it is possible that fermentation was highly efficient, with good nutrient preservation during the anaerobic period. As discussed by Kung Jr. et al. (2018), well-fermented silages with high lactic acid production may become more susceptible to aerobic deterioration due to the accumulation of substrates that can be easily metabolized by yeasts. Furthermore, Muck et al. (2018) highlight that the presence of acetic acid and low yeast populations favor aerobic stability, which was not observed in the present study, as these populations did not decrease significantly. Therefore, the higher DMLae in the FC + 10% TD treatment may be a possible consequence of its high initial fermentative efficiency.

Including *Tithonia diversifolia* (TD) in the ensiling of elephant grass cv. BRS Capiaçu proved beneficial, as it increased CP content without negatively affecting other quality parameters. However, greater fermentation-related losses were observed. This indicates that, although *T. diversifolia* has high nutritional potential, its benefits in silage systems are highly dependent on adequate dry matter content at ensiling. Therefore, special attention should be given to adverse climatic conditions during ensiling, as they can hinder achieving adequate dry matter (DM) levels and increase the susceptibility of wetter silages to undesirable fermentation losses. When the objective is to produce silage with higher protein content and improved aerobic stability, the ensiling of wilted elephant grass cv. BRS Capiaçu combined with 20% *T. diversifolia* is recommended. Nevertheless, appropriate preensiling management is essential to ensure higher forage DM content.

Acknowledgments

The authors thank the Federal Institute of Southeast Minas Gerais, Barbacena campus, for the infrastructure provided and the Center for Nuclear Energy in Agriculture at the University of São Paulo for conducting the analyses.

The Article Processing Charge for the publication of this research was funded by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) (ROR identifier: 00x0ma614).

Funding

This study was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), no grant identification number is available. No specific research funding was received for the experiment.

Conflicts of Interest

No potential conflicts of interest have been reported by the author(s).

References

Ajao, A. A., A. N. Moteetee, and A. Gray. 2017. "*Tithonia diversifolia* (Hemsl, Asteraceae: Heliantheae), Uma Planta Invasora De Significativa Importância Etnofarmacológica: Uma Revisão." *South African Journal of Botany* 113: 396–403. https://doi.org/10.1016/j.sajb.2017.09.017.

AOAC - Association of Official Analytical Chemistry. 1995. *Official Methods of Analysis* 16th, ed. AOAC International.

Bueno, A. V. I., G. L. D. Vigne, C. O. Novinski, C. Bayer, C. C. Jobim, and P. Schmidt. 2020. "Natamycin as a Potential Silage Additive: A Lab Trial Using Sugarcane to Assess Greenhouse Gas Emissions." *Revista Brasileira de Zootecnia* 49: e20200017. https://doi.org/10.37496/rbz4920200017.

Gutiérrez, D., A. Morales, A. Elías, R. G. García López, and L. Sarduy. 2014. "Chemical Composition and In Situ Ruminal Degradability of Dry Matter in Mixed Silages of *Tithonia diversifolia*: *Pennisetum purpureum* Cv. Cuba CT. 169, Inoculated with VITAFERT." *Cuban Journal of Agricultural Science* 48, no. 4: 379–385, https://cjascience.com/index.php/CJAS/article/view/567.

Heinritz, S. N., S. D. Martens, P. Avila, and S. Hoedtke. 2012. "O Efeito DA Adição de Inoculante e Sacarose na Qualidade da Silagem De Leguminosas Forrageiras Tropicais Com Ensilabilidade Variável." *Animal Feed Science and Technology* 174, no. 3-4: 201–210. https://doi.org/10.1016/j.anifeedsci.2012.03.017.

Holguín, V. A., M. Cuchillo, J. Mazabel, S. Quintero, and J. Mora-Delgado. 2017. "Valor Nutritivo e Estabilidade Aeróbica da Ensilagem De Pennisetum Purpureum Com Inclusões de *Tithonia diversifolia*." *Zootecnia Tropical* 35, no. 3-4: 163–170, https://ve.scielo.org/scielo.php?script=sci_arttext&pid=S0798-72692017000300009.

Instituto Nacional de Meteorologia (INMET). 2022. "Estado do Clima no Brasil em 2022." Brasília, DF: INMET, 2023. Disponível em: Portal INMET – Estado do Clima no Brasil em 2022. Acesso em: 1 jun. 2024.

Jobim, C. C., L. G. Nussio, R. A. Reis, and P. Schmidt. 2007. "Avanços Metodológicos na Avaliação da Qualidade da Forragem Conservada." *Revista Brasileira de Zootecnia* 36: 101–119. https://doi.org/10.1590/S1516-35982007001000013.

Junges, D., P. Schmidt, C. O. Novinski, and J. L. P. Daniel. 2013. "Aditivo Contendo Bactérias Homo e Heteroláticas NA Qualidade da Fermentação DA Silagem De Milho." *Acta Scientiarum. Animal Sciences* 35: 371–377. https://doi.org/10.4025/actascianimsci.v35i4.18833.

K. O. Marques, A. Jakelaitis, K. C. Guimarães, and L. S. Pereira. 2021. "Perfil Agronômico, Fermentativo e Bromatológico DA Silagem Obtida do Consórcio Entre Milho e Soja." *Research, Society and Development* 10, no. 1: e41410111925. https://doi.org/10.33448/rsd-v10i1.11925.

