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Fermentation profile, nutritional value and aerobic stability of mixed elephant grass and butterfly pea silages

Perfil fermentativo, valor nutricional e estabilidade aeróbia de silagens mistas de capim-elefante e cunhã

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Abstract: The aim was to evaluate the effect of butterfly pea inclusion on the fermentation dynamics, nutritional quality and aerobic stability of mixed elephant grass silages. Butterfly pea levels (0, 20, 40, 60 and 80% on a natural matter basis) were added to elephant grass silages. A completely randomized design was adopted, with 5 treatments and 3 replications, totaling 15 experimental silos, which were opened after 30 days of fermentation. The inclusion of butterfly pea in elephant grass silages resulted in a quadratic effect for permeability, density, maximum pH, final pH, time to reach maximum temperature and aerobic stability ($P<0.05$). Butterfly pea inclusion levels increased dry matter recovery, pH, dry matter, organic matter, ether extract, crude protein and total digestible nutrients ($P<0.001$) and reduced gas and effluent losses, mineral matter, neutral detergent fiber, acid detergent fiber, hemicellulose, cellulose, lignin and total carbohydrates ($P<0.001$). Inclusions of 40, 60 and 80% of butterfly pea provided temperature increases at 10, 20, 30 and 40 hours. The inclusion of butterfly pea with levels of up to 80% reduces fermentation losses, allows for a nutritional increase and increase in aerobic stability of silages.

Keywords: fermentation dynamics; legume silage

Resumo: Objetivou-se avaliar o efeito da inclusão de cunhã na dinâmica fermentativa, qualidade nutricional e estabilidade aeróbia de silagens mistas de capim-elefante. Níveis de cunhã (0, 20, 40, 60 e 80% na matéria natural) foram adicionados às silagens de capim-elefante. Adotou-se o delineamento inteiramente casualizado, com 5 tratamentos e 3 repetições, totalizando 15 silos experimentais, que foram abertos após 30 dias de fermentação. A inclusão de cunhã nas silagens de capim-elefante resultou em efeito quadrático para permeabilidade, densidade, pH máximo, pH final, tempo para atingir a temperatura máxima e estabilidade aeróbia (P<0,05). Níveis de inclusão de cunhã aumentaram

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a recuperação de matéria seca, pH, matéria seca, matéria orgânica, extrato etéreo, proteína bruta e nutrientes digestíveis totais (P<0,001) e reduziram as perdas por gases e efluentes, matéria mineral, fibra em detergente neutro, fibra em detergente ácido, hemicelulose, celulose, lignina e carboidratos totais (P<0,001). As inclusões de 40, 60 e 80% de cunhã proporcionaram aumentos de temperatura às 10, 20, 30 e 40 horas. A inclusão de cunhã em teores de até 80% reduz as perdas na fermentação, permite incremento nutricional e aumento da estabilidade aeróbica das silagens.

Palavras-chave: dinâmica da fermentação; silagem de leguminosas

1. Introduction

Tropical grasses are widely used for making silage. Elephant grass (*Pennisetum purpureum* Schum) is a grass widely used in ruminant production in tropical regions due to its great potential for dry matter production, high regrowth capacity, good adaptation to different climatic conditions and well accepted by animals[1].

However, although elephant grass has a nutritional value considered ideal for fermentation, it has a low dry matter content, which directly influences the increase in buffering capacity and water-soluble carbohydrates[2]. These characteristics compromise the fermentation efficiency in silage, as high moisture content favors the occurrence of secondary fermentation, caused by bacteria of the genus *Clostridium* sp.[3]. In addition, the high content of degraded soluble carbohydrates results in the production of butyric acid and ammonia release, affecting silage quality and reducing its nutritional value [4], with losses of the most digestible fraction of the plant causing the increase in fiber fraction components during effluent percolation[5].

The use of absorbent additives upon ensiling tropical grasses has been one of the main technologies adopted to increase the dry matter content and thus reduce the losses often found in these silages[6]. Among the commonly used additives, legumes have been gaining a prominent role, due to their physical and chemical characteristics that improve the fermentation and nutritional characteristics of silage. In this perspective, the use of butterfly pea (*Clitoria ternatea* L.) becomes an excellent option for ensiling elephant grass.

