




Chemical composition, fermentative characteristics, and in situ ruminal degradability of elephant grass silage containing *Parkia platycephala* pod meal and urea

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Abstract

This study aimed to evaluate the effects of the addition of *Parkia platycephala* pod meal (PP) and urea on the chemical composition, fermentation characteristics, and in situ ruminal degradability of elephant grass silage. A completely randomized design with a 4 × 2 factorial arrangement was adopted, with four levels of pod meal (0, 10, 20, and 30%) and two levels of urea (0 and 1.5%) on as fed basis. The produced silages were evaluated in terms of dry matter (DM), crude protein (CP), mineral matter (MM), neutral detergent fiber (NDF), acid detergent fiber (ADF), hemicellulose, ammonia nitrogen, pH, short chain fatty acids, and in situ ruminal degradability. For the study of degradation, four Santa Ines rumen fistulated sheep, with average weight of 45 ± 2.5 kg were used. The association of the additives increased ($p < 0.05$) DM, CP, DM degradability, fractions *a*, *b*, and effective degradability at all passage rates (2, 5, and 8%/h) and reduced NDF and ADF contents. DM disappearance increased ($p < 0.05$) during the incubation time, especially for the silages containing the two additives. The interaction in the rumen environment is essential for microbial multiplication. Thus, the use of additives such as PP and urea contributed to the availability of digestible fractions of the feed and greater use by ruminal microorganisms. The association of *Parkia platycephala* with urea improves the fermentation characteristics, chemical composition, and degradability of elephant grass silage.

Keywords Dry matter · Neutral detergent fiber · pH · Short chain fatty acids

Introduction

Elephant grass (*Pennisetum purpureum* Schum.) is considered one of the most important tropical forages because of its high potential for biomass production, easy adaptation, and good acceptability by animals. In addition, it is widely used for silage production due to its high productivity (Rocha et al. 2006; Sifeeldein et al. 2018). When not intended for storage, it is used by cutting, mostly unplanned, resulting in uneven use of the planted material, causing a supply of low-quality material.

However, due to the high moisture content and low soluble carbohydrates content of the elephant grass during storage in

the silo, undesired fermentation or loss of nutrients from the ensiled mass may occur (Gonçalves et al. 2006), with higher production of acetic and butyric acids, ammonia nitrogen, amides, and amines (Ferrari Júnior et al. 2009). This may be fixed by using silage additives as they may affect the fermentation pattern, improve the chemical composition of the silage, decrease losses, increase stability, improve fiber digestibility, and increase dry matter content (Bergamaschine et al. 2006; Santos et al. 2010). Moisture absorbers make it possible to make silage of young forage plants, as they are usually sources of carbohydrates, cereals, meals, and others, used to increase the silage DM content, reduce effluent production, and increase the nutritive value of the silage (Lopes et al. 2007).

Among the moisture absorbing additives, pod meal can be used because they have high levels of soluble carbohydrates and high levels of dry matter, besides being rich in protein (Mota et al. 2015). In this context, pods of *Parkia platycephala*, which has a dry matter content of approximately 77.25% and 69.26% of soluble carbohydrates (Barbosa et al.

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2015), may be an alternative to improve chemical and fermentative characteristics of silages. *P. platycephala* is a forage resource locally available in semiarid regions, being a potential alternative to reduce feed expenditure and highly acceptable by ruminants (Ramos et al. 1985). In addition, the use of additives, such as urea, can improve silage quality and decrease losses of DM and soluble carbohydrates, providing better chemical composition in treated silages (Lopes et al. 2007). Under the action of urease, the urea is transformed into ammonium, which upon binding to water, forms ammonium hydroxide which is capable of solubilizing cell wall components, especially hemicellulose, reducing the fibrous fraction of the material (Morais et al. 2017).

Given the above, it was hypothesized that the *Parkia platycephala* pod meal would modify the chemical composition, increasing the dry matter and soluble carbohydrates content of the silages, which would provide improved fermentative characteristics and in association with urea would increase the dry matter degradability of the silages. Thus, we aimed to evaluate the effects of the addition of *Parkia platycephala* pod meal and urea on the chemical composition, fermentative characteristics and rumen in situ degradability of elephant grass silages.

Material and methods

Experiment location

The study was carried out at the Federal University of Piauí (UFPI), located in Bom Jesus, Piauí, Brazil, at latitude 9° 4' 28" south and longitude 44° 21' 31" west, and an average altitude of 277 m. The average annual rainfall is 875.1 mm and the mean temperatures are 25.4 °C minimum and 28.2 °C maximum. During the grass cutting period, the average air relative humidity was 50.76% and the average maximum and minimum temperatures were 32.68 and 21.50 °C, respectively (INMET 2018). The study was approved by the Animal Use Ethics Committee (CEUA / UFPI) under the protocol no. 394/17.

Experimental design and treatments

A completely randomized design in a factorial arrangement (4 × 2) was used, with four levels of inclusion of *Parkia platycephala* pod meal (PP: 0, 10, 20, and 30% as fed) and two levels of urea (U: 0 and 1.5% as fed), totaling eight treatments with five replications each. The treatments correspond to eight different types of silage as shown in Table 1.

Table 1 Experimental treatments used to evaluate the chemical composition, fermentative characteristics, and in situ ruminal degradability of elephant grass silage containing *Parkia platycephala* pod meal and urea

Treatments	Elephant grass (%)	<i>Parkia platycephala</i> (%)	Urea (%)
T1	100	0.00	0.00
T2	90.0	10.0	0.00
T3	80.0	20.0	0.00
T4	70.0	30.0	0.00
T5	98.5	0.00	1.5
T6	88.5	10.0	1.5
T7	78.5	20.0	1.5
T8	68.5	30.0	1.5

Silage production

The Elephant grass (*Pennisetum purpureum*, cv. Napier), from an established area, at 60 days of regrowth, was manually harvested and processed into 2-cm fragments in a forage chopper and shredder machine model GTM-2001sb GARTHEN®. Mature pods of *Parkia platycephala* were harvested in a naturally occurring area, in the city of Valença, Brazil. The pods were ground in a DMP type machine (disintegrator, grinder, and chopper) using a 10-mm sieve, in order to obtain the meal.

