

Fermentation dynamics, nutritional quality, and heating capacity of mixed silages of elephant grass (*Pennisetum purpureum* Schum) and Leucaena (*Leucaena leucocephala*)

Dinâmica fermentativa, qualidade nutricional e capacidade de aquecimento de silagens mistas de capim elefante (Pennisetum purpureum Schum) e leucena (Leucaena leucocephala)

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ABSTRACT

Leucaena has been used to make mixed silages to obtain nutritional enrichment of the silages. Thus, the inclusion of Leucaena as an additive in mixed elephant grass silages can reduce fermentation losses, and increase the nutritional value and aerobic stability of the mixed silage without changing the fermentation profile. This study evaluated the fermentation profile, nutritional composition, and aerobic stability of elephant grass silages combined with different levels of Leucaena. A total of five inclusion levels of Leucaena (0, 20, 40, 60, and 80% on a dry matter basis) were added to elephant grass silages. A completely randomized design was adopted, with 5 treatments and 3 repetitions, totaling 15 experimental silos that were opened after 30 days of sealing. Fermentation profile, chemical composition, and aerobic stability were analyzed. A descriptive analysis of temperature and pH peaks during aerobic stability was performed. The increase in the inclusion of Leucaena in the composition of silages reduced gas and effluent losses, neutral and acid detergent fiber, cellulose, lignin, total and fiber carbohydrates, and total digestible nutrients, and resulted in increased dry matter, ether extract, and crude protein. A quadratic effect of treatments was found for the temperature to reach the maximum pH (P=0.009). Aerobic stability remained constant after 40% Leucaena inclusion in the composition of elephant grass silages reduces fermentation losses, promotes a nutritional increase, and increases the aerobic stability of the silages.

Keywords: Dry matter. Effluent losses. Fermentation. Forage conservation. Semiarid.

RESUMO

A leucena tem sido utilizada para a confecção de silagens mistas para o enriquecimento nutricional das silagens. Assim, a inclusão da leucena como aditivo em silagens mistas de capim-elefante pode reduzir as perdas fermentativas e aumentar o valor nutricional e a estabilidade aeróbia das silagens, sem alterar o seu perfil fermentativo. Objetivou-se avaliar o perfil fermentativo, composição nutricional e estabilidade aeróbia de silagens de capim elefante associadas com níveis crescentes de leucena. Um total de cinco níveis de leucena (0, 20, 40, 60 e 80% em base da matéria seca) foram incluídos em silagens de capim elefante. Adotou-se um delineamento inteiramente casualizado, com 5 tratamentos e 3 repetições, totalizando 15 silos experimentais, os quais foram abertos após 30 dias de ensilagem. Foram analisados o perfil fermentativo, a composição química e a estabilidade aeróbica. Foi realizada uma análise descritiva dos picos de temperatura e pH durante a estabilidade aeróbia. O aumento da inclusão da leucena na composição das silagens reduziu as perdas por gases, perdas por efluentes, fibra em detergente neutro, fibra em detergente ácido, celulose, lignina, carboidratos totais, carboidratos fibrosos e nutrientes digestíveis totais e aumentou os de teores de matéria seca, extrato etéreo e proteína bruta. Foi observado efeito quadrático dos tratamentos sobre a temperatura para atingir o pH máximo (P=0.009). A estabilidade aeróbia permaneceu constante a partir de 40% de inclusão de leucena na composição das silagens de capim elefante. A inclusão da leucena em até 80% na composição de silagens de capim elefante reduz as perdas fermentativas, promove incremento nutricional e aumento da estabilidade aeróbia das silagens.

Palavras-chave: Matéria seca. Perdas por efluente. Fermentação. Conservação de forragem. Semiárido.

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Introduction

Elephant grass (*Pennisetum purpureum* Schum) has become an important forage resource for the development of livestock in dryland regions. In this sense, one of the ways to preserve this grass is by ensiling. However, one of the difficulties in ensiling this grass is related to the high moisture content (83.7% on a natural matter basis; (Garcez et al., 2021)), low content of water-soluble carbohydrates (12.7% on a dry matter basis; (Li et al., 2019)), and dry matter losses due to oxidation and carbon dioxide production (Garcez et al., 2021).

To obtain silage with good fermentation and nutritional patterns, it is necessary to know the factors that alter the dynamics of dry matter and nutrient losses (Kim et al., 2021). Likewise, care about aerobic stability is essential to achieve the productive efficiency of silage. In this context, losses during the fermentation process can reduce dry matter recovery, since the surface of the silo tends to increase oxygen penetration, causing oxidation of organic matter and reduction of dry matter (Borreani et al., 2018).