Butterfly pea is a legume widely distributed in tropical and subtropical regions, presenting good adaptability to the climate and soil of the Brazilian semi-arid region, with high dry matter production in this region (approximately 4.2 tons per hectare)[7]. In addition to persistence, this legume forage has high nutritional value, with high levels of dry matter (351.2 g/kg in fresh matter), crude protein (162.3 g/kg in dry matter) and neutral detergent fiber (617.0 g/kg in dry matter) [8], and bioactive antioxidant and bactericidal compounds, which can modify the fermentation of silages, improving the quality of the ensiled mass[9]. Based on the above, it is possible to infer that the use of butterfly pea as an absorbent additive in the ensiling process of tropical grasses, such as elephant grass, makes it possible to increase dry matter levels and thus reduce fermentative losses, improving the silage's nutrition.

Therefore, based on the hypothesis that butterfly pea can guarantee the preservation and quality of elephant grass silage, the aim was to evaluate the effects of including butterfly pea levels on the fermentative dynamics, nutritional characteristics and aerobic stability of elephant grass silage.

2. Material and methods

The experiment was conducted at the Federal Universidade Federal do Vale do São Francisco (UNIVASF), Campus of Agricultural Sciences, Petrolina, Pernambuco, Brazil (silage process) and at the Brazilian Agricultural Research Corporation - Embrapa Semiárido, Petrolina, Pernambuco, Brazil (Laboratory analysis). According to Köppen's climate classification is hot semiarid(10). During the experimental period, maximum and minimum temperatures of 33.56 °C and 26.14 °C, with relative humidity between 73.56% and 58.10% respectively, with average evapotranspiration of 4.06 mm and average annual rainfall of 376 mm.

Five levels of butterfly pea (0; 20; 40; 60 and 80% on a natural matter basis) were evaluated in elephant grass silages, in a completely randomized design, with 5 treatments and 3 replications, totaling 15 experimental silos.

Elephant grass cv. Camerom (*Pennisetum purpureum* Schum) used to make silage came from a pre-planted grass field after 60 days of regrowth, manually cut at 10 cm from the ground, approximately 190 cm high. Butterfly pea (*Clitoria ternatea* Linn) came from an experimental area already established 36 months ago and used as a protein bank. Branches with 15 mm thickness (measured with a digital caliper) were harvested. The cuts were made 120 cm from the ground. The material was processed in a stationary forage machine (PP-35, Pinheiro Máquinas, Itapira, São Paulo, Brazil). Samples of elephant grass and butterfly pea were evaluated for average particle size using the "State Particle Size Separator" (SPSS) sieve set with 19.0; 8.0 and 4.0 mm mesh size and a bottom box(11). Samples of processed material (300g) were collected for chemical analysis (Table 1).

Table 1 Particles and chemical composition of elephant grass and butterfly pea before ensiling.

Particle size	Elephant grass	Butterfly pea
>19 mm	24.23	46.58
9 – 19 mm	47.15	42.02
4 – 8 mm	15.43	5.52
< 4 mm	12.07	4.36
Chemical composition (g/kg DM)		
Dry matter*	287.68	362.34
Mineral matter	66.07	62.65
Organic matter	933.92	937.34
Ether extract	21.70	28.43
Crude protein	60.78	161.35
Neutral detergent fibre	770.38	589.09
Acid detergente fibre	487.88	395.59

Hemicellulose	282.50	193.50
Cellulose	466.49	373.22
Lignin	21.39	22.37
Total carbohydrates	851.44	747.56
Non-fibrous carbohydrates	81.06	185.46
Total digestible nutrients	339.13	466.03

*DM- Dry matter; *in g/kg fresh matter*

The material was mixed according to the treatments and ensiled in experimental polyvinyl chloride (PVC) silos (10 cm in diameter, 50 cm in height), equipped with a Bunsen valve to allow gases to escape during fermentation. To quantify the effluents produced, 1 kg dry sand was deposited at the bottom of the silos, protected by a cotton fabric, preventing the ensiled material from coming into contact with the sand, allowing the effluent to drain. After sealed, silos remained in a covered shed for 30 days.

