DOI: 10.5433/1679-0359.2019v40n5Supl1p2397

Nutritional composition of 'Ponta Negra' forage sorghum silage enriched with dried *Leucaena leucocephala* forage

Composição nutricional da silagem de sorgo forrageiro Ponta Negra aditivada com forragem de leucena (*Leucaena leucocephala*) desidratada

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Abstract

The aim of this study was to evaluate fermentation and chemical characteristics and the *in situ* degradability of sorghum silages enriched with dried Leucaena. The experiment was conducted as completely randomized design with four treatments (0 - control silage, silage without leucaena; 10.0, 20.0, or 30.0% inclusion of Leucaena) and five replicates, totaling 20 experimental units. The inclusion levels of leucaena influenced (P <0.05) the pH values, and for each 1% inclusion there was a reduction of 0.0023 points in the pH value, and in all silages presented values. Dry matter recovery did not show effect (P > 0.05), with all silages having values above 90%. Gas loss decreased (P < 0.05) as the level of leucine increased in sorghum silage. A linear increase was observed for dry matter (P ≤ 0.0001) and crude protein (P = 0.0008) contents in response to Leucaena inclusion. There was a linear (P < 0.05) linear effect on neutral detergent fiber, hemicellulose and acid detergent fiber. As leucaena inclusion levels increased, for each 1% inclusion a reduction of 0.179 was observed; 0.059 and 0.119% for the values of neutral detergent fiber, hemicellulose and acid detergent fiber respectively. The inclusion of leucaena influenced (P < 0.05) the increase of silage lignin levels, probably due to the higher lignin content of the legume. The soluble fraction (a) of the silages increased along with the Leucaena inclusion levels, with highest values observed at the inclusion of 20 and 30% of the legume, respectively. Potential degradation (A) increased linearly with the levels of Leucaena added to the silage; the highest value for this variable was observed at 30% inclusion. Effective degradability (ED) increased up to the inclusion level of 20% (46.77%). Degradation rate (c) decreased markedly with the use of 30% Leucaena. The use of the forage part of Leucaena dried for three hours leads to reduced gas production, increased

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Received: Apr. 12, 2018 - Approved: June 08, 2019

dry matter and crude protein contents, and decreased fiber contents. The degradability of dry matter increases up to the inclusion level of 20%.
Key words: Leguminous. *Sorghum bicolor*. Wilting.

Resumo

O objetivo deste trabalho foi avaliar as características fermentativas e químicas e a degradabilidade *in situ* de silagens de sorgo enriquecidas com Leucaena desidratada. O experimento foi conduzido em delineamento inteiramente casualizado, com quatro tratamentos (0 - silagem controle, silagem sem leucena; 10,0, 20,0 ou 30,0% de inclusão de leucena) e cinco repetições, totalizando 20 unidades experimentais. Os níveis de inclusão da leucena influenciaram ($P < 0,05$) os valores de pH, sendo que para cada 1% de inclusão houve redução de 0,0023 pontos no valor do pH, sendo que em todas as silagens apresentaram valores ideias. A recuperação de matéria seca não apresentou efeito ($P > 0,05$), sendo que todas as silagens apresentaram valores acima de 90%. A perda de gases reduziu ($P < 0,05$), à medida que aumentou o nível de leucina na silagem de sorgo. A linear increase was observed for dry matter ($P \leq 0, 0001$) and crude protein ($P = 0.0008$) contents in response to Leucaena inclusion. Observou-se efeito ($P < 0,05$) linear decrescente a fibra em detergente neutro, hemicelulose e fibra em detergente ácido. À medida que se elevou os níveis de inclusão da leucena, para cada 1% de inclusão foi observada redução de 0,179; 0,059 e 0,119% para os valores de fibra em detergente neutro, hemicelulose e fibra em detergente ácido, respectivamente. A inclusão de leucena influenciou ($P < 0.05$) o aumento dos níveis de lignina da silagem, provavelmente pelo maior teor de lignina da leguminosa. A fração solúvel (a) das silagens aumentou juntamente com os níveis de inclusão de leucena, com maiores valores observados na inclusão de 20 e 30% da leguminosa, respectivamente. A degradação potencial (A) aumentou linearmente com os níveis de Leucaena adicionados à silagem; o maior valor para essa variável foi observado em 30% de inclusão. A degradabilidade efetiva (DE) aumentou até o nível de inclusão de 20% (46,77%). Taxa de degradação (c) diminuiu acentuadamente com o uso de 30% de Leucena. O uso da parte forrageira de Leucaena seca por três horas leva à redução na produção de gás, aumento do teor de matéria seca e proteína bruta e diminuição do teor de fibras. A degradabilidade da matéria seca aumenta até o nível de inclusão de 20%.
Palavras-chave: Leguminosa. *Sorghum Bicolor*. Emurchecimento.

