Research Paper

Fermentation losses and aerobic stability of elephant grass silages containing *Parkia platycephala* pod meal and urea

Pérdidas de fermentación y estabilidad aeróbica de ensilajes de pasto elefante que contienen harina de vaina de Parkia platycephala y urea

LUANA ANDRADE DA COSTA¹, RICARDO LOIOLA EDVAN¹, LEÍLSON ROCHA BEZERRA², DARKLÊ LUÍZA DE SOUZA JÁCOME¹, JOSÉ PIRES DE CARVALHO NETO¹, ALEX RODRIGUES DE SOUSA¹, MAYRA FERNANDA ALVES DE MACEDO¹, OTÁVIO TAVARES MEDEIROS¹, TAIRON PANNUNZIO DIAS-SILVA¹ AND MARCOS JÁCOME DE ARAÚJO¹

¹Universidade Federal do Piauí, Bom Jesus, Piauí, Brasil. <u>ufpi.br</u> ²Universidade Federal de Campina Grande, Patos, Paraíba, Brasil. <u>portal.ufcg.edu.br</u>

Abstract

Fermentation losses and aerobic stability of elephant grass silages with inclusion of 4 levels of *Parkia platycephala* pod meal (PP) (0, 10, 20 and 30 %) and 2 levels of urea (0 and 1.5 %) on as-fed basis were evaluated. The silages were analyzed for gas and effluent losses, dry matter recovery (DMR), pH and aerobic stability (pH and temperature) to determine effects of interactions between levels of urea and PP on gas losses, dry matter recovery, temperature and pH of the silages. The combination of urea and PP increased gas losses and reduced the DMR. PP reduced effluent losses and urea increased aerobic stability of the silages. pH values decreased with inclusion of PP and increased with use of urea. Silages containing 20 and 30 % PP showed greater stability over time, especially when associated with urea. PP with urea were effective in reducing effluent losses, maintaining DMR and allowing higher aerobic stability, with stability rupture from 40 h with the inclusion of 20 % of PP in elephant grass silages.

Keywords: Aerobic degradation, dry matter recovery, effluents, pH.

Resumen

Se evaluaron las pérdidas por fermentación y la estabilidad aeróbica de ensilajes de pasto elefante con inclusión de 4 niveles de harina de vaina (PP) de *Parkia platycephala* (0, 10, 20 y 30 %) y 2 niveles de urea (0 y 1.5 %) sobre la base del alimento en fresco. Los ensilajes se analizaron en cuanto a pérdidas de gas y efluentes, recuperación de materia seca, pH, nitrógeno amoniacal y estabilidad aeróbica (pH y temperatura) para determinar los efectos de las interacciones entre los niveles de urea y PP en las pérdidas de gas, recuperación de materia seca (DMR), temperatura y pH de los ensilajes. La combinación de urea y PP aumentó las pérdidas de gas y redujo la DMR. El PP redujo las pérdidas de efluentes y la urea aumentó la estabilidad aeróbica de los ensilajes. Los valores de pH disminuyeron con la inclusión de PP y aumentaron con el uso de urea. Los ensilajes que contenían 20 y 30 % de PP mostraron mayor estabilidad en el tiempo, especialmente cuando se asociaron con urea. La combinación de PP con urea fue efectiva para reducir las pérdidas de efluentes y mantener la recuperación de materia seca y permitir una mayor estabilidad aeróbica, con ruptura de la estabilidad a partir de las 40 h con la inclusión de 20 % de PP en los ensilajes de pasto elefante.

Palabras clave: Degradación aeróbica, efluentes, pH, recuperación de materia seca.

Correspondence: Tairon Pannunzio Dias-Silva, Universidade Federal do Piauí, CEP 64900-000, Bom Jesus, PI, Brazil. Email: <u>Pannunzio@ufpi.edu.br</u> Elephant grass (*Cenchrus purpureus*) is one of the most important tropical forages cultivated in many regions of the world due to its high production potential, quality, acceptability and vigor (<u>Fonseca and Martuscello 2010</u>). Using surplus elephant grass for silage is an alternative to direct feeding to provide a feed with high nutritional value (<u>Santos et al. 2008; Andrade et al. 2010</u>).

