https://doi.org/10.22319/rmcp.v15i1.6379

Article

Microsilages elephant grass BRS Capiaçu added with commercial microbial consortium on different days of regrowth

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Abstract:

This study aimed to evaluate whether bacterial inoculation improves the fermentative, microbiological, and chemical characteristics of silages of the elephant grass cv. BRS Capiaçu on different regrowth days. The experimental design was completely randomized and set up in a 3x2 factorial arrangement (three regrowth days, with and without inoculant), with four replications. There was a significant interaction between the regrowth days and inoculant on the pH, ammoniacal nitrogen ($N\text{-}NH_3$), and effluent losses (EL) of the silages. Inoculation decreased the EL with the advance of regrowth days and increased the dry matter recovery index compared to the silages without inoculant. The population of molds and yeasts decreased when inoculation was adopted to the forage harvested after 85 d. There was a significant interaction between the dry matter (DM), crude protein (CP) and neutral detergent fiber corrected for ash and protein

(NDFap) contents of the silages. Inoculation in the grass harvested after 85 d increased the DM contents of the silage. The highest CP contents were observed in the silages after 85 d. The NDFap contents of the grasses harvested after 110 and 135 d were higher than those of the grass harvested after 85 d. The NDFap contents of the silages without inoculant increased with the harvest age. The BRS Capiaçu forage silage harvested at 110 d demonstrated favorable performance for silage production. However, the influence of inoculant use was low for the characteristics evaluated.

Keywords: Biological inputs, *Pennisetum purpureum*, Quality.

Received: 05/01/2023

Accepted: 18/09/2023

Introduction

Elephant grass (*Pennisetum purpureum* Schum) stands out among the tropical grasses used for silage due to its high production capacity, nutritive value, adaptability to the local edaphoclimatic conditions, number of varieties, easy cultivation, and high acceptability by animals [(1)].

The low soluble solids and dry matter contents associated with the high buffering power of this grass negatively influence the fermentation process during ensilage and cause losses that compromise silage quality[(2)]. From this perspective, new cultivars have been developed to improve the characteristics of elephant grass, e.g., the cultivar BRS Capiaçu. Released in 2016 by Embrapa Gado de Leite, the cultivar BRS Capiaçu has stood out due to its high dry matter yield (72t ha^{-1} yr^{-1}), producing about 30 % more forage mass (300t MV ha^{-1} yr^{-1}), showing more soluble carbohydrates and crude protein contents in relation to other elephant grass cultivars, and being a less expensive alternative than maize as a perennial crop that does not require annual seed purchase[(3,4,5)].

Biological inputs are widely used as bacterial inoculants in the ensilage of elephant grass to improve the population of lactic acid bacteria, which decrease the pH and intensify fermentation, thus reducing losses caused by undesirable microorganisms and increasing the nutrient quality of silages[(6,7,8)].

Furthermore, the harvest age of elephant grass during ensilage influences the development of microbial populations since the low moisture content and the high concentration of soluble carbohydrates are necessary for the development of lactic acid

bacteria[9,10]. Therefore, balancing forage production and quality is essential to producing BRS Capiaçu grass silages.

As a forage recently released on the market, studies on the cultivar BRS Capiaçu, especially for its use as silage, are still required to provide appropriate conditions for fermentation. In this scenario, this study aimed to identify whether bacterial inoculation improves the fermentative, microbiological, and chemical characteristics of the silage of the elephant grass (*Pennisetum purpureum* Schum.) cultivar BRS Capiaçu on different regrowth days.

Material and methods

Treatments and ensilage management

The experiment was conducted in Teresina, Piauí, Brazil (latitude: 5º 2'28.41 S, longitude: 42º 47'0.08 W, at an elevation of 67 m asl.) from March 2019 to March 2021. The elephant grass (*Pennisetum purpureum* Schum) used in the experiment was subjected to manual uniformization at a mean height of 10 cm from the ground, followed by fertilization with 50 kg of N ha^{-1}, 60 kg of K_2O ha^{-1}, and 60 kg of P_2O_5 supplied as urea, potassium chloride, and single superphosphate, respectively. Except for phosphate fertilization, all other nutrients were resupplied after 47 d according to soil analysis and the recommendations of Embrapa (2008). The grass was irrigated daily using a micro-sprinkler system from March to June 2019.