The ensiled material was manually homogenized according to each treatment and then placed in experimental PVC silos (3 kg of capacity), compacted to an average density of 473 kg m³, and properly sealed. During the silage making process, samples of approximately 500 g of the grass and *Parkia platycephala* pod meal were collected, which were identified, packed in plastic bags, and immediately stored in a freezer to later determine the chemical composition (Table 2).

After 50 days of fermentation, the silos were opened and, after discarding approximately 10 cm from the upper and lower layers of the silage, the median portion was used to determine dry matter, mineral matter, crude protein, neutral detergent fiber, acid detergent fiber, hemicellulose, N-NH₃, pH, organic acids (lactic, acetic, butyric, and propionic), and rumen in situ degradability.

Fermentative characteristics

The pH was evaluated according to the methodology cited by Jobim et al. (2007), diluting 25 g of fresh silage in 100 mL of distilled water and reading in digital pH meter after 1 h of sitting.

For the determination of ammonia nitrogen (N-NH₃), 10 g of the fresh silage sample was weighed and ground in a

Table 2 Chemical composition of elephant grass and *Parkia platycephala* pod meal at the time of ensiling

Item (% DM)	Elephant grass	<i>Parkia platycephala</i> pod meal
Dry matter (%AF)	22.27	85.57
Mineral matter	5.32	1.54
Organic matter	94.68	98.46
Ether extract	2.40	1.23
Crude protein	6.87	8.50
Neutral detergent fiber	73.27	20.20
Acid detergent fiber	42.76	10.55
Hemicellulose	30.51	9.65
Total carbohydrates	85.42	88.73
Non-fibrous carbohydrates	12.14	68.53

AF, as fed; DM, dry matter

blender with 60 mL of distilled water until the material was aqueous, then filtered on paper filter. The N-NH₃ was determined by distilling 2 mL of each sample with the addition of 5 mL of 2 N KOH in a Kjeldhal apparatus. The distillate was received in 10 mL of 2% H₃BO₃ to a final volume of 50 mL, followed by titration with sulfuric acid solution (0.02 N), according to the technique of Mizubuti et al. (2009).

For the determination of the organic acids (lactic, acetic, propionic, and butyric), 10 g of fresh silage was ground in an industrial blender for 1 min with distilled water and then filtered on paper filter (Whatman no. 54). From the filtrate, 10 mL of extract was acidified with 50 µL of 50% H₂SO₄ and then filtered through fast filter paper. Two milliliters of this filtrate received 1 mL 20% metaphosphoric acid solution and 0.2 mL of 1% phenic acid solution, used as internal standard. The samples were centrifuged at maximum rotation for 15 min. The supernatant was put in *Eppendorf* tubes and placed in a freezer for further analysis. The identification and quantification of organic acids were performed by high-performance liquid chromatography (Shimadzu, Japan), brand THERMO, model UV/VISIBLE coupled to the Ultra Violet Detector (UV) using a wavelength of 210 nm. A 30 cm × 4.5 mm diameter HPX-87H (BIORAD) column with a flow rate of 0.6 mL/min and a pressure of 73 kgf was used. Mobile phase: water in 0.05 MM of sulfuric acid and the injected volume was 10 µL (Kung Jr. and Ranjit 2001).

Chemical composition analysis and in situ ruminal degradability

Approximately 500 g of samples from the material before and after ensiling was collected, dried in a forced-air oven (60 °C, 72 h), and ground in a Willey mill (Marconi, Piracicaba, SP,

Brazil) using 1-mm mesh sieves for the determination of dry matter (DM) (Method 967.03-AOAC, 1990), crude protein (CP) (Method 981.10-AOAC, 1990), and mineral matter (MM) (Method 942.05-AOAC, 1990).

The NDF analysis procedures were performed in accordance with the methodology of Van Soest et al. (1991) with modifications proposed by the Ankom device manual (Ankom²⁰⁰ Technology Corporation, Macedon, NY, USA): all the samples were treated with heat-stable alpha-amylase (Termamyl 2X, Novozymes) without the use of sodium sulfite and 8 M urea solution. The ADF was measured according to the methods of the AOAC 1990 (Method 954.01-AOAC, 1990). There was no procedure for protein or ash correction in NDF and ADF analysis. Hemicellulose was calculated as NDF minus ADF (Van Soest and Robertson 1980). To estimate total carbohydrates (TCH), the following equation proposed by Sniffen et al. (1992) was used: TCH (%) = 100 - (%CP + %EE + %MM); and the non-fibrous carbohydrates (NFC) content were estimated using the equation recommended by Mertens (1997): NFC (%) = 100 - (%MM - %CP - %EE - %NDF). The chemical analyses of the material were performed at the Animal Nutrition Laboratory of the Federal University of Piauí, Bom Jesus (Piauí, Brazil).