Optimal nutritional value of silage from elephant grass harvested at 60-70 days is associated with high moisture in the grass, low levels of soluble carbohydrates and high buffering capacity, factors that inhibit the fermentation process and make it difficult to obtain good quality silage. This favors development of Clostridium bacteria and increases loss of nutrients from the large amount of effluent produced during undesirable secondary fermentation (Andrade et al. 2010; Pacheco et al. 2014). Wilting before the silage process has been shown to be beneficial for fermentation, in particular to increase lactic acid production (Santos et al. 2010). Additives have been tested in elephant grass silage to modify fermentation dynamics, inhibit growth of undesirable microorganisms during the fermentation process, reduce losses, improve aerobic stability, increase nutritional value of the silage and provide soluble carbohydrates for fermentation (Santos et al. 2008; Andrade et al. 2010; Rezende et al. 2011).

Moisture absorbing additives allow ensiling young forage plants, which are typically sources of carbohydrates. Cereals and feed meal can be used to raise dry matter content of silages, reduce effluent production and increase nutritional value of silages (Lopes et al. 2007). As an example, Parkia platycephala pods can be used because they contain 77 % dry matter, which can be used as an alternative to improve chemical and fermentation characteristics of silage. The genus Parkia, belongs to the subfamily Mimosoideae, and is found mainly in tropical rainforests. Parkia platycephala is a legume native to arid and semi-arid regions. Fallen pods are consumed by animals and contain high levels of soluble carbohydrates, about 69 % of DM (Barbosa et al. 2015), and about 8 % of crude protein with good biomass yield (Magalhães et al. 2014).

Use of urea in silages is based on its transformation into ammonia (NH₃), which reacts with water to form ammonium hydroxide, raising the pH and metabolism of microorganisms that cause secondary fermentation, such as yeasts, enterobacteria and *Clostridium* bacteria (Siqueira et al. 2007). Urea increases the aerobic stability of the silage (Rocha et al. 2006).

It was hypothesized that *Parkia platycephala* pod meal (PP) reduces fermentation losses, mainly by reducing effluents, and together with urea, increases aerobic stability of silages, extending the preservation period after opening of these silos. The aim in this study was to evaluate fermentation losses and aerobic stability of elephant grass silages with inclusion of different levels of PP and urea.

Materials and Methods

Experimental location and treatments

The experiment was carried out in the Federal University of Piauí (UFPI), located in Bom Jesus Piauí, Brazil (09°04'28" S, 44°21'31" W; 277 masl). The average annual rainfall is 875 mm and during forage harvesting the mean maximum and minimum temperatures were 32.7 and 21.5 °C, respectively (Inmet 2018).

The experimental design was completely randomized in a factorial arrangement (4×2) with 4 levels of inclusion of PP (0, 10, 20 and 30 % as-fed basis) and 2 levels of urea (0 and 1.5 % as-fed basis) with 5 replications. The treatments corresponded to 8 different types of silage:

T1=100 % elephant grass + 0 % PP + 0 % urea (Control) T2=90 % elephant grass + 10 % PP + 0 % urea T3=80 % elephant grass + 20 % PP + 0 % urea T4=70 % elephant grass + 30 % PP + 0 % urea T5=98.5 % elephant grass + 0 % PP + 1.5 % urea T6=88.5 % elephant grass + 10 % PP + 1.5 % urea T7=78.5 % elephant grass + 20 % PP + 1.5 % urea T8=68.5 % elephant grass + 30 % PP + 1.5 % urea

