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In situ and *in vitro* degradation parameters of elephant grass silage with sugarcane bagasse

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Abstract

The objective of this study was to evaluate the chemical composition and *in situ* degradability of elephant grass silage dry matter with increasing levels of sugarcane bagasse. A completely randomized design was adopted with five treatments: 0, 5, 10, 15, and 20% inclusion of sugarcane bagasse on an as-fed matter basis, with five replications, totalling 25 experimental units. The increasing proportion of sugarcane bagasse analysed was favourable only in increasing the concentration of dry matter and fibre of silages and in maintaining a considerable crude protein content. In the *in situ* degradation assay, the addition of sugarcane bagasse in silage decreased the ruminal degradability of dry matter, and as the passage rates increased, the effective degradability decreased. In the *in vitro* gas production assay, the total production of gases was decreased with the inclusion of sugarcane bagasse, as did the degradability of organic matter and neutral detergent fibre. The recommendation for the inclusion of sugarcane bagasse should be analysed according to the desired objective, emphasizing that values higher than 5% can greatly compromise the final nutritive value of elephant grass silage.

Keywords: conservation of forage, by-product, *in vitro* gas production, methane production, nutritional value

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Introduction

Forage production is limited due to the seasonality of production and most producers do not carry out any planning to supplement the herds in periods of scarcity. Elephant grass is a grass of the genus *Pennisetum*, which presents high forage production (~80,000 kg/ha/year) with good nutritional value and a high leaf/stem ratio (Pompeu *et al.,* 2006; Desta *et al.,* 2016). This grass can be used in the silage process, but its moisture content can be a problem in the fermentation process (Wu *et al.,* 2020). To obtain an effective silage from elephant grass, research has been carried out through the use of moisture absorbent additives, aiming at adjusting the dry matter content of silage and improving nutritional quality.

Several agro-industrial residues with high dry matter content and nutritional value have been used as a way to absorb moisture from tropical grass silages, arousing interest in alternative foods for ruminants (Bergamashine *et al.,* 2006; Cândido *et al.,* 2007; Rêgo *et al.,* 2010; Monteiro *et al.,* 2011; Negrão *et al.,* 2016). These techniques have facilitated improvements, however, the increase in complementary alternatives for adequate silage has informed the correct choice of additives based on the fermentative and economic issues of silage.

Sugarcane bagasse, resulting from the physical process of separation of fibre and soluble sugars, is rich in cell wall with reduced digestibility, crude protein, reserve carbohydrates, and energy (Costa *et al.,* 2015). However, as the largest residue of the Brazilian agroindustry, it becomes an

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economic measure to ensure the supply of feed during drought periods because its harvest coincides with the scarcity of forage. Thus, bagasse is widely evaluated as a bulky supplement in ruminant diets (Pessoa *et al.,* 2013; Gunun *et al.,* 2016; Freitas *et al.,* 2019), and can be used as an alternative additive in the manufacture of silages (Siqueira *et al.,* 2019).

Studies on the components and degradability of silages are essential to define the proportion of additives so that they become effective in the quantitative aspects of digestion, combined with the factors of the fermentative profile of silage, aiming at more efficient feeding systems, with lower silage losses. Within this context, it is of great importance to define the ideal levels of inclusion of this by-product, aiming to ensure silage with good nutritional value. The objective of this study was to determine the chemical composition, *in situ* degradability, and *in vitro* gas production parameters of elephant grass silages supplemented with sugarcane bagasse.

Materials and Methods

The experiment was conducted in the Forage sector located at the Centre for Agricultural and Environmental Sciences of the Federal University of Maranhão (CCAA/UFMA), in Chapadinha-MA, located at latitude 03°44'33" S; longitude 43°21'21" W. The rainy season of the region corresponds to the months of January–June and the dry period is from July–December.

The forage species used was elephant grass (*Pennisetum purpureum* Schum), from weeding established since 2013 in the Forage Sector of CCAA/UFMA. A cut was made with 60 days of regrowth; average height, 1.80 m; then the material was crushed into forage of 3-cm particle sizes. A completely randomized design was adopted with five treatments: 0, 5, 10, 15, and 20% inclusion of sugarcane bagasse (SCB) on as as-fed basis, with five replications, totalling 25 experimental units.

