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Phenolic acids and ruminal parameters of different varieties of sugarcane *in natura* or ensiled

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ABSTRACT. This study aimed to determine and measure the phenolic acids in four varieties of sugarcane (RB 855536, RB 765418, SP 80-1842 and SP 80-1816) as fresh and ensiled, and assess the influence of these acids on *in situ* degradability of dry matter and neutral detergent fiber. The experimental design was completely randomized in a factorial design. For the fraction of dry matter (DM), varieties RB 855536 and SP 80-1842 had average 17.80% higher compared to others. The SP80-1842 and RB 855536 varieties did not differ ($p > 0.05$), with an average of DM effective degradability of 33.70%. For silage, the highest levels of *p*-coumaric acid were found in the variety RB 855536, which was 24.64% higher than the varieties RB 765418 and SP 80-1842 (average 12.75%) and 52.60% of the variety SP 80-1816 (average 8.02%), since for the *in natura* form, varieties did not differ between each other ($p > 0.05$), and had an average of 8.86%. Silage variety SP 80-1816 has ferulic acid content 40.82% higher than the other varieties, with an average of 4.63%. The examination of the concentrations of phenolic acids *p*-coumaric and ferulic are not sufficient to justify the effect of microbial action on the varieties studied in different forms of processing.

Keywords: digestibility, fermentation, lignin, silage, nutritional value.

Ácidos fenólicos e parâmetros ruminais de diferentes variedades de cana-de-açúcar *in natura* ou ensilada

RESUMO. Objetivou-se determinar e mensurar os ácidos fenólicos em quatro variedades de cana-de-açúcar (RB 855536, RB 765418, SP 80-1842 e SP 80-1816) na forma *in natura* e ensilada, bem como avaliar a influência destes ácidos sobre a degradabilidade *in situ* da matéria seca e fibra em detergente neutro. O delineamento experimental foi inteiramente casualizado em esquema fatorial. Para a fração da matéria seca (MS), as variedades RB 855536 e SP 80-1842 tiveram médias 17,80% superior em relação às demais. As variedades SP 80-1842 e RB 855536, não diferiram entre si ($p > 0,05$) com média de degradabilidade efetiva da MS de 33,70%. Para a silagem, os maiores teores de ácido *p*-cumárico foram constatados na variedade RB 855536 que foi 24,64% superior às variedades RB 765418 e SP 80-1842 (média de 12,75%) e 52,60% à variedade SP 80-1816 (média de 8,02%), sendo que para a forma *in natura,* as variedades não diferiram entre si ($p > 0,05$), média de 8,86%. A silagem da variedade SP 80-1816 apresentou teor de ácido ferúlico 40,82% superior às demais variedades, média 4,63%. A verificação das concentrações dos ácidos fenólicos *p*-cumárico e ferúlico não são suficientes para justificar o efeito da ação microbiana sobre as variedades estudadas nas diferentes formas de processamento.

Palavras-chave: digestibilidade, fermentação, lignina, silagem, valor nutricional.

Introduction

The main obstacle in the use of sugarcane (*Saccharum officinarum*) in ruminant diets is related to the low crude protein and fiber fraction, since it causes a reduction in consumption, mainly due to its low degradability and digestibility (Gomes et al., 2011), making this difficult to balance the diet with nutrients.

The ruminal degradation of the cell wall of forage plants is closely linked to its chemical composition. Among the constituents of the cell wall, lignin is considered to have the most negative effect (Van Soest, 1994; Deschamps & Ramos, 2002; Detmann et al., 2009) .Therefore, understanding the concentration of lignin precursors, phenolic acids and their association with the cell wall constituent carbohydrates, hemicellulose and cellulose, is essential to understand the relevant limitations related to the fiber degradation of tropical forages.

The main phenolic acids related to lignification and interconnection between cell wall carbohydrates with lignin are determined by the method of high-performance liquid chromatography (HPLC) (Deschamps & Ramos, 2002). Thus, there is a high scientific interest in understanding the structure and function of phenolic acids that compose the lignin fraction of forage species, however, low attention has been directed to quantify the composition of phenolic acids. Hence, the importance of phenolic acids in the nutrient release process, in a long term, requires a quantitative analysis method employed to determine the effect of the composition of phenolic acids in the plant constitution.

