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Strategic Ensilage of Signal Grass Pastures in Two Seasons in a Tropical Region

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Abstract: In tropical regions, grass silage can be produced from the pasture in the rainy season to feed animals during the dry season. We evaluated the chemical composition and fermentation characteristics of ensiled signal grass (*Urochloa decumbens* Stapf. Basilisk) fertilized with nitrogen (N) or intercropped with calopo (*Calopogonium mucunoides* Desv.) with and without microbial inoculant. We used a 4 × 2 factorial scheme in a randomized block design, with two blocks and two treatments per block, considering $p > 0.05$. We studied signal grass fertilized with 0 kg ha^{-1} of N (0 N), 50 kg ha^{-1} of N (50 N), or 100 kg ha^{-1} of N (100 N), or intercropped with calopo legume (LEG), with (I) or without (WI) inoculant, in two seasons. During the dry–water transition, lower concentrations of butyric acid were observed in 50 N and LEG silages (2.77 and 2.55 g kg^{-1} dry matter, DM) (inoculated) compared to control (7.77 g kg^{-1} DM). During the water–dry transition, higher concentrations of crude protein were observed in 100 N and LEG silages (71.90 and 54.6 g kg^{-1} DM) than in 0 N (46.3 g kg^{-1} DM). The signal grass–calopo intercropping is an alternative to nitrogen fertilization, as it provides forage with a higher protein content and silage with satisfactory fermentative characteristics.

Keywords: *Calopogonium mucunoides*; chemical composition; microbial population; nitrogen fertilization; organic acids; *Urochloa decumbens*

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1. Introduction

One of the main limitations to pasture-based animal production in tropical regions is the variation in the growth of forage plants throughout the year owing to climate fluctuations. Based on this fact, grass silage produced from the pasture in the rainy season is an alternative for feeding animals in the dry season.

Species of *Urochloa* occupy extensive areas in tropical regions. However, high moisture and low soluble carbohydrate contents limit the fermentation of signal grass (*Urochloa decumbens* Staf. cv. Basilisk) during ensiling, which hinders a rapid decline in pH [1]. These characteristics enable fermentation by bacteria of the genus *Clostridium*, resulting in silages of reasonable quality [2].

The dry matter (DM) content and nutritive value of silage can be improved using additives (chemical, microbial, and moisture sequestrants) during ensiling [3]. Microbial additives aim to improve silage fermentation and inhibit the growth of microorganisms that are harmful to its quality [2].

Nitrogen stands out for maximizing the dry biomass productivity of grasses, the proportion of leaves, and forage accumulation [4]. Therefore, it is the main nutrient required for maintaining pasture productivity and quality [5]. However, the costs of chemical fertilizers for treating extensive pasture areas represent a high cost in the pasture animal production system. Thus, grass–legume intercropping provides direct benefits by increasing the nutritive value of forage [6], which is one of the most important factors in

reducing costs in animal production, and indirect benefits, such as replacing nitrogen in the soil over the years and improving the sustainability of the system [7].

Calopo (*Calopogonium mucunoides* Desv.) is a legume from the Brazilian Cerrado and is well adapted to acidic and low-fertility soils. Furthermore, it has good biological nitrogen fixation ability [8], which increases the availability of this nutrient for plant growth and the crude protein content for grazing.

Numerous studies have demonstrated that nitrogen fertilization affects the productivity and nutritive value of tropical grasses in monoculture as well as the effects obtained using grass–legume mixed pastures. However, studies on the effects of nitrogen fertilization on the quality of signal grass (*U. decumbens*) silages compared to those from intercropped pastures are lacking.

Therefore, we hypothesized that the supply of nitrogen via chemical fertilization or legume would positively alter the chemical composition of signal grass and, combined with the use of an inoculant, produce silages with better fermentative characteristics than without an inoculant. Then, our objective was to evaluate the fermentative characteristics and chemical composition of ensiled signal grass intercropped with calopo or fertilized with doses of nitrogen (50 or 100 kg ha^{-1}), treated or not with a microbial inoculant.

2. Materials and Methods

2.1. Location, Treatments, and Experimental Design

The experiment was conducted at the Laboratory of Forage Crops and Silage Microbiology, Animal Science Department, Universidade Federal de Viçosa, UFV, Viçosa Campus. The city is located in Minas Gerais State at 657 m altitude, geographically defined by the coordinates 20°45′20″ south latitude and 42°52′40″ west longitude. The climate type is Cwa, according to KÖEPPEN [9], with two seasons: from May to October (dry) and from November to April (rainy). The average annual precipitation is 1341 mm, and the maximum and minimum temperatures are 26.8 °C and 15.7 °C, respectively. The climatic conditions during the growth period of silage plants are presented in Table 1.

Table 1. Precipitation and temperature (T°) during growth periods of signal grass (*Urochloa decumbens* cv. Basilisk) (from post-grazing to ensiling date).

Growth Period	Item			
	Precipitation (mm)	Maximum T° (°C)	Mean T° (°C)	Minimum T° (°C)
1. Dry–water transition (27 June 2018 to 14 November 2018)	360.2	25.7	18.8	14.3
2. Water–dry transition (24 January 2019 to 28 March 2019)	286.8	29.9	22.9	18.8

The experimental area had 16 paddocks measuring 1300 m^2 each, containing signal grass (*U. decumbens*) that was established approximately 24 years before. In the rainy season, grazing management was conducted at a pre-grazing height of 25 cm and a post-grazing height of 15 cm, whereas deferred grazing was practiced in the dry season.