Sugarcane bagasse was homogenized in a 100 L plastic container to facilitate handling and avoid soil contamination. Then, the material was packed in PVC silos of 0.10 m diameter and 0.30 m height, adapted with Bunsen valves on the lids to facilitate the escape of gases from fermentation. In each silo, an average of 1.4 kg of the fresh mixture was placed, adopting a compaction of 600 kg/m^3. Before ensiling, a 400-g collection of elephant grass and sugarcane bagasse was performed to determine the chemical composition (Table 1).

After ensiling, the silos were maintained at room temperature and the opening procedure was performed 60 d after sealing. The silages were characterized according to dry matter content (DM - ID 930.15), organic matter (OM - ID 942.05), and crude protein (CP=6.25xN; - ID 988.05) by the methods described in AOAC (1995). Neutral detergent fibre (NDF) contents were determined according to the methods of Van Soest *et al*. (1991), using stable term alpha-amylase and sodium sulphite. Acid detergent fibre (ADF) contents were determined according to the method of Goering and Van Soest (1970). Acid detergent lignin (ADL) was estimated according to the method of Van Soest (1973) using the H_2SO_4 method at 72%. These measurements of fibrous fractions were estimated sequentially, with the aid of the ANKOM 220 fibre analyser (ANKOM Technology Corporation, Macedon, NY, USA), using the same sample in filter bags.

At the time of silo opening, samples were also collected to determine pH. Approximately 9 g of the material was homogenized with 60 mL of distilled water, and after resting 50 min, with the aid of a digital pH meter, pH readings were measured. Dry matter degradability (DMD) was estimated using the *in situ* technique (Mehrez and Orskov, 1977). We used a male, cross-breed, adult sheep, fistulated as suggested by Tomich and Sampaio (2004), weighing 60 kg, fed chopped elephant grass. The silages produced were ground to 2-mm particles and packed in nylon bags with dimensions of 12 × 8 cm, and porosity 50 µm (Nocek, 1988). In the degradability assay, a completely randomized design with a 4 × 5 factorial arrangement (four incubation times and five levels of inclusion) was adopted. The incubation times were 6, 24, 72, and 96 h.

To determine the disappearance of the material at time zero, the bags were placed in a water bath at 39 ºC for 1 h. After the removal of the bags from inside the rumen at each time, the nylon bags were slightly washed in running water, packed in plastic bags, and frozen until the last incubation, when they were washed in a washing machine for three cycles, for 10 min, including the bags containing the same amount of sample to determine the loss of readily soluble material (time zero). After washing, the bags were dried in a greenhouse with forced air circulation at 60 ºC until constant weight.

The *in situ* degradation parameters of DM (a, b, and c) were estimated using the Ørskov and McDonald model (1979), modified by Sampaio (1995)

$$DP = A–B.e^{-c.t}, \quad (1)$$

in which A = maximum degradation potential, B = potentially degradable fraction, c = degradation rate, and t = time. The effective degradability (ED) of dry matter (DM) was calculated by adopting three rates of ruminal passages, 2, 5, and 8% h^{-1}, using the equation described by Ørskov and McDonald (1979):

$$DE = a+(b*c/c+k), \quad (2)$$

in which: a = soluble fraction; b = potentially degradable fraction, c = degradation rate, and k = passage rate.

The degradation and products of *in vitro* ruminal fermentation were estimated using the gas production technique (Theodorou *et al.,* 1994) adapted to a semi-automatic system (Mauricio *et al.,* 1999), modified by Bueno *et al.* (2005), using a pressure transducer and data store (Pressure Press Data 800, LANA, CENA-USP, Piracicaba, Brazil). The total gas production was calculated using the equation defined for the laboratory conditions:

$$V = 7.365 \times p, \quad (3)$$

where V is the volume of gases produced (mL), and p is the measured pressure (psi).