There is an interest in combining forage plants as mixed silages to improve nutritional quality and reduce fermentation losses during the process (Drouin et al., 2021). In this scenario, woody forages, such as Leucaena (*Leucaena leucocephala*), have been used as an additive for silage making (Rodrigues et al., 2020) as a viable source of dry matter and crude protein, to obtain nutritional enrichment of the silages produced (Zhang et al., 2019).

Given the nutritional characteristics of elephant grass and Leucaena, we hypothesized that the inclusion of Leucaena as a nutrient additive to compose mixed elephant grass silages enables the reduction of fermentation losses, nutritional increase, and aerobic stability of the silage without changing the fermentation profile. Therefore, the objective was to evaluate the fermentation profile, nutritional composition, and aerobic stability of elephant grass silages combined with increasing levels of Leucaena.

Material and Methods

The experiment was conducted at the Laboratory of Animal Requirement and Metabolism (LEMA) belonging to the Agricultural Sciences Campus of the Federal University of the São Francisco Valley (UNIVASF), Petrolina, state of Pernambuco, Brazil (9° 19' 28" South latitude, 40° 33' 34" West longitude, 393 m altitude). The climate is hot and semi-arid, with a rainy season (BSh) (Köppen & Geiger, 1928), and an average annual rainfall of 376 mm.

Levels of Leucaena inclusion (0, 20, 40, 60, and 80% on a dry matter basis) were evaluated in elephant grass silage, in a completely randomized experimental design, with 5 treatments and 3 repetitions, totaling 15 experimental silos. Elephant grass (cv. Cameron) used for making the silages came from a planted grass field and was harvested after 60 days of regrowth, cut manually at 10 cm from the ground. The Leucaena came from an experimental area used as a protein bank, planted five years ago, with the upper third of the plants manually harvested. The collected material was processed in a stationary forage machine (Nogueira Pecus 9004, Saltinho - SP, Brazil) and then samples of elephant grass and Leucaena were evaluated for

Table 1 – Particles and chemical composition of elephant grass and Leucaena before ensiling

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Particle size >19 mm 9-19 mm 4-8 mm < 4 mm Chemical composition (g/kg DM) Dry matter* Mineral matter Organic matter Ether extract Crude protein Neutral detergent fiber Acid detergente fiber Hemicellulose Cellulose	Elephant grass	Leucaena				
Particle Size	(%)	(%)				
>19 mm	23.41	32.01				
9-19 mm	47.69	41.88				
4-8 mm	15.98	13.68				
< 4 mm	12.07	10.87				
Chemical composition (g/kg DM)						
Dry matter*	289.75	349.16				
Mineral matter	66.03	74.96				
Organic matter	933.96	925.03				
Ether extract	14.34	51.52				
Crude protein	50.45	265.96				
Neutral detergent fiber	763.70	601.33				
Acid detergente fiber	479.62	351.70				
Hemicellulose	284.08	249.63				
Cellulose	440.68	319.08				
Acid detergent lignin	38.93	32.61				
Total carbohydrates	869.17	607.55				
Non-fibrous carbohydrates	105.47	6.22				
Fibrous carbohydrates	763.70	601.33				
Total digestible nutrients	343.81	457.46				
DM. 1						

DM: dry matter; *in g/kg natural matter.

average particle size (Table 1) using the Penn State Particle Size Separator (PSPSS), with diameters of 19.8 and 4 mm of porosity and a bottom box (Heinrichs & Kononoff, 2013). Samples of the material before ensiling (original material) were collected for further laboratory analysis (Table 1).

The material was manually mixed according to the treatment levels, on a dry matter basis. Soon after mixing, the material was ensiled in experimental silos (10 cm in diameter, 50 cm in height, and 326.99 cm³) made of polyvinyl chloride (PVC), equipped with a Bunsen valve to allow the escape of gases from fermentation. For drainage and quantification of effluents, 1 kg dry sand was deposited at the bottom of the experimental silos, protected by cotton fabric, avoiding contact of the ensiled mass with the sand, allowing the effluent to drain. Silos were weighed before and after forage deposition. Silos were opened after 30 days of fermentation, and the 10 cm of silage at the top and bottom of the silos were disregarded.

Silos were weighed empty, after ensiling and weighed again 30 days after sealing, upon opening. Effluent losses (EL), gas losses (GL), and dry matter recovery (DMR) were estimated according to Amorim et al. (2020):

$$GL = \left(\left(SWC - SWO \right) / FMC \times MSi \right) \times 1000 \tag{1}$$

where SWC = total silo weight at closure, SWO = total silo weight at opening, FMC = forage mass at closure, and; DMCC = forage dry matter concentration at closure.

$$EL = \left[\left((ESWO - SW) - (ESWC - SW) \right) / FMC \right] \times 1000$$
 (2)

where ESWO = empty silo weight + sand weight + screen at opening; SW = empty silo weight, ESWC = empty silo weight + sand weight + screen at closure and,

$$FMC = forage \ mass \ at \ closure.DMR = \begin{pmatrix} (FMO \ x \ DMO) \ / \\ (FMC \ x \ DMC) \end{pmatrix} x \ 100 \quad (3)$$

where DMR = dry matter recovery rate; FMO = forage mass at opening; DMO = dry matter at opening; FMC = forage mass at closure and DMC = dry matter at closure.