Silage preparation

Elephant grass cultivar 'Napier' was manually harvested after 60 d regrowth and chopped in a forage chopper/grinder (model GTM-2001sb GARTHEN®) into 2 cm fragments. Mature pods of PP were harvested from local trees in Valencia, PI and air dried. Dry pods were ground in a disintegrator/chopper/grinder using a 10 mm sieve to obtain the meal. Samples of approximately 500 g of elephant grass and PP were collected, packed in labelled plastic bags and stored in a freezer before pre-drying in a forced ventilation oven at 60 °C for 72 h. Dry samples were ground in a Wiley mill to pass a 1 mm sieve for the determination of the chemical composition (Table 1) according to AOAC (2012) to determine dry matter (DM) (method 967.03), ash (method 942.05), crude protein (CP) (method 981.10) and ether

extract (EE) (method 920.29). To quantify the neutral detergent fiber (NDF), the methodology of Van Soest et al. (1991) was used with modifications proposed in the Ankon device manual (Ankon Technology Corporation, USA). Acid detergent fiber (ADF) was determined according to the method of Robertson and Van Soest (1981). Total carbohydrates (TCHO) were estimated using the equation proposed by Sniffen et al. (1992):

Non-fiber carbohydrates (NFC) were estimated using the equation recommended by Mertens (<u>1997</u>):

Plant materials for each treatment were manually homogenized and compressed to a density of 473 kg/ m³ in experimental PVC silos of approximately 3 kg with 1 kg of sand in the bottom of each silo separated from the forage by a layer of cotton fabric. Silos were closed and sealed with plastic adhesive tape to exclude air and Bunsen valves were inserted in each silo to allow escape of gasses and loss of dry matter (DM) from the fermentation process to be quantified. Silos were stored at room temperature from 21 °C to 38 °C for 50 d.

Table 1. Chemical composition of elephant grass and PP atthe time of ensiling.

Variable (% DM)	Elephant grass	PP
Dry matter (DM) as-fed	22.27	85.57
Ash	5.32	1.54
Organic matter (OM)	94.68	98.46
Ether extract (EE)	2.40	1.23
Crude protein (CP)	6.87	8.50
Neutral detergent fiber (NDF)	73.27	20.20
Acid detergent fiber (ADF)	42.76	10.55
Hemicellulose	30.51	9.65
Total carbohydrates (TCHO)	85.42	88.73
Non-fiber carbohydrates (NFC)	12.14	68.53

Fermentation losses

Fermentation losses of silages were quantified by weight difference of the mini silos before and after ensiling, and the respective dry matter contents estimated through equations according to Schmidt (2006).

Gaseous losses were quantified by subtracting the weight of the full silo before opening from the weight

directly after ensiling using the following equation:

$$G = \frac{[(WFen - Wen) \times DMen] - [(WFao - Wen) \times DMao] \times 100}{[(WFen - Wen) \times DMen]}$$

where:

G is gaseous losses in % of DM;

WFen is weight of the full silo at ensiling (kg);

Wen is total weight (silo + lid + sand + screen + cotton fabric) at ensiling (kg);

DMen is DM content of the forage at ensiling (%); WFao is weight of the full silo at opening (kg); and DMao is DM content in the forage at opening (%).

Production of effluents was calculated by using the sand as a collector at the bottom of silos, considering the total weight at opening and before ensiling in relation to the amount of fresh forage ensiled:

EL (kg/t GM) =
$$\frac{(EFW \times 1000)}{GMen}$$

where:
EL is effluent losses;
GM is green mass;
EFW is effluent weight (weight

EFW is effluent weight (weight of empty silo after opening-weight of empty silo before filling); and

GMen is weight of green mass of ensiled forage.

DM recovery was estimated through the equation:

$$DMR (\%) = \frac{(FMao \times DMao) \times 100}{(FMac \times DMac)}$$
where:
DMR is dry matter recovery index;
FMao is forage mass at opening;
DMao is DM content at opening;
FMac is forage mass at closure; and
DMac is DM content of the forage at closure.

After 50 days of fermentation, silos were weighed and opened and approximately 10 cm from the upper and lower layers of the silages that could have been spoiled in the ensiling process were discarded. Silage samples were manually removed from the silos and stored in plastic bags for homogenization and transferred to an air-conditioned room at 25.23 ± 0.4 °C with an average air relative humidity of 63.23 ± 5.91 %. The samples were spread to allow greater penetration of air into the mass. Silage temperatures were taken every hour with a digital thermometer (INCOTERM[®]) inserted at 10 cm depth in the center of the silage mass, as proposed by Kung Jr. et al. (2003) and Bernardes et al. (2007). Surface temperatures were measured by an infrared digital thermometer with laser targeting (BENETECH[®]).