Methane was determined using a gas chromatograph (Shimadzu GC2014, Tokyo, Japan), and calculated according to Longo *et al.,* (2006):

$$CH_4 \text{ mL} = (\text{total gases, mL + head space, 85 mL}) \times CH_4\,\% \quad (4)$$

The values for gas and methane production were expressed on the basis of truly degraded organic matter (mL/g TDOM) and degraded dry matter (mL/g DDM). The ratio between TDOM (mg) and gas production (mL) in 24 h was used as microbial synthesis efficiency index (PF: partition factor, Blümmel *et al.,* 1997).

The data were submitted to homoscedasticity and normality tests. Data variance was analysed using the GLM procedure of the SAS package and a comparison of means was performed using Duncan's test at 5% probability. The parameters, a, b, and c, and the *in situ* degradation curves of the nutrients of the silages were determined using the interactive Gauss–Newton method using the PROC NLIN procedure of SAS (2000). Regression analysis at 5% probability was used to explore the effects of the increasing addition of sugarcane bagasse in silages using the PROC REG procedure of the statistical package, SAS, version 9.0 (2000).

Results

The dry matter content was greater (difference of 351.2 g kg^{-1} as-fed) and the crude protein content was smaller (difference of 28.8 g kg^{-1} dry matter) in sugarcane bagasse compared to elephant grass (Table 1).

Table 1 Chemical composition (g kg^{-1} DM) of elephant grass and sugarcane bagasse prior to ensiling

Item	DM	Chemical components (g kg^{-1} DM)							
	(g kg^{-1})	OM	Ash	CP	NDF	ADF	ADL	HEM	CEL
Elephant grass	202.0	919.4	80.63	71.8	694.7	527.4	77.7	167.3	449.7
Sugarcane bagasse	553.2	980,0	20.03	43.0	803.1	735.0	157.1	68.1	577.9

DM (dry matter); OM (organic matter); CP (crude protein); NDF (neutral detergent fibre); ADF (acid detergent fibre); ADL (acid detergent lignin); HEM (hemicellulose); CEL (cellulose)

According to the regression analysis, the increasing levels of sugarcane bagasse (SCB) in elephant grass silage (Table 2) showed a positive linear effect ($P <0.05$) on the levels of DM, NDF, ADF and ADL (Table 2). For every 1% of SCB addition, there was an increase of 10.7, 5.75, 7.55, and 3.15 percentage points in the levels of DM, NDF, ADF and ADL of silages, respectively. SCB levels showed a negative linear effect ($P <0.05$) on the CP content of silages, with a decrease of 0.66 percentage points for each 1% of SCB addition. Gas losses in the fermentation process (GLF) tended to present negative linear behaviour ($P <0.05$) and pH values increased positively and linearly ($P <0.05$) as levels of SCB increased in silage (Table 2).

Table 2 Chemical composition and fermentative characteristics of elephant grass silage with increasing levels of sugarcane bagasse

Item	Levels of SCB in the silage (%)								
	0	5	10	15	20	Equation*	R^2	VC	SEM
DM	193.3	357.1	407.8	416.9	433.3	Y=253.7+10.7BC	0.72	5.69	20.5
OM	930.9	927.5	941.2	939.8	944.5	Y = 928.86+0.79BC	0.59	0.42	1.67
Ash	69.1	72.5	58.7	60.3	55.5	Y= 71.14-0.79BC	0.59	6.15	1.67
CP	118.1	116.8	116.7	110.8	104.4	Y= 119.8-0.66BC	0.80	7.00	2.07
NDF	659.8	703.8	745.1	769.4	770.9	Y= 672.2+5.75BC	0.87	1.41	9.98
ADF	573.6	626.9	674.4	705.4	723.2	Y=585.1+7.55BC	0.91	2.27	12.8
ADL	76.2	122.7	137.5	143.9	144.4	Y=93.4+3.15BC	0.61	11.48	6.52
HEM	82.85	76.98	70.70	65.45	46.30	Y=85.3-1.69BC	0.52	18.01	3.77
CEL	497.4	504.2	536.9	561.5	578.8	Y= 467.24+5.952BC	0.77	3.67	8.23
GLF	0.036	0.033	0.030	0.029	0.021	Y=0.0369-0.0007BC	0.51	19.00	0.01
pH	3.84B	4.82A	4.82A	4.83A	5.01A	Y=4.19+0.047BC	0.60	2.27	0.09