Introduction

The unavailability of forage in acceptable quality and quantity in certain periods of the year is one of the major obstacles in pasture-based ruminant production systems. During the off-season, the pasture has a low carrying capacity and pastured animals consequently lose weight, since the consumed material has a high fiber content and low levels of nitrogenous compounds, which limit its degradation (DETMANN et al., 2014). To overcome this negative impact of forage seasonality, alternatives should be adopted for the preservation of the forage harvested during the rainy period.

Silage making is a forage preservation technique characterized by fermenting forage in an anaerobic medium. To be ensiled, the forage plant must have certain characteristics so that the resulting silage has good nutritional value, e.g. high soluble carbohydrate content; dry matter content between 30 and 35%; and low buffer capacity (McDONALD, 1981). Corn is considered the ideal plant for silage making as it meets the aforementioned requirements. However, it is a very demanding crop in terms of soil-climatic conditions, which may restrict its production in certain locations. Sorghum is another great alternative forage plant to be used in the ensiling process, second only to corn (VIEIRA et al., 2004).

Sorghum carries some advantages of extreme importance, such as tolerance to dry environmental conditions and nutrient-poor soil as well as high regrowth capacity (ZAGO, 1999). Furthermore, sorghum has a great potential for grazing in a rotational-stocking system (SIMILI et al., 2011). The forage sorghum variety BRS Ponta Negra is a small-sized plant with good forage production and high water-utilization efficiency, as reported by Perazzo et al. (2013). Nevertheless, its low protein content is a limiting factor in animal production, as protein is the most expensive nutrient in the feeding system.

An option for producers who wish to mitigate this issue is the use of additives rich in crude protein, such as leguminous species. Leguminous species are not good candidates for single crop silage making because of their high buffer capacity, low soluble-carbohydrate content, and elevated moisture. According to Tobi et al. (2007), leguminous species may, however, be used in the proportion of 30 to 50% of the silage without compromising the fermentation process. Evangelista et al. (2005) evaluated sorghum silage with added *Leucaena* and recommended its addition at the level of 40%, but the authors also observed an increase in pH and a simultaneous reduction in the dry matter content of the silage resulting from the elevated moisture content in the legume.

To mitigate this issue, *Leucaena* forage can be subjected to a wilting period so that its dry matter content is elevated. Another noteworthy aspect of the drying process is that it may contribute to reducing the concentrations of mimosine in *Leucaena*, considering that this amino acid is degraded when the plant is exposed to a drying period, since endogenous enzymes potentiate amino acid breakdown (LYON, 1985).

This study was conducted to evaluate the fermentation characteristics and nutritional value of sorghum silage variety BRS Ponta Negra enriched with dried *Leucaena* forage.

Material and Methods

The experiment was carried out in the Forage Crops section of the Center for Agricultural and Environmental Sciences at the IV campus of the Federal University of Maranhão, located in Chapadinha, MA, Brazil (03°44′33″ S, 43°21′21″ W). The experimental period was January 2016 to July 2016. Experimental procedures were approved by the university's Ethics Committee for Animal Experimentation under number 23115.011059/2015-26.