Soil correction with limestone and fertilization was performed as described by Chaves et al. [10]. Calopo was sown (4.8 kg ha^{-1} of pure live seeds) in the intercropping paddocks in December 2017. Urea was applied as the source of chemical nitrogen, on 6 March 2017 and 2018, before the pastures were sealed for deferred grazing.

The chemical composition of the forage before ensiling was evaluated using a randomized block design with four treatments: 0 N (signal grass without nitrogen fertilization), 50 N (signal grass fertilized with 50 kg ha^{-1} of N), 100 N (signal grass fertilized with 100 kg ha^{-1} of N), and LEG (signal grass intercropped with calopo), in two blocks with two treatments per block, totaling 16 experimental units.

Before ensiling, four samples were collected from the intercropped pastures, using 0.25 m^2 frames, to estimate the percentages of grass and legume, and 91% and 91.2% of

grass and 9.0% and 8.8% of calopo were found, on a dry matter basis, during the first (2018) and second (2019) growth periods, respectively.

In the first (2018) and second (2019) growing periods, the paddocks were not grazed for approximately 140 and 63 days, respectively, before ensiling. The average heights of the sward recorded at the time of harvest were 66.2, 68.9, 73.7, and 64.4 cm, and 74.9, 77.8, 77.4, and 80.5 cm for treatments 0 N, 50 N, 100 N, and LEG, in 2018 and 2019, respectively.

2.2. Silage Preparation and Experimental Design

Two silage production trials were carried out: in period 1, with ensilage in November 2018 (dry–water transition), and in period 2, with ensilage in March 2019 (water–dry transition). Forage from each paddock was harvested at 5 cm above the ground using a backpack brush cutter. The forage was crushed using a stationary forage machine to a 1.0–2.0 particle size. Approximately 16 kg of forage was harvested from each paddock, and the inoculant was applied to half of the forage.

The commercial inoculant (Sil-All 4 × 4, Lallemand Animal Nutrition®, Patos de Minas, MG, Brazil), containing *Lactiplantibacillus plantarum, Pediococcus acidilactici, Lactobacillus Ligilactobacillus salivarius,* and *Enterecoccus faecium* and four enzymes (amylase, cellulase, hemicellulase, and xylanase), was used. The inoculant was diluted in 100 mL water and sprayed onto the forage. The application rate was 10^5 log CFU g^{-1} forage, as recommended by the manufacturer. The same amount of water was applied to mounds that did not receive the inoculant. After homogenization, the forage was ensiled in silos (plastic buckets, 12 kg capacity) with a density of approximately 600 kg FM/m^3. The buckets were then sealed and stored at room temperature (24 °C) for 60 days.

The silages were evaluated using a 4 × 2 factorial scheme in a randomized block design, with two blocks and two treatments per block. We studied signal grass fertilized with 0 kg ha^{-1} of N (0 N), 50 kg ha^{-1} of N (50 N), 100 kg ha^{-1} of N (100 N), and intercropped with calopo (LEG), with (I) or without (WI) inoculant, in two seasons, totaling 32 experimental units for each trial.

2.3. Fermentative Characteristics

Forage pH before ensiling and silage pH were measured using a digital peagameter (Tecnal, Piracicaba, SP, Brazil) with an aqueous extract containing 25 g sample/225 mL Ringer Solution (Oxoid™, Hampshire, UK), as described by Kung Jr. [11]. A 15 mL aliquot of the aqueous extract was collected, filtered through Whatman 54 filter paper (Whatman, Florham, NJ, USA), and placed in tubes, to which 100 µL of sulfuric acid solution (50%) was added. Subsequently, the samples were stored frozen until analysis of lactic acid (LA), acetic acid (AA), propionic acid (PA), butyric acid (BA), and ethanol using a high-performance liquid chromatography model SPD-10 AVP device (Shimadzu®, Tokyo, Japan), with an ultraviolet detector set at a wavelength of 210 nm [12], and ammonia nitrogen (NH_3-N) [13].

2.4. Quantification of Microbial Populations

A 10 mL aliquot of the aqueous extract (25 g of sample/250 mL of sterile saline solution) was serially diluted, ranging from 10^{-2} to 10^{-7}, to quantify the population of microorganisms in the forage and silage before ensiling. Microorganisms were cultivated in sterile Petri dishes in a selective culture medium for three microbial groups: MRS agar (MerckKGaA, Darmstadt, Germany) was used to cultivate lactic acid bacteria (LAB), violet-red bile agar (Oxoid, Basingstoke, UK) to cultivate enterobacteria, and potato dextrose agar (PDA: Difco, São Paulo, SP, Brazil) added with 1.5% tartaric acid 10% (w/v) to cultivate molds and yeasts by using the pour-plate technique.

The plates were incubated in a TE-391 B.O.D. Incubator (Tecnal, Piracicaba, SP, Brazil) oven, with a temperature and period determined for each group of microorganisms: enterobacteria, 37 °C for 24 h; LAB, 37 °C for 48 h; and yeasts and molds, 25 °C for 72 and 120 h, respectively. At the end of the incubation time, counting was performed using a manual colony counter (Model CP 608; Phoenix Luferco, Araraquara, SP, Brazil). Plates with

30–300 colony-forming units (CFUs) were counted. For data evaluation and interpretation, the results were converted to a logarithmic scale (Log10 CFU).