For the ruminal degradability trial, four Santa Ines non-castrated rams were used, animals had an average body weight of 45.0 ± 2.5 kg and were ruminally cannulated (Permanent silicone ruminal cannula with an internal diameter of 7.62 cm—Kehl® Industria e Comercio Ltda ME, São Carlos, São Paulo, Brazil) and were kept in individual stalls (1.6 m²) with feed through and water available ad libitum. Silage samples were dried in a forced air oven (60 °C, 72 h) and then ground in a Willey mill (2-mm sieve). Non-woven textile (NWT) bags (100 g/cm²) of 4 × 5 cm were manufactured, as recommended by Valente et al. (2011). Samples were placed in individual bags following the ratio of 20 mg DM/cm² of surface (Nocek 1988) in duplicate. For all experimental procedures, bags were heat-sealed. Bags were placed in the dorsal sac of the reticulo-rumen of each animal. Bags were removed at 6, 12, 24, 48, 72, and 96 h of incubation. Incubation times were evaluated in a reverse order so all bags were taken from the reticulo-rumen at same time. After that, bags were hand washed thoroughly in cold running water until the rinsing water was clear. After that, the bags were oven-dried (60 °C for 72 h) followed by 105 °C for 1 h, placed in dissecator, and then weighed to determine DM disappearance (Detmann et al. 2001). In order to determine the losses of material at time zero (0-h samples), bags containing samples were washed in water together with all the others bags. After washing, the bags were subjected to the same procedures as incubated bags. Animals were clinically healthy and dewormed as a result of the EPG (eggs per gram) test performed periodically. The animals received water ad libitum. The animals were fed a total mixed ration (TMR) presented a

Table 3 Fermentative characteristics of elephant grass silage containing *Parkia platycephala* pod meal (PP) and urea (U)

Urea (%AF)	<i>Parkia platycephala</i> pod meal (%AF)				Mean	SEM	<i>p</i> value		
	0	10	20	30			PP	U	PP × U
pH									
0	3.40bA	3.71aA	3.60aA	3.56aA	3.57	0.27	< 0.0001	< 0.0001	< 0.0001
1.5	8.70aA	3.85aB	3.53aB	3.63aB	4.93				
Mean	6.04	3.78	3.57	3.60					
N-NH ₃ (%total nitrogen)									
0	5.25bA	3.48aB	2.55bC	3.36aB	3.66	0.24	< 0.0001	< 0.0001	< 0.0001
1.5	15.96aA	1.74bB	1.88bB	1.42bB	5.25				
Mean	10.60	2.61	2.22	2.40					
Lactic acid (%DM)									
0	1.51	1.72	2.17	2.13	1.88b	0.16	0.01	0.05	0.71
1.5	2.00	2.21	2.22	2.90	2.33a				
Mean	1.75	1.96	2.20	2.52					
Acetic acid (%DM)									
0	0.62	1.08	1.22	1.18	1.02	0.09	0.11	0.18	0.66
1.5	1.06	1.19	1.24	1.30	1.19				
Mean	0.84	1.14	1.23	1.24					
Propionic acid (%DM)									
0	0.32	0.52	0.54	0.55	0.48b	0.04	0.31	0.01	0.46
1.5	0.62	0.62	0.63	0.66	0.63a				
Mean	0.47	0.57	0.58	0.61					
Butyric acid (%DM)									
0	0.13	0.17	0.16	0.17	0.16b	0.01	0.36	0.001	0.78
1.5	0.20	0.21	0.20	0.21	0.20a				
Mean	0.16	0.19	0.18	0.19					

Means followed by different lowercase letters in the columns differ for urea and uppercase in the rows differ for the level of *Parkia platycephala* by the Fisher's LSD test ($p < 0.05$); AF, as fed; DM, dry matter

60:40 forage (elephant grass) to concentrate (corn ground, soybean meal, and mineral supplement) ratio. The TMR was formulated to meet the nutritional requirements of sheep according to NRC (2007).

The in situ degradability of DM was determined using the weight difference for each component between the weighing performed before and after rumen incubation and was expressed as a percentage. After obtaining the coefficients a , b , and c , they were entered into the following equation proposed by Ørskov and McDonald (1979) to calculate the potential degradability (PD):

$$PD (\%) = a + b \times (1 - e^{-ct})$$

where PD = fraction degraded at time t ; a = rapidly soluble fraction (%); b = potentially degradable insoluble fraction (%); c = fraction b degradation rate (%/h); and t = time (h).

The non-linear parameters a , b , and c were estimated using interactive Gauss-Newton procedures. After determining the model parameters, the effective degradability (ED) of the dry

matter (DM) was calculated using the following model:

$$ED = a + [(b \times c)/(c + k)]$$

where k corresponds to the estimated passage rate of the particles in the rumen.

Feed passage rates of 2, 5, and 8%/h were considered for low, medium, and high consumption, according to the model proposed by Ørskov and McDonald (1979).

Statistical analysis

All analyses were performed using the MIXED Procedure of SAS (Version 9.0, Institute Inc., Cary, NC, USA). For the composition trial, the model used was:

$$Y_{ijk} = \mu + PP_i + U_j + (PP \times U)_{ij} + e_{ijk},$$

where Y_{ijk} is the dependent variable; μ is the overall mean; PP_i is the fixed effect of *Parkia platycephala* level ($i = 1$ to 4); U_j

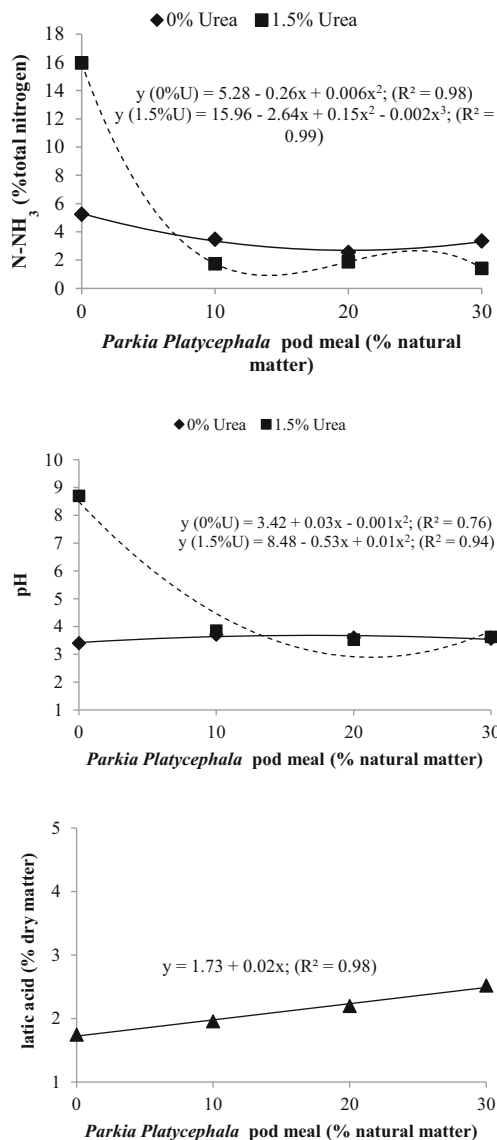


Fig. 1 a pH, b ammonia nitrogen (N-NH₃; % total nitrogen), and c lactic acid concentration in elephant grass silages as a function of the level of *Parkia platycephala* pod meal