The forage sorghum (*Sorghum bicolor* var. Ponta Negra) was planted in January 2016 in a 400-m^2 area. The forage material was harvested manually, to 10 cm of the level of the ground, at 120 days of growth. Subsequently, the material was chopped to particles of 2-3 cm in a forage chopper coupled to a tractor. *Leucaena* was harvested at 50 days of age, at a height of 1 m above the soil. Woody branches measuring up to 1 cm in diameter were collected, as described by Ferreira et al. (2016). After harvest, the material was milled and placed on plastic tarps to dry in the sun for 3 h.

To facilitate homogenization, *Leucaena* was added at its respective ratios to sorghum inside plastic drums and the material was stirred for a few minutes. Next, the material was ensiled in experimental silos made from PVC tubes of 0.10 m diameter and 0.35 m length, using wooden pestles for compaction at a density of 600 kg m^{-3} (fresh matter).

Sixty days after being sealed, the silos were opened and discarded 10 cm from the top and bottom of the silo and a 500 g sample of the silage was collected for chemical and in situ degradability analyses. Sub-samples were collected to determine the pH, with readings performed using a pH meter following the methodology described by Bolsen et al. (1992). Gas losses and dry matter recovery were calculated based on equations proposed by Schmidt (2006).

Collected samples were pre-dried in a forced ventilation oven at 55ºC for 72 h. Subsequently, the material was ground through a Willey mill with screen sieves of 1 mm diameter. Concentrations of dry matter (method 930.15) and crude protein (method 954.01) were determined by following procedures described in AOAC (1990). Neutral detergent fiber (NDF), acid detergent fiber (ADF), and lignin content were obtained as described by Van Soest et al. (1991) in the material before ensilage and later in the silage samples (Table 1).

Table 1. Chemical composition of the before ensiling.

Composição	'Ponta Negra' sorghum	Leucaena
Dry Matter[1]	33.06	41.20
Crude protein[2]	9.89	20.11
Neutral detergent fiber[2]	56.75	41.54
Acid detergent fiber[2]	23.88	22.25
Lignin[2]	3.17	8.99

[1]% of fresh matter, [2] % of dry matter.

In situ degradability was determined in a Santa Inês sheep with an average live weight of 60 kg, fistulated in the rumen, as proposed by Tomich et al. (2004). The animal received a diet with a 60:40 roughage-to-concentrate ratio. The roughage source was chopped elephant grass. The feed was supplied in a trough at 08:00 and 16:00 h.

Five grams of the sample were placed in 20 cm × 10 cm nylon bags with 50 µm porosity. The area occupied by the sample inside the bag was 20 g of dry matter cm^{-2} (NOCEK, 1988). Incubation periods were 0, 6, 24, 72, and 96 h, and bags were weighed in duplicates and placed in the rumen in descending order for simultaneous removal.

To determine the disappearance of the material at zero time (soluble fraction a), the bags were heated in a bath for 1 h at a temperature of 39 °C (MAKKAR, 1999). Afterwards, the soluble fraction bags were washed in running water together with the sample bags incubated in the rumen until the water ran clear. Next, they were dried in a forced ventilation oven for 72 h at 55 ºC. After pre-drying, bags were weighed to calculate dry matter disappearance (%).

The in situ degradability parameters of dry matter (a, b, and c) were estimated using the model proposed by Ørskov and McDonald (1979) that was modified and simplified by Sampaio (1995), as follows: $PD = A - B \times e^{-ct}$, where A = maximum degradability potential; B = potentially degradable fraction; c = rate of degradability of fraction B; and t= time.

The effective degradability (ED) of DM was estimated considering three rates of rumen passage, 2, 5, and 8% h^{-1}, by the equation described by Ørskov and McDonald (1979): $ED = a+(b \times c/(c+k))$, where a = soluble fraction; b = potentially degradable fraction; c = rate of degradability of fraction b; and k = rate of passage.

The data were subjected to a regression analysis to examine the effects of inclusion levels using the PROC REG procedure, whereas degradation parameters were estimated using the PROC NLIN procedure of the Gauss-Newton algorithm of SAS software version 9.0 (2000).