*Equation at 5% probability level; SCB (sugarcane bagasse); VC (variation coefficient); SEM (standard error of mean); DM (dry matter); OM (organic matter); CP (crude protein); NDF (neutral detergent fibre); ADF (acid detergent fibre); ADL (acid detergent lignin); HEM (hemicellulose); CEL (cellulose); GLF (gas losses by fermentation process)

There was no interaction effect (*P >0.05*) on the disappearance of dry matter as a function of SCB inclusion levels and incubation times (Table 3). The inclusion of SCB negatively influenced *(P >0.05*) the disappearance of DM from silages.

Table 3 Disappearance of elephant grass silage dry matter with increasing levels of sugarcane bagasse

Item	Levels of SCB in the silage (%)						
Time (h)	0	5	10	15	20	Mean	VC (%)
6	38.55	25.30	18.40	18.85	18.00	23.82D	
24	53.05	36.27	28.35	28.87	29.40	35.19C	
72	67.97	49.27	41.47	39.92	36.12	46.95B	6.74
96	72.17	54.47	46.32	44.95	44.00	52.38A	
Mean	57.93A	41.33A	33.63C	33.15C	31.88C		

*Averages followed by capital letters in the rows and lowercase in the columns do not differ from each other using Duncan's test at 5% probability; SCB (sugarcane bagasse); VC (variation coefficient)

The inclusion of SCB in silage negatively influenced the soluble fraction (a), potential degradability (A), and degradable rumen fraction (B) (Table 4). The effective degradability for the passage rates 2, 5, and 8% also tended to present negative linear behaviour with the inclusion of SCB in silages. However, there was a negative quadratic effect for the degradation rate (C).

Table 4 Parameters of ruminal degradability of dry matter of elephant grass silage with increasing levels of sugarcane bagasse

Item	Levels of SCB in the silage (%)				
Time (h)	0	5	10	15	20
a’	33.80	27.29	17.52	14.79	13.81
A	74.74	59.95	53.16	49.48	45.85
B	42.42	38.55	38.32	34.04	30.72
C102	2.72	1.92	1.73	1.94	2.14
R^2	90.58	97.79	94.55	96.08	93.23
ED 2(%)	57.39	43.29	34.05	31.87	30.37
ED 5 (%)	48.22	36.35	26.68	24.49	23.41
ED 8 (%)	44.19	33.61	23.86	21.56	20.57

SCB (sugarcane bagasse); Soluble fraction (a'); potential degradability (A); rumen degradable fraction (B); degradation rate (C); coefficient of determination (R^2) and effective degradability (ED for passage rates of 2, 5 and 8%/h) related to the models of degradation of DM of silages

The inclusion of sugarcane bagasse showed negative linear behaviour (*P <0.05*) as the inclusion increased for the production of gases based on dry matter (PGDM) and organic matter (PGOM) (Table 5). The production of methane gases based on dry matter (CH_4DM) and organic matter (CH_4OM) also showed a negative linear effect (*P <0.05*). The percentage of CH_4 showed a linear decrease with the 10, 15, and 20% inclusions of sugarcane bagasse as an additive in elephant grass silage. The true degraded organic matter (TDOM) also showed a negative linear effect (*P <0.05*) and the neutral detergent fibre degradability (NDFD) tended towards a negative linear effect at 10, 15, and 20% inclusions of sugarcane bagasse. The partition factor (PF) remained low with values of 1.23–1.42.