Approximately 50 g of silage was taken every 8 hours (3 samples per day) from each treatment during 4 days of air exposure, to determine pH following the methodology described by Jobim et al. (2007). Aerobic stability was based on an increase in silage temperature of 2 °C in relation to the environment after opening of silos (Moran et al. 1996). Number of hours for the elevation of silage temperature to 2 °C in relation to room temperature, number of hours to reach maximum temperature and maximum temperature in silages exposed to air from 0 to 4 d were measured as proposed by O'Kiely et al. (1999).

Statistical analyses

All data were analyzed using the MIXED procedure of the SAS software as a 4×2 factorial design. Effects of level of inclusion of PP on fermentation losses and aerobic stability were evaluated using orthogonal contrasts to determine the linear and quadratic effects. The contrasts were significant when the P-value was ≤ 0.05 . Effect of urea and days of air exposure were compared through the probability of the difference (PDIFF) using Fisher's LSD test. Statistical differences were declared significant at P-value<0.05 and trends accepted if P-value<0.1.

Residues were plotted against predicted values and used to verify the assumptions of the model such as homoscedasticity, independence and normality of errors. A measurement was considered an outlier and removed when the studentized residue was outside the range of \pm 2.5.

Results

Fermentation losses

Inclusion of PP promoted linear reduction of effluent losses and DMR (Table 2). Silage with 1.5 % of urea with 0, 20 % and 30 % PP showed the lowest DMR. PP levels and urea levels had significant effects (P<0.0001) on gaseous losses and DMR. A linear increase was observed in gaseous loss with increasing PP without and with urea. The interaction (P=0.32) between PP and urea levels did not influence effluent losses (Table 2). Silages containing urea showed higher effluent losses.

Aerobic stability

The temperature and pH of silages were affected (P<0.0001) by the interaction PP \times U \times Day (Table 3). A quadratic effect was observed with temperatures increasing proportionally to levels of PP. All silages, except the one with 20 % PP, showed higher fermentation temperatures when urea was added, with higher values being recorded on the third day.

pH values increased over the days regardless of level of inclusion of additives (Table 3). Silages with no PP nor urea showed the highest pH values depending on hours of air exposure, while silage with 30 % PP retained a pH value close to 4 (Figure 1).

Table 2. Fermentation losses of	elephant grass	s silages conta	ining urea and I	Parkia platycephala	pod meal (PP).
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Urea (% AF)	PP (% GM)				Mean	SEM	P-value			
-	0	10	20	30			PP	U	PP×U	
		Gas (%	% DM)							
0	3.24 ^{Db}	9.54^{Ca}	15.38^{Ba}	18.92 ^{Aa}	11.77	0.89	< 0.0001	< 0.0001	< 0.0001	
1.5	8.41 ^{Ca}	9.81 ^{Ca}	16.05^{Ba}	19.12 ^{Aa}	13.35					
Mean	5.82	9.67	15.72	19.02						
		Effluent (kg /t GM)							
0	53.86	32.60	32.01	7.08	31.38b	3.08	< 0.0001	0.02	0.32	
1.5	61.25	37.25	30.40	13.34	35.56a					
Mean	57.55	34.92	31.20	10.21						
0	87.55 ^{Aa}	84.24^{Ba}	80.74^{Ca}	78.05^{Da}	82.64	0.60	< 0.0001	< 0.0001	< 0.0001	
1.5	82.42 ^{Bb}	84.82 ^{Aa}	78.80 ^{cb}	77.05 ^{Da}	80.77					
Mean	84.98	84.53	79.77	77.54						

Means followed by different lowercase letters in the columns differ for the level of urea and by different uppercase letters in the rows differ for the level of PP by Fisher's LSD test (P < 0.05); AF=as-fed; GM=fresh matter; DM=dry matter.

Stability rupture occurred immediately after exposure to air in silages with PP and those containing 20 and 30 % PP had higher temperature variations over the hours of aerobic activity (Figure 2). Silages with 0 and 10 % PP without urea and 0 % PP + 1.5 % U had the maximum temperatures immediately after opening and kept aerobic stability after 96 hours of air exposure. Treatments without urea and 0, 10 and 30 % PP showed rupture of stability immediately after air exposure.

Table 3. Temperature (°C) and pH of elephant grass silages containing urea (U) and *Parkia platycephala* pod meal (PP) during four days (D) of air exposure.

Urea (% AF)	Day	Parkia platycephala pod meal (% AF)				Mean	ean SEM P-value				value		
		0	10	20	30	-		PP	U	D	PP×D	U×D	PP×U×D
		Temperature (°C)											
0	1st	25.06 ^{Ac}	24.50^{Bc}	24.79Abc	25.05 ^{Ac}	24.85							
	2nd	26.51^{Ba}	25.84 ^{сь}	26.62^{Bab}	27.08 ^{Ab}	26.51							
0	3rd	26.31^{Ca}	26.47^{Ca}	26.81^{Ba}	27.62 ^{Aa}	26.80							
	4th	25.93 ^{сь}	26.00 ^{сь}	26.32 ^{вь}	27.16 ^{Ab}	26.35							
Mean		25.95α	25.70β	26.14α	26.73a		0.06	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
	1st	24.58 ^{Ac}	23.94^{Bc}	24.04^{Bd}	24.18^{Bd}	24.19							
15	2nd	25.33 ^{Bb}	27.00 ^{Aa}	26.74 ^{Ab}	24.80 ^{Cc}	25.97							
1.5	3rd	25.85^{Da}	26.86^{Ca}	28.00^{Ba}	28.56 ^{Aa}	27.32							
	4th	25.56^{Cab}	26.04^{Bb}	25.68^{BCc}	27.71 ^{Ab}	26.25							
Mean		25.33β	25.96a	26.12α	26.32β								
			pН										
	1st	3.74 ^{Ac}	3.76 ^{Ac}	3.84 ^{Ab}	3.93 ^{Aa}	3.82							
0	2nd	4.08^{Ac}	3.83 ^{Ac}	3.88 ^{Ab}	4.02 ^{Aa}	3.95							
0	3rd	5.35 ^{Ab}	4.20^{Bab}	4.06^{Bab}	4.13^{Ba}	4.43							
	4th	5.82 ^{Aa}	4.54^{Ba}	4.40^{Ba}	4.19^{Ba}	4.75							
Mean		4.76β	4.08β	4.05α	4.07α		0.08	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
	1st	6.43 ^{Ac}	3.80^{Bc}	3.76^{Bc}	3.77 ^{Bb}	4.44							
15	2nd	6.65 ^{Ac}	4.04^{Bc}	3.83^{Bc}	3.75 ^{Bb}	4.57							
1.3	3rd	7.07 ^{Ab}	4.70^{Bb}	4.64^{Bb}	3.94^{Cab}	5.10							
	4th	8.41 ^{Aa}	6.26^{Ba}	5.35 ^{Ca}	4.37 ^{Aa}	6.10							
Mean		7.14α	4.70α	4.40α	3.96a								

Means followed by different lowercase letters in the columns differ for day and uppercase in the rows differ for the level of *Parkia platycephala* and Greek letters in the columns differ for urea by the Fisher's LSD test (P < 0.050). AF=as-fed.



Figure 1. pH of elephant grass silages containing PP and urea after air exposure.



Figure 2. Temperature (°C) of elephant grass silages containing PP and urea with hours of air exposure.

Discussion

Results showed that gaseous losses increased with addition of PP in elephant grass silage regardless of use of urea. This effect may be related to increase in soluble carbohydrates from PP as described by Barbosa et al. (2015). When elephant grass was ensiled with urea and without PP, gaseous losses were higher than in silage

made with only elephant grass, possibly because of the higher percentage of water retained, promoting changes in the fermentation process (<u>Rezende et al. 2011</u>), together with further development of gas producing microorganisms in the presence of urea.