Silos were weighed empty, after ensiling and weighed again 30 days after ensiling, upon opening. After weighing, the top and bottom layers (10 cm) of the silage were discarded. Density (D), effluent losses (EL), gas losses (GL), and dry matter recovery (DMR) were estimated according to Jobim et al.(12). Porosity (POR, in μm), and permeability (*K,* in μm^2) were estimated according to Williams(13). To evaluate the fermentation 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), pH according AOAC(14), ammonia nitrogen (NH3-N, in % total N), and buffering capacity (BC, in E.mgNaOH/100g DM) were evaluated according to Mizubuti et al.(15).

Aerobic stability (AE, expressed in hours) was evaluated using the methodology of Kung Junior(16) in which plastic containers with a capacity of 4 L were used representing each experimental unit, the containers had approximately 2 kg forage, kept in a closed room, at a controlled temperature at 24±1°C. Silage spoilage was recognized when internal temperature exceeded that of the surrounding environment by 2°C(2). Internal temperature was measured at two hour-intervals for 96 hours with a digital thermometer (GULterm 180 – Gulton do Brasil Ltda.), inserting the stainless-steel tip into the center of the silage. During the stability test, the pH was measured at 6-hour intervals(17). The maximum pH recorded after opening the silos (maximum pH), final 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), maximum difference between silage temperature and the room temperature (DTS, in °C), the sum of the maximum difference between silage temperature and the room temperature (ΣDT, in °C), and the time for silage temperature showing an upward trend (STUT, in hours) were analyzed according to Tao et al.(18).

Chemical analyses were performed using the procedures described by the Association of Analytical Chemists(14) for determination of dry matter (DM; method 967.03), mineral matter (MM; method 942.05), crude protein (CP; method 981.10) and acid detergent fiber

(ADF; method 973.18). The ether extract (EE) content was analyzed using a fat extractor (ANKOM TX-10, Macedon – NY, United States)[19]. Neutral detergent fiber (NDF) and lignin (LIG) were determined according to Van Soest et al.[20]. Total carbohydrates (TC) were estimated according to Sniffen et al.[21]. Non-fiber carbohydrate (NFC) content were estimated according to Hall[22]. Hemicellulose (HEM) were estimated according to AOAC[11]. To determine lignin, samples were washed with 72% sulfuric acid for cellulose solubilization, and obtaining acid-digested lignin (ADL), according to the methodology proposed by Van Soest et al.[20]. Cellulose (CEL) was determined by the difference between the ADF – ADF. The content of total digestible nutrients (TDN) was estimated according to Undersander et al.[23].

A descriptive analysis of temperature and pH peaks during aerobic stability was performed according to Araújo et al.[17]. Data were tested by analysis of variance and regression at the level of 5% probability using PROC REG from the Statistical Analysis System Software (SAS University). 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 = μ + Tj + eij, where: μ = overall mean; Tj = effect of butterfly pea; eij = residual error.

3. Results

There was a decreasing linear effect on GL (*P=0.040*) and EL (*P<0.001)* with a reduced of 1.394% and 2.091% for each 1% butterfly pea added in elephant grass silages (Table 2). There was an increasing linear effect on DMR (*P<0.001*) with an increase of 0.138% for each 1% butterfly pea added in elephant grass silages (Table 2).

Quadratic effect was observed for *K* (*P=0.009*; Table 2), with a minimum point of 802.35 μm^2 in *K* with the inclusion of 50.34% butterfly pea in elephant grass silages. Regarding POR, a decreasing linear effect (*P<0.001*) was observed in silages, with a reduction of 0.079 μm for each 1% inclusion of butterfly pea in elephant grass silages (Table 2).