2.5. Chemical Composition

Samples of approximately 300 g were collected and subjected to partial moisture removal in a forced air oven at 55 °C to evaluate the chemical composition of the silage and forage before ensiling. Subsequently, the samples were processed in a knife mill, type "Willey" (Tecnal, Piracicaba, SP, Brazil) with a 1 mm sieve, to be analyzed for dry matter (DM; method 934.01), crude protein (CP; method 984.13), and acid detergent fiber (ADF) and lignin content (method 973.18), according to AOAC [14]. Neutral detergent fiber (NDF) contents were determined with the addition of thermostable α-amylase, according to Mertens et al. [15]. The residues from the NDF and ADF were subjected to ash analysis [15] and nitrogenous compounds [16] to obtain the corrected NDFap, ADFap, and ADIN contents. The indigestible neutral detergent fiber content (iNDF) was determined according to the methodologies described by Detmann et al. [17], and the water-soluble carbohydrate contents were determined as described by Nelson [18].

2.6. Statistical Analysis

The data obtained for forage before ensiling were analyzed in a randomized block design, with two blocks and two treatments per block, and subjected to analysis of variance (ANOVA), and the means were compared using Tukey's test. Silage data were analyzed using a factorial scheme (4 × 2) in a randomized block design, with two blocks and two treatments per block. The different management strategies, inoculants, and interactions between the factors were considered fixed effects and the blocks and the errors were considered random effects in the model. After analysis of the data variance, the significant interactions between the factors were broken down, and the means were compared using the F and Tukey tests, using PROC MIXED in SAS version 9.4 (SAS Institute Inc., Cary, NC, USA), adopting 0.05 as the critical probability level for type I error.

3. Results

3.1. Trial 1: Ensilage in November 2018 (Dry–Water Transition)

3.1.1. Chemical Composition and Microbial Population of Forage before Ensiling

The effects of management on the chemical composition, microbial population, and pH of the signal grass before ensiling are presented in Table 2. The variables DM (p = 0.5801), ADF (p = 0.3331), WSC (p = 0.7665), enterobacteria (p = 0.4203), and pH (p = 0.6442) were unaffected ($p > 0.05$) by the treatments. In contrast, CP levels (p = 0.0002), NDFap (p = 0.0095), NDIN (p = 0.0025), ADIN (p = 0.0235), and the populations of LAB ($p < 0.0001$), yeast (p = 0.6670), and mold (p = 0.6469) were affected ($p < 0.05$) by pasture management (Table 2).

The average DM and WSC contents of the forage were 213 g kg^{-1} of FM and 22.71 g kg^{-1} of DM, respectively. The LEG treatment provided a higher CP content in the signal grass (106 g kg^{-1} DM) than the other treatments. Lower NDFap concentrations were obtained in LEG than in the 50 N and 100 N treatments. All management strategies using a nitrogen source increased the NDIN concentration. Higher concentrations of ADIN were obtained in LEG than in the 0 N and 100 N treatments.

The treatments did not affect forage pH before ensiling, with an average value of 5.97. All N-source treatments increased the LAB population relative to that of the control. The enterobacteria, yeast, and mold populations were unaffected ($p > 0.05$) by the treatments, with averages of 7.27, 5.64, and 5.50 log CFU g^{-1} of FM, respectively.

Table 2. Chemical composition, microbial population, and pH of signal grass with different managements before ensiling (dry–water transition).

Item [1]	Management [2]				SEM [3]	*p*-Value
	0 N	50 N	100 N	LEG		
DM (g kg^{-1} FM)	220	206	213	214	3.8136	0.5801
CP (g kg^{-1} DM)	66.5 ^{b}	73.5 ^{b}	80.7 ^{b}	106 ^{a}	2.4916	0.0002
NDFap (g kg^{-1} DM)	653 ^{ab}	662 ^{a}	674 ^{a}	621 ^{b}	6.5540	0.0095
ADF (g kg^{-1} DM)	367	367	384	368	3.7312	0.3331
WSC (g kg^{-1} DM)	21.3	22.3	23,3	24.1	0.9530	0.7665
NDIN (%TN)	19.3 ^{b}	25.1 ^{a}	25.7 ^{a}	23.1 ^{a}	0.7961	0.0025
ADIN (%TN)	6.78 ^{b}	7.31 ^{ab}	6.90 ^{b}	8.51 ^{a}	0.2375	0.0235
LAB (log CFU g^{-1} FM)	5.53 ^{c}	6.55 ^{a}	6.18 ^{b}	6.20 ^{b}	0.0966	<0.001
ENT (log CFU g^{-1} FM)	7.18	7.64	7.16	7.09	0.1368	0.4203
Yeast (log CFU g^{-1} FM)	5.54	5.59	5.59	5.85	0.0871	0.6670
Mold (log CFU g^{-1} FM)	5.50	5.41	5.58	5.52	0.0778	0.6469
pH	5.85	6.05	5.92	6.04	0.0671	0.6442

[1] DM, dry matter; FM, fresh material; CP, crude protein; NDFap, neutral detergent fiber corrected for ash and protein; ADF, acid detergent fiber; WSC, water-soluble carbohydrates; NDIN, neutral detergent insoluble nitrogen; ADIN, acid detergent insoluble nitrogen; TN, total nitrogen; LAB, lactic acid bacteria; ENT, enterobacteria. [2] 0 N, without nitrogen fertilizer; 50 N, 50 kg ha^{-1} of N; 100 N, 100 kg ha^{-1} of N; LEG, signal grass–calopo intercropping. [3] SEM, standard error of the mean. Means followed by the same letter in the line do not differ ($p > 0.05$) from each other using the Tukey test.

3.1.2. Fermentation Characteristics and Microbial Population of Silages

There was an effect of the management (M) × inoculant (I) interaction ($p < 0.05$) for the acetic acid ($p = 0.0275$), butyric acid ($p = 0.0008$), ethanol (0.0096), and NH_3-N (0.0215) contents. In contrast, pH and lactic acid (LA) were unaffected ($p > 0.05$) by the treatments studied, with average values of 4.86 and 24.6 g kg^{-1} DM, respectively (Table 3).