A completely randomized design was set up in a 2x3 factorial arrangement. The treatments corresponded to the bacterial inoculant combinations and the grass regrowth days, identified as follows: Factor 1) Bacterial inoculation when ensilage: presence and absence of inoculant; Factor 2) Days of grass regrowth: 85, 110, and 135 d. Based on this arrangement, six treatments were generated, and each was evaluated with four replications, totaling twenty-four experimental units.

The forage was harvested after 85, 110 and 135 d of regrowth from an area of 60 m^2 already established, delimiting 20 m^2 for each evaluated age. The plants were cut manually, with a cleaver, at a height of 10 cm from the ground and chopped into fragments of 1 to 2 cm, in a stationary shredder. After this process, chopped forage was manually homogenized with the silage additive according to each treatment and placed in plastic trays.

The lyophilized bacterial inoculant SILOTRATO® was applied during the ensilage of the BRS Capiaçu grass following the recommendations of the manufacturer (two grams per

ton of green mass), such as ensuring product quality until the expiration date, exclusive use for animal feed and non-toxicity. The bacterial inoculant was composed of various homofermentative lactic acid bacteria, facultative homofermentative bacteria, and facultative heterofermentative bacteria, and 5 % of an enzyme complex with a count limit of 10^{10} CFU.g^{-1}, according to each harvest age and control treatment (without inoculant application).

Cylindrical experimental silos made of polyvinyl chloride (PVC) were used in the assays, each measuring 50 cm in length and 10 cm in width. Each silo received 1.3 kg of dry sand, which was separated from the forage by a shading screen to allow quantifying the effluent produced.

After complete homogenization, the grass was deposited in the silos and compacted with the aid of a wooden piston by adopting a density of 600 kg m^{-3} of natural matter per silo. After being filled, the silos were closed with tap covers containing Bunsen valves, sealed with adhesive tape, and weighed. Then, the silos were stored at ambient temperature and opened 83 d after ensilage.

Fermentative losses and dry matter recovery index

The dry matter losses through gas and effluent and the dry matter recovery index (DMRI) were quantified by the weight difference according to the equations described by Schmidt *et al*[11]. The gas losses were obtained according to equation 1:

$$PG = [(PsChf - PsCha)/(MVFE \times MSFE)] \times 100 \quad (1)$$

where: PG= gas losses, PsChf= filled silo weight at the beginning of ensilage (kg), PsCha= filled silo weight at the end of ensilage (kg), MVFE = ensiled forage fresh matter (kg), MSFE= ensiled forage dry matter (%) discounting the weight of the sand added to the silo.

The effluent losses were obtained by equation 2:

$$EL\ (kg/t\ of\ MV) = [(PVf - Ts) - (PVi - Ts)]/MFi \times 100 \quad (2)$$

where: EL= effluent losses, PVf= empty silo weight + sand weight at the end of ensilage (kg), Ts= silo tare, PVi= empty silo weight + sand weight at the beginning of ensilage (kg), MFi= forage mass at the beginning of ensilage (kg).

The dry matter recovery rate was estimated using equation 3:

$$DMRI(\%) = (MFf \times MSf)/(MFi \times MSi) \times 100 \quad (3)$$

where: DMRI= dry matter recovery index (%), MFf= forage matter at the end of ensilage (kg), MSf= dry matter at the end of ensilage (%DM), MFi= forage matter at the beginning of ensilage (kg), MSi = forage dry matter content at the beginning of ensilage (%DM).

pH and ammoniacal nitrogen

Before ensilage, the chemical composition of the BRS Capiacu grass was analyzed at each harvest age (Table 1).

Table 1: Chemical composition of the BRS Capiaçu grass at different harvest ages

Item	Regrowth ages (days)		
	85	**110**	**135**
DM	16.8	21.9	26.0
OM	91.0	92.1	91.9
ASH	9.0	7.9	8.1
CP	6.1	5.4	3.9
EE	1.3	1.3	1.6
NDFap	72.9	71.8	71.0
ADF	52.8	56.5	51.6
NFC	10.7	14.6	15.4
HEM	20.1	15.3	19.4

DM= dry matter, OM= organic matter, ASH= ashes; CP= crude protein, EE= ether extract, NDFap= neutral detergent fiber corrected for ash and protein, ADF= acid detergent fiber, NFC= non-fiber carbohydrates, HEM= hemicellulose.