Table 2 **Losses and fermentative profile of elephant grass silages with butterfly pea inclusion levels.**

Variables	Butterfly pea levels (%)					SEM	P-value	
	0	20	40	60	80		L	Q
GL[1]	24.00	23.57	20.00	21.53	18.05	1.11	0.040	<0.001
EL[2]	16.85	14.42	12.42	11.99	7.61	1.52	<0.001	<0.001
DMR[3]	92.23	95.41	100.54	100.50	101.36	1.79	<0.001	0.001
K[4]	864.76	830.08	800.89	805.78	824.70	11.31	0.019	0.009
POR[5]	71.48	70.59	67.03	66.25	65.74	0.42	<0.001	0.050

D[6]	423.22	397.42	403.70	416.43	440.54	11.29	0.164	0.031
pH[7]	3.52	3.74	3.89	3.88	3.89	0.04	<0.001	0.012
T	27.83	27.50	28.16	27.50	27.83	0.25	0.998	0.998

*GL= gas losses (%DM); EL= effluent losses (kg/t FM); DMR= dry matter recovery (%DM); **K**= Permeability (μm^2); POR= Porosity (μm); D= Density (kg/m^3); T= temperature (°C); SEM= standard error of the mean; L= linear; Q= quadratic; Equations: $^1\hat{y}$= 25.612 – 1.394x, R^2 = 0.79; $^2\hat{y}$= 18.931 - 2.091x, R^2= 0.94; $^3\hat{y}$= 93.667 + 0.138x, R^2= 0.62; $^4\hat{y}$= 866.314 – 2.540x + 0.0252x^2, R^2= 0.98; $^5\hat{y}$= 71.389 – 0.0792x, R^2= 0.90; $^6\hat{y}$= 420.716 – 1.2500x + 0.019x^2, R^2= 0.95; $^7\hat{y}$= 3.611 + 0.0044x, R^2= 0.76. Significance at 5% of probability.*

Dens showed a quadratic effect (*P=0.031*; Table 2), with a minimum point of 400.13 kg.m^3 with the inclusion of 32.93% butterfly pea in elephant grass silages. pH of the silages increased linearly by 0.004 for every 1% butterfly pea included in the elephant grass silage (*P<0.001*; Table 2). The temperature of elephant grass silages was not affected (*P=0.998*; Table 2) by the inclusion of butterfly pea, with a mean value of 27.76 °C.

Butterfly pea levels provided a quadratic effect at maximum pH (*P=0.018*) and final pH (*P=0.021*) of silages (Table 3) during exposure to oxygen. A maximum point of 4.40 was found at the maximum pH recorded with the inclusion of 47.18% butterfly pea (Table 3).

Regarding the final pH, the maximum point was 4.36 with the inclusion of 48.10% butterfly pea (Table 3). There was no effect of butterfly pea inclusion on the time to reach maximum pH of silages (*P>0.05*), with a mean value of 46.4 hours (Table 3).

Table 3 **Aerobic stability of elephant grass silage with butterfly pea inclusion levels.**

Variables	Butterfly pea levels (%)					SEM	P-value	
	0	20	40	60	80		L	Q
pH maximum[1]	3.67	4.16	4.42	4.31	4.06	0.17	0.118	0.018
TpH maximum	48.00	44.00	48.00	48.00	44.00	2.00	0.541	0.605
pH final[2]	3.67	4.03	4.42	4.31	4.04	0.17	0.087	0.021
MT	27.00	26.83	26.50	27.16	27.00	0.30	0.739	0.405
T Final	26.33	26.33	26.00	26.00	26.33	0.25	0.692	0.325
TMT[3]	6.00	37.33	24.00	24.00	32.66	1.22	<0.001	<0.001
DST	2.66	2.66	2.00	2.00	2.33	0.36	0.275	0.352
ΣDT	18.33	18.33	13.66	14.00	19.66	2.97	0.863	0.172
AS[4]	6.00	26.00	24.66	24.00	32.66	1.52	<0.001	0.003