Table 3. Fermentation characteristics (g kg^{-1} MS) and microbial population (log CFU g^{-1} FM) of signal grass silages under different managements, with and without microbial inoculant (dry–water transition).

Inoculant [1]	Management [2]	Item [3]							
		pH	LA	AA	BA	ET	NH_3-N (%TN)	LAB	ENT
WI	0 N	4.90	21.5	13.5 ^{bA}	7.90 ^{aA}	10.38 ^{aA}	13.7 ^{bcA}	7.63	3.17
	50 N	4.85	26.9	14.8 ^{abA}	5.97 ^{aA}	9.41 ^{abA}	12.5 ^{cB}	8.08	3.91
	100 N	4.77	20.9	20.0 ^{aA}	2.33 ^{bB}	6.47 ^{bA}	18.1 ^{aA}	7.98	ND
	LEG	4.90	24.3	17.8 ^{abA}	7.60 ^{aA}	10.75 ^{aA}	16.0 ^{abA}	7.54	3.04
I	0 N	4.91	27.9	13.4 ^{bA}	7.77 ^{aA}	7.85 ^{aB}	13.4 ^{bA}	8.00	2.58
	50 N	4.86	22.9	15.5 ^{abA}	2.77 ^{bB}	4.88 ^{bB}	16.7 ^{aA}	7.88	2.84
	100 N	4.95	24.8	12.8 ^{bB}	4.87 ^{abA}	7.65 ^{abA}	16.9 ^{aA}	7.62	3.41
	LEG	4.76	27.6	20.3 ^{aA}	2.55 ^{bB}	7.50 ^{abB}	16.9 ^{aA}	8.03	2.98
SEM [1]		0.0646	1.331	1.0998	0.6616	0.5945	0.4499	0.127	0.204
General average for inoculant (I)									
WI		4.85	23.10	16.46	5.94	9.25	15.06	7.81	3.37 ^{A}
I		4.87	26.00	15.33	4.48	6.97	15.96	7.87	2.91 ^{B}
General average for management (M)									
0 N		4.90	24.69	13.44	7.83	9.11	13.51	7.81	2.82
50 N		4.86	24.90	15.07	4.37	7.14	14.56	7.99	3.30
100 N		4.86	22.57	16.96	3.59	7.06	17.50	7.80	3.41
LEG		4.83	25.93	19.08	5.07	9.12	16.46	7.79	3.01
p-value									
I		0.8384	0.1762	0.3672	0.0188	0.0005	0.1319	0.613	0.007
M		0.9199	0.5868	0.0130	0.0004	0.0127	0.0002	0.780	0.102
M × I		0.5875	0.1563	0.0275	0.0008	0.0096	0.0215	0.166	0.127

[1] WI, without inoculant; I, with inoculant; SEM, mean standard error; M, management; and M × I, management x inoculant interaction. [2] 0 N, without nitrogen fertilization; 50 N, 50 kg ha^{-1} of N; 100 N, 100 kg ha^{-1} of N; LEG, signal grass–calopo intercropping. [3] LA, lactic acid; AA, acetic acid; BA, butyric acid; ET, ethanol; NH_3-N, ammonia nitrogen; TN, total nitrogen; LAB, lactic acid bacteria; ENT, enterobacteria. Means followed by the same lowercase letter in the management column do not differ ($p > 0.05$) from each other by the Tukey test, and capital letters in the inoculant column do not differ ($p > 0.05$) from each other by the F test.

In non-inoculated silages, management with 100 N provided an acetic acid content (AA) higher than 0 N but similar to that of the 50 N and LEG treatments. In contrast, a higher AA content was observed in inoculated LEG silages, without differing from that obtained with 50 N. When evaluating the effect of using inoculants in each management system, there was a reduction in the AA concentration (20.0 to 12.8 g kg^{-1} DM) only with 100 N.

Lower butyric acid (BA) concentrations and ethanol production were obtained only with 100 N among the non-inoculated silages. In the inoculated silages, both 50 N and LEG reduced BA concentration compared to that obtained with 0 N, and there was a reduction in ethanol production with 50 N. The use of the inoculant reduced ($p < 0.05$) the BA concentrations in the 50 N and LEG treatments and the ethanol (ET) concentrations in the 0 N, 50 N, and LEG treatments compared to those obtained with the non-inoculated silages.

Using 100 N in the non-inoculated silages resulted in a higher concentration of NH_3-N than with the 0 N treatment. However, management with chemical N or LEG resulted in higher concentrations of ammonia than those in the control.

The LAB population was unaffected ($p > 0.05$) by the treatments, whereas the use of an inoculant reduced ($p = 0.0077$) the enterobacteria population. There were no mold or yeast populations in the silages evaluated in this study.

3.1.3. Chemical Composition of Silages

There were effects of the M × I interaction for NDIN ($p = 0.0375$) and ADIN ($p = 0.0110$) and an effect of management on CP ($p = 0.0547$) and NDFap ($p = 0.0309$). The DM, ADF, and iNDF concentrations were unaffected ($p > 0.05$) by the factors evaluated (Table 4).

Higher CP content was obtained in the LEG silage than in the 0 N silage, and lower NDFap content was observed with 100 N, compared with 50 N.

Table 4. Chemical composition (g kg^{-1} DM) of signal grass silages under different managements, with or without microbial inoculant (dry–water transition).