is the fixed effect of urea level ($j = 1$ to 2); $(PP \times U)_{ij}$ is the fixed effect of the interaction; and e_{ijk} is the random residual error. When the $PP \times U$ interaction was significant, the F test for the PP effect in each U level was calculated using the SLICE option of LSMEANS. The effects of the PP inclusion level were evaluated using orthogonal contrasts to determine the linear, quadratic, and cubic effects (CONTRAST statement of SAS). The contrasts were significant when the p value was < 0.05 . For the effects of the urea levels, the estimated means using LSMEANS were compared using Fisher's LSD test. Significant differences were declared at $p < 0.05$.

The data used (observed) to estimate the degradation parameters were analyzed by the interactive method, using the PROC NLIN of SAS for non-linear models. For data on in situ degradability, the model above including the effect of

incubation time as repeated measurements over time (REPEATED option) was used. The DM disappearance data from different silages were submitted to analysis of variance, with means comparison by the Fisher's LSD test. Studentized residuals were plotted against predicted values to verify model assumptions. No outliers (studentized residuals $> |2.5|$) were identified.

Results

Fermentative characteristics

A significant effect of the interaction ($p < 0.0001$) between urea and PP levels was observed on the silage pH values (Table 3). Elephant grass silage without urea presented maximum value of 3.65 at the level of 15% PP ; while in the silage with 1.5% urea was observed minimum value of 1.46 at 26.5% of PP (Fig. 1a). The highest pH value was recorded in the silage without PP and with urea (0% PP + 1.5% U).

A quadratic effect ($p < 0.0001$) of the PP level was observed on the N-NH₃ values of the silages, so that when elephant grass was ensiled without urea, a minimum value of 2.46% was observed at a PP level of 21.7% (Table 3). In the silages treated with urea, a higher value of N-NH₃ was found when they contained only elephant grass, reducing as PP was added (Fig. 1).

There was a linear increase in lactic acid concentrations as the PP increased (Fig. 1). A fixed effect ($p < 0.01$) of the urea levels on lactic acid was observed, presenting lower values in the silages containing 1.5% urea. However, the elephant grass silages (0% PP + 0% U) presented the lowest value of lactic acid.

The concentrations of propionic ($p = 0.01$) and butyric acid ($p = 0.001$) presented higher values in the silages containing 1.5% urea.