*MT= maximum temperature (°C); T Final= final temperature (°C); TMT= time to reach maximum temperature (h); DTS= maximum difference between silage temperature and the environment temperature (°C); **Σ**DT= sum of the maximum difference of the silage temperature in relation to the environment (°C); AS= aerobic stability (h); SEM= standard error of the mean; L= linear; Q= quadratic; Equations: $^1\hat{y}$= 3.674 + 0.0309x – 0.00033x^2, R^2= 0.99; $^2\hat{y}$= 3.640 + 0.0302x – 0.000314x^2, R^2= 0.95; $^3\hat{y}$= 12.229 + 0.657x – 0.0057x^2, R^2= 0.40; $^4\hat{y}$= 9.257 + 0571x – 0.0040x^2, R^2= 0.75. Significance at 5% of probability.*

There was no effect of butterfly pea inclusion on MT, final T, DST and ΣDT (*P>0.05*; Table 3) of silages. TMT was quadratically influenced (*P<0.001*; Table 3) by the levels of butterfly pea inclusion in elephant grass silages, with a maximum point of 31.12 hours with the inclusion of 57.50% butterfly pea. Quadratic effect was also verified for AS (*P=0.003*; Table 3), with a maximum point of 29.99 hours with the inclusion of 72.65% butterfly pea in silages.

In this study, the inclusion of 40, 60 and 80% butterfly pea resulted in temperature increases after 10, 20, 30 and 40 hours (Figure 1A). When these silages were exposed to an aerobic environment, pH increases were observed before the silages reached the maximum pH value (Figure 1B).

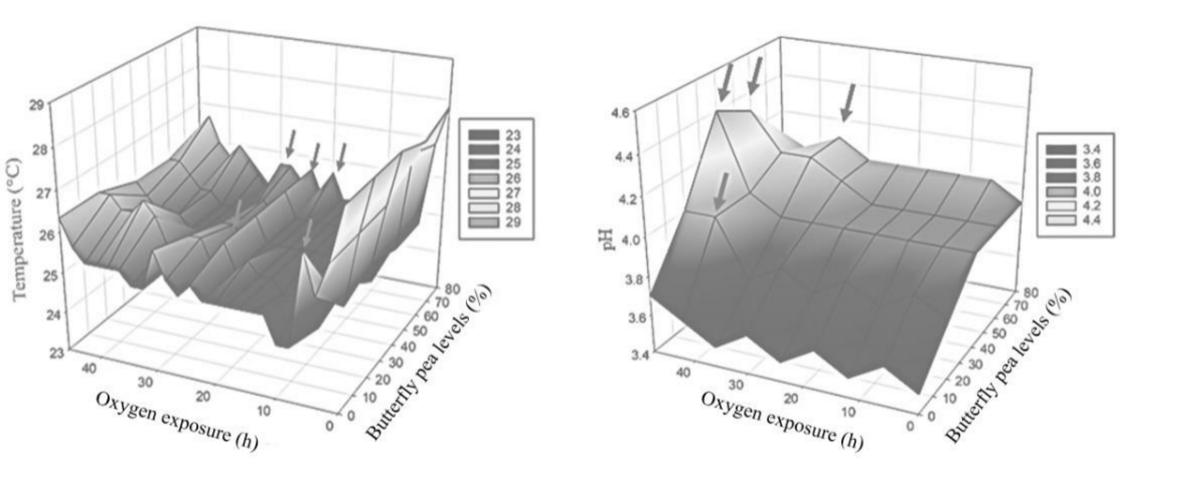

Figure 1 **Distribution of temperature (A) and pH (B) elevations of elephant grass silages with butterfly pea inclusion levels during aerobic stability.**

The inclusion of butterfly pea resulted in an increasing linear effect on the content of DM, OM, EE, CP, LIG, and TDN (*P<0.001*; Table 4), with increases of 0.79 g/kg FM; 0.20; 0.08; 1.05; 1.87 and 1.05 g/kg DM, respectively, for each 1% butterfly pea included in elephant grass silages (Table 4). There was a decreasing linear effect for MM, NDF, ADF, HEM, CEL, and TC (*P<0.001*; Table 4), with reductions of 0.20; 1.50; 0.47; 1.02; 0.38; and 1.11 g/kg DM, respectively, for each 1% butterfly pea included in elephant grass silages (Table 4). There was no effect of including butterfly pea (*P>0.05*; Table 4) on the NFC content of silages, with a mean value of 172.48g/kg DM.