Results and Discussion

Leucaena inclusion in the sorghum silage had a positive effect ($P < 0.05$) on pH, which rose linearly (Table 2). At every 1% inclusion of the legume, the pH increased by 0.0023 units, with an overall mean of 4.03. Dry matter recovery (DMR) was not influenced ($P > 0.05$) by the *Leucaena* levels in the silage, averaging 93.47%. The pH increase is attributed to the low soluble carbohydrates content and elevated buffer capacity of *Leucaena* (LIU et al., 2011). According to McDonald (1981), 80% of the resistance to a pH decline in forages with high buffer capacity are due to the presence of organic acids like citric and malic acids, whereas only 20% are attributed to high protein content, regardless of the high concentration of cations (K^+, Ca^{2+}, and Mg^{2+}). Despite the pH increase in the enriched silages, its values were within the expected range for experiments with leguminous species, which show higher pH values for the stabilization of the fermentation process (EPIFANIO et al., 2016).

Table 2. pH values, dry matter recovery and gas production in sorghum silage with forage levels of Leucena.

Variables	Leucaena of levels (%)				Equation	R^2	Linear	SEM
	0	10	20	30				
pH	4.00	4.04	4.02	4.08	Y=3.99+0.0023x	0.51	0.0018	0.009
Dry matter Recovery[1]	93.63	91.78	95.58	92.89	Y=113.01	-	0.6142	0.488
Gas production[1]	0.11	0.09	0.06	0.04	Y=0.1068-0.002x	0.80	<0.0001	0.007

[1] % of dry matter, R^2- Determination coefficient, P-value = Probability by regression model. , SEM- standard error mean.

Jaster (1995) submitted that the expected pH value for silages of leguminous plants is 4.0 to 4.5. It can be inferred that the 'Ponta Negra' sorghum silages did not have their fermentation process compromised by the addition of the *Leucaena* forage, since no development of clostridial bacteria occurred. Another fact that reinforces this assertion is that no dry matter losses were recorded; additionally, gas losses were reduced in the silages as the *Leucaena* inclusion levels were increased. These results may be explained by the reduction of gas-producing bacteria such as enterobacteria and clostridia (McDONALD, 1981) (Table 2).

This response is because the *Leucaena* plant had been dried, which prevented dry matter losses because of the less development of enterobacteria that would result in a low-quality fermentation process (EDVAN et al., 2013; FERREIRA et al., 2016). Therefore, the inclusion of the dried legume ensures good fermentation of the sorghum silage in addition to possibly improving the production performance of sheep consuming it (TJANDRAATMADJA et al., 1993).

Evangelista et al. (2005) worked with sorghum silages enriched with *Leucaena* forage that were not subjected to wilting (10.0, 20.0, 30.0, and 40.0% inclusion) and observed a reduction in the silage dry matter content that was explained by the higher moisture content of *Leucaena* when compared with that of sorghum. A different result was observed in the present study, where the dry matter content of the silages was increased. The explanation for this occurrence was that *Leucaena* forage had been subjected to a drying period of 3 h, which was sufficient time for the material to reach a higher dry matter content than that of sorghum (41.20 vs. 33.06%, respectively).

The presence of *Leucaena* influenced ($P < 0.05$) gas production (GP) in the sorghum silage. Every 1% inclusion of the plant in the silage led to a 0.002% reduction in the production of gases originating from the fermentation process.

The dry matter content increased linearly (P < 0.05) with the levels of *Leucaena* added to the silage. A 18.79% increase was observed for this variable with the inclusion of 30% *Leucaena,* in relation to control silage. Dry matter content remained within the ideal range (of 27.0 to 35.0%) suggested by McDonald et al. (1991), which ensured a good fermentation process, as can be seen in Table 2. The dry matter content of unenriched sorghum silage decreased by 5.59% in relation to that of the same material prior to ensiling, which is below the acceptable maximum limit of 10%, according to Faria (1986).

Dry matter is a parameter of great importance in the ensiling of forage plants, since elevated moisture enables the proliferation of bacteria of the genus *Clostridium*, which results in a compromised fermentation process as these bacteria convert the soluble carbohydrates into butyric acid. As a consequence, dry matter is lost, leading to a decrease in nutritional value (PAHLOW et al., 2003).