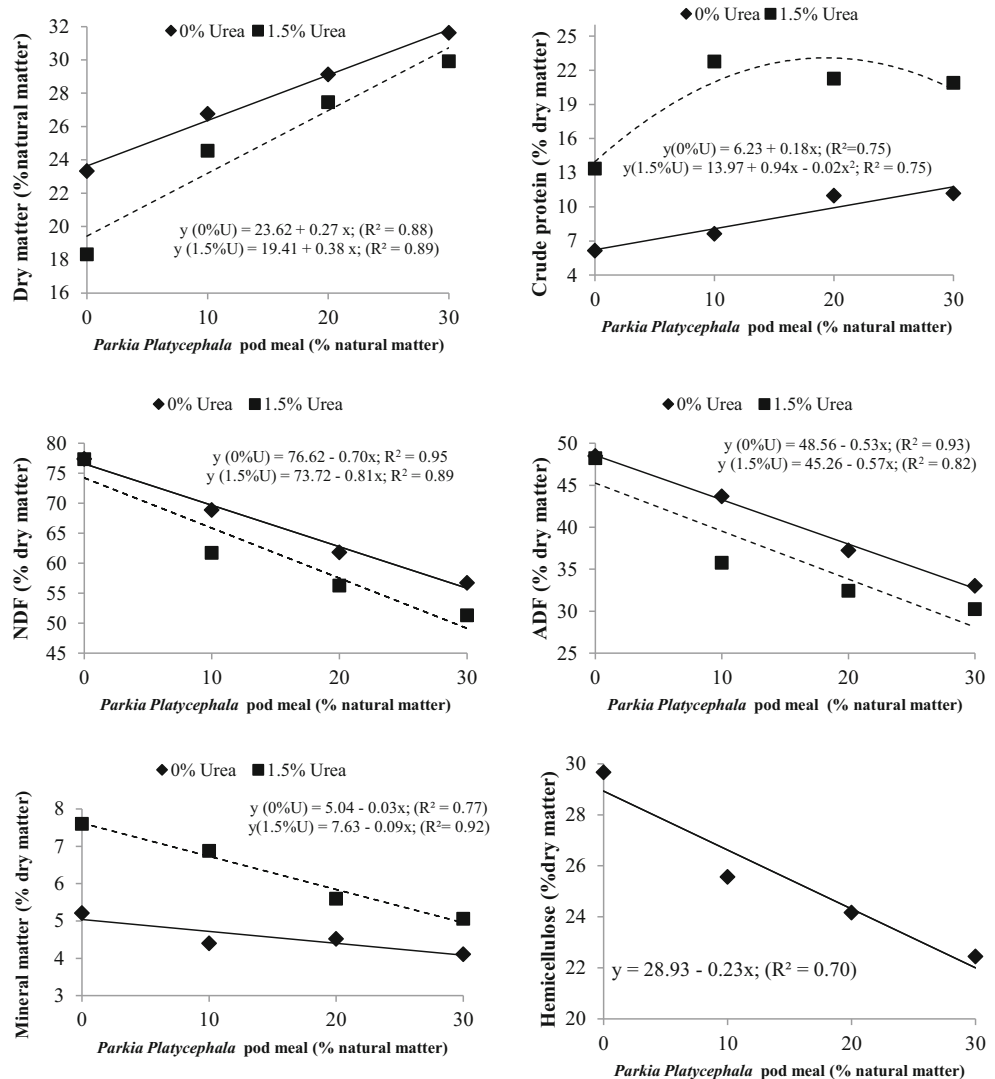
Chemical composition

The silages containing PP without urea showed ($p < 0.01$) higher DM, NDF, and ADF values than the silages with urea. CP and MM contents were higher in the silages that contained PP and urea (Fig. 2).

Parkia platycephala pod meal levels promoted linear increase of the CP contents of the silages without urea (Table 4; Fig. 2). Whereas in the silages with urea, the levels of PP promoted a quadratic effect (Table 4; Fig. 2) on CP contents, with the highest value (25% CP) found at the level of 23.5% of PP .

There was a linear decrease of MM with the addition of PP in the elephant grass silages, with an estimated decrease of 0.03 and 0.09% of the MM content for each unit of PP added

Fig. 2 a Concentrations of dry matter, b crude protein, c neutral detergent fiber (NDF), d acid detergent fiber (ADF), e mineral matter, and f hemicellulose in elephant grass silages containing *Parkia platycephala* pod meal (PP) and urea, as a function of the PP level



in the silages without and with urea, respectively (Table 4; Fig. 2).

The regression analysis revealed a linear reduction of NDF and ADF values with the inclusion of PP (Table 4; Fig. 2).

In situ ruminal degradability of dry matter

There was a higher disappearance of the DM of elephant grass silages that contained PP and urea during the incubation period (Fig. 3).

An effect of the interaction ($p < 0.03$) between urea and PP levels was observed on the DM degradability as well as on fractions *a* and *b* (Table 5). Evaluating the effect of the PP on the degradability of the DM, it can be observed a linear increase, with estimated increases of 0.70% and 0.78% in the silages without and with urea, respectively, for each unit of PP added. The highest degradability of the DM was found in the treatment containing 30% PP with urea (Table 5; Fig. 5).

The fraction *b* was affected by the interaction ($p < 0.01$) between urea and PP, being that in the silages without the addition of urea, there was an increase of 0.65% for each unit of PP added (Fig. 4). On the other hand, in the silages with urea, a quadratic effect was observed, with a minimum value of 37% at the level of 14.5% of PP (Fig. 4).

The effective degradability of the silages was affected by the interaction ($p < 0.01$) between urea and PP (Table 6).

Discussion

The state of symbiosis in the rumen environment is essential. Therefore, a factor that directly interferes is the quality of the diet, since the fermentative parameters are important for the multiplication of microorganisms, which act on fiber degradability favoring greater nutrient assimilation and animal productivity.

Table 4 Chemical composition of elephant grass silage containing *Parkia platycephala* pod meal (PP) and urea (U)

Urea (%AF)	<i>Parkia platycephala</i> pod meal (%AF)				Mean	SEM	<i>p</i> value		
	0	10	20	30			PP	U	PP × U
Dry matter (%AF)									
0	23.33aD	26.77aC	29.13aB	31.64aA	27.72	0.48	< 0.0001	< 0.0001	0.01
1.5	18.33bD	24.55bC	27.47bB	29.92bA	25.07				
Mean	20.83	25.66	28.30	30.78					
Crude protein (%DM)									
0	6.16bB	7.65bB	11.00bA	11.19bA	9.00	0.80	< 0.0001	< 0.0001	< 0.0001
1.5	13.37aC	22.78aA	21.27aAB	20.92aB	19.58				
Mean	9.76	15.21	16.13	16.06					
Mineral matter (%DM)									
0	5.21bA	4.40bBC	4.52bB	4.11bC	4.56	0.18	< 0.0001	< 0.0001	< 0.0001
1.5	7.60aA	6.88aB	5.60aC	5.06aD	6.28				
Mean	6.40	5.64	5.06	4.58					
Neutral detergent fiber (%DM)									
0	77.41aA	68.91aB	61.81aC	56.74aD	66.22	1.52	< 0.0001	< 0.0001	0.0002
1.5	77.36aA	61.73bB	56.31bC	51.34bD	61.68				
Mean	77.38	65.31	59.06	54.04					
Acid detergent fiber (%DM)									
0	48.50aA	43.70aB	37.27aC	33.04aD	40.63	1.09	< 0.0001	< 0.0001	< 0.0001
1.5	48.22aA	35.79bB	32.44bC	30.27bD	36.68				
Mean	48.36	39.75	34.85	31.66					
Hemicellulose (%DM)									
0	29.92	25.20	24.53	23.83	25.87a	0.52	< 0.0001	0.14	0.16
1.5	29.43	25.93	23.80	21.07	25.06a				
Mean	29.67	25.57	24.17	22.45					

Means followed by different lowercase letters in the columns differ for urea and uppercase in the rows differ for the level of *Parkia platycephala* by the Fisher's LSD test ($p < 0.05$); AF, as fed; DM, dry matter

The higher pH in urea-treated silages (0% PP + 1.5% U) can be attributed to the increased ammonia concentration in

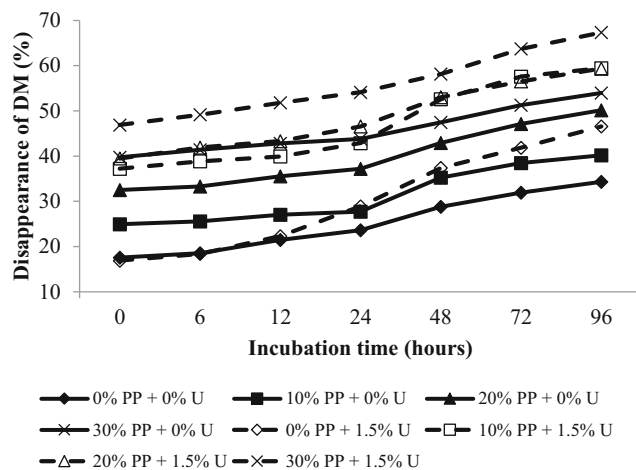


Fig. 3 Disappearance of dry matter (DM) of elephant grass silage containing *Parkia platycephala* pod meal and urea as a function of the incubation time

these silages, as this is a substance with alkalizing capacity, making it difficult to reduce pH (Kung Jr. et al. 2003; Siqueira et al. 2007). However, according to Rocha et al. (2006), it is not an indicative of undesirable fermentations in silage. The better pH value of 3.85 for silages was recorded in the treatment containing 10% PP + 1.5% U, which is within the range considered optimal (3.8 to 4.2) for adequate fermentation (McDonald et al. 1981).

The lowest N-NH₃ values were found in the treatments with both urea and PP. The absence of additives in the silages increased the N-NH₃ value, which was probably due to excessive breakdown of protein into ammonia during the fermentation process. According to McDonald et al. (1991), the N-NH₃ content is associated with the fermentative quality of the silage, as this compound is derived from the degradation of the protein fraction by proteolytic enzymes secreted especially by bacteria of the genus *Clostridium*, and should be below 10%. Except for the silages without PP and with urea (0% PP + 1.5% U), the N-NH₃ concentrations of the silages in the

Table 5 Ruminal degradation parameters of the dry matter (DM) of elephant grass silage containing *Parkia platycephala* pod meal (PP) and urea (U)

Urea (%AF)	<i>Parkia platycephala</i> pod meal (% AF)				Mean	SEM	<i>p</i> value		
	0	10	20	30			PP	U	PP × U
	DM degradability (%)								
0	25.17Db	31.30Cb	39.81Bb	45.77Ab	35.51	1.78	<0.0001	<0.0001	0.03
1.5	30.32Ca	46.96Ba	48.60Ba	55.87Aa	45.44				
Mean	27.75	39.13	44.21	50.82					
	Fraction <i>a</i> (%)								
0	17.33Da	24.19Cb	32.20Bb	40.17Ab	35.97	0.17	<0.0001	<0.0001	<0.0001
1.5	15.93Db	36.24Ca	39.44Ba	47.56Aa	40.88				
Mean	16.63	30.21	35.82	43.86					
	Fraction <i>b</i> (%)								
0	25.98Db	32.98Cb	39.47Ba	45.45Aa	35.97	0.68	<0.0001	<0.0001	<0.0001
1.5	40.22Ba	38.92BCa	37.80Ca	46.58Aa	40.88				
Mean	33.10	35.95	38.63	46.01					
	Fraction <i>c</i> (%/h)								
0	1.16	0.77	0.64	0.38	0.74b	0.001	<0.0001	<0.0001	0.67
1.5	1.57	1.03	1.03	0.60	1.06a				
Mean	1.37	0.90	0.84	0.49					

Means followed by different lowercase letters in the columns differ for urea and uppercase in the rows differ for the level of *Parkia platycephala* by the Fisher's LSD test ($p < 0.05$); AF, as fed

Fig. 4 Ruminal degradation parameters of the dry matter (DM) (a degradability of (DM), b fraction *a*, c fraction *b*, and d fraction *c*) of elephant grass silages containing *Parkia platycephala* pod meal and urea (U) as a function of the level of *Parkia platycephala*

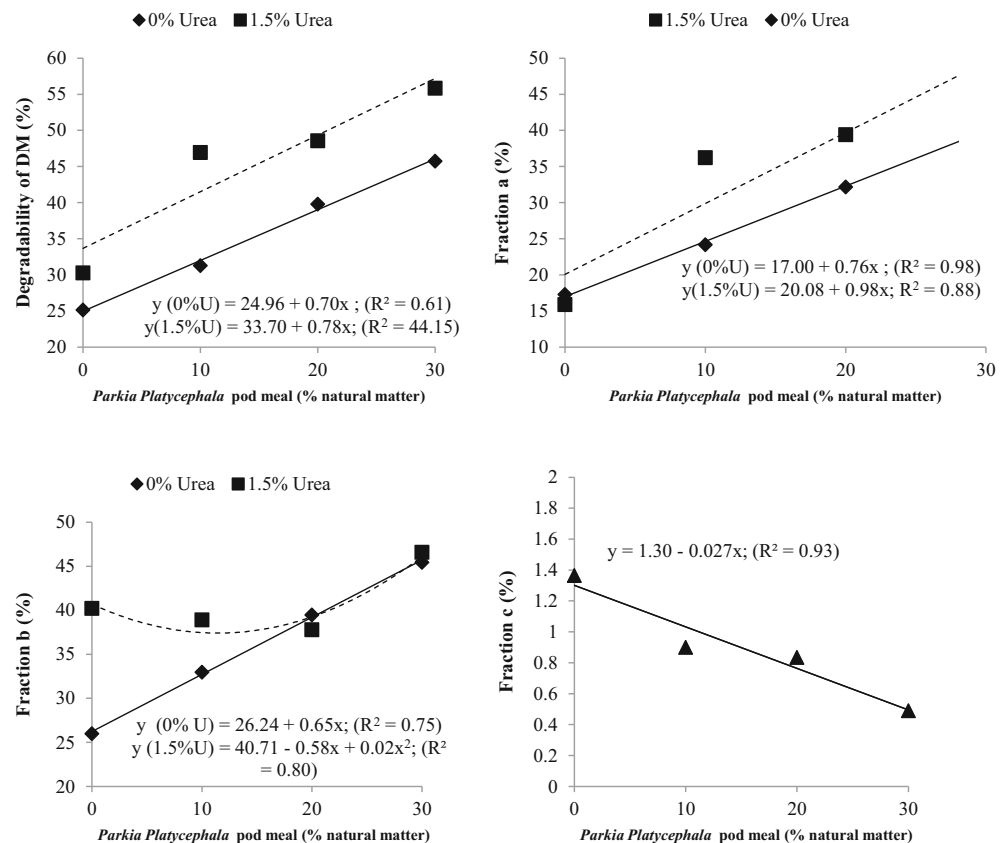


Table 6 Effective degradability of the dry matter (DM) of elephant grass silage containing *Parkia platycephala* pod meal (PP) and urea (U)