Table 4 **Chemical composition of elephant grass with butterfly pea inclusion levels.**

Variables g/kg DM	Butterfly pea levels (%)					SEM	P-value	
	0	20	40	60	80		L	Q
DM[1]	285.13	294.07	329.62	337.46	342.61	4.23	<0.001	0.050
MM[2]	83.30	79.63	72.92	71.62	66.64	1.99	<0.001	0.717

OM[3]	916.69	920.36	927.07	928.37	933.35	1.99	<0.001	0.717
EE[4]	20.26	22.81	24.84	26.04	27.59	0.30	<0.001	0.032
CP[5]	59.94	81.46	101.60	121.31	145.09	1.24	<0.001	0.400
NDF[6]	754.25	727.58	689.77	658.98	638.51	16.12	<0.001	0.754
ADF[7]	475.97	471.90	469.25	454.83	436.55	5.34	<0.001	0.072
HEM[8]	278.28	255.68	220.52	204.15	201.96	13.51	<0.001	0.266
CEL[9]	450.66	447.28	447.39	434.87	418.25	5.28	<0.001	0.076
LIG[10]	18.28	19.96	21.80	24.62	25.31	1.34	<0.001	0.552
TC[11]	911.46	887.76	866.26	845.48	820.64	1.28	<0.001	0.754
NFC	157.21	160.17	176.49	186.49	182.13	16.57	0.177	0.742
TDN[12]	350.41	369.08	395.55	417.10	431.44	11.28	<0.001	0.754

**g/kg Fresh matter; DM= dry matter; MM= mineral matter; OM= organic matter; EE= ether extract; CP= crude protein; NDF= neutral detergent fiber; ADF= acid detergent fiber; HEM= hemicellulose; CEL= cellulose; LIG= lignin; TC= total carbohydrates; NFC= non-fibrous carbohydrates; TDN= total digestible nutrientes; SEM= standard error of the mean; L= linear; Q= quadratic; Equations:* [1] $\hat{y} = 286.113 + 0.792x$, $R^2 = 0.90$; [2] $\hat{y} = 83.092 - 0.207x$, $R^2 = 0.97$; [3] $\hat{y} = 916.908 + 0.207x$, $R^2 = 0.97$; [4] $\hat{y} = 20.730 + 0.0895x$, $R^2 = 0.97$; [5] $\hat{y} = 59.851 + 1.0577x$, $R^2 = 0.99$; [6] $\hat{y} = 753.842 - 1.500x$, $R^2 = 0.99$; [7] $\hat{y} = 480.887 - 0.479x$, $R^2 = 0.88$; [8] $\hat{y} = 272.956 - 1.0209x$, $R^2 = 0.92$; [9] $\hat{y} = 455.136 - 0.386x$, $R^2 = 0.82$; [10] $\hat{y} = 16.378 + 1.872x$, $R^2 = 0.98$; [11] $\hat{y} = 911.110 - 1.119x$, $R^2 = 0.99$; [12] $\hat{y} = 350.710 + 1.050x$, $R^2 = 0.99$. *Significance at 5% of probability.*

4. Discussion

Silage losses occur throughout the production process, being directly influenced by the moisture content of the silage[5]. Elephant grass contains a low dry matter content and reduced amount of soluble carbohydrates, which causes large effluents losses and the growth of undesirable bacteria, such as those of the genus *Clostridium*[24].

Losses (gases and effluents) occurring during fermentation are inevitable and can be minimized with a forage combination that balances the moisture content of the silage. With the increase in EL, nutrients are leached, causing nutritional damage to the final product[25]. Gas losses is related to the type of fermentation that occurs inside the silo, so the low values of gas losses demonstrate that in the ensiling process there was little participation of fermentation by enterobacteria and clostridial bacteria, which resulted in a decrease in secondary fermentations[26]. Thus, the inclusion of butterfly pea allowed the reduction of EL and GL and increased the DMR. This fact was also observed by Almeida et al.[27] by including 30% butterfly pea hay in the composition of mixed corn silages with and without cobs and by Lemos et al.[8] when evaluating silages of different varieties of elephant grass associated with butterfly pea. According to these authors, the inclusion of legumes in silage increases the dry matter content, reduces losses and secondary fermentations and recovers a greater proportion of ensiled mass, providing advantages for the ensiling process.