To estimate the permeability (*K*, in μ m²) the equation by Williams (1994) was applied:

$$K = (726 - 0.368DM - 0.737\rho - 94.0 (DM / \rho,))$$
(4)

where ρ = density; DM = dry matter.

Silage porosity (POR, in μm) was determined according to Richard et al. (2004):

$$POR = 1 - \rho_{MN} x \begin{cases} \left[(1 - DM) / \rho_a \right] + \\ \left[(DM \ x \ OM) / \rho_{OM} \right] + \\ \left[(DM \ x \ (1 - OM)) / \rho_{MM} \right] \end{cases}$$
(5)

where ρ_{MN} = material density in natural matter (g/cm³); ρ_{a} water density (1 g/cm³); ρ_{om} = organic matter density (1.6 g/cm³); ρ_{MM} = mineral matter density (2.5 g/cm³); DM = dry matter; and OM = organic matter.

The density of the ensiled mass (DENS, in kg/m³) was obtained through the equation:

$$DENS = m / V \tag{6}$$

where m= weight of the ensiled mass, kg; V= volume of the ensiled material.

For the evaluation of the fermentative profile, the internal temperature (T, in °C), and temperature of the silo panel (TP, in °C) were measured at the time of opening with the aid of a digital infrared thermometer (Benetech, Rio de Janeiro – RJ, Brazil) and pH according to the methodology of Silva & Queiroz (2002). Aerobic stability (AS, in hours) was assessed following the methodology of Costa et al. (2021). The internal temperature of the silages was measured at 1-h intervals, for 120 h. During the stability test, the pH was monitored at 6-hour intervals until 96 h of exposure to air (Araújo et al., 2020).

The maximum pH recorded after opening the silos (maximum pH), time to reach maximum pH (maximum TpH, in hours), maximum temperature after opening the silos (MT, in °C), time to reach maximum temperature (TMT, in hours), the maximum difference between silage temperature and the environment temperature (DTS, in °C), the sum of the maximum difference of the silage temperature to the environment (Σ DT, in °C), and the time for the silage temperature showing an upward trend (STUT, in hours) were analyzed according to Tao et al. (2021).

Quantifications were performed for contents of dry matter (DM, method: 967.03), mineral matter (MM, method: 942.05), crude protein (CP, method: 981.10) (Association of Official Analytical Chemists, 2016), ether extract (EE) (American Oil Official Method Chemists' Society, 2017), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL) (van Soest, 1994), hemicellulose (HEM = NDF - ADF) and cellulose (CEL = ADF - ADL). The content of total digestible nutrients (TDN) was estimated according to Harlan et al. (1991). Total carbohydrates (TC) (Sniffen et al., 1992) and non-fibrous carbohydrates (NFC) (Hall, 2003) were estimated using the equations:

$$TC = 100 - (\% CP + \% EE + \% MM)$$
(7)

$$NFC = 100 - (\% CP + \% EE + \% MM + NDF)$$
 (8)

A descriptive analysis of temperature and pH peaks during aerobic stability was performed according to Wilkinson & Davies (2012). Data were analyzed using PROC GLM from the Statistical Analysis System University Software subjected to analysis of variance and regression at the level of 5% probability for type I error. The significance of the parameters estimated by the models and the coefficients of determination were used as a criterion for selecting regression models. The following statistical model was used:

$$Y = \mu + Tj + eij \tag{9}$$

where: μ = overall mean; Tj = effect of leucaena inclusion; eij = residual error.

Results

The inclusion of increasing levels of Leucaena with elephant grass for silage making had a negative linear effect on GL (P = 0.002) and EL (P = 0.032). There was no effect

of Leucaena inclusion levels on *K*, POR, RMS, and DENS of silages (P>0.05; Table 2). There was a positive linear effect of Leucaena inclusion on the silage pH (P < 0.001) and silo TP (P < 0.001; Table 2), with an increase of 0.01 on the pH scale and 0.01 °C for every 1% inclusion of Leucaena in silage composition. A negative linear effect of Leucaena inclusion was found on the temperature of silages (P < 0.001; Table 2).