Table 5 Gas and methane production, degradability, and the partition factor of elephant grass silage with increasing levels of sugarcane bagasse

Item	Levels of SCB in the silage (%)					Equation	R^2	VC (%)	SEM
	0	5	10	15	20				
PGDM (mL/g DM)	120.77	97.65	91.27	88.93	82.17	Y=110.6-1.51x	0.62	7.31	2.47
PGOM (mL/g OM)	290.83	227.02	210.03	203.20	186.37	Y=261.13-4.06x	0.64	7.21	6.46
CH4DM (mL/g DM)	8.07	8.47	6.80	6.82	5.30	Y=8.72-0.16x	0.47	19.99	0.331
CH4OM (mL/g OM)	19.43	19.67	15.70	15.58	12.07	Y=20.60-0.40x	0.43	19.76	0.786
CH4 (%)	6.63	8.70	7.48	7.10	6.58	Y=8.07	-	19.09	0.299
TDOM (g/kg)	358.14	319.10	289.88	252.48	228.23	Y=353.66-6.446x	0.56	14.60	11.25
NDFD (g/kg)	150.48	158.35	157.82	146.18	108.64	Y=168.94	-	38.13	10.54
PF (mg TDOM/mL PG)	1.23	1.42	1.38	1.25	1.23	Y=1.37	-	13.08	0.0346

*Averages followed by the same letters do not differ from each other using Duncan's test at 5% probability; VC (variation coefficient); SEM (standard error of mean); PGDM (production of gases based on dry matter); PGOM (production of gases based on organic matter); CH_4DM (production of methane gases based on dry matter); CH_4OM (production of methane gases based on organic matter); CH_4 (percentage of methane concentration as a function of total gas volume); TDOM (truly degraded organic matter); NDFD (neutral detergent fibre degradability); PF (partition factor)

Discussion

The DM contents of elephant grass in the present study are close to those reported by Cândido *et al.* (2007) and Wu *et al.* (2020), who observed DM values of 167 and 170 g.kg^{-1}, respectively. This fact shows that elephant grass has a high moisture content at the time of ensiling. According to McDonald *et al.,* (1991), the forage plant must contain dry matter contents of 28–35% to ensure adequate lactic acid fermentation and lower effluent and nutritional losses due to inhibition of undesirable microorganisms, such as *Clostridia*, which produce butyric acid. Sugarcane bagasse (SCB) presented higher DM content when compared to elephant grass *in situ*, with values close to 503.0 g.kg^{-1} reported by Ribeiro *et al.* (2017).

The CP levels of SCB in this study were low, agreeing with the data reported by Carvalho *et al.* (2006) and Gomes *et al.* (2013) of 23.2 and 37.8 g.kg^{-1}, respectively. Sugarcane, from which the bagasse is derived, is known to be a source of food with a low protein content (Costa *et al.,* 2015). Carvalho *et al.* (2006) observed high levels of NDF, ADF, and lignin for fresh sugarcane bagasse equal to 590.2, 383.4, and 73.4 g.kg^{-1}, respectively, which were lower than those of the present study. The chemical composition of sugarcane bagasse can be influenced by the variety and type of processing for sugar extraction (Hozhabri and Singhal, 2006).

The increasing linear behaviour of SCB on the DM content of silage can be justified by the high DM content of the bagasse used, which can characterize it as an additive of high hygroscopicity. Results from Candido *et al.* (2007) showed that for each 1% of addition of passion fruit by-product, an increase of 0.68 percentage points in the DM content of silages was verified. A study conducted by Carvalho *et al.* (2007a) worked with elephant grass silages supplemented with coffee hulls; for each 1% coffee hull inclusion, there was an increase of 0.77 percentage points in the DM content of the silages. We emphasize that the high rate of DM increment observed in the current study (1.07%) is due to the fact that SCB was higher in DM content than passion fruit by-product or coffee hulls.

The inclusion of 10, 15, and 20% SCB provided higher DM levels than those recommended by McDonald (1981) of 28–35% DM. At the time of silage compaction, it was observed that these treatments (10, 15, and 20% SCB) were most difficult to compact. Silages >40% DM may compromise the compaction process and consequently affect the fermentation in silos; low DM content of the silage may favour the development of harmful microorganisms, such as *Clostridia*, which in addition to losses by effluents, results in lower quality silages (Muck *et al.,* 2018).

The reduction in the CP content of the silages can be explained by the low CP content of the additive used, which provided a dilution effect of the protein components of elephant grass. Neiva Jr. *et al.* (2007), using sugarcane bagasse with low protein content (26.3 $g.kg^{-1}$), observed a marked decrease in protein from 96.8 to 81.0 $g.kg^{-1}$ DM with the addition of sugarcane bagasse as an additive to the silage.