According to Randby et al.[28], porosity is directly related to the aeration rate in the silo, consequently, it will influence the degree of silage spoilage. The greater the porosity, the easier it is for air to enter the silo, causing the proliferation of inappropriate microorganisms. In this sense, it is necessary to reduce these values and obtain anaerobic conditions.

Silage density is directly influenced by particle size and compaction of the ensiled mass. Despite the higher density observed for elephant grass silage containing 80% butterfly pea, in relation to the control treatment (0% butterfly pea), all silages presented a density below the established density (between 500 - 600 kg/m^3). According to Costa et al.[29], low densities favor increased losses and make DMR difficult, promoting a reduction in silage quality. However, despite the lower density obtained in this study, the inclusion of butterfly peas reduced fermentative losses and increased the dry matter content of the silages. However, more studies are needed with intermediate levels of butterfly pea inclusion in elephant grass silages, subjected to a longer storage period in silos, so that the behavior of this variable can be observed.

The pH value is an important indicator for evaluating the fermentation quality of the silage[30]. The addition of butterfly pea contributed to the increase in the pH of elephant grass silages, with a variation between 3.52 - 3.89. This effect was expected due to the buffering capacity of legumes, which have high levels of orthophosphate, organic acid salts, as well as a high protein content and low soluble carbohydrate content[31]. However, despite the increase in the pH of silages with the inclusion of legumes, only with the inclusion of levels above 20% of butterfly pea was it possible to achieve pH values found within the limit (3.8–4.2) considered ideal for well-preserved silages, and which limits the action of proteolytic enzymes in the ensiled mass, which reduces the development of enterobacteria and *Clostridium*[27]. Possibly, the low pH values obtained in silages with 0 and 20% butterfly pea may be associated with the presence of strong acids in the silage, since during ensiling, microorganisms can convert $NO3^-$ into NO_2, which reacts with water to form HNO3[32]. Thus, we can infer that future studies will be carried out with the evaluation of organic acids and nitrogen dioxide in the silages tested here.

Aerobic stability of silage consists of the resistance of the forage mass to the spoilage process after opening the silo, when mass is exposed to air[33]. Loss of aerobic stability generally occurs as a function of increasing temperature and high pH values. These increases are caused by microorganisms that metabolize lactic acid present in silage and residual carbohydrates to acetic acid, CO2 and water[34, 35]. During the process of stability loss, temperature peaks are noticeable as the ensiled mass is exposed to the aerobic environment. These high temperatures occur through microbial activities producing heat. In this study, it was possible to observe that silage of elephant grass alone was the first to reach 2 °C above room temperature and obtained higher DTS, promoting an increase in temperature in silages, thus resulting in lower stability. This result may be related to the development of aerobic microorganisms, such as fungi, yeasts and molds[36].

The association of butterfly pea with elephant grass in the composition of mixed silages was beneficial, as the legume acted as an absorbent additive and improved the DM content of the silages, going from 285.13 g/kg (0% butterfly pea) to 342.61 g/kg (80% butterfly pea). Although the inclusion of butterfly pea provided an increase in the DM content of the silages, only with the inclusion of levels above 20% was it possible to obtain dry matter contents between the limit established by McDonald et al.[37] to obtain good quality silage (between

30-35% dry matter). Inferior results were reported by Lemos et al.[8] who, when evaluating elephant grass silages combined with butterfly peas, found a dry matter content of 278.9 g/kg.

The increase in EE content in silages is related to the higher proportion of this nutrient in butterfly pea, compared to elephant grass (Table 1). Similar values were reported by Araújo et al.[38] who reported an increase in EE levels when including forage peanuts in elephant grass silages. The authors emphasized that the association of grasses and legumes helps to balance the energy value of silages, which is important in rumen fermentation, fiber digestibility and passage rate. According to Marques et al.[39], so that feed intake is not limited by ruminants, it must have EE values below 5%. Thus, according to our results, all silages could be used to feed ruminants as they would maximize the ruminant's intake, which would not be affected by limitations due to high energy concentration.