Thus, the objective was to determine and measure the phenolic acids in four varieties of sugarcane (RB 855536, RB 765418, SP 80-1842 and 80-1816 SP) *in natura* and ensiled, as well as to evaluate the influence of these acids on degradability *in situ* of dry matter and fiber fraction.

Material and methods

The sugarcane used in this study was acquired in Epamig - Experimental Farm of Mocambinho, in the city of Jaíba, State Minas Gerais. This region lies at an altitude of 452 m, an average temperature of 25.5°C, with a minimum of 18.7°C and maximum 32.3°C, insolation of 2,987 hours per year, relative humidity 65.5 % and average rainfall range of 800 mm per year.

The treatments provided consisted in four varieties of sugarcane, RB855536, RB765418, SP 80-1842 and 80-1816 SP, both *in natura* and ensiled for each variety. The samples were gathered in order to make the tests to be performed when the sugarcane was ripe (180 days), due to its higher sucrose content, combined with the best features for animal consumption.

The cuts of the varieties were performed manually, 10 cm from ground level, with a part held *in natura* and immediately entire minced (2 cm), weighed, and carried to forced ventilation ovens for 72 hours at 55° removal of C. After the incubator, they were put in sieves of 1 mm to perform the laboratory analysis and frills 5 mm to achieve the degradability test.

The remaining part was used in the silage production, without additives or inoculants, on which they were chopped into particles of approximately 2 cm, ensiled and compacted with the help of a wood piece, in an experimental silos equipped with a '*Bunsen*' valve and a bottom compartment containing sterile sand, separated by a nylon screen of the ensiled material, for receiving effluent.

To estimate the silage density, the silos were weighed before and after being filled, the weight of the ensiled material was compared with the area occupied by the forage into the silo, in order to keep approximately 500 kg of dry matter per cubic meter. Four varieties of sugarcane were ensiled with 4 repetitions each, in a total of 16 silos. After 60 days of fermentation, the silos were opened, and after the removal and homogenization of its contents, the samples were immediately weighed and taken to forced ventilation ovens for 72 hours at 55ºC. After the incubator, it was performed the same procedures described for the *in natura* sugar cane.

The dry matter content (DM) was determined accordingly with the Association of Official Analytical Chemists (AOAC, 1990). The fiber concentrations of neutral detergent fiber (NDF), acid detergent fiber (ADF), and lignin were determined by a sequential method in accordance with the techniques described by Van Soest, Robertson, and Lewis (1991). For the determination of lignin, it was used sulfuric acid at 72%. The hemicellulose content was calculated by the difference between NDF and ADF. The cellulose was calculated by the ADF difference by lignin, corrected for ash.

For the evaluation of ruminal degradation kinetics, it was used two crossbred bullocks with ruminal cannulation and an average weight of 450 kg. The animals received 3.0 kg concentrate/head/day, containing 88% DM, 14% of CP and 70% of total digestive nutrients TDN, divided into two equal parts, in the morning and evening. Besides the concentrate, the animals received diets based on ensiled sugarcane. It was used the technique of degradability *in situ,* with synthetic non-woven fabric bags (TNT, weight 100), measuring 7.5 x 15 cm, with an approximate porosity of 50 μm, according to Casali et al. (2009), with quantity of samples following a ratio of 20 mg DM cm^{-2} of bag surface area (Nocek, 1988).

Then, the bags were placed in nylon bags measuring 20 x 30 cm, along with 100 g lead weights. The bags were tied with a nylon cord, leaving a free length of 1 m so that they had free movement in the solid and liquid phases of the rumen. The bags were deposited in the region of the ventral sac of the rumen for 0, 6, 12, 24, 48, 72 and 96 hours, and the remaining end of the nylon cord was tied to the cannula. The bags were placed in reverse order, starting with 96 hours, and the samples relating at time 0 hour have been set in the rumen for five min at the end. Thereafter, all samples were removed and washed in cold water, aiming at the interruption of ruminal fermentation.

Subsequently, the samples were placed in ovens at 55°C for 72 hours and afterwards, cooled in a desiccator and weighed.

The remaining residues in the non-woven bags (TNT) collected in the rumen were analyzed for dry matter (DM) and neutral detergent fiber (NDF). The degradation percentage was calculated by the ratio of food remaining in the bags after ruminal incubation. The NDF was analyzed according to Van Soest et al. (1991), without using α-amylase and with the use of autoclave for 40 min at 90°C for one hour.