According to Singh and Oosting (1992), NDF levels can be used to classify feed quality. The authors indicate that bulky foods with NDF values of <450 $g.kg^{-1}$ DM can be classified as high quality, those with values of 450–650 $g.kg^{-1}$ DM are average, and those with values >650 $g.kg^{-1}$ DM are low quality. In the present study, all silages presented values >659.8 $g.kg^{-1}$ due to the chemical composition of the silage components. The increasing linear behaviour of the fibrous fraction (NDF and ADF of silages with sugarcane bagasse) can be explained by the higher concentration of fibrous content present in the by-product when compared to elephant grass *in situ*. Candido *et al.* (2007) and Costa *et al.* (2016), evaluating the addition of agroindustry residues (passion fruit and babassu meal, respectively) to elephant grass silage reported that fibrous concentrations in the final silage were strongly related to NDF and ADF contents of the additives. In studies conducted by Ferreira *et al.* (2007) and Carvalho *et al.* (2007) adding cashew and coffee husk by-product, respectively, to elephant grass silages, observed an increasing linear effect of ADF, similar to that observed in the current study.

The accretion of the contents of the ADF and lignin may limit the digestibility and intake of silage, since it is the indigestible fraction of the food that forms complexes with structural carbohydrates and this hinders exposure to microbial enzymatic attack. The high concentration of lignin, a phenolic compound, in forage is negatively correlated with the degradability and digestibility of feed (Van Soest, 1994).

The lower levels of hemicellulose in sugarcane bagasse may have influenced the reduction of 0.169% in silage with increasing levels of bagasse by providing a dilution effect. In addition to the dilution effect, the reduction in hemicellulose content may have been the result of its use in order to increase the fermentative capacity of silage. Hemicellulose is considered as a fermentable substrate, and when the material is ensiled, hemicellulases work to break down the hemicellulose to release xylose and arabinose, which are used in the fermentation process (Ribeiro *et al.,* 2008).

The reduction in gas production during the silage fermentation process may be associated with the low fermentability of the high fibre content of sugarcane bagasse and its action as a moisture sequester in silage. The reduction in moisture has as a consequence the probable decrease in the production of bacteria of the genus, *Clostridium*, a main cause of the reduction in nutritional value by the production of gases (McDonald, 1981). This fact may have influenced the effective fermentation of the ensiled material. The high moisture content of forage at the time of ensiling can generate a large amount of effluent, which reduces the content of soluble components and concentrates the less digestible constituents (Cajarville *et al.,* 2012).

The pH is one of the main factors to influence the extent of fermentation and the quality of ensiled forage, as it indicates the fermentation capacity through microbiological activity and the production of organic acids. A higher pH value is associated with the high dry matter content, since the DM content in forage is a determinant of water activity (Aw), which in turn has a large influence on microbiological activity in silage (Jobim *et al.,* 2007). The increase in the dry matter content of silage, through the addition of SCB, promoted reductions in water activity (Aw) and microbial proliferation. Consequently, there was an influence on the fermentation quality of silage due to the lower activity of microorganisms in general, including lactic acid bacteria, which are fundamental for the drop in pH in silage (Pauly, 2009). As the incubation time increased, there was an increase in the disappearance of dry matter, and 96 h provided disappearance of 45.47% in relation to 6-h fermentation. The longer the residence time of the material in the rumen, the greater the disappearance of dry matter. This fact occurs until reaching a plateau, and according to Sampaio *et al.,* (1988), this point would be reached by 96 h. The 0 and 5% levels of SCB inclusion in silage did not differ from each other in DM disappearance and can be explained by the lower levels of NDF and ADF observed in these treatments.

It can be inferred that the reduction in the soluble fraction is associated with the lower soluble carbohydrate content and the higher amount of cell wall material when using, since these components are insoluble in water (Gomes *et al.,* 2013). The study of Gomes *et al.,* (2013) with fistulated sheep reported values of potential degradability (A) for sugarcane bagasse of 34.15%. The result of Carvalho *et al.* (2007c) for this same variable was 41.36%, a value higher than that reported by Gomes *et al.* (2013). The values reported by the authors mentioned above were lower than those observed in the present study, since the authors evaluated sugarcane bagasse as-fed.