According to Lemos et al.[8], legumes tend to contain a higher nitrogen content in leaf tissues compared to grasses, which certainly elucidates the high CP content found in elephant grass silage when butterfly pea are included, increasing from 59.94 g/kg (0% butterfly pea) to 145.09 g/kg (80% butterfly pea) of crude protein. Similar results were reported by Rodrigues et al.[40] who increased the levels of crude protein (8.76% - 11.53%) of pearl millet silages with the inclusion of leucaena in its composition. The results obtained with the inclusion of butterfly pea in tested silages are above the level necessary to ensure adequate rumen fermentation (7% of crude protein[41]), without compromising the efficient use of fibrous carbohydrates in silages. Adequate levels of CP serve as an indication of lower proteolysis during fermentation of the ensiled material. This fact may be due to the lower activity of *Clostridium*[42] and, consequently, lower concentration of butyric acid in the silages.

The progressive inclusion of butterfly pea in elephant grass silage promoted a reduction in MM, unlike OM, which showed an increase in content. The OM content is estimated from the MM content, with an inversely proportional relationship, elucidating the increase in OM for the highest levels of butterfly pea in the silage.

The addition of butterfly pea to the silage favored the increase in TDN, however, it is below the 50% desirable for silages[43]. The lower TDN levels obtained are due to the lower concentrations of EE from the forage plants used in the composition of the silages studied here, which possibly contributed to the reduction of the TDN in the silages, since the EE concentration provides 2.25 times more energy than carbohydrate. In this sense, the nutritional composition of a silage is dependent on the nutrient concentrations in the forage plant that will be used in the ensiling process[43].

The NDF and ADF contents indicate the quantity and quality of fiber present in the forage. However, the high NDF content limits DM intake[44]. In this study, as increasing levels of butterfly pea were added, NDF and ADF contents reduced, which can be explained by the structural composition of legumes, which contain lower fiber content. According to Hawu et al.[45] the reduction in fibrous fractions may have occurred due to the hydrolysis of hemicellulose into monosaccharides, which provide extra carbohydrates for the generation of lactic acid throughout fermentation. The observed NDF and ADF results are above the maximum limit recommended by Van Soest[46] which is 60% NDF and that recommended by Gülümser et al.[47], which is 30% of ADF for roughage that will be used in ruminant diets. Thus silages that have a high concentration of fibrous carbohydrates cause slow digestion in the rumen[48]. Therefore, it is necessary to synchronize carbohydrates and proteins in the ruminant diet to ensure microbial efficiency.

In this study, cellulose, and hemicellulose contents decreased with the inclusion of the legume, showing that silage added with butterfly pea, regarding the proportions of the fiber components, is beneficial. The increase in these components can limit the digestibility of nutrients, inhibiting the activity of rumen microorganisms, thus affecting the nutritional quality of the silage[49]. However, there was an increase in the lignin content in silages as butterfly pea levels increased, which was expected, since the cell wall of legumes has a higher concentration of lignin compared to grasses[27].

As the levels of butterfly pea increased, the TC content decreased. This is possibly because this component is influenced by crude protein; butterfly pea has considerable values of this nutrient; this directly influenced the reduction in total carbohydrates.

5. Conclusion

The inclusion of wedge in levels of up to 80% to compose mixed elephant grass silages reduces fermentation losses, increases aerobic stability, promotes a nutritional increment and reduces the low-quality fiber content of silages.

Conflict of interest

The authors declare no competing interests.

Author contributions

Silva, C.S., Miranda, A.S., Novaes, J.J.S., Araújo, C.A., Macedo, A., Araújo, J.S., Lima, D.O.: Formal Analysis, Investigation, Methodology; Emerenciano Neto, J.V., Araújo, G.G.L., Campos, F.S.: Project Management, Supervision, Visualization; Gois, G.C.: Writing (original draft), Writing (proofreading and editing)

Data availability

Further information on the data and methodologies will be made available by the author for correspondence, as requested.

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