Crude protein contents also increased linearly (P < 0.05) as the levels of the dried legume were elevated, with every 1% inclusion providing a 0.073% increase in the concentration of this component (Table 3). *Leucaena* inclusion led to an increase in the crude protein content of the silages, and this result was due to the higher protein value of the *Leucaena* forage in comparison with that of forage sorghum ‘Ponta Negra’. Sorghum has a low crude protein content (6.3%) (FERNANDES et al., 2009; LIMA-OROZCO et al., 2012). Silages with crude protein levels below 8% limit the development of rumen microorganisms and reduce the degradation of neutral detergent fiber (LAZZARINI et al., 2009). It is thus clear that *Leucaena* addition had a positive effect on the crude protein content of the silages.

Table 3. Chemical composition of sorghum silages supplemented with Leucena forage levels.

Variables	Leucaena of levels (%)				Equation	R^2	P value	SEM
	0	10	20	30				
Dry Matter[1]	27.47	30.18	30.86	33.83	Y=27.61+0.197x	0.87	<0.0001	0.609
Crude protein[2]	10.59	12.40	12.93	12.86	Y=11.092+0.073x	0.56	0.0008	0.090
Neutral detergent fiber[2]	67.55	65.22	64.15	61.88	Y=67.40-0.179x	0.78	<0.0001	0.586
Hemicellulose[2]	31.35	31.27	30.45	29.58	Y=31.57-0.059x	0.52	0.0024	0.240
Acid detergent fiber[2]	36.20	33.95	33.70	32,31	Y=35,83-0,119x	0,77	<0,0001	0,395
Lignin[2]	4.75	5.69	5.79	5.94	Y=4.99+0.036x	0.45	0.0045	0.157

[1] % of fresh matter, [2] % of dry matter, R^2- Determination coefficient, P-value = Probability by regression model linear, SEM-standard error mean.

According to Pereira et al. (2004), in the fermentation process, a small part of the protein is degraded to non-protein nitrogen. This degradation may be potentiated when a crop with low dry-matter and soluble-carbohydrate content and high buffer capacity is ensiled. Therefore, the data indicate that the level of proteolysis was minimal, and it did not affect the crude protein content of the silages in response to the addition of *Leucaena* forage

Neutral detergent fiber (NDF) decreased linearly (P < 0.05) by 0.179% with every 1% inclusion of *Leucaena* in the silage. The hemicellulose and acid detergent fiber (ADF) content also decreased linearly (P < 0.05) with the inclusion of the legume in the sorghum silage. At 30% inclusion, these two variables decreased by 5.65 and 10.74%, respectively, in relation to that in the silage without *Leucaena*. The opposite effect was seen (P < 0.05) in the lignin content of the silage, which increased linearly by 0.036% for every 1% inclusion of *Leucaena*. Values ranged from 4.75% for the silage without the legume to 5.94% for the silage with 30% *Leucaena*.

Neutral detergent fiber (NDF) decreased with the inclusion of *Leucaena* in the silage owing to the dilution effect, since the NDF content of the *Leucaena* forage was lower than that of sorghum. Jahanzad et al. (2016) evaluated silage of pearl millet enriched with soybean and observed a reduction in the NDF content, which may be explained by the fact that the legume was less fibrous than pearl millet. The elevated NDF content has a negative correlation with the forage nutritional value and with dry matter intake, considering that a limitation occurs as a result of the physical rumen-fill effect (PIRES et al., 2006).

The reduction in hemicellulose content is attributed to the presence of hemicellulases in the material, which caused the breakdown of hemicellulose into arabinose and xylose—carbohydrates used in the fermentation process that are converted to organic acids (McDonald, 1981). Defining the fermentation capacity based solely on the amount of soluble carbohydrates may underestimate the fermentation potential of the crop, since hemicellulose is broken down by the action of hemicellulases and its products are fermented and contribute to the formation of organic acids (RIBEIRO et al., 2008). The hydrolysis of hemicellulose generates openings on the cell wall between the fiber bonds that probably allow the microorganisms to have access to the cell content (McDONALD et al., 1991).