When the silos were opened, the samples were separated and split into three aliquots, the first of which was used fresh soon after homogenization to determine the pH according to Silva and Queiroz[12]. The ammoniacal nitrogen (N-NH_3) was determined according to Ferreira *et al*[13] based on the silage extract.

Chemical composition

After thawing, the second aliquot was pre-dried in a forced-air oven at 55 °C and ground to pass through a 1 mm sieve in a Wiley knife mill. The subsamples were analyzed for dry matter (DM; method 934.01), ash (method 942.05), crude protein (CP; method 978.04), and ether extract (EE; method 920.39) according to AOAC[14]. Neutral detergent fiber corrected for ash and protein (NDFap), acid detergent fiber (ADF) and hemicellulose

were determined by the sequential method according to the procedures described by Van Soest *et al*[15] adapted for autoclave (0.5 atm/1h) using TNT bags with a porosity of 100 µm[16].

Microbiological profile

The third aliquot was used to evaluate the microbiological profile of the silages by quantifying the microbial populations of *Lactobacillus* sp., *Clostridium* sp., filamentous fungi, and yeasts. The entire microorganism analysis was performed in a laminar flow cabinet.

The microbial populations in the silage were quantified by preparing an aqueous suspension with a fresh silage sample (25g) in 225 mL of peptone water, which was manually homogenized for three minutes. After homogenization, decimal dilutions were prepared in sterile tubes containing 9 mL of the solution and then sown in duplicate in sterile Petri dishes at dilutions of 10^{-1}, 10^{-2}, and 10^{-3}. The count of *Lactobacillus* was performed by adding 20 mL of MRS agar to the plates (*Lactobacillus* MRS agar). After homogenization and solidification of the culture medium, 10 mL of the same agar was added to form the overlayer. The dishes were then incubated at 35 ± 2 °C for 72 h in a bacteriological incubator.

The bacterial count of the genus *Clostridium* was performed by adding 20 mL of *Clostridium perfringens* agar and a 0.85 % egg yolk/saline emulsion at a proportion of 1:1 in the Petri dishes. Then, the inoculation was performed with 0.1 mL of the corresponding dilutions. Subsequently, 10 mL of the same agar was added to form the overlayer. Finally, the dishes were incubated at 35 ± 2 °C in anaerobiosis for 48 h in a bacteriological incubator. The filamentous fungi and yeasts were counted by adding 20 mL of Potato Dextrose Agar (PDA) with 10 % tartaric acid in the Petri dishes. After the culture medium solidified, 0.1 mL of the corresponding dilutions was added to the dishes, which were then incubated at 37 ± 2 °C for 120 h in a bacteriological incubator. The microorganisms were counted after incubation, and the results were expressed as log CFU g^{-1}[17].

Statistical analysis

The data referring to fermentative losses, chemical composition, and the microbiological profile were analyzed using the least squares method, by the GLM procedure, and by performing the analysis of variance and the SNK means comparison test through the

PROC NLIN procedure of the SAS software (Statistical Analysis System, version 9.0) at a significance level of 0.05.

The statistical model used was as follows:

$$Y_{ijk}=\mu+\alpha_i+\beta_j+(\alpha*\beta_{ij})+e_{ijk} \quad (4)$$

where:
Y_{ijk}= dependent variable,
μ= overall mean,
α_i= inoculation effect (fixed effect; i = presence and absence when ensilage), β_j = effect of the grass regrowth days (fixed effect; j = 85, 110, and 135 d),
$\alpha*\beta_{ij}$= effect of the interaction between the bacterial inoculant and the grass regrowth days,
e_{ijk}= random error associated with each observation.

Results

There was a significant interaction ($P<0.05$) between the regrowth days and inoculation on the fermentative characteristics of pH, ammoniacal nitrogen (N-NH_3), and effluent losses (EL) of the BRS Capiaçu grass silage (Table 2). The silage harvested after 85 d showed the lowest ($P<0.05$) pH (3.5), which increased to 3.79 when the inoculant was applied, an effect observed only for the silage of the forage harvested at the shortest age (85 d). The silage harvested after 135 d showed the lowest ($P<0.05$) N-NH_3 content (1.50 %) in relation to the forages harvested after 85 and 135 d.

No difference was observed in the N-NH_3 values of the silages regarding inoculation ($P>0.05$), with a mean of 1.95 % N-NH_3. Regarding the losses of ensiled mass, the effluent losses (EL) of the BRS Capiaçu grass silages were, on average, 145.53 kg t^{-1}. However, when the inoculant was applied to the forage harvested at 135 d, the effluent losses decreased.