Inoculant [1]	Management [2]	Item [3]						
		DM (g kg^{-1} FM)	CP	NDFap	ADF	NDIN (%TN)	ADIN (%TN)	iNDF
WI	0 N	202	45.8	651	380	18.3 ^{aB}	7.96 ^{aB}	202.30
	50 N	205	47.7	640	384	17.6 ^{aA}	10.4 ^{aA}	202.90
	100 N	189	51.9	656	380	21.9 ^{aA}	9.97 ^{aB}	189.06
	LEG	212	52.6	640	385	18.3 ^{aA}	8.83 ^{aA}	221.97
I	0 N	205	45.8	665	394	22.9 ^{aA}	11.4 ^{bA}	201.15
	50 N	207	50.6	679	386	17.5 ^{bA}	9.97 ^{bA}	194.05
	100 N	182	48.7	636	386	22.4 ^{aA}	15.2 ^{aA}	196.36
	LEG	217	53.4	642	384	16.8 ^{bA}	8.96 ^{bA}	191.70
SEM [1]		6.8256	1.2053	5.1616	2.2901	0.8888	0.8469	0.5350
General average for inoculant (I)								
WI		202	49.5	647	382	19.0	9.30	205
I		203	49.6	656	388	19.9	11.4	196
General average for management (M)								
0 N		203	45.8 ^{b}	658 ^{ab}	387	20.6	9.69	202
50 N		206	49.2 ^{ab}	668 ^{a}	385	17.5	10.2	198
100 N		186	50.3 ^{ab}	638 ^{b}	383	22.1	12.6	193
LEG		214	52.9 ^{a}	641 ^{ab}	385	18.4	8.90	207
p-value								
I		0.9022	0.9576	0.2509	0.2968	0.2705	0.0026	0.1713
M		0.0967	0.0547	0.0309	0.9464	0.0008	0.0031	0.4371
M × I		0.9439	0.6587	0.6011	0.7256	0.0375	0.0110	0.1696

[1] WI, without inoculant; I, with inoculant; SEM, mean standard error; M, management; and M × I, management x inoculant interaction. [2] 0 N, without nitrogen fertilization; 50 N, 50 kg ha^{-1} of N; 100 N, 100 kg ha^{-1} of N; LEG, signal grass–calopo intercropping. [3] DM, dry matter; FM, fresh material; CP, crude protein; NDFap, neutral detergent fiber corrected for ash and protein; ADF, acid detergent fiber; NDIN, neutral detergent insoluble nitrogen; ADIN, acid detergent insoluble nitrogen; TN, total nitrogen; iNDF, indigestible neutral detergent fiber. Means followed by the same lowercase letter in the management column do not differ ($p > 0.05$) from each other using the Tukey test, and capital letters in the inoculant column do not differ ($p > 0.05$) from each other by the F test.

In the inoculated silages, a lower NDIN content was obtained in the 50 N and LEG silages compared to the 0 N and 100 N treatments, while a higher ADIN content was observed in the 100 N treatment compared to the others. The use of the inoculant increased the NDIN content at 0 N and the ADIN content at 0 N and 100 N.

3.2. Trial 2: Ensilage in March 2019 (Water–Dry Transition)

3.2.1. Chemical Composition and Microbial Population before Ensiling

Management affected ($p < 0.05$) the concentrations of DM ($p = 0.0016$), CP ($p < 0.0001$), ADIN ($p < 0.0001$), LAB ($p = 0.00031$), yeast ($p = 0.0407$), and mold ($p = 0.0692$) populations (Table 5). Treatment with an N source reduced the DM content; however, it increased the CP content of signal grass, which was higher in LEG, and reduced ADIN levels compared with those of the control. Management did not affect NDFap, ADF, WSC, or NDIN, with average values of 647, 378, and 22.3 g kg^{-1} of DM and 23.8%, respectively.

Management did not affect the pH and the population of enterobacteria in the forage before ensiling, with recorded values of 6.1 and 7.32 log CFU g^{-1} of fresh material (Table 5), respectively. A higher LAB population was observed in the 100 N and LEG treatments than in the 0 N and 50 N treatments, which presented higher yeast populations than the 0 N and 50 N treatments. Lower mold populations were recorded in the 100 N and LEG treatments than in the 0 N treatment.

Table 5. Chemical composition, microbial population, and pH of forage with different managements before ensiling (water–dry transition).

Item [1]	Management [2]				SEM [3]	*p*-Value
	0 N	50 N	100 N	LEG		
DM (g kg^{-1} FM)	264 ^{a}	233 ^{b}	211 ^{b}	218 ^{b}	6.2796	0.0016
CP (g kg^{-1} DM)	48.9 ^{d}	62.9 ^{c}	76.5 ^{b}	93.5 ^{a}	2.8344	<0.0001
NDFap (g kg^{-1} DM)	665	648	648	628	5.5211	0.1314
ADF (g kg^{-1} DM)	383	380	373	376	3.0402	0.7221
WSC (g kg^{-1} DM)	21.9	24.7	24.5	18.1	1.0562	0.0749
NDIN (%TN)	23.9	23.4	22.8	24.9	0.5604	0.6816
ADIN (%TN)	11.9 ^{a}	7.32 ^{c}	8.87 ^{b}	8.77 ^{b}	0.4453	<0.0001
LAB (log CFU g^{-1} FM)	7.47 ^{bc}	7.00 ^{c}	8.06 ^{a}	7.80 ^{a}	0.1226	0.0031
ENT (log CFU g^{-1} FM)	7.17	6.98	7.62	7.49	0.1034	0.0959
Yeast (log CFU g^{-1} FM)	6.02 ^{a}	5.89 ^{a}	5.72 ^{b}	5.67 ^{b}	0.0407	0.0004
Mold (log CFU g^{-1} FM)	5.64 ^{a}	5.52 ^{ab}	5.08 ^{c}	5.29 ^{bc}	0.0692	0.0063
pH	6.03	6.03	6.16	6.16	0.0491	0.6445

[1] DM, dry matter; FM, fresh material; CP, crude protein; NDFap, neutral detergent fiber corrected for ash and protein; ADF, acid detergent fiber; WSC, water-soluble carbohydrates; NDIN, neutral detergent insoluble nitrogen; ADIN, acid detergent insoluble nitrogen; TN, total nitrogen; LAB, lactic acid bacteria; ENT, enterobacteria. [2] 0 N, without nitrogen fertilizer; 50 N, 50 kg ha^{-1} of N; 100 N, 100 kg ha^{-1} of N; LEG, signal grass–calopo intercropping. [3] SEM, standard error of the mean. Means followed by the same letter in the line do not differ ($p > 0.05$) from each other using the Tukey test.

3.2.2. Fermentation Characteristics and Microbial Population of Silages

There was no effect ($p > 0.05$) of the M × I interaction on the fermentation characteristics or microbial populations of the silages (Table 6).

The LA ($p = 0.0196$) and AA ($p = 0.0113$) contents were affected ($p < 0.05$) only by management (M) (Table 6). The lowest concentration of LA was observed with 50 N, compared to the other treatments. Silage with 0 N presented an AA concentration similar to that with the 50 N and 100 N treatments and a lower concentration compared to that with the LEG treatment.

The pH, BA, ET, and NH_3-N were unaffected ($p > 0.05$) by the factors evaluated, with average values of 4.94, 5.69, and 4.62 g kg^{-1} DM and 17.32%, respectively (Table 6).

Only the mold population was affected ($p = 0.0300$) by the use of the inoculant, with a lower population recorded in the inoculated silages. The LAB, ENT, and YST

populations were unaffected ($p > 0.05$) by the treatments evaluated, with average values of 7.92, 4.14, and 3.54 log CFU g^{-1} of FM, respectively (Table 6).

Table 6. Fermentation characteristics (g kg^{-1} MS) and microbial population (log CFU g^{-1} of FM) of signal grass silages under different managements, with or without microbial inoculant (water–dry transition).

Inoculant [1]	Management [2]	Item [3]							
		pH	LA	AA	BA	ET	NH_3-N (%TN)	LAB	ENT
WI	0 N	4.90	26.2	13.5	6.11	3.33	17.3	8.02	4.06
	50 N	4.99	22.9	17.6	5.44	4.95	16.1	7.99	3.78
	100 N	4.74	32.6	16.6	6.41	3.73	18.4	7.86	4.50
	LEG	5.08	29.1	20.9	6.14	5.40	18.9	7.82	4.95
I	0 N	4.86	29.1	14.5	3.99	3.48	15.1	7.99	3.64
	50 N	5.04	19.2	14.6	3.76	4.43	15.9	7.93	4.18
	100 N	4.86	26.1	16.9	5.98	4.90	19.5	7.86	4.16
	LEG	5.04	28.4	21.6	7.81	6.60	17.1	7.88	3.84
SEM [1]		0.0724	1.3673	1.0899	0.5612	0.4951	0.6424	0.0525	0.2854
General average for inoculant (I)									
WI		4.93	27.4	17.0	6.02	4.35	17.7	7.92	4.32
I		4.95	25.7	16.8	5.38	4.85	16.9	7.92	3.95
General average for management (M)									
0 N		4.88	27.7 [a]	13.8 [b]	5.05	3.40	16.2	8.00	3.85
50 N		5.01	21.1 [b]	16.3 [ab]	4.60	4.69	16.0	7.96	3.98
100 N		4.80	29.9 [a]	16.7 [ab]	6.19	4.32	18.9	7.86	4.32
LEG		5.06	27.7 [a]	20.8 [a]	6.97	5.99	18.0	7.85	4.39
p-value									
I		0.7622	0.3495	0.8492	0.5510	0.5657	0.4121	0.9394	0.3785
M		0.0879	0.0196	0.0113	0.3944	0.2308	0.0940	0.3725	0.7294
M × I		0.8694	0.2334	0.6766	0.5958	0.8567	0.5088	0.9203	0.6111

[1] WI, without inoculant; I, with inoculant; SEM, mean standard error; M, management and M × I, management x inoculant interaction. [2] 0 N, without nitrogen fertilization; 50 N, 50 kg ha^{-1} of N; 100 N, 100 kg ha^{-1} of N; LEG, signal grass–calopo intercropping. [3] LA, lactic acid; AA, acetic acid; BA, butyric acid; ET, ethanol; NH_3-N, ammonia nitrogen; TN, total nitrogen; LAB, lactic acid bacteria; ENT, enterobacteria; FM, fresh material. Means followed by the same lowercase letter in the column do not differ ($p > 0.05$) from each other by the Tukey test.

3.2.3. Chemical Composition of Silages

There was no effect ($p > 0.05$) of M × I interaction on the chemical composition of the silages (Table 7). The average DM content of the silages was 225 g kg^{-1} FM.

The CP contents observed in the silages were affected by the use of an inoculant (I) ($p = 0.0403$) and management (M) ($p < 0.0001$). The highest CP content was recorded in inoculated silage compared to non-inoculated silage. The highest value observed for management was for the 100 N treatment.

The concentrations of NDFap ($p = 0.0073$) and ADF ($p = 0.0225$) in the silage were only affected by management (M). The 0 N silages presented higher fiber concentrations only in relation to the 100 N treatment. Moreover, we observed an effect ($p < 0.05$) of management on the iNDF content of silages, with a lower value only at 100 N, compared to that with 0 N (Table 7).

The NDIN variable was affected ($p < 0.0001$) by management, with a lower value observed in the 100 N silages than in the others (Table 7). The concentration of ADIN in the silage was affected by management ($p < 0.0001$) and inoculants ($p = 0.0011$). The lowest ADIN content was observed in the inoculated silage, whereas the 100 N treatment resulted in silage with a lower concentration of ADIN compared with that of the other treatments.

Table 7. Chemical composition (g kg^{-1} DM) of signal grass silages under different managements, with or without microbial inoculant (water–dry transition).

Inoculant [1]	Management [2]	Item [3]						
		DM (g kg^{-1} FM)	CP	NDFap	ADF	NDIN (%TN)	ADIN (%TN)	iNDF
WI	0 N	248	44.0	676	399	18.4	8.98	21.7
	50 N	227	48.4	661	381	22.5	9.86	21.2
	100 N	214	69.6	635	381	13.4	7.44	19.1
	LEG	215	53.7	639	393	19.5	11.1	19.7
I	0 N	243	48.7	699	399	17.1	7.64	21.4
	50 N	220	55.8	663	390	19.8	8.78	21.8
	100 N	214	74.3	634	376	13.0	6.02	18.7
	LEG	221	55.5	671	394	18.9	10.7	20.7
SEM [1]		5.5876	2.6594	6.4942	2.9545	0.8807	0.6042	0.5320
General average for inoculant (I)								
WI		226	53.9 ^{B}	653	388	18.4	9.35 ^{A}	20.4
I		225	58.6 ^{A}	667	390	17.2	8.29 ^{B}	20.7
General average for management (M)								
0 N		245	46.3 ^{c}	688 ^{a}	399 ^{a}	17.7 ^{b}	8.21 ^{b}	21.6 ^{a}
50 N		224	52.1 ^{bc}	662 ^{ab}	386 ^{ab}	21.2 ^{a}	9.32 ^{b}	21.5 ^{ab}
100 N		214	71.9 ^{a}	635 ^{b}	378 ^{b}	13.2 ^{c}	6.73 ^{c}	18.9 ^{b}
LEG		218	54.6 ^{b}	655 ^{ab}	394 ^{ab}	19.2 ^{ab}	10.9 ^{a}	20.2 ^{ab}
p-value								
I		0.8888	0.04043	0.1522	0.7367	0.0908	0.0021	0.7268
M		0.7765	<0.0001	0.0073	0.0225	<0.0001	<0.0001	0.0297
M × I		0.9620	0.8486	0.5316	0.7557	0.6164	0.6607	0.8816

[1] WI, without inoculant; I, with inoculant; SEM, mean standard error; M, management and M × I, management x inoculant interaction. [2] 0 N, without nitrogen fertilization; 50 N, 50 kg ha^{-1} of N; 100 N, 100 kg ha^{-1} of N; LEG, signal grass–calopo intercropping [3] DM, dry matter; FM, fresh material; CP, crude protein; NDFap, neutral detergent fiber corrected for ash and protein; ADF, acid detergent fiber; NDIN, neutral detergent insoluble nitrogen; ADIN, acid detergent insoluble nitrogen; TN, total nitrogen; iNDF, indigestible neutral detergent fiber. Means followed by the same lowercase letter in the column comparing managements do not differ ($p > 0.05$) from each other using the Tukey test, and a capital letters in the column comparing inoculants do not differ ($p > 0.05$) from each other by the F test.

4. Discussion

4.1. Chemical Composition and Microbial Population before Ensiling

At the time of ensiling, forage DM concentration is an important factor affecting fermentation and silage quality. In the first growth period (Table 2), forage DM concentrations were below those recommended for adequate fermentation (25–35% DM) [19]. In the second period, the 50 N, 100 N, and LEG treatments reduced the DM content of plants (Table 5), which was associated with the stimulation of new tissue and leaf production by nitrogen [20]. The DM content presented by the control before ensiling was close to the acceptable range for ensiling, although it had the lowest CP and highest ADIN contents. It could be recommended to wilt the material before ensiling to increase the dry matter concentration of grass silage; however, this could increase silage losses and production costs.

Nitrogen is an essential constituent of plant proteins and chlorophyll [21]. Several studies have demonstrated the efficiency of nitrogen from chemical fertilization [22,23] or the inclusion of legumes [24] in increasing the concentration of CP in tropical grass pastures. In our study, pastures fertilized with 50 and 100 kg ha^{-1} of N presented a higher protein content than unfertilized pastures in the second period of this study, indicating the benefit of nitrogen fertilizer application several months before harvesting forage for silage.

Signal grass–calopo intercropping had a higher protein content than unfertilized pastures and those fertilized with nitrogen during both periods (Tables 2 and 5). This result could be attributed to the participation of calopo, a legume with high biological nitrogen

fixation [8], which may have contributed to increasing the availability of N for the grass during intercropping, even at a low percentage in pastures.

The low proportions of calopo in the intercropped pastures (9.0% and 8.8% in the first and second periods, respectively) may have occurred because the signal grass suppressed legume growth. Even so, intercropping resulted in a higher protein content up to 15 months after sowing calopo, indicating a longer-term benefit when compared to pastures fertilized with two doses of N and pastures without any nitrogen source.

Regarding fibers, the NDFap and ADF concentrations were within the expected range for perennial grasses in a tropical climate, which is higher than that for grasses in a temperate climate.