In the stability test, the maximum pH of silages was not changed (P > 0.05). However, the TpH had a quadratic effect (P = 0.009) on leucaena inclusion (Table 3). For example, for silages with 40% inclusion of Leucaena TpH was reached 30 h before (86.00 h) than for silages of elephant grass with no inclusion of Leucaena (116.00 h). The pH at the end of the exposure period showed a quadratic behavior with higher values for the silage of elephant grass alone (0%) (P = 0.005; Table 3). There was a quadratic effect

Table 2 - Losses and fermentative profile of elephant grass silages with Leucaena inclusion levels

Variables —	Leucaena levels (%)					CEM.	P-value	
	0	20	40	60	80	SEM	L	Q
GL (%DM) ¹	20.27	19.44	18.28	17.43	17.00	0.66	0.002	0.661
EL (kg/t NM) ²	33.16	31.09	29.02	24.10	22.11	4.37	0.032	0.873
DMR (%DM)	93.36	93.80	90.26	91.91	91.32	1.08	0.111	0.457
<i>K</i> (μm²)	857.61	864.90	870.17	845.94	839.51	10.89	0.140	0.193
POR (µm)	71.02	69.83	68.64	67.46	66.27	0.04	0.998	0.998
DENS (kg/m ³)	420.67	439.86	457.00	444.61	450.04	10.91	0.096	0.193
рН³	3.44	3.68	3.97	4.68	4.33	0.22	0.003	0.388
T (°C) ⁴	28.00	27.33	27.33	26.00	26.00	0.24	<0.001	0.998
TP (°C)⁵	27.50	29.83	28.83	29.50	29.50	0.33	0.006	0.037

GL: gas losses; EL: effluent losses; DMR: dry matter recovery; *K*: permeability; POR: porosity; DENS: density; pH: hydrogenionic potential; T: temperature; TP: temperature of the silo panel; SEM: standard error of the mean; L: linear effect; Q: quadratic effect. Significance at 5% of probability. Equations: $^{1}\hat{y} = 20.2000 - 0.0428x$, $R^2 = 0.97$; $^{2}\hat{y} = 33.7180 - 0.1454x$, $R^2 = 0.97$; $^{3}\hat{y} = 3.4647 + 0.0140x$, $R^2 = 0.78$; $^{4}\hat{y} = 28.0000 + 0.0267x$, $R^2 = 0.88$; $^{5}\hat{y} = 28.3000 + 0.0183x$, $R^2 = 0.38$.

Table 3 – Aerobic stability of elephant grass silage with Leucaena inclusion levels

Variables —	Leucaena levels (%)					CEM	P-value	
	0	20	40	60	80	SEM	L	Q
Maximum pH	5.81lz	5.08	4.63	5.07	5.02	0.47	0.315	0.239
Maximum TpH (h) ¹	116.00	106.00	86.00	96.00	114.00	7.09	0.547	0.009
pH final ²	5.21	3.74	4.07	4.15	4.71	0.28	0.531	0.005
Maximum temperature (°C)	26.33	25.66	25.66	25.66	25.66	0.33	0.235	0.310
Final temperature (°C)	24.00	24.00	24.33	23.66	24.00	0.21	0.061	0.242
TMT (h) ³	44.55	39.65	39.71	44.72	54.68	1.19	< 0.001	< 0.001
DTS (°C)⁴	3.00	1.66	1.00	1.00	1.33	0.33	0.004	0.009
ΣDT (°C)⁵	41.96	-3.30	-8.60	-11.36	-9.80	4.75	< 0.001	< 0.001
STUT (h)6	22.66	22.33	23.00	23.16	23.00	0.18	0.027	0.812
Aerobic stability (h) ⁷	39.33	90.00	120.00	120.00	120.00	2.98	<0.001	<0.001

Maximum TpH: time to reach maximum pH; TMT: time to reach maximum temperature; DTS: the maximum difference between silage temperature and the environment temperature; Σ DT: the sum of the maximum difference of the silage temperature to the environment; STUT: silage temperature showing an upward trend; SEM: standard error of the mean; L: linear effect; Q: quadratic effect. Significance at 5% of probability. Equations: ${}^{1}\hat{y} = 118.6857 - 1.2986x + 0.0154x^{2}$, $R^{2} = 0.86; {}^{2}\hat{y} = 5.0379 - 0.0571x + 0.0007x^{2}$, $R^{2} = 0.78; {}^{3}\hat{y} = 44.5524 - 0.3686x + 0.0062x^{2}$, $R^{2} = 0.90; {}^{4}\hat{y} = 2.9714 - 0.0771x + 0.0007x^{2}$, $R = 0.99; {}^{5}\hat{y} = 37.8457 - 1.9332x + 0.0172x^{2}$, $R^{2} = 0.92; {}^{6}\hat{y} = 22.5333 + 0.0075x$, $R^{2} = 0.50; {}^{7}\hat{y} = 59.6000 + 0.9567x$, $R^{2} = 0.73$.

of Leucaena inclusion on DTS and Σ DT of silages, with higher temperatures for silage of elephant grass only (0%) (P < 0.05; Table 3). The inclusion of Leucaena showed a positive linear effect on STUT (P = 0.027) and AE (P < 0.001) of silages (Table 3).