Urea (%AF)	<i>Parkia platycephala</i> pod meal (% MN)				Mean	SEM	<i>p</i> value		
	0	10	20	30			PP	U	PP × U
2%/h									
0	26.87Db	33.06Cb	41.80Bb	47.40Ab	37.28	0.62	< 0.0001	< 0.0001	< 0.0001
1.5	33.25Ca	49.40Ba	50.67Ba	58.02Aa	47.84				
Mean	30.06	41.23	46.24	52.71					
5%/h									
0	22.37Db	28.46Cb	36.70Bb	43.37Ab	32.73	0.40	< 0.0001	< 0.0001	< 0.0001
1.5	25.38Da	42.88Ca	45.28Ba	52.48Aa	41.51				
Mean	23.88	35.67	40.99	47.93					
8%/h									
0	20.76Db	27.00Cb	35.13Bb	42.22Ab	31.28	0.30	< 0.0001	< 0.0001	< 0.0001
1.5	22.44Da	40.68Ca	43.41Ba	50.78Aa	39.33				
Mean	21.60	33.84	39.27	46.50					

Means followed by different lowercase letters in the columns differ for urea and uppercase in the rows differ for the level of *Parkia platycephala* by the Fisher's LSD test ($p < 0.05$); AF, as fed; NM, natural matter

present study were considered low, indicating good quality and well-preserved silages.

The linear increase of lactic acid in silages containing PP and urea (Table 3) may indicate a rapid increase of acid-lactic bacteria, showing that occurred efficient colonization and good adaptation of the bacteria to the silages, both due to the increase of soluble carbohydrates from the PP and to the increase of the DM content. According to Ferreira et al. (2004), the increase in lactic acid production may be a result of the greater availability of soluble carbohydrates, with consequent maintenance of low pH. Acetic acid values were above the critical level (0.8%) (Muck 1988), suggesting a good preservation of the ensiled mass due to its power feature of inhibiting yeast growth.

The linear increase of DM according to the addition of PP is justified by the fact that PP has a high DM content (85.57%; Table 2). Increases of 0.27% and 0.38% were observed for each unit of PP added to the silages at the levels of 0% and 1.5% of urea, respectively (Fig. 2). Similar results were found by Mota et al. (2015), when evaluating the fermentative characteristics of elephant grass silage with different additives. Additives with high DM content act by increasing the DM content of the ensiled material by absorbing the excess of forage moisture, thus improving microbial fermentation and nutritional value in tropical grasses silage (Zanine et al. 2006; Santos et al. 2010; Mota et al. 2015).

The levels of PP inclusion of 20 and 30%, whether or not associated with urea, provided DM values within the reference range (28 to 40%) (Jobim et al. 2009), as necessary for the predominance of lactic fermentation and inhibition of undesirable fermentation. Urea, on the other hand, reduced silage

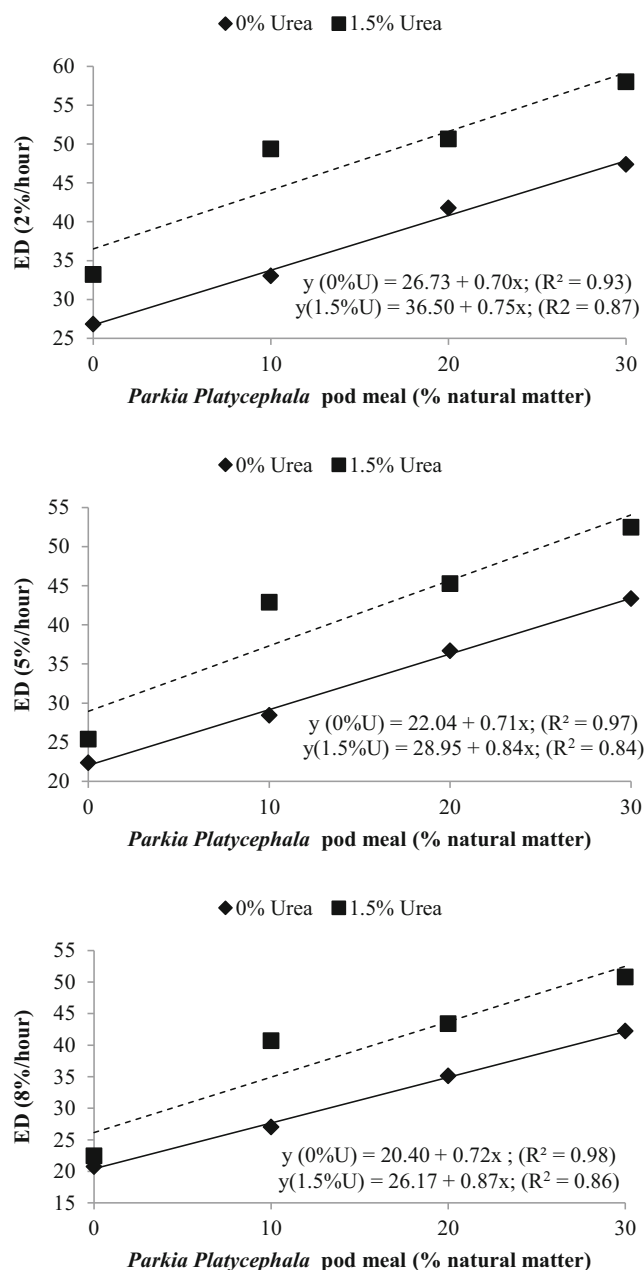
DM values (Table 4; Fig. 2), which coincides with Siqueira et al. (2007), who evaluated the inclusion of 1.5% of urea in sugar cane silage and verified a decrease in the DM content of the treated silages.