Table 2: Fermentative characteristics of BRS Capiaçu grass silages at different harvest ages and bacterial inoculation

Item	Inoculant	Harvest ages (days)			Mean	SEM	*P*-value		
		85	110	135			Inoculant	Harvest age	Inoculant x harvest age
pH	With	3.79^{Ab}	4.23^{Aa}	4.26^{Aa}	4.10	0.07	0.4002	<0.0001	0.0095
	Without	3.50^{Bc}	4.49^{Aa}	4.13^{Ab}	4.04				
Mean		3.65	4.20	4.36					
NH_3-N, % TN	With	1.97^{Aa}	2.17^{Aa}	1.72^{Aa}	1.95	0.08	0.5128	0.0005	0.0327
	Without	2.50^{Aa}	2.10^{Aa}	1.50^{Ab}	2.03				
Mean		2.23	2.13	1.61					
Effluent losses, kg t^{-1}	With	165.14^{Aa}	154.46^{Ab}	93.58^{Bc}	137.73	5.42	0.1753	<0.0001	0.0014
	Without	150.95^{Aa}	150.44^{Aa}	135.19^{Aa}	145.53				
Mean		158.04	152.45	114.39					
Gas losses, % of DM	With	2.48	0.41	0.44	1.11A	0.18	0.8266	0.0025	0.5000
	Without	1.83	0.85	0.40	1.03A				
Mean		2.16a	0.63^{b}	0.42b					
Dry matter recovery, % of DM	With	73.99	89.12	87.29	83.47A	2.18	0.5732	<0.0001	0.1143
	Without	75.14	83.60	89.09	82.61A				
Mean		74.57^{b}	86.36^{a}	88.19^{a}					

SEM= standard error of the mean.

Means followed by the same lowercase letter in the row and uppercase letter in the column do not differ by the SNK test at a 5% significance level.

The gas losses (PG) were higher (P<0.05) in the BRS Capiaçu grass silage harvested after 85 d (2.16 %), whereas inoculation did not reduce (P>0.05) this parameter. The highest DMRI (P>0.05) was obtained in the silages of the forages harvested after 110 and 135 d, 15.08 % higher than the DMRI of the forage harvested after 85 d (Table 2).

The population of lactic acid bacteria (LAB) was higher (P<0.05) in the silage of the BRS Capiaçu grass forage harvested after 85 and 110 d (5.9 $\log_{10}$ CFU g^{-1}). However, inoculation did not decrease (>0.05) the population of LAB (Table 3).

There was significant interaction (P<0.05) of the regrowth days and inoculation on the population of molds and yeasts of the BRS Capiaçu grass silage. The population of molds and yeasts was, on average, 4.0 $\log_{10}$ CFU g^{-1}. However, when the inoculant was applied to the forage, the concentration of molds and yeasts was observed between the ages of 85 and 135 d, while in the treatments without application of inoculants, no significant differences (P>0.05) were observed between the assessed ages. No populations of *Clostridium* spp. were detected (Table 3).

There was significant interaction (P<0.05) of the regrowth days and inoculation on the dry matter (DM), ash, crude protein (CP) and neutral detergent fiber corrected for ash and protein (NDFap) of the BRS Capiaçu grass silages. The DM content of the silages increased (P<0.05) with the harvest age, ranging from 29.36 % in the silage of the forage harvested after 85 d to 34.15 % in the forage harvested after 135 d. Inoculation increased (P<0.05) the DM content of the forage harvested after 85 d from 27.33 % to 29.36 % (Table 4).

Table 3: Microbiological profile of BRS Capiaçu grass silages at different harvest ages and bacterial inoculation

Item (log_{10} CFU g^{-1})	Inoculant	Harvest ages (days)			Mean	SEM	*P*-value		
		85	110	135			Inoculant	Harvest age	Inoculant x harvest age
Lactic acid bacteria	With	6.0	5.4	3.8	5.1^{A}	0.25	0.2575	0.0005	0.8815
	Without	6.2	5.9	4.8	5.5^{A}				
Mean		6.1^{a}	5.7^{a}	4.0^{b}					
Molds and yeasts	With	3.4^{Ab}	$3.8A^{ab}$	4.6^{Aa}	3.9	0.21	0.8008	0.5663	0.0137
	Without	5.0^{Aa}	3.7^{Aa}	3.3^{Aa}	4.0				
Mean		4.2	3.7	4.0					

SEM= standard error of the mean.