The inclusion of 40 and 60% Leucaena resulted in elevations in temperatures at 80 h and 60 h (Figure 1A). In the exposure of silages to the aerobic environment, there were increases in pH before the silages had the maximum pH (Figure 1B).

The increased inclusion of Leucaena in the elephant grass silage composition resulted in positive linear effect on the contents of DM (P < 0.001), EE (P < 0.001), CP (P < 0.001) and TDN (P < 0.001) and a reduction in the contents of NDF (P < 0.001), ADF (P < 0.001), CEL (P < 0.001), ADL (P < 0.001), CHO (P < 0.001) and FC (P < 0.001). There was no effect of the inclusion of Leucaena on the levels of MM, OM, HEM, and NFC (P > 0.05; Table 4).

Discussion

The dynamics of GL and EL are directly related to the dynamics of fermentation of silages. For elephant grass silage making, one of the objectives is to reduce these losses and reduce the risk of fermentation by *Clostridium* (Borreani et al., 2018; Muck et al., 2018). These objectives were achieved with the inclusion of Leucaena, possibly because of the increase of DM and the osmotic pressure in the ensiled mass, avoiding the proliferation of *Clostridium*

bacteria, consequently maintaining the pH at suitable values and decreasing the percolation of nutrients through losses by effluents (König et al., 2017; Borreani et al., 2018).

To present good aerobic stability, it is crucial to maintain a good silo sealing capacity to avoid the exposure of silage to oxygen, paying attention to the size and uniformity of the particle size and compaction (Wilkinson & Davies, 2012). The similar results of POR, K, and Dens were reflected directly in DMR, which was also not affected by the inclusion of Leucaena. The rate of entry of oxygen into the ensiled mass, along with porosity, permeability and density are the factors that directly affect the silage during the fermentation period.

Exclusive silages or silages with the combination of legumes have a higher pH when compared to grass silages, which was observed in the present study. For example, silages with greater inclusion of Leucaena (60 and 80% inclusion) presented pH values above the limit (3.8-4.2) recommended by McDonald et al. (1991). This effect is attributed to the high buffering capacity of Leucaena (Gandra et al., 2017) associated with its low content of soluble carbohydrates (Andrade et al., 2018).

During the beginning of fermentation, the presence of residual oxygen is common, promoting the heating of the ensiled mass and the presence of undesirable microorganisms. In this sense, oxygen favors the proliferation of microorganisms; and after oxygen depletion, the temperature of the ensiled mass tends to decrease (Vu et al., 2019). This may explain

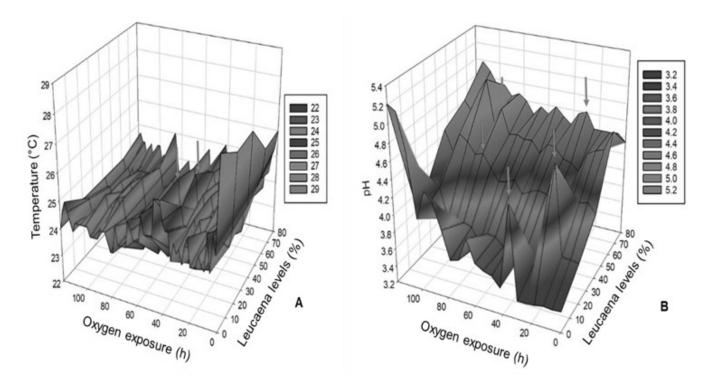


Figure 1 – Distribution of temperature (A) and pH (B) elevations before the onset of deterioration.

Table 4 - Chemical composition of elephant grass silage with Leucaena inclusion levels