It was verified that the increases in the passage rates in silages with a lower proportion of SCB promoted a reduction in the degradation rate. This fact can be justified due to the reduction of the time that the material remained in the rumen, thus causing insufficient time for the total use of the material by the ruminal microbial population (Garcez *et al.,* 2016). The higher proportion of slowly degraded constituents provided by the SCB increased the residence time in the rumen, which reduced the passage rate of the fibre.

The *in vitro* gas production technique was chosen to evaluate the effect of the inclusion of sugarcane bagasse with elephant grass silage in order to evaluate the degradation and fermentation products of the substrates. In general, this *in vitro* gas production procedure considers the conversion of all major carbohydrate sources (monosaccharides, polysaccharides, pectins, starch, cellulose, and hemicellulose) into CO_2 and CH_4 (Mizubuti *et al.,* 2014). The inclusion of SCB in elephant grass silage decreased gas production as a result of the lower *in vitro* fermentation of silages. The lower fermentation correlates with the lower utilization of nutrients, which implies that the inclusion of SCB negatively supported ruminal fermentation *in vitro*. According to Zhong *et al.,* (2016), differences in nutritional value will result in different fermentation characteristics. As the addition of SCB influenced the final nutritive value of silages, this may be the main explanation for the *in vitro* fermentative behaviour of the evaluated silages.

The amount of gas produced depends on the amount of fermented substrate and the amount and molar proportion of short-chain fatty acids produced (Pashaei *et al*., 2010). Wascheck *et al.* (2010) reported that lower gas production may be associated with higher protein levels, resulting in the formation of ammonium bicarbonate from CO_2, reducing the contribution to gas production. However, the protein contents of elephant grass silages decreased linearly as sugarcane bagasse increased and this lower production of gases was not associated with higher protein levels.

Enteric methane in ruminants can be considered as a secondary product of feed fermentation metabolism by microorganisms in the rumen. The composition of diets and consumption are the main factors that affect methane production by ruminants (Archimède *et al.,* 2011), and the increasing inclusion of sugarcane bagasse in elephant grass silage was conducive to the decrease in methane production (Table 5). According to Zhong *et al.* (2016), ruminal microorganisms perform the initial breakage and convert the components of the diet into short-chain fatty acids and sometimes ammonia, releasing carbon dioxide and hydrogen, the latter being the main substrates for ruminal methanogenesis. Higher concentrations of NDF associated with lower CP levels cause changes in the profile of short-chain fatty acids, with higher acetic acid formation, thus increasing methane production per degraded forage unit (Macome *et al.,* 2017).

According to Codognoto *et al.* (2014), with a good amount of fibre and good digestibility, there is a decrease in methane production, which may be the explanation for the results of the current study. The decrease in methane production (CH_4, %) was well evidenced at 20% SCB inclusion in elephant grass silage and may also be associated with the lower observed value of NDF degradability. Another factor that can influence methane production is the rate of degradation of dietary fibre (Johnson and Johnson, 1995). Generally, forage feeds rich in fibre will generate higher proportions of acetate:proprionate, and as a consequence will increase the amount of methane produced. The partition factor (PF) was not influenced by the increasing addition of sugarcane bagasse in the silages evaluated, and these were slightly below the values reported by Makkar (2004) (2.74– 4.41 mg DOM/mL of gases).

The inclusion of up to 20% sugarcane bagasse promoted an increase in the dry matter content above the levels recommended by McDonald *et al.* (1991), with a reasonable decrease in crude protein. However, increases in NDF and ADF negatively compromised the DM degradability, causing a decrease in gas production. A moderate inclusion of sugarcane bagasse was conducive to the fermentation of elephant grass silage without major detriment to the nutritional value of the silage.

Conclusion

The inclusion of 5% sugarcane bagasse in elephant grass silage is sufficient to adjust the dry matter concentration of the silage without compromising the nutritional value of the preserved food.

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