Means followed by the same lowercase letter in the row and uppercase letter in the column do not differ by the SNK test at a 5% significance level.

Table 4: Chemical composition of BRS Capiaçu grass silages at different harvest ages and bacterial inoculation

Item (%)	Inoculant	Harvest ages (days)			Mean	SEM	*P*-value		
		85	110	135			Inoculant	Harvest age	Inoculant x harvest age
DM	With	29.36^{Ac}	30.55^{Ab}	34.15^{Aa}	31.35	0.38	0.5097	<0.0001	0.0223
	Without	27.33^{Bc}	31.67^{Ab}	34.21$_{Aa}$	31.07				
Mean		28.35	31.11	34.21					
Ash	With	8.66^{Aa}	6.97^{Bb}	7.56^{Ab}	7.73	0.12	0.0088	<0.0001	0.0053
	Without	8.97^{Aa}	8.29^{Ab}	7.39^{Ac}	8.21				
Mean		8.81	7.63	7.47					
CP	With	5.21^{Aa}	3.54^{Bb}	3.32^{Ab}	4.02	0.14	0.0297	<0.0001	<0.0001
	Without	5.37^{Aa}	4.61^{Ab}	2.91^{Bc}	4.28				
Mean		5.26	4.08	3.12					
EE	With	1.32	0.89	0.79	1.00^{B}	0.04	0.0497	<0.0001	0.1510
	Without	1.29	1.01	1.02	1.11^{A}				
Mean		1.30^{a}	0.95^{b}	0.90^{b}					
NDFap	With	68.59^{Ab}	72.08^{Aa}	71.91A^{a}	70.86	0.42	0.1345	<0.0001	0.0214

	Without	68.83^{Ac}	70.70^{Ab}	71.96^{Aa}	70.49				
Mean		68.71	71.39	71.93					
ADF	With	47.67	49.20	49.94	48.94^{A}	0.20	0.0406	<0.0001	0.8647
	Without	46.64	48.62	49.25	48.17^{B}				
Mean		47.15^{b}	48.91^{a}	49.60^{a}					

DM= dry matter, CP= crude protein, EE= ether extract, NDFap= neutral detergent fiber corrected for ash and protein, ADF= acid detergent fiber, SEM= standard error of the mean.

Means followed by the same lowercase letter in the row and uppercase letter in the column do not differ by the SNK test at a 5% significance level.

The highest ash, CP, and EE contents (P<0.05) were observed in the silage of the BRS Capiaçu grass harvested after 85 d. In contrast, inoculation of the forage harvested after 110 d resulted in the lowest (P<0.05) ash content (6.97 % *vs* 8.29 %). Inoculation resulted in the lowest (P<0.05) EE content in the silage (1.0 %) in relation to the silage without inoculation (1.11 %). Inoculation resulted in equivalence (P>0.05) in the CP content of the silages of the forages harvested after 110 and 135 d (3.43 %), although lower (P<0.05) than the silage of the forage harvested after 85 d (5.21 %). The CP content of the forage without inoculation decreased (P<0.05) with the advance of regrowth days (Table 4).

The NDFap contents of BRS Capiaçu grass forage silage harvested at 110 and 135 d (88.45 % and 72 %) were higher (P<0.05) than those of forage silage harvested at 85 d (84.81 % and 68.59 %). The NDFap contents of uninoculated silages increased (P<0.05) with harvest age (Table 4).

The ADF contents were lower (P<0.05) in the silage of the forage harvested after 85 d. Inoculant application resulted in the highest (P<0.05) ADF conten t in the silage (48.94 %) in relation to the absence of inoculant (48.17 %) (Table 4).

Discussion

The conservation of forage in the ensiling process is based on the principle of conservation in an anaerobic environment, where the absence of oxygen in the silo predisposes to the increase of lactic acid bacteria (LAB), which emit pH and prevent the development of undesirable microorganisms that harm the quality of the silage[18].

The application or not of inoculant did not influence the population of molds and yeasts in the forage silage. The silages of the forages harvested at younger ages (85 and 110 d) showed a greater population of LAB, favoring the fermentation of the forage harvested after 85 d due to its lowest pH. According to Kung *et al*[19], the possible explanations for flaws in the use of LAB-based inoculants include the intense competition of the epiphytic flora and soluble carbohydrates, excess oxygen, and problems during inoculation.

The low pH of the silages (<4.5) favored the absence of *Clostridium* spp. in this study. According to Pahlow *et al*[20], these bacteria demand high pH values for their development. The presence of undesirable microorganisms is mainly associated with flaws during fermentation.

The absence of *Clostridium* ssp. in the silages of the present study, responsible for proteolysis during ensilage, contributed to the low N-NH_3 concentrations obtained in the silages. Furthermore, the fact that the pH values of the silages were below 4.5 increases the fermentation efficiency and reduces protein hydrolysis in non-protein nitrogen

compounds [21]. Similar results were obtained for the silage of the elephant grass cv. Roxo with bacterial inoculation[22].

The low PG values can be attributed to the absence of *Clostridium* spp. bacteria in the silages of the present study, the main ones responsible for CO_2 production and other acids. Inoculation was unfavorable in reducing the pH due to the reduced losses observed in this study. In all silages, the dry matter contents (DM) increased from as the age regrowth days increased. According to Van Soest[23], the increase in DM is mainly due to the high effluent losses resulting from the low DM contents before ensilage, which was observed in the present study. With regard to inoculant application, an increase in DM content was observed only at 85 d of cutting age, treatment with the lowest DM content.

Microbial inoculation reduced the proteolytic activity of the silages, resulting in a rapid pH reduction since proteolytic bacteria develop better in silages with higher pH values. Therefore, the high pH value in the forages harvested after 110 and 135 d (4.20 and 4.36) favored CP reduction compared to the silages harvested after 85 d, which showed the highest CP and the lowest pH (3.65). The BRS Capiaçu grass silages showed CP contents lower than the 7 % minimum proposed by Church[24] as necessary to sustain microbial activity in the rumen, indicating the need for protein supplementation in order to meet the nutrient requirements of ruminants.

Inoculation in the BRS Capiaçu grass forage resulted in the lowest EE content in relation to the silage without the inoculant. However, these silages showed less than 8 % of EE, which is recommended by McGuffey and Schingoethe[25] to prevent reductions in food consumption and limited ruminant performance. However, the low EE proportion impacts the energy value of silages, considering the calorific value of lipids in relation to other organic compounds.

According to Wilson[26], tropical grasses require support structures represented by the cell wall. Therefore, the older the plant age, the greater the proportion of cell wall components and the lower the cell content. These statements justify the results of the BRS Capiaçu silages harvested after 85 d, which showed the lowest contents of fibrous constituents (NDFap and ADF) and the highest contents of non-fiber constituents (CP and EE) compared to the regrowth days of 110 and 135 d.

Inoculation in the BRS Capiaçu grass forage resulted in the highest ADF content in relation to the non-inoculated sample. A similar behavior was observed by others[7,27], who mention increased ADF contents (48.35 % and 46.86 %) in silages of the elephant grass cultivars Napier and Cameron with bacterial inoculant. Inoculation in the silages of the BRS Capiaçu grass may have increased the cellulose contents through the absence of activity in the enzymatic complex of the inoculant, solubilizing cell wall constituents[28], and increasing the ADF contents.

Conclusions and implications

The BRS Capiaçu forage silage harvested at 110 d demonstrated favorable performance for silage production. However, the influence of inoculant use was low for the characteristics evaluated. These results indicate that the BRS Capiaçu cultivar naturally can have good ensiling capacity and the use of inoculants can be ineffective as it depends on several factors, such as forage management, concentration of epiphytic bacteria and the inoculant, in addition to environmental conditions. Therefore, to more comprehensively evaluate the potential of using inoculants, it is necessary to use specific inoculants in the BRS Capiaçu cultivar. These investigations can provide valuable insights into the effectiveness and economic viability of using inoculants to optimize the fermentation and quality of BRS Capiaçu silage harvested at different ages.

Acknowledgments

Thanks to the Postgraduate Program in Tropical Animal Science of the Federal University of Teresina, Piauí, Brazil, the National Council for Scientific and Technological Development (CNPq, Brasília, DF, Brazil), and the Federal Institute of Maranhão (Caxias, Maranhão, Brazil).

Conflicts of Interest

The authors have no conflicts of interest to declare.

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