Variables (g/kg dry matter)	Leucaena levels (%)						P-value	
	0	20	40	60	80	SEM	L	Q
Dry matter*1	297.87	310.38	309.61	325.91	334.53	2.90	<0.001	0.415
Mineral matter	65.66	66.63	67.07	61.14	64.63	2.17	0.298	0.875
Organic matter	930.53	930.15	925.91	937.52	932.25	1.60	0.059	0.337
Ether extract ²	13.62	15.42	19.65	19.46	24.65	1.42	<0.001	0.666
Crude protein³	49.64	80.25	120.74	144.75	201.88	4.98	<0.001	0.078
Neutral detergent fiber⁴	783.59	733.94	690.32	651.48	614.48	9.99	<0.001	0.440
Acid detergent fiber⁵	512.28	460.98	430.21	413.94	365.01	11.07	<0.001	0.652
Hemicellulose	271.30	272.95	260.13	237.47	250.01	12.42	0.075	0.802
Cellulose ⁶	474.44	424.60	393.75	378.80	331.00	10.93	<0.001	0.636
Acid detergent lignin ⁷	37.83	36.38	36.45	35.13	34.00	0.37	<0.001	0.606
Total carbohydrates [®]	871.05	837.68	792.53	774.63	708.82	5.90	<0.001	0.120
Non-fibrous carbohydrates	87.46	103.74	102.17	123.21	94.33	10.53	0.342	0.117
Fibrous carbohydrates ⁹ Total	783.59	733.94	690.35	651.41	614.48	9.99	<0.001	0.440
digestible nutrients ¹⁰	329.88	364.64	395.15	422.40	448.26	6.99	<0.001	0.440

*in g/kg natural matter; SEM: standard error of the mean; L: linear effect; Q: quadratic effect. Significance at 5% of probability. Equations: ${}^{1}\hat{y} = 297.8940 + 0.4444x$, R² = 0.93; ${}^{2}\hat{y} = 13.3478 + 0.1304x$, R² = 0.92; ${}^{3}\hat{y} = 45.6642 + 1.8448x$, R² = 0.98; ${}^{4}\hat{y} = 778.9048 - 2.1037x$, R² = 0.99; ${}^{5}\hat{y} = 504.8037 - 1.7079x$, R² = 0.97; ${}^{6}\hat{y} = 467.0561 - 1.6633x$, R² = 0.97; ${}^{7}\hat{y} = 37.7475 - 0.0446x$, R² = 0.94; ${}^{8}\hat{y} = 874.4495 - 1.9376x$, R² = 0.97; ${}^{9}\hat{y} = 778.9048 - 2.1037x$, R² = 0.99; ${}^{10}\hat{y} = 333.1666 + 1.4726x$, R² = 0.99.

the reduction of silage T with the inclusion of Leucaena. On the other hand, the silage TP increased with the inclusion of Leucaena, and this result may be related to the oxidation of the silo panel. As soon as the silo is sealed and the aerobic phase is completed, the temperature inside the silo tends to decrease and stabilize until the silo is opened under conditions of fermentation and stable preservation (Williams & Shinners, 2012).

Silo opening favors the proliferation of aerobic microorganisms. These microorganisms start their activity through the metabolization of lactic acid and residual carbohydrates as an energy source, an effect that favors the increase in silage pH when they are exposed to the aerobic environment (Weirich et al., 2018; Gayer et al., 2019). Thus, the change in time for silages to reach the maximum pH is related to the rate of proliferation of aerobic microorganisms and the amount of lactic acid and residual carbohydrates available.

TMT is related to the capacity to accumulate and dissipate heat from the mass of silage, an effect observed

in this study, in which silages of elephant grass only (0%) presented higher DTS, which leads to a higher heating capacity of the silages, such as the Σ DT, thus decreasing AS. This effect may be associated with the proliferation of fungi in elephant grass silages, which includes yeasts and molds causing silage degradation (Vu et al., 2019), and when exposed to the aerobic environment, these microorganisms assume the dominance of the medium. In addition to the direct association of temperature peaks with the population of organisms in the environment, the first temperature rise is related to the presence of yeasts and aerobic acetic acid bacteria, while the second rise is the result of the development of fungi (Wilkinson & Davies, 2012).

The increase in the content of dry matter, ether extract, and crude protein in silages is directly related to the nutritional characteristics of Leucaena which, in combination with elephant grass, improved the nutritional value of mixed silages. Results of dry matter are within the range described by Amorim et al. (2020) for a good fermentation of silages, which should be between 28 and 40% DM. The increase in CP content is also related to the nutritional value of Leucaena, providing values of crude protein above the minimum necessary to adequate ruminal fermentation, which is 7% according to van Soest (1994), supporting the positive contributions of the inclusion of Leucaena for improved silage quality.

The alteration in the contents of NDF, ADF, CEL, ADL, and FC are also directly related to fermentation losses, a process that results in the percolation of soluble fractions and increased concentrations of the cell wall (Ramos et al., 2021).

Conclusion

The inclusion of Leucaena up to 80% in the composition of elephant grass silages reduces fermentation losses,

References

American Oil Official Method Chemists' Society – AOCS. Official methods and recommended practices. 7th ed. Urbana: AOCS; 2017.

Amorim DS, Edvan RL, Nascimento RR, Bezerra LR, Araújo MJ, Silva AL, Mielezrski F, Nascimento KS. Fermentation profile and nutritional value of sesame silage compared to usual silages. Ital J Anim Sci. 2020;19(1):230-9. http://dx.doi.org/10.1080/1828051X.2020.1724523.