Kung L. Jr., R. D. Shaver, R. J. Grant, and R. J. Schmidt. 2018. "Revisão de Silagem: Interpretação de Componentes Químicos, Microbianos e Organolépticos de Silagens." *Journal of Dairy Science* 101, no. 5: 4020–4033. https://doi.org/10.3168/jds.2017-13909.

Kung, L., and N. K. Ranjit. 2001. "The Effect of Lactobacillus Buchneri and Other Additives on the Fermentation and Aerobic Stability of Barley Silage." *Journal of Dairy Science* 84: 1149–1155. https://doi.org/10.3168/jds.S0022-0302(01)74575-4.

Kung, L., D. B. Grieve, J. W. Thomas, and J. T. Huber. 1984. "Added Ammonia or Microbial Inocula for Fermentation and Nitrogenous Compounds of Alfalfa Ensiled at Various Percents of Dry Matter." *Journal of Dairy Science* 67: 299–306. https://doi.org/10.3168/jds.S0022-0302(84)81302-8.

McDonald, P., A. R. Henderson, and S. J. E. Heron. 1991. *The Biochemistry of Silage* 2nd, ed. 340. Chalcombe Publications.

Medina, M. G., D. E. García, M. E. González, L. J. Cova, and P. Moratinos. 2009. "Variables Morfo-Estructurales y de Calidad De LA Biomasa de *Tithonia diversifolia* en LA Etapa Inicial De Crecimiento." *Zootecnia Tropical* 27, no. 2: 121–134, https://ve.scielo.org/scielo.php?pid=S0798-72692009000200003&script=sci_arttext.

Morales, A., R. Rodríguez, D. Gutiérrez, A. Elías, S. Gómez, and L. Sarduy. 2016. "Avaliação da Inclusão De VITAFERT No Valor Nutritivo De Silagens De *Tithonia diversifolia e Pennisetum purpureum*." *Revista Cubana de Ciências Agrárias* 50, no. 4: 619–630, https://cjascience.com/index.php/CJAS/article/view/666.

Moran, J. P., Z. G. Weinberg, G. Ashbell, Y. Hen, and T. R. Owen . 1996. "A comparison of two methods for the evaluation of the aerobic stability of whole crop wheat silage." in *International Silage Conference Aberystwyth. Proceedings* 162–163. Vol. 11. University of Wales.

Muck R. E., E. M. G. Nadeau, T. A. McAllister, F. E. Contreras-Govea, M. C. Santos, and L. Kung Jr. 2018. "Silage Review: Recent Advances and Future Uses of Silage Additives." *Journal of Dairy Science* 101: 3980–4000. https://doi.org/10.3168/jds.2017-13839.

Okoye, C. O., Y. Wang, L. Gao, et al. 2023. "O Desempenho De Bactérias Do ácido láctico NA Produção De Silagem: Uma Revisão DA Biotecnologia Moderna Para Melhoria da Silagem." *Pesquisa Microbiológica* 266: 127212. https://doi.org/10.1016/j.micres.2022.127212.

Oliveira, J. S., J. E. C. Miranda, J. C. Carneiro, P. S. D. Oliveira, and V. M. A. Magalhães 2015. *Como Medir a Matéria Seca (MS%) Em Forragem Utilizando Forno De Micro-Ondas. Comunicado Técnico*. Embrapa Gado de Leite, Juiz de Fora. vol. 77.

Pereira, A. V., F. D. S. Ledo, M. J. F. Morenz, et al. 2016. "BRS Capiaçu: Cultivar De Capim-Elefante De Alto Rendimento Para Produção De Silagem.

Ribeiro, K. G., I. A. de Souza, J. P. S. Rigueira, A. S. Cezário, S. de Campos Valadares Filho, and O. G. Pereira. 2020. "Campo Grande Stylo and Elephant Grass Mixed Silages Treated with Microbial Inoculant." *Semina: Ciências Agrárias* 41, no. 5: 1729–1738. https://doi.org/10.5433/1679-0359.2020v41n5p1729.

Sosa, H. G. 2024. *Capim-Elefante e Titônia Para Produção De Silagem NA Região Do Alto Paraná DA Argentina (Dissertação De Mestrado)*. Universidade Tecnológica Federal Do Paraná, Campus Pato Branco.

Tendonkeng, F., B. Fogang Zogang, C. Sawa, B. Boukila, and E. T. Pamo. 2014. "Inclusion of *Tithonia diversifolia* in Multinutrient Blocks for West African Dwarf Goats Fed Brachiaria Straw." *Tropical Animal Health and Production* 46, no. 6: 981–986. https://doi.org/10.1007/s11250-014-0597-2.

Van Soest, P. J. 1994. *Nutritional Ecology of the Ruminant* 488. Cornell University Press. https://doi.org/10.7591/9781501732355.

Woolford, M. K. 1990. *The Silage Fermentation* 350. Marcel Dekker.

Zapata, A., and B. E. Silva. 2010. *Reconversión Ganadera y Sistemas Silvopastoriles en el Departamento de Risaralda y el Eje Cafetero de Colombia* 112. CARDER, CIPAV.https://cipav.org.co/sdm_downloads/sistemas-silvopastoriles-aspectos-teoricos-y-practicos/.