The collected data were adjusted for a non-linear regression by the Gauss-Newton method (Neter, Wasserman, & Kutner, 1996), using Statistical Analysis System (SAS, 2004) software, according to the equation proposed by Ørskov and McDonald (1979): $Y = a+b(1-e^{-ct})$, where Y = cumulative degradation of the nutritional component analyzed after time t; a = the intercept degradation curve when t = 0, which corresponds to the water soluble fraction of the nutritional component analyzed; b = degradation potential of water insoluble fraction in the nutritional component analyzed; a + b = potential degradation of the nutritional component analyzed as time is not a limiting factor; c = degradation rate by fermentative action of b; t = incubation time.

Once calculated, the coefficients a, b and c were applied to the equation proposed by Ørskove McDonald (1979): DE = a+(b*c/c+k), where: DE = actual ruminal degradation of the nutritive component analyzed; k = food pass rate. It was assumed the estimated particle passage rates in the rumen of only 5% $hour^{-1}$, as suggested by the Agricultural and Food Research Council (AFRC, 1993).

The NDF degradability was estimated using the model proposed by Mertens and Loften (1980): Rt = B x e-ct + I, in which Rt = fraction degraded at time t; B = insoluble fraction potentially degradable and I = indigestible fraction. After adjusting the NDF degradation equation, the standardization of fractions were proceeded, as proposed by Waldo, Smith, and Cox (1972), using the equations: BP = B/(B+I) × 100; IP = I/(B+I) × 100, where: BP = standardized potentially degradable fraction (%); IP = standardized indigestible fraction (%); B = insoluble fraction potentially degradable and I = indigestible fraction. To calculate the NDF degradability the following model was used: DE = BP x c/(c+k), where BP is the potentially degradable fraction (%) standardized.

The tests of degradability *in situ* were conducted in a completely randomized design (CRD) in a factorial 4 x 2 (4 varieties in 2 ways of use: *in natura* and silage), with four repetitions. The animals were adapted to the diet for 14 days. The variables were analyzed using the GLM procedure of SAS (2004).

For the determination of phenolic acids by high-performance liquid chromatography, the following phenolic compounds were evaluated: ferulic acid and *p*-coumaric acid, *m*-coumaric acid and *o*-coumaric acid. The composite samples were taken for each variety *in natura* and ensiled. After homogenization, the samples were brought to forced ventilation oven at approximately 35°C (room temperature) for 72 hours, then milled in a 1 mm sieve and stored in a suitable container, for later carrying out the extraction of phenolic acids.

To remove the soluble low molecular weight compounds, two methods were used, as follows:

1 - Extraction with 80% ethanol;

For the extraction with 80% ethanol, 3 g of samples were placed in nylon bags measuring 5 × 7 cm, with 50-micron porosity, also, three replicates were used for each treated material. The samples were kept under stirring in one liter of solution for two hours at room temperature. After this period, the samples were washed in water and left to dry in an oven 60°C (Deschamps, 1999).

2 - Extraction with neutral detergent;

The samples treatments with a neutral detergent (ND) were also carried out with three replications in the same packaging system previously described. The samples were then subjected to extraction in neutral detergent for 40 min in an autoclave at 120°C (Deschamps, 1999).

3 - Witness – non-extraction;

After the extraction step (without extraction with 80% ethanol and ND), for phenolic acid solubilization, 50 mg of the sample were extracted in a test tube with 5 ml of NaOH 1 mol L^{-1} for 24 hours in an incubator at 20°C. Then, the material was filtered through glass fiber filter and washed with Mille-Q water, and the filtrate was acidified to pH 2.5 with the addition of 0.7 ml solution of HCl: H_2O (1:1). The final volume was adjusted to 10 mL with water, and 2 mL was removed for further analysis. After a night in the refrigerator, the material was centrifuged in an Ependorf centrifuge (13.000 rpm) for 5 min, and left in the freezer until the time of analysis. After defrosting, to be injected into the chromatograph, the samples were again centrifuged (13,000 rpm for 5 min). Then, approximately 0.5 mL was transferred to the automatic injector HPLC vials. The injection volume was 5 microliters. The liquid chromatographer by brand Shimadzu consists in a system with two pumