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Effect of wilting time and enzymatic-bacterial inoculant on the fermentative profile, aerobic stability, and nutritional value of BRS capiaçu grass silage

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ABSTRACT - The objective of this study was to evaluate the effects of wilting times and application of an enzymatic-bacterial inoculant on the fermentative profile and nutritional characteristics of BRS capiaçu grass silage in a semi-arid region. Four wilting times treatments (control, 6, 24, and 30 h), with or without the addition of an enzymatic-bacterial inoculant, were analyzed as a split-plot completely randomized design with eight replications. Parameters of the rumen degradability test were analyzed using a split-plot completely randomized block design with four replications. There was no interaction between wilting times and inoculant application on pH, ammoniacal nitrogen (NH_3-N), and aerobic stability of BRS capiaçu silage. Aerobic stability was reduced by 1.2 h for every 1-h increase in wilting time. Inoculant application reduced the pH values by 2.59% and extended the aerobic stability of the silage by 19 h. There was a significant interaction of wilting times and inoculant application on the levels of malic, succinic, lactic, and acetic acids. Inoculant application increased the contents of dry matter, ash, crude protein, insoluble neutral detergent fiber, and total carbohydrates by 3.63, 6.13, 7.73, 6.39, and 9.97% compared with non-inoculated silages, respectively. Wilting times for up to 30 h and application of enzymatic-bacterial inoculant improves the fermentative profile and chemical composition and reduces dry matter losses of silage of BRS capiaçu grass harvested at 100 days of regrowth.

Keywords: enzyme complex, *Lactobacillus buchineri*, *Pennisetum purpureum*, semi-arid, volatile organic compounds

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1. Introduction

Under adequate agronomic management, the BRS capiaçu grass (*Pennisetum purpureum* Schum.) produces high amounts of dry matter (DM) per unit area (above 45 t ha^{-1}) with good nutritive value (i.e., 70-80 g kg^{-1} crude protein as fed and 500 g kg^{-1} digestibility of DM as fed; Pereira et al., 2017; Monção et al., 2019, 2020). The BRS capiaçu grass, released at the end of 2015 by Embrapa Gado de Leite, is one of the most productive tropical forages in the world and has been used by cattle farmers in Brazil, mainly for silage production. Even harvesting BRS capiaçu grass at the recommended age for silage production (90-120 d; Pereira et al., 2017; Monção et al., 2019), the low DM content of the forage

(<200 g kg^{-1} as fed) can result in DM losses, which is not recommended for grass-based silages (Kung Jr. et al., 2018). Ensiling high-moisture forages increases the risk of butyric acid fermentation and effluent production, thereby resulting in high fermentative losses, which compromises the quality and nutritive value of the silage (Gomes et al., 2019).

Wilting time of forages before ensiling is common in many countries (Edmunds et al., 2014). The main reasons for wilting are to improve the quality of fermentation (Marsh, 1979) and reduce environmental pollution and nutrient losses in the form of gases and effluents. However, silage quality can be negatively affected due to the proliferation of undesirable microorganisms if the harvested forage is exposed to the sun for prolonged periods (Pahlow et al., 2003). Extended wilting times can also affect aerobic stability and the nutritive value of silages (Wilkinson and Davies, 2013; Brüning et al., 2018). Low-moisture silages (less than 60%) are more prone to aerobic instability due to the lower concentration of acetic acid, which has antifungal properties (Brüning et al., 2018). Therefore, due to high temperatures (annual average between 22-26 °C), air speed (1 m s^{-1}), solar radiation (~200 W m^{-2}), and evaporation (~8 mm; Medeiros et al., 2005; Silva et al., 2010) in the semi-arid region, it is necessary to know the ideal period of exposure of BRS capiaçu grass to the sun with a focus on the fermentative profile and nutritive value.

Moreover, Wilkinson and Davies (2013) reported that less heat is required to raise the temperature of the drier material than for the wetter material. Therefore, applying an enzymatic-bacterial inoculant during the ensiling of BRS capiaçu grass can reduce DM losses and aerobic deterioration of silage (Gomes et al., 2019). Furthermore, the inoculant can improve the DM digestibility as it contains enzymes such as hemicellulases, cellulases, and amylases (Muck et al., 2018; Li et al., 2019; Bureenok et al., 2019).

Based on the above, the objective of this study was to evaluate the effects of wilting times and application of an enzymatic-bacterial inoculant on the fermentative profile and nutritional characteristics of BRS capiaçu grass silage in a semi-arid region.

2. Material and Methods

The procedures for the care and handling of animals used in the experiment were in accordance with the guidelines of the Brazilian College of Animal Experimentation (COBEA) and were approved by the Ethics, Bioethics, and Animal Welfare Committee (CEBEA) (protocol no. 175/2018).

2.1. Treatments and silage management

On August 1st, 2019, an area (~400 m^2) planted with BRS capiaçu in 2016 (rows spaced 1.2 meters apart) on an experimental farm (geographic coordinates: 15°52'38" S, 43°20'05" W) in Brazil, was prepared for cutting and ensiling.

The climate of the region, according to the Köppen's classification (Köppen, 1948), is the Aw type with summer rains and dry periods well defined in winter. The average annual rainfall is 800 mm, with an annual average temperature of 27 °C and 60% humidity. The climate is tropical mesothermal, almost megahermic, due to its altitude and it being subhumid and semiarid, with irregular rains, causing long periods of drought.

After the standardization cut in August, 10 t of cattle manure (pH of 8.4; 217 g of moisture, 488 g of DM, 11 g kg^{-1} of N, and 13 g kg^{-1} of P) and 15 kg ha^{-1} of N in the form of urea (46% N) were applied per hectare based on soil analysis. Overhead irrigation was used (flow rate 1.25 m^3/h; 17.36 mm/h; 20 m range (radius)) for 2 h.

As recommended by Monção et al. (2019, 2020), the BRS capiaçu grass at 100 days of growth was manually cut close to the ground using a sickle, and 12 piles were made (1×1 m). The forage was left in the field for 6, 24, and 30 h, and, subsequently, ensiled, following completely randomized design in split-plot scheme with eight replications. Three random piles of BRS capiaçu grass (unwilted; control)

were homogenized and chopped immediately after harvesting in a stationary forage chopper (JF, 40 P, Itapura, São Paulo, Brazil) to a 2-mm size. During the period of wilting times, the air temperature, relative humidity, and wind speed were measured using data logger (Table 1). On average, plants were 3.43 m and contained 341 g kg^{-1} of leaf blades, 594 g kg^{-1} of stem + leaf sheaths, and 65 g kg^{-1} of senescent material (g kg^{-1} of DM).

During the ensiling of BRS capiaçu grass, according to each light wilting time and the control treatment, the lyophilized enzymatic-bacterial inoculant (SILOTRATO®) was sprayed according to the manufacturer's recommendations (2 g of the product per ton of green forage mass). The enzyme-bacterial inoculant used was composed of *Lactobacillus curvatus*, *L. acidophilus*, *L. plantarum*, *L. buchneri*, *L. lactis*, *Pediococcus acidilactici*, and *Enterococcus faecium*, in concentrations of 10^{10} CFU g^{-1} and 5% of enzymatic complex based on cellulose. The warranty levels had been met by the manufacturer. All treatments received the same volume of dechlorinated water (2 mL kg^{-1}). The inoculant was evaluated for enzyme activity and bacterial composition, irrespective of the manufacturer's information. The experimental silos were made of polyvinyl chloride (PVC) of known weight measuring 50 cm length and 10 cm in diameter. The bottom of the silos contained 10 cm of dry sand (400 g), which was separated from the forage by foam to allow quantifying the amount of effluent produced. After complete homogenization of the forage, the resulting material was deposited in the silos and compacted using a wooden plunger. For each treatment, the silage density (550 kg of organic matter m^{-3}) was quantified as recommended by Ruppel et al. (1995). After filling, the silos were closed with PVC lids fitted with Bunsen-type valves, sealed with adhesive tape, and weighed. The silos were stored at room temperature and opened 60 days after ensiling.

Table 1 - Climatic conditions during the trial period

Item	Wilting time, h (clock time)			
	0 (08:00)	6 (14:00)	24 (08:00)	30 (14:00)
Minimum temperature (°C)	22.50	30.60	20.80	29.40
Average temperature (°C)	22.80	32.40	20.80	30.70
Maximum temperature (°C)	23.20	32.80	22.40	32.30
Relative humidity (%)	68.00	35.00	71.00	39.00
Solar radiation (KJ m^{-2})	23.90	3642.20	26.00	3133.80
Wind speed (m s^{-1})	1.50	3.70	1.60	5.20

2.2. Dry matter losses

The DM losses in the silage in the form of gas and effluent were quantified by weight difference according to Jobim et al. (2007). Effluent losses were calculated according to equation 1, as follows:

$$E = (Wop - SWen)/(GREM) \times 1000, \quad (1)$$

in which E = effluent production (kg/ton of green mass), Wop = set weight (full bucket + lid + wet sand + foam) at silo opening (kg), SWen = set weight (full bucket + lid + dry sand + foam) at ensiling (kg), and GREM = green forage mass ensiled (kg).

Dry matter losses in the form of gases were calculated according to equation (2):

$$G = [(Wen - SWen)*DMen] - [(Wop - SWen)*DMop] \times 100 / [(Wen - SWen)*DMen], \quad (2)$$

in which G = gas losses (% of DM), Wen = weight of the full bucket at ensiling (kg), DMen = forage dry matter content at ensiling, and DMop = forage dry matter content at silo opening. The DM recovery for each silo was calculated based on the initial and final weights and the DM contents of the forages and silages according to Jobim et al. (2007).

2.3. Aerobic stability

Aerobic stability was determined by placing a homogeneous silage sample (approximately 3 kg) from each mini-silo in another new mini-silo, which was kept in a controlled temperature room at 25±1 °C. Silage temperature was measured every hour with the aid of a temperature data logger inserted into the center of the mass for nine days. Room temperature was also measured every hour with the aid of a temperature data logger placed near the mini-silo. Aerobic stability was calculated as the time taken by the silage upon exposure to air to show a 2 °C increase in temperature above room temperature (Moran et al., 1996).

2.4. pH, ammoniacal nitrogen, and volatile organic compounds

The determination of pH, ammoniacal nitrogen (NH_3-N), and organic acids (Pryce, 1969) were obtained from the silage extract. For production of silage extract, the fresh silage was placed in a hydraulic press with a capacity of 24 tons. The pH was measured using a potentiometer (DM-22, Digimed, São Paulo, SP, Brazil), and the NH_3-N was determined according to the technique described by Noel and Hambleton (1976). Volatile fatty acid contents were estimated by gas chromatography-mass spectrometry (GCMS Shimadzu® 20A System, Kyoto, Japan) with a capillary column (Rezex ROA Column 30 cm × 9 mm; 60 m, 0.25 mm ø, 50 µL; UV Detector - 210 nm; Column Temperature 60 °C) according to manufacturer's recommendations. For each acid, stock solution containing five analytes was prepared and diluted to appropriate different concentrations, and calibration curves were established.

2.5. Chemical composition

Samples of fresh material and silages were pre-dried in a forced-ventilation oven at 55 °C and ground to pass in a 1-mm screen (Wiley knife mill). Subsamples were analyzed for ash (method 942.05), ether extract (EE; method 920.39), and crude protein (CP; method 978.04), as described by AOAC (1990) (Table 2). The neutral detergent fiber (NDF) and the acid detergent fiber (ADF) were determined by the sequential method according to procedures described by Van Soest et al. (1991), using a TECNAL® TE-149 fiber analyzer (Piracicaba, SP, Brazil). Cellulose was solubilized in 72% sulfuric acid, and the lignin content was obtained from the resulting weight difference (Goering and Van Soest, 1970). Total carbohydrates (TC) were obtained by the following equation: TC = 100 − (% CP + % ash + % EE) according to the methodology described by Sniffen et al. (1992). The content of non-fibrous carbohydrates was calculated as NFC = 100 − (CP + NDFa + EE + ash). Total digestible nutrients were estimated according to Weiss (1998).

Table 2 - Chemical composition of fresh forage before silage according to light wilting times

Item	Wilting time (h)			
	0	6	24	30
Dry matter (DM; g kg^{-1} as fed)	242.6	272.8	329.2	333.7
Ash (g kg^{-1} of DM)	98.2	94.0	112.6	106.6
Crude protein (g kg^{-1} of DM)	80.6	64.9	88.6	63.6
Ether extract (g kg^{-1} of DM)	24.7	14.3	10.6	6.5
Neutral detergent fiber (g kg^{-1} of DM)	729.7	749.0	703.9	691.5
Acid detergent fiber (g kg^{-1} of DM)	466.2	464.9	454.7	479.1
Lignin (g kg^{-1} of DM)	67.4	71.0	44.7	61.0
iNDF (g kg^{-1} of DM)	395.4	414.9	352.3	374.5
Total carbohydrates (g kg^{-1} of DM)	796.5	830.5	784.5	823.2
Non-fibrous carbohydrates (g kg^{-1} of DM)	66.8	81.5	80.6	131.7
Total digestible nutrients (g kg^{-1} of DM)	422.0	404.3	417.1	414.7

iNDF - indigestible neutral detergent fiber.

2.6. Ruminal parameters

Four rumen-cannulated crossbred steers with an average weight of 500±70 kg were used to evaluate the ruminal kinetics of DM and NDF from BRS capiaçu grass silages. The animals received 4.0 kg of concentrate (240 g kg^{-1} CP and 700 g kg^{-1} of TDN) in two equal amounts in the morning and afternoon and silage of BRS capiaçu grass *ad libitum*. The *in-situ* degradability test was performed using 7.5 × 15 cm non-woven bags (100 g m^{-2}; Pore size 60 microns) according to Casali et al. (2009). The number of samples was based on the sample size to bag surface area ratio of 20 mg of DM cm^{-2} (Nocek, 1988). Samples were placed in the ventral sac region of the rumen for 0, 3, 6, 12, 24, 48, 72, 96, 120, and 144 h. Zero-time bags were not incubated in the rumen but were washed in running water (20 °C) similarly to the incubated bags. All samples were removed and washed in cold water (20 °C) to stop fermentation. Subsequently, the samples were oven-dried at 55 °C for 72 h, cooled in a desiccator, and weighed. The obtained residues in the non-woven bags were analyzed for DM and NDF contents. The percentage disappearance was calculated from the proportion of feed remaining after incubation.

Data were adjusted to a non-linear regression model using the Gauss-Newton method in SAS software (Statistical Analysis System, version 9.0), according to the equation proposed by Orskov and McDonald (1979): $Y = a + b (1 - e^{-ct})$, in which Y = disappearance (%) at time t; a = intercept of degradation curve when t = 0, which corresponds to the water-soluble fraction of the analyzed nutritional component; b = potential degradation of the water-insoluble fraction of the analyzed nutritional component; a + b = potential degradation of the analyzed nutritional component when time is not a limiting factor; c = fractional degradation rate of disappearance of fraction b in the rumen; and t = incubation time. Once calculated, the coefficients a, b, and c were applied to the equation proposed by Ørskov and McDonald (1979): ED = a + (b × c/c + k), in which ED = effective ruminal degradation of the analyzed nutritional component and k = passage rate. Estimated rumen passage rates (2, 5, and 8% h^{-1}) were assumed as suggested by the AFRC (1993). The DM and NDF disappearances at time zero (fraction a) were used to estimate the lag time (LT) according to Goes et al. (2017). Parameters a, b, and c were obtained by the Gauss-Newton algorithms: LT = [-ln(a' -a-b)/c].

The NDF degradability was estimated using the model proposed by Mertens and Loften (1980): $Rt = B \times e^{-ct} + I$, in which R = fraction degraded at time t, B = potentially digestible insoluble fraction, and I = indigestible fraction. After adjusting the NDF degradability equation, fractions were standardized as proposed by Waldo et al. (1972), using the equations: Bp = B/(B + I) × 100 and Ip = I/(B + I) × 100, in which Bp = standardized potentially digestible fraction (%) and Ip = standardized indigestible fraction (%). The effective NDF degradability was calculated according to the model: ED = Bp × c/(c + k).

2.7 Statistical analysis

Data were subjected to analysis of variance using the IML, GLM, and REG procedures of SAS. The Shapiro-Wilk and Bartlett's tests were used to examine the normality of residues and homoscedasticity of variances, respectively. Data on the fermentative profile and chemical composition were analyzed according to the model:

$$Y_{ijk} = \mu + Ino_i + e_{ij} + TE_k + Ino_i \times TE_k + e_{ijk}, \quad (3)$$

in which Y_{ijk} = observed response of wilting time in subplot k added or not with inoculant in plot i in the repetition j; μ = overall mean; Ino_i = effect of the application or not of inoculant i, with i = 1 and 2; e_{ij} = experimental error associated with plots, assumed to be normally distributed with zero mean and unit variance; TE_k = effect of wilting time k, with k = 1, 2, 3, and 4; $Ino_i \times TE_k$ = effect of the interaction between the i-th level of inoculant with the k-th wilting time; and e_{ijk} = experimental error associated with all observations (Y_{ijk}), independent, assumed to be normally distributed with zero mean and unit variance.

The ruminal degradability test was conducted in a split-plot randomized block design with eight treatments (plots) and ten incubation times (subplots) and four blocks. Animals were blocked by weight. The following statistical model was used:

$$Y_{ijk} = \mu + T_i + B_j + e_{ij} + P_k + T_i \times P_{ik} + e_{ijk}, \quad (4)$$

in which Y_{ijk} = observed response of incubation time (P) in the subplot k of the treatment (T_j) in block j; μ = overall mean; T_i = effect of the treatment i, with i = 1, 2, 3, 4, 5, 6, 7, and 8; B_j = effect of block j, with j = 1, 2, 3, and 4; e_{ij} = experimental error associated with plots, assumed to be normally distributed with zero mean and unit variance; P = effect of incubation time k, with k = 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10; TP_{ik} = effect of the interaction between the i-th level of treatment with the k-th incubation time; and e_{ijk} = experimental error associated with plots, assumed to be normally distributed with zero mean and unit variance.

The means for the use of inoculants and their interactions were compared by the F test. Comparisons between wilting times were performed by partitioning the sum of the squares into orthogonal linear contrasts and quadratic effects, with subsequent adjustments to the regression equations. For all statistical procedures, α = 0.05 was used as the maximum tolerable limit of type-I error.

3. Results

There was no interaction between wilting times and application or not of inoculant on pH values (P = 0.57), NH_3-N (P = 0.16), and aerobic stability (P = 0.72) of BRS capiaçu silage (Table 3). The mean pH responded quadratically to wilting time, with a maximum point at 15.87 h of wilting. Aerobic stability was reduced by 1.2 h for every 1-h increase in wilting time. There was no difference of wilting times (P = 0.57) and use of inoculant application (P = 0.45) on NH_3-N, with an average of 7.99% of total nitrogen (TN). Inoculant application reduced the pH values by 2.59% (P<0.01) and extended the aerobic stability (P = 0.02) of the silage by 19 h. There was a significant interaction between wilting times and application or not of inoculant on gas losses (P<0.01). On the one hand, the highest gas losses in unwilted silages (time 0 h) were found in materials without inoculant. On the other hand, there was no difference between silages added or not with inoculant (mean of 2.72% of DM), regardless of wilting

Table 3 - pH, ammoniacal nitrogen (NH_3-N), and losses during fermentation of BRS capiaçu silage managed at different wilting times associated with enzymatic-bacterial inoculant in the semiarid region

Item	Inoculant	Wilting time (h)				SEM	P-value			
		0	6	24	30		Time L	Time Q	Inoculant	Time × inoculant
pH[1]	Without	4.10	4.33	4.38	4.20	0.04	0.04	<0.01	<0.01	0.57
	With	4.01	4.23	4.34	4.05					
NH_3-N (% TN)	Without	9.01	7.63	8.11	7.51	0.3	0.15	0.57	0.45	0.16
	With	8.12	7.37	8.60	7.60					
Gases losses (% of DM)	Without[2]	3.72A	2.89A	3.93A	2.16A	0.26	<0.03	<0.01	0.28	0.01
	With[3]	2.79B	2.69A	3.11A	1.58A					
Effluent losses (kg t^{-1})[4]	Without	85.02	52.05	49.54	49.27	5.31	<0.01	0.01	0.09	0.99
	With	79.53	44.70	44.24	41.38					
Dry matter recovery (% of DM)	Without[5]	85.07B	90.69A	91.24A	89.09B	1.51	<0.01	<0.01	0.25	<0.01
	With[6]	92.41A	93.04A	94.64A	95.48A					
Aerobic stability (h)[7]	Without	132.00	152.00	108.00	112.00	16.11	0.01	0.93	0.02	0.72
	With	168.00	152.00	132.00	128.00					

TN - total nitrogen; DM - dry matter; Time L - linear effect; Time Q - quadratic effect; Time × inoculant - interaction between wilting time and inoculant; SEM - standard error of the mean.
Means in the same column with different letters differed (P<0.05).
Regression equations:
[1] $\hat{y} = 3.54 + 0.05*X - 0.002*X^2$, $R^2 = 0.99$;
[2] $\hat{y} = 3.28 + 0.07X - 0.0033X^2$, $R^2 = 0.27$;
[3] $\hat{y} = 2.53+0.12*X - 0.005*X^2$, $R^2 = 0.63$;
[4] $\hat{y} = 69.62 - 0.92*X$, $R^2 = 0.58$;
[5] $\hat{y} = 85.48 + 0.91*X - 0.03*X^2$, $R^2 = 0.94$;
[6] $\hat{y} = 92.41 + 0.10*X$, $R^2 = 0.99$;
[7] $\hat{y} = 153.59 - 1.20*X$, $R^2 = 0.92$, in which X is the wilting time (h); * significant by the t test (P<0.05).

time. The means for gas losses responded quadratically to wilting time, reaching their maximum points at 10.60 and 12.00 h for treatments without and with inoculant, respectively.

There was no interaction between wilting times and application or not of inoculant on effluent losses (P = 0.99). There was no difference between silages without and with inoculants (P = 0.09), mean of 55.71 kg green mass/ton. Effluent losses decreased by 0.92 kg green mass/ton for every 1-h increase in wilting time for treatments without and with inoculant, respectively. There was an interaction between wilting time and application or not of the inoculant on DM recovery (P<0.01). The highest DM recovery was observed at times 0 and 30 h in silages with inoculants. At times 6 and 24 h, there was no difference between silages, regardless of inoculant application. The mean DM recovery of silage without inoculant responded quadratically to wilting time, with a maximum point at 15.16 h. The DM recovery in silages with inoculant increased by 0.10% for every 1-h increase in wilting time.

There was an interaction between wilting time and application or not of inoculant on the levels of malic (P<0.01), succinic (P<0.01), lactic (P<0.01), and acetic (P<0.01) acids. Within wilting times 0 and 6 h, there was no difference between the means (14.0 g kg^{-1} of DM) without and with the application of inoculant on the lactic acid content. In the wilting times of 24 and 30 h, the lactic acid content in the silage with inoculant was on average 25.90% higher compared with that in silage without inoculant (mean of 11.3 g kg^{-1} of DM). There was no interaction between factors on the concentrations of tartaric (P = 0.66) and butyric (P = 0.39) acids, lactic:acetic acid ratio (P = 0.71), and ethanol (P = 0.16). Butyric acid content decreased (P = 0.01) by 0.004% for every 1-h increase in wilting time (Table 4).

There was no interaction between wilting time and application or not of inoculant on chemical composition traits (P = 0.71), except for NFC content (P<0.01; Table 5). The DM content decreased

Table 4 - Fermentation profile of BRS capiaçu silage managed at different wilting times associated with enzymatic-bacterial inoculant in the semiarid region

Item	Inoculant	Wilting time (h)				SEM	P-value			
		0	6	24	30		Time L	Time Q	Inoculant	Time × inoculant
Tartaric acid (g kg^{-1} of DM)	Without	0.40	0.50	0.40	0.40	<0.01	0.39	0.06	0.41	0.66
	With	0.40	0.50	0.50	0.40					
Malic acid (g kg^{-1} of DM)	Without[1]	0.60A	0.40A	1.80A	1.80A	0.10	<0.01	0.41	<0.01	<0.01
	With[2]	0.30A	0.40A	1.10B	0.30B					
Succinic acid (g kg^{-1} of DM)	Without[3]	1.00A	0.90B	1.50A	2.10A	<0.01	<0.01	0.01	0.2	<0.01
	With[4]	0.80B	1.20A	1.50A	1.70B					
Lactic acid (g kg^{-1} of DM)	Without[5]	12.40A	14.60A	12.60B	10.00B	0.06	0.02	0.01	<0.01	<0.01
	With[6]	13.20A	15.80A	14.50A	16.00A					
Acetic acid (g kg^{-1} of DM)	Without[7]	3.00B	2.60B	3.50B	4.30B	0.30	0.01	0.06	<0.01	<0.01
	With[8]	4.00A	3.90A	5.40A	10.40A					
Lactic:acetic acid ratio[9]	Without	4.37	5.66	3.66	2.32	0.36	<0.01	<0.01	<0.01	0.71
	With	3.44	4.11	2.70	1.54					
Butyric acid[10] (g kg^{-1} of DM)	Without	1.90	1.40	0.80	0.00	0.04	0.01	0.15	0.53	0.39
	With	0.90	1.10	1.40	0.00					
Ethanol (g kg^{-1} of DM)	Without	0.37	0.40	0.36	0.19	0.06	0.16	0.10	0.83	0.16
	With	0.26	0.30	0.49	0.31					

Time L - linear effect; Time Q - quadratic effect; Time × inoculant - interaction between wilting time and inoculant; SEM - standard error of the mean. Means in the same column with different letters differed (P<0.05).
Regression equations:
[1] $\hat{y} = 0.40 + 0.005*X$, $R^2 = 0.89$;
[2] $\hat{y} = 0.10 + 0.01X - 0.003X^2$, $R^2 = 0.59$;
[3] $\hat{y} = 0.80 + 0.004*X$, $R^2 = 0.86$;
[4] $\hat{y} = 0.90 + 0.003*X$, $R^2 = 0.93$;
[5] $\hat{y} = 13.70 - 0.009X$, $R^2 = 0.44$;
[6] $\hat{y} = 14.10 + 0.005X$, $R^2 = 0.29$;
[7] $\hat{y} = 2.60 + 0.004X$, $R^2 = 0.77$;
[8] $\hat{y} = 3.20 + 0.02*X$, $R^2 = 0.70$;
[9] $\hat{y} = 4.02 + 0.15*X - 0.01*X^2$, $R^2 = 0.97$;
[10] $\hat{y} = 1.40 - 0.004*X$, $R^2 = 0.66$, in which X is the wilting time (h); * significant by the t test (P<0.05).

by 0.20% for every 1-h increase in wilting time (P<0.01). The means for ash content responded quadratically to wilting time, with a maximum point at 15.90 h. The CP content decreased by 0.05%, while EE content increased by 0.01% for every 1-h increase in wilting time. The contents of NDF, ADF, lignin, iNDF, and TDN in the BRS capiaçu grass silage were not affected by wilting time. Inoculant application increased the contents of DM (P = 0.01), ash (P<0.01), CP (P = 0.05), iNDF (P = 0.03,) and TC (P = 0.02) by 3.63, 6.13, 7.73, 6.39, and 9.97% compared with the treatment without inoculant, respectively. There was no difference in NFC content between treatments at times 0 and 30 h, regardless of inoculant application. The highest levels of NFC at 6 and 24 h were observed in inoculated silages. The mean NFC in silages without and with inoculant responded quadratically to wilting time, reaching their maximum points at 11 and 7.5 h, respectively.

There was no interaction between wilting time and application or not of inoculant on the variables of ruminal degradability of DM (P = 0.68; Table 6). There was no effect of wilting time and application or not of inoculant on the readily soluble fraction (fraction a), potentially digestible insoluble fraction (fraction b), degradation rate of fractions b and c, potential degradability, and effective degradability (k = 5 and 8% h^{-1}) of BRS capiaçu grass silage. The effective degradability (k = 2% h^{-1}; P = 0.05) of the DM decreased by 0.10% for every 1-h increase in wilting time.

There was no interaction between wilting time and application or not of inoculant on NDF degradability parameters (P = 0.57; Table 7). The Bp fraction and effective degradability of

Table 5 - Chemical composition of BRS capiaçu silage managed at different wilting times associated with enzymatic-bacterial inoculant in the semiarid region (dry matter basis)

Item	Inoculant	Wilting time (h)				SEM	P-value			
		0	6	24	30		Time L	Time Q	Inoculant	Time × Inoculant
Dry matter[1] (% of DM as fed)	Without	25.85	27.44	30.81	31.97	0.42	<0.01	0.02	0.01	0.12
	With	26.78	28.64	31.94	33.09					
Ash[2] (% of DM)	Without	9.54	10.99	10.62	10.29	0.22	<0.01	<0.01	<0.01	0.47
	With	10.36	11.42	11.03	11.34					
Crude protein[3] (% of DM)	Without	8.13	8.24	7.39	6.29	0.38	<0.01	0.09	0.05	0.10
	With	8.97	8.31	7.34	7.95					
Ether extract[4] (% of DM)	Without	2.10	1.66	1.75	1.55	0.12	0.01	0.63	0.24	0.71
	With	1.87	1.58	1.82	1.47					
Neutral detergent fiber (% of DM)	Without	68.61	71.06	69.16	68.7	0.92	0.56	0.17	0.22	0.21
	With	68.2	68.27	70.37	67.83					
Acid detergent fiber (% of DM)	Without	45.9	49.43	47.43	47.66	0.95	0.40	0.37	0.98	0.22
	With	46.78	43.70	48.84	47.11					
Lignin (% of DM)	Without	5.44	5.67	6.33	6.70	0.94	0.77	0.15	0.66	0.40
	With	5.01	5.40	6.72	6.35					
iNDF (% of DM)	Without	37.59	40.51	37.25	39.13	0.60	0.72	0.92	0.03	0.09
	With	37.44	37.80	37.38	38.61					
Total carbohydrates[5] (% of DM)	Without	80.21	79.09	80.22	81.86	0.51	<0.01	0.12	0.02	0.11
	With	78.79	78.69	79.80	79.23					
Non-fibrous carbohydrates (% of DM)	Without[6]	11.60A	8.03B	11.06A	12.36B	0.67	<0.01	0.12	0.02	<0.01
	With[7]	10.59A	12.18A	10.67A	14.38A					
Total digestible nutrients (% of DM)	Without	44.21	41.47	42.98	43.18	1.08	0.66	0.12	0.60	0.11
	With	43.41	43.08	42.25	42.73					

DM - dry matter; iNDF - indigestible neutral detergent fiber; Time L - linear effect; Time Q - quadratic effect; Time × inoculant - interaction between wilting time and inoculant; SEM - standard error of the mean.
Means in the same column with different letters differed (P<0.05).
Regression equations:
[1] $\hat{y} = 26.54 + 0.20*X$, $R^2 = 0.99$;
[2] $\hat{y} = 10.14 + 0.14*X - 0.0044*X^2$, $R^2 = 0.64$;
[3] $\hat{y} = 8.55 - 0.05*X$, $R^2 = 0.99$;
[4] $\hat{y} = 1.86 - 0.01*X$, $R^2 = 0.40$;
[5] $\hat{y} = 79.10 + 0.042*X$, $R^2 = 0.72$;
[6] $\hat{y} = 11.03 - 0.44*X + 0.02*X^2$, $R^2 = 0.77$;
[7] $\hat{y} = 11.42 - 0.15*X + 0.01*X^2$, $R^2 = 0.44$, in which X is the wilting time (h); * significant by the t test (P<0.05).

Table 6 - Ruminal kinetics of dry matter of BRS capiaçu silage managed at different wilting times associated with an enzymatic-bacterial inoculant in the semiarid region

Item	Inoculant	Wilting time (h)				SEM	P-value			
		0	6	24	30		Time L	Time Q	Inoculant	Time × inoculant
Fraction a (% of DM)	Without	19.18	17.12	17.88	15.49	1.22	0.44	0.63	0.65	0.33
	With	17.63	17.77	17.23	19.21					
Fraction b (% of DM)	Without	31.70	31.20	34.07	33.34	3.65	0.31	0.79	0.32	0.25
	With	41.47	34.64	37.11	27.70					
Degradation rate, c (% h^{-1})	Without	2.00	1.50	1.75	2.00	<0.01	0.41	0.12	0.31	0.17
	With	1.75	2.00	1.75	2.00					
Potential degradability (% of DM)	Without	50.88	48.32	51.95	48.84	4.31	0.28	0.91	0.40	0.68
	With	59.10	52.41	54.34	46.90					
Effective degradability (k = 2% h^{-1})[1] (% of DM)	Without	32.68	32.58	32.17	31.76	2.06	0.05	0.16	0.33	0.07
	With	40.83	31.38	33.75	32.05					
Effective degradability (k = 5% h^{-1}) (% of DM)	Without	26.47	26.01	25.81	24.72	1.61	0.07	0.14	0.35	0.19
	With	31.60	25.01	26.49	26.49					
Effective degradability (k = 8% h^{-1}) (% of DM)	Without	24.18	23.37	23.40	21.94	1.44	0.10	0.18	0.39	0.35
	With	27.62	22.72	23.69	24.31					

DM - dry matter; k - passage rate (AFRC, 1993); Time L - linear effect; Time Q - quadratic effect; Time × inoculant - interaction between wilting time and inoculant; SEM - standard error of the mean.
Regression equation:
[1] $\hat{y} = 34.96 - 0.10*X$, $R^2 = 0.42$, in which X is the wilting time (h); * significant by the t test (P<0.05).

Table 7 - Ruminal kinetics of neutral detergent fiber from BRS capiaçu silage managed at different wilting times associated with enzymatic-bacterial inoculant in the semiarid region

Item	Inoculant	Wilting time (h)				SEM	P-value			
		0	6	24	30		Time L	Time Q	Inoculant	Time × inoculant
Fraction Bp[1] (% of DM)	Without	44.69	45.40	36.70	41.35	2.32	<0.01	0.34	0.05	0.21
	With	48.73	56.95	46.31	44.25					
Degradation rate, c (% h^{-1})	Without	2.00	2.25	2.25	1.75	<0.01	0.39	0.37	0.39	0.57
	With	2.75	2.00	2.75	1.75					
Colonization time (h)	Without	11.86	8.20	8.19	8.46	1.1	0.07	0.09	0.01	0.06
	With	5.99	8.49	7.32	6.25					
Fraction Ip[2] (% of DM)	Without	55.32	54.60	63.31	58.66	2.69	<0.01	0.34	0.05	0.21
	With	51.27	43.06	53.69	55.75					
Effective degradability (k = 2% h^{-1})[3] (% of DM)	Without	20.69	23.87	22.00	19.59	2.03	0.02	0.36	0.15	0.33
	With	29.40	26.99	24.02	21.84					
Effective degradability (k = 5% h^{-1}) (% of DM)	Without	11.18	14.15	13.33	10.99	1.58	0.09	0.31	0.18	0.22
	With	17.99	15.01	14.65	12.34					
Effective degradability (k = 8% h^{-1}) (% of DM)	Without	7.66	10.09	9.57	7.65	1.25	0.13	0.29	0.19	0.20
	With	12.97	10.40	10.61	8.60					

Fraction Bp - standardized potentially degradable fraction; Fraction Ip - standardized indegradable fraction; k - passage rate (AFRC, 1993); Time L - linear effect; Time Q - quadratic effect; Time × inoculant - interaction between wilting time and inoculant; SEM - standard error of the mean.
Regression equations:
[1] $\hat{y} = 49.11 - 0.24*X$, $R^2 = 0.60$;
[2] $\hat{y} = 50.88 + 0.24*X$, $R^2 = 0.60$;
[3] $\hat{y} = 25.67 - 0.14*X$, $R^2 = 0.87$, in which X is the wilting time (h); * significant by the t test (P<0.05).

NDF (k = 2% h^{-1}) decreased by 0.24 and 0.14% for every 1-h increase in wilting time, respectively. Inoculant application increased the Bp fraction (P = 0.05) by 14.32% and reduced the lag time and the standardized indigestible fraction (Ip; P = 0.01) by 23.59 and 12.13% for every 1-h increase in wilting time for treatments without and with inoculant, respectively.

4. Discussion

Forage plants must have adequate DM content at ensiling, low buffering capacity, and at least 8% of soluble carbohydrates content (DM basis) for adequate fermentation (Oude Elferink et al., 2000). Water-soluble carbohydrates are the primary source of nutrients for microorganisms such as homo- and heterofermentative bacteria, which produce lactic, acetic, succinic, and propionic acids (lactic acid bacteria, LAB). In this study, the light wilting of BRS capiaçu grass, after 30 h of exposure, increased the DM content in 18.43% compared with time without wilting. This increase was essential to adjust the DM content to the recommended range (25-35%) proposed by Kung Jr. et al. (2018) for proper fermentation of grasses in the silo. Despite silage with less than 25% DM, BRS capiaçu grass managed with 100 days of regrowth showed good-quality silage in terms of fermentation profile and nutritional value.

There was a significant reduction in gases and effluent losses and greater DM recovery with increasing DM content due to wilting time. Low DM content in silages favors the growth of bacteria of the genus *Clostridium*, responsible for butyric acid production. The light wilting of BRS capiaçu grass contributed to the linear reduction of butyric acid concentration with increasing DM content. Moreover, the enzymatic-bacterial inoculant increased the DM content of the silage by 3.63% due to the reduction in pH. Accordingly, manually harvested BRS capiaçu grass with 100 days (3.43 m high) in a semi-arid region should be inoculated to minimize DM losses and increase the DM content of silage.

Moreover, inoculated silages had improved DM recovery and longer aerobic stability compared with silage without inoculation. This is justified by the greater production of acetic acid by LAB, such as the strains of *Lactobacillus buchneri* and *Propionibacterium acidipropionici* that produce acetic acid, which is capable of reducing the number of fungi and yeasts, thereby increasing the aerobic stability of silage. The strains of *Lactobacillus plantarum*, *L. acidophilus*, *L. curvatus, L. plantarum*, *L. lactis, Pediococcis acidilactici*, and *Enterococcus faecium* present in the enzymatic-bacterial inoculant led to the highest concentration of lactic acid in the silage. According to Kung Jr. et al. (2018), the low pKa of lactic acid (mean of 3.8) contributes to a rapid decline in the pH of the ensiled mass, thereby favoring desirable fermentation to the detriment of the growth of bacteria of the genus *Clostridium*. It explains the lower pH in inoculated BRS capiaçu grass silage in comparison with silage without inoculant (mean of 4.25).

Light wilting increased the ash content due to the mass concentration of DM. This response was also observed in inoculated silages. However, CP reduced linearly with wilting time. The highest moisture loss in inoculated silages, which is associated with less proteolysis, contributed to the higher CP content in relation to silage without inoculant (mean of 7.51%).

Wilting time and inoculant application in the silage did not affect the fibrous fraction (NDF, ADF, lignin). However, inoculated silages had lower contents of iNDF compared with the silage without inoculant. These results allow us to infer that the activity of the enzyme complex of the inoculant led to the breakage of bonds between lignin and hemicellulose, thereby favoring the degradation of fibers by fibrolytic bacteria present in the rumen (Jung and Deetz, 1993). Despite the increased concentration of NFC in inoculated silages with increasing wilting time, there was no effect of treatments on the content of total digestible nutrients (mean of 42.91%). The wilting times of BRS capiaçu grass before ensiling did not alter the ruminal kinetics parameters of DM. This behavior is justified by the reduction of protein content and EE with increasing wilting time. The effective DM degradability is associated with the readily soluble fraction represented by the rapidly fermenting soluble carbohydrates in cell contents and the middle lamella of the plants. In general, the potential degradability of DM was low for BRS capiaçu grass silage (mean of 51.59%). This result is associated with the high content of iNDF

present in the forage harvested after 100 days of regrowth. Monção et al. (2019) studied different harvesting age of BRS capiaçu grass and found an average of 39.5% for iNDF content. This result is high and can compromise the productive performance of the animals. According to Detmann et al. (2014), the dry matter intake is linear and correlates negatively with the content of iNDF in diets.

Well-managed BRS capiaçu grass in the semiarid region is suitable for silage production and has adequate characteristics for silage fermentation when harvested from 90 to 120 days (3.5 meters high) as recommended by Monção et al. (2019, 2020). This adequate fermentation of the ensiled mass is associated with the manual harvesting in the field and the time for processing until the silo is closed. Therefore, there will always be wilting with increasing the DM content. In this study, wilting for at least 6 h increased the DM content. In practical terms, this minimum amount of time is necessary to ensure adequate fermentation of BRS capiaçu grass because there is not always control of the cutting height of grass on farms or under conditions of cultivation without irrigation. When mechanically harvested (unwilted), the inoculant should be applied to reduce DM losses due to the rapid decline in the pH of the ensiled mass, as observed in this study. The factors that influence the fermentative capacity of the ensiled mass are adequate DM levels (25 to 38%), soluble carbohydrate content above 6% of DM, and low buffer capacity (McDonald et al., 1991). If these factors are not met by the forage, the use of the inoculant will not guarantee adequate fermentation and conservation of the ensiled mass.

5. Conclusions

Light wilting for up to 30 h and the application of an enzymatic-bacterial inoculant improves the fermentative profile and chemical composition and reduces dry matter losses of silage of BRS capiaçu grass harvested at 100 days of regrowth. Moreover, it does not alter the potential degradability of dry matter despite reducing the effective degradability of the fibrous fraction.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

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