Acid detergent fiber (ADF) behaved similarly to NDF, because *Leucaena* had a lower concentration of the former component than did sorghum, leading to the dilution effect. Pereira et al. (2004) observed a similar response in a study on the effect of adding *Leucaena* forage to corn silages (10.0, 20.0, 30.0, and 40.0% inclusion). The lignin content showed the opposite effect, increasing as the levels of *Leucaena* added to the silage were increased. This finding is explained by the higher lignin content of the legume when compared with sorghum, and this result may compromise the nutritional value of silage (LAZZARINI et al., 2009).

The soluble fraction (a) of the silages increased along with the *Leucaena* inclusion levels, with the highest values observed at the inclusion of 20 and 30% of the legume, respectively. The potentially digestible fraction (b) had a similar response to that shown by the soluble fraction, except in the treatment with 20% inclusion of *Leucaena*, where this parameter decreased. The degradation rate, in turn, decreased as the proportions of *Leucaena* in the sorghum silage were increased, with the lowest value (0.87%) observed at 30% inclusion (Table 4).

The increase of the soluble fraction in response to *Leucaena* inclusion is caused by the reduction of NDF and increase in CP content, since silages with more *Leucaena* have a higher amount of non-protein nitrogen, i.e. a highly soluble fraction. Another factor that might have contributed to this finding is the higher concentration of protein, which ensures greater development of the microbial mass stemming from the higher utilization of protein by the rumen microorganisms, which ultimately leads to improved microbial protein synthesis (GIANG et al., 2016).

Potential degradation (A) increased linearly with the levels of *Leucaena* added to the silage; the highest value for this variable was observed at 30% inclusion. Effective degradability (ED) increased up to the inclusion level of 20% (46.77%). At 30% inclusion of *Leucaena*, this variable declined to 45.82% when the rate of passage of 2%/h was used as reference. As the rate of passage was increased, ED decreased, in all silages (Table 4).

Table 4. Parameters of *in situ* degradation, potential degradation and effective degradation of dry matter of sorghum silages with Leucena forage levels.

Levels (%)	a(%)	b(%)	c(%/h)	A	R^2	Efective degradability		
						2 % h^{-1}	5 % h^{-1}	8 % h^{-1}
0	18.90	40.23	3.52	59.13	95.76	44.55	35.52	31.19
10	22.60	39.37	2.46	61.97	97.37	44.32	35.58	31.86
20	27.50	56.69	1.03	84.19	95.70	46.77	37.18	33.97
30	27.00	62.28	0.87	89.28	98.21	45.82	36.19	33.08

Soluble fraction (a), Potentially degradable insoluble fraction (b), Fractional breakdown rate (c), potential degradation (A), coefficient of determination (R^2), and effective degradability for the passage rates of 2, 5, and 8% h^{-1}.

Degradation rate (c) decreased markedly with the use of 30% *Leucaena*. This result is likely related to the increasing lignin content observed with the increasing levels of the legume added, since lignin content is a limiting factor for the degradability of the protein in the rumen that reduces the speed at which microorganisms degrade the material (JUNG; ALLEN, 1995). By contrast, this lower rate of degradation may also be associated with the elevated concentration of slowly degradable protein in *Leucaena* that reduces the degradability of the material, as was reported by Santos et al. (2017). Effective degradability decreased as the rate of passage was increased, which is explained by the shorter duration for which the microorganisms remained adhered to the material inside the rumen (ØRSKOV, 1988).

Conclusion

The use of the forage part of *Leucaena* dried for 3 h leads to reduced gas production, increased dry matter and crude protein content, and decreased fiber content. The degradability of dry matter increases up to the inclusion level of 20%.

It is recommended to use the forage part of *Leucaena* up to the inclusion level of 20% in silages of the forage sorghum variety Ponta Negra.

Acknowledgment

This project was financed by FAPEMA and FOPAMA.

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