In the silages containing urea, the protein contents were higher when compared with the silages without urea due to the high NPN content (46%), thus contributing to the increase of the nitrogen content of the final product. In the research developed by Rocha et al. (2006), it was observed a positive effect of urea doses on CP content of elephant grass silages, but the authors point out that these results do not mean that it would be ideal to add high doses of this additive, as they would exceed the nutritional needs of ruminants. The chemical treatment with urea increases the nitrogen content increasing its availability to the rumen microorganisms, allowing a higher efficiency on substrate degradability, due to the increase of the synthesized crude protein content, by the addition of non-protein nitrogen (Silva et al. 2016).

The treatment containing elephant grass only (0% PP + 0% U) presented a CP value (6.16%) lower than the recommended minimum, due to the low CP content of elephant grass (6.87% of DM; Table 2) prior to ensiling.

The silages with urea presented higher MM content, and the lowest values were found in the treatment with 30% PP. Probably this effect occurred due to the loss of cellular constituents during fermentation, resulting in proportional increase of the values. Dias et al. (2014) found lower organic matter content in sugarcane silages that were not treated with urea, and attributed that fact to the higher production of gases and effluents from the higher extent of undesirable fermentations.

Fig. 5 Effective degradability (ED) of the dry matter (**a** 2%/h, **b** 5%/h, and **c** 8%/h) of elephant grass silage containing *Parkia platycephala* pod meal and urea (U) as a function of the level of *Parkia platycephala*



According to the NDF and ADF values, the silages containing urea presented lower fiber content when compared with the silages without urea, except for treatments without PP. The use of urea in roughages promotes changes in the fibrous fraction resulting in reduced NDF and ADF content due to the solubilization of hemicellulose and lignin and cellulose, respectively (Silva et al. 2016). This is due to the action of ammonium hydroxide, which is formed after urease converts urea into ammonia that binds to water, promoting alkaline hydrolysis of the fibrous fraction (Rocha et al. 2006). Even though there was a linear decrease in hemicellulose ($p < 0.0001$) as PP levels increased, it was estimated a decrease of 0.23% in

hemicellulose content for each unit of PP added (Fig. 2). This decrease may be associated with the dilution effect proportional to the inclusion of PP, which presented low hemicellulose value (9.65% of DM; Table 2) when compared with the elephant grass (30.51% of DM; Table 2).

The increase in DM disappearance of the silage containing both PP and urea was proportional to the PP inclusion levels, being higher in the silages treated with urea, except for those without PP and with urea. The lower fibrous fraction contents of the PP (Table 2) may have contributed to this effect, which was potentiated by the urea. This difference in rumen degradation of the silage DM may be an important factor with impact on animal consumption.

The PP levels promoted linear increase of the soluble fraction (*a*) and potentially degradable fraction (*b*) in the absence of urea (Table 5; Fig. 5). This was probably due to the high non-fibrous carbohydrate content present in PP (Table 2). These results corroborate data reported by Alves et al. (2007), who found the value of 70% of digestibility of the soluble fraction of *Parkia platycephala* pods, evidencing the high potential of this additive for inclusion in ruminant diet.

Urea provided increased digestibility of the roughage materials due to the effect of ammonia on the cell wall by disrupting ester bonds between cell wall components and phenolic acids and to depolymerization of lignin in addition to increasing fermentable carbohydrate content (Silva et al. 2016). Accordingly, the highest degradability of fraction *a* was found in the treatments with 30% PP, since the *Parkia platycephala* pods have high NFC content (Barbosa et al. 2015).

The use of urea provided greater degradability of fraction *b* when silages contained 0% and 10% of PP. However, when PP participation increased to 20 and 30%, the effect of urea disappeared (Table 5). The combination of 20% PP + 1.5% U resulted in the lowest fraction *b* among the silages with added urea (Table 5).

A linear increase of ED was observed as the PP level increased in the silage, being higher in the silages with urea (Fig. 5). The silages containing urea had higher effective DM degradability rates when compared with the ones without additives (Fig. 5). These results suggest a better nutritive value of these silages when compared with the others evaluated.

It was observed that there were changes in elephant grass silages with the inclusion of additives. Regarding the fermentative characteristics, there was an increase in pH, N-NH₃, and butyric acid in urea-treated silages without PP, while PP increased lactic acid contents. Regarding the chemical composition, PP increased the DM contents and together with urea increased CP. The NDF and ADF contents were reduced in the presence of urea. Regarding the rumen degradability of the silages, there was an increase in the DM disappearance in those treated with PP and urea, besides the increase of fractions *a* and *b* and ED of the DM. These results are in agreement with the literature, as observed by Lopes et al. (2007), who associated urea and moisture absorbing additives in sugarcane silages, and obtained improved nutritional value, and higher consumption and digestibility of the silages with additive.

Conclusions

The use of *Parkia platycephala* pod meal associated with urea improves the fermentative characteristics and chemical composition of elephant grass silage, promoting an increase in the dry matter and crude protein contents and reducing the levels

of fiber, consequently increasing potential and effective degradability.

Compliance with ethical standards

The study was approved by the Animal Use Ethics Committee (CEUA / UFPI) under the protocol no. 394/17.

Conflict of interest The authors declare that they have no conflict of interest.

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