In silages that contained urea, effluent losses were higher, probably because of enhanced biochemical reactions producing metabolic water with percolation through the ensiled mass. According to Balieiro et al. (2009) some additives can alter the structural integrity of plant cells and their ability to retain water, leading to rupture and leakage of cellular contents. Effluent losses reduced with inclusion of PP, demonstrating its capacity to absorb moisture from the silage. Many additives with high DM content, such as coffee hulls (Bernardino et al. 2005), wheat meal (Zanine et al. 2006), cassava meal (Oliveira 2009) and Parkia platvcephala pods (Barbosa et al. 2015), have been used to reduce moisture content of silage and thereby reduce losses by effluents. Reduction of losses by effluents also promotes reduction of nutrient losses by percolation with the effluent. Effluent loss values observed in this study were high when compared with results reported in the literature. Rezende et al. (2011) reported that plant respiration and growth of microorganisms produce water and contribute to formation of effluents. However, in this experiment, increased production of effluent may be due to the low dry matter content of elephant grass.

DMR reduced as PP was added to the silage. This reduction is associated with higher gaseous losses in treatments with PP. However, Barbosa et al. (2015) obtained an increase of DMR with inclusion of Parkia platycephala pods, which increased dry matter contents and promoted increased availability of soluble carbohydrates for fermentation inside silos with consequent reduction of CO, production. According to McDonald et al. (1991), fermentation by Clostridium bacteria involves decarboxylation and/or oxidation with high losses by gas (CO₂). The DMR values were considered sufficient to provide high DM recovery of the ensiled material. According to Pedroso et al. (2005), DM recovery is highly correlated with gaseous losses confirming the results obtained in this study, in which DMR was inversely proportional to gaseous losses.

Highest silage temperature was recorded for mixtures of elephant grass with PP, possibly due to increased fermentation of soluble carbohydrates present, showing higher heat production due to the elevated metabolism of microorganisms present. According to Lima et al. (2015), silages rich in soluble carbohydrates are more susceptible to aerobic deterioration. The increase in temperature of silages after opening of silos is due to the growth of aerobic microorganisms, which utilize organic acids and other soluble nutrients as an energy source, resulting in loss of nutrients (Rezende et al. 2011).

Inclusion of PP provided a suitable environment for

development of lactic acid bacteria and pH increases after opening silos is related to degradation of lactic acid by aerobic microorganisms for energy and loss of other organic acids by volatilization (Amaral et al. 2008). Increase of pH does not imply poorer quality silage (Rocha et al. 2006). Lower pH does not ensure that activity of undesirable microorganisms is prevented during fermentation unless pH reduction is achieved quickly (Oliveira 2009; Rezende et al. 2008). The final pH of the silage is related to length of the fermentation period and it becomes more difficult to reduce pH value as fermentation proceeds (Siqueira et al. 2007). Treatments with no inclusion of urea and inclusion of 10, 20 and 30 % PP and the treatment with 30 % PP and 1.5 % urea all presented mean pH values within the range recommended by McDonald (1981) for good preservation of ensiled material. However, only the treatment with 30 % PP without urea reached the maximum value after 88 h of air exposure. Silages containing urea had high pH after 96 h of air exposure.

Aerobic stability is important to retain nutrients. Rezende et al. (2011) evaluated the aerobic stability of sugarcane silages treated with lime and found the highest pH values in the treated silages due to the strong alkaline nature of the additive. Stability rupture immediately after exposure to air of silages with 0, 10 and 30 % of PP was probably due to the fast metabolism of yeasts during the first hours of aerobic activity and other spoilage microorganisms which caused the temperature to vary significantly during that period (Santos 2013). Treatments with 1.5 % urea showed higher aerobic stability, probably because of the effects of urea on development of microorganisms (Jobim et al. 2008). The highest DM contents and inclusion of urea may have hindered proliferation of microorganisms due to reduction of moisture in the silages. Bernardes et al. (2007) found that silages of tropical grasses exposed to air have a prevalence of aerobic bacteria due to fermentation stability at pH above 4.5, the high moisture content and the absence of substrates.

Conclusions

The combination of PP with urea is effective in decreasing losses by effluents as well as in keeping DMR at appropriate levels to maintain the characteristics of a good silage. Silages containing PP and urea were more stable when exposed to air, with stability rupture from 40 hours with the inclusion of 20 % of PP.

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(Note of the editors: All hyperlinks were verified 25 January 2023).

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