Andrade WR, Moura MMA, Rocha VR Jr, Costa RF, Santos LHT, Silva MM. Quality of sorghum silage with leucaena. Acta Sci Anim Sci. 2018;41(1):e36493. http:// dx.doi.org/10.4025/actascianimsci.v41i1.36493.

Araújo CA, Santos APM, Monteiro CCF, Lima DO, Torres AM, Santos CVS, Silva JJ. Effect of silage time on chemical composition, fermentative profile and aerobic stability of corn silages (*Zea mays*). Div J. 2020;5(1):547-61. http://dx.doi.org/10.17648/diversitas-journal-v5i1-1035.

Association of Official Analytical Chemists – AOAC. Official methods of analysis. 20th ed. Gaithersburg: AOAC International; 2016.

Borreani G, Tabacco E, Schmidt RJ, Holmes BJ, Muck RE. Silage review: factors affecting dry matter and quality losses in silages. J Dairy Sci. 2018;101(5):3952-79. http://dx.doi. org/10.3168/jds.2017-13837. PMid:29685272.

Costa DM, Carvalho BF, Bernardes TF, Schwan RF, Ávila CLS. New epiphytic strains of lactic acid bacteria improve the conservation of corn silage harvested at late maturity.

promotes a nutritional increase, and increases the aerobic stability of the silages.

Conflict of Interest

The authors declare that they have no competing interests.

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Anim Feed Sci Technol. 2021;274(1):114852. http://dx.doi. org/10.1016/j.anifeedsci.2021.114852.

Drouin P, Tremblay J, Renaud J, Apper E. Microbiota succession during aerobic stability of maize silage inoculated with *Lentilactobacillus buchneri* NCIMB 40788 and *Lentilactobacillus hilgardii* CNCM-I-4785. MicrobiologyOpen. 2021;10(1):e1153. http://dx.doi.org/10.1002/mbo3.1153. PMid:33369186.

Gandra JR, Oliveira ER, Gandra ERS, Takiya CS, Goes RHTB, Oliveira KMP, Silveira KA, Araki HMC, Orbach ND, Vasquez DN. Inoculation of *Lactobacillus buchneri* alone or with *Bacillus subtilis* and total losses, aerobic stability, and microbiological quality of sunflower silage. J Appl Anim Res. 2017;45(1):609-14. http://dx.doi.org/10.1080/09712119.2016.1249874.

Garcez AF No, Santos TM, Silva J, Fernandes SR. Effect of whey permeate and *Lactobacillus buchneri* on biomass conservation, chemical characteristics and aerobic stability of elephant grass silage. Waste Bio Val. 2021;12(2):879-93. http://dx.doi.org/10.1007/s12649-020-01035-z.

Gayer TO, Kasper NF, Tadielo LE, Krolow RH, Azevedo EB, Oaigen RP, Castagnara DD. Different dry matters content used for the conservation of annual ryegrass (*Lolium multiflorum* Lam.) in anaerobic environment. Afr J Agric Res. 2019;14(6):369-78. http://dx.doi.org/10.5897/AJAR2018.13675.

Hall MB. Challenges with non-fiber carbohydrate methods. J Anim Sci. 2003;81(12):3226-32. http://dx.doi. org/10.2527/2003.81123226x. PMid:14677880.

Harlan DW, Holter JB, Hayes HH. Detergent fiber traits to predict productive energy of forages fed free choice to non lacting dairy cattle. J Dairy Sci. 1991;74(4):1337-53. http://dx.doi.org/10.3168/jds.S0022-0302(91)78289-1. PMid:1650381.

Heinrichs J, Kononoff P. Evaluating particle size of forages and TMRs using the new Penn State Forage Particle Separator [Internet]. Pennsylvania: Pennsylvania State University, College of Agricultural Sciences, Cooperative Extension DAS; 2013 [cited 2021 Aug 11]. Available from: http://storti.com/documenti/doc_app/evaluating-particlesize-of-forages.pdf.

Kim D, Lee KD, Choi KC. Role of LAB in silage fermentation: effect on nutritional quality and organic acid production: an overview. AIMS Agric. Food. 2021;6(1):216-34. http://dx.doi.org/10.3934/agrfood.2021014.

König W, Lamminen M, Weiss K, Tuomivirta TT, Muñoz SS, Fritze H, Elo K, Puhakka L, Vanhatalo A, Jaakkola S. The effect of additives on the quality of white lupin-wheat silage assessed by fermentation pattern and qPCR quantification of clostridia. Grass Forr Sci. 2017;72(4):757-71. http://dx.doi.org/10.1111/gfs.12276.