The WSC concentrations in the signal grass did not vary with management during either period. However, a linear reduction in soluble carbohydrate contents was observed with increasing N doses in several studies [23,25]. The initial concentration of WSC in the forage affects the decrease in the pH of the ensiled material directly because WSC is the main substrate used by homo- and heterofermentative bacteria, which produce lactic acid and other organic acids during ensiling [26].

Therefore, a low concentration of WSC in plants intended for ensiling is not desirable, given that its fermentation products, mainly LA, are essential for decreasing the pH and preserving the silage in the silo. In the present study, WSC concentrations in both periods were much lower than the 60–80 g kg^{-1} DM recommended by Woolford [27] to ensure adequate fermentation inside the silo. This result can be attributed to the characteristics of tropical grasses, which present low concentrations of WSC for fermentative processes at the time of ensiling [1].

Among microbial groups, the LAB population is of the greatest interest during the fermentation process because they are responsible for lactic acid production. Therefore, the epiphytic population of these microorganisms can affect the ensiling process [3,19]. Although treatments with an N source increased the LAB population in both periods, there are no explanations based on the literature for how nitrogen fertilization can affect the population of these microorganisms.

4.2. Fermentation Characteristics and Microbial Populations of Silages

The inoculant did not efficiently reduce the pH of the silages during either experimental period. Moreover, Rigueira et al. [28] did not observe an effect of the inoculant on the pH of Marandu grass (*Urochloa brizantha*) silages with and without the inclusion of different proportions of *Stylosanthes* cv. Campo Grande (*S. capitata* + *S. macrocephala*) (10, 20, and 30%). On average, the pH values of the silages in our study (Tables 3 and 6) were higher than the pH range (4.3–4.7) obtained for perennial grasses by Kung Jr. et al. [19].

The pH depends on the amount of organic acid produced by the microorganisms present in the silage, and its rapid decrease in the ensiled material, associated with the anaerobic conditions inside the silo, enables the silage to be stored without deterioration for long periods. LA is the strongest organic acid that contributes most to reducing pH and preserving silage, whereas AA, BA, and NH_3-N contribute to a higher pH [19]. Therefore, the high pH values observed in our study could be attributed to the low LA and high NH_3-N contents (Tables 3 and 6). Results similar to those of our study were observed by Silva et al. [29], who did not find any effect of microbial inoculants on the LA concentration.

The rapid growth and dominance of fermentation by LAB inoculants depend on factors such as the ability of the inoculated bacteria to grow in the silo, the ideal amount of substrate, and the ability of the inoculant bacteria to compete with the population of epiphytic bacteria [30]. In our study, the bacteria in the inoculant did not surpass the natural LAB population, as the factors evaluated did not affect the LAB population in the silage (Tables 3 and 6). This result may be related to the low WSC contents observed in the signal grass during the two experimental periods (Tables 2 and 5), which may have limited the growth of the LAB population from the microbial inoculant, as well as from the forage.

Insufficient concentrations of WSC resulted in low LA production and high pH, as seen in Tables 3 and 6, which enables the growth of undesirable microorganisms, such as *Clostridium* [31], which produces BA and excess NH_3-N, among other undesirable products in silage. Regardless of low DM and WSC contents and high pH values in the two experimental periods, the concentrations of BA were within the range for perennial grass silages [19]. The decrease in the population of enterobacteria in the inoculated silages, and also the BA content (50 N and LEG) and the AA content (100 N), indicate an action of the inoculant in suppressing undesirable microorganisms, such as *Clostridium*, which harm the quality of the silage.

Acetic acid concentrations of up to 30 g kg^{-1} DM are acceptable [19] because AA has antifungal characteristics and can reduce the yeast population in silage [19,32]. Thus, the average value of AA, 16.02 g kg^{1} DM, in the dry–rainy period (2018) appears to have been sufficient to control mold and yeast (Table 3).

4.3. Chemical Composition of Silages

The preservation of forage nutrients indicates good fermentation quality in the silo [33]. Typically, more than half of the true protein is fermented by microorganisms and plant proteolytic enzymes into non-protein nitrogen during ensiling, leading to the inefficient use of silage nitrogen by animals [3]. The higher CP contents in the inoculated silages in the second period of our study (Table 7) demonstrated the effect of the microbial inoculant in reducing proteolysis by spoilage microorganisms, according to Kung Jr. et al. [19].

In the dry–water transition, management with LEG provided a higher crude protein content in the silage, although without differing from treatment with 100 N and 50 N, while management with 100 N decreased the NDF content in relation to 50 N (Table 4). In the water–dry transition, management with 100 N provided the highest crude protein content in the silage and decreased the NDF content in relation to 0 N (Table 7). These results can be attributed to the positive effect of nitrogen in increasing protein content and causing a dilution effect on fiber content.

In the water–dry transition, the reduction in NDFi, observed in 100 N signal grass silages, shows the benefit of nitrogen in reducing the indigestible part of the fiber, improving the availability of energy in the forage. In a previous study, Leite et al. [23] observed a linear reduction in NDFi in Marandu grass fertilized with increasing doses of nitrogen. All NDFi values observed in our study were lower than 270 g/kg DM, considered a limit value for maintaining the quality of signal grass, without compromising the consumption and performance of the animals [34].

5. Conclusions

Signal grass intercropped with calopo provides a better chemical composition before ensiling than other systems.

The silage of signal grass intercropped with calopo or fertilized with nitrogen, especially when inoculated, has a higher protein content and better fermentative characteristics, and can be produced from surplus forage in different seasons of the year in tropical pastures.

Microbial inoculants suppress the spoilage microorganisms' growth and subsequent products that reduce silage quality.

The effectiveness of these practices may vary depending on specific conditions; further research and practical evaluations are needed to confirm their viability in different contexts.

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