Köppen W, Geiger R. Klimate der Erde. Gotha: Verlag Justus Perthes; 1928. Wall-map 150 cm × 200 cm.

Li D, Ni K, Zhang Y, Lin Y, Yang F. Fermentation characteristics, chemical composition and microbial community of tropical forage silage under different temperatures. Asian-Australas J Anim Sci. 2019;32(5):665-74. http://dx.doi.org/10.5713/ ajas.18.0085. PMid:30056673.

McDonald P, Henderson AR, Heron SJE. The biochemistry of silage. Marlow, UK: Chalcomb Publications; 1991.

Muck RE, Nadeau EMG, Mcallister TA, Contreras-Govea FE, Santos MC, Kung L Jr. Silage review: recent advances and future uses of silage additives. J Dairy Sci. 2018;101(5):3980-4000. http://dx.doi.org/10.3168/jds.2017-13839. PMid:29685273.

Ramos BLP, Pires AJV, Cruz NT, Santos APS, Nascimento LMG, Santos HP, Amorim JMS. Perdas no processo de ensilagem: uma breve revisão. Res Soc Dev. 2021;10(5):e8910514660. http://dx.doi.org/10.33448/rsd-v10i5.14660.

Richard TL, Veeken AH, Wilde V, Hamelers HVM. Air-filled porosity and permeability relationships during solid-state

fermentation. Biotechnol Prog. 2004;20(5):1372-81. http:// dx.doi.org/10.1021/bp0499505. PMid:15458320.

Rodrigues WM, Sales ECJ, Monção FP, Marques OFC, Rigueira JPS, Assis Pires DA, Gomes VM. pH, gas losses, effluents and nutritional valueof millet silages [*Pennisetum glaucum* (L.) R. Br.] with different levels of leucaena (*Leucaena Leucocephala* (Lam.) de Wit) in semiarid. Braz J Dev. 2020;6(4):22001-16. http://dx.doi.org/10.34117/ bjdv6n4-389.

Silva DJ, Queiroz AC. Análise de alimentos: métodos químicos e biológicos. 2a ed. Viçosa: Editora UFV; 2002.

Sniffen CJ, O'Connor JD, van Soest PJ, Fox DG, Russell JB. A net carbohydrate and protein system for evaluating cattle diets: II. Carbohydrate and protein availability. J Anim Sci. 1992;70(11):3562-77. http://dx.doi.org/10.2527/1992.70113562x. PMid:1459919.

Tao X, Ji C, Chen S, Zhao J, Wang S, Li J, Sun F, Shao T. Fermentation quality and aerobic stability of Napier grass ensiled with citric acid residue and lactic acid bacteria. Trop Grassl-Forrajes Trop. 2021;9(1):52-9. http://dx.doi. org/10.17138/tgft(9)52-59.

van Soest PJ. Nutritional ecology of the ruminant. 2nd ed. Ithaca: Cornell University Press; 1994. http://dx.doi. org/10.7591/9781501732355.

Vu VH, Li X, Wang M, Liu R, Zhang G, Liu W, Xia B, Sun Q. Dynamics of fungal community during silage fermentation of elephant grass (*Pennisetum purpureum*) produced in northern Vietnam. Asian-Australas J Anim Sci. 2019;32(7):996-1006. http://dx.doi.org/10.5713/ ajas.18.0708. PMid:30744340.

Weirich DT, Neres MA, Hunoff CA, Ströher SM, Nath CD, Sunahara SMM, Sarto JRW, Oldoni T. Microbiological profile and aerobic stability of Tifton 85 bermudagrass silage with or without vacuum and microbial inoculants. Biosci J. 2018;34(1):151-61. http://dx.doi.org/10.14393/BJ-v34n1a2018-33628.

Wilkinson JM, Davies DR. The aerobic stability of silage: key findings and recent developments. Grass Forr Sci. 2012;68(1):1-19. http://dx.doi.org/10.1111/j.1365-2494.2012.00891.x.

Williams AG. The permeability and porosity of grass silage as affected by dry matter. J Agric Eng Res. 1994;59(2):133-40. http://dx.doi.org/10.1006/jaer.1994.1070.

Williams SD, Shinners KJ. Farm-scale anaerobic storage and aerobic stability of high dry matter sorghum as a biomass feedstock. Biom Bioen. 2012;46(1):309-16. http://dx.doi. org/10.1016/j.biombioe.2012.08.010.

Zhang YC, Li DX, Wang XK, Lin YL, Zhang Q, Chen XY, Yang FY. Fermentation dynamics and diversity of

bacterial community in four typical woody forages. Ann Mic. 2019;69(3):233-40. http://dx.doi.org/10.1007/s13213-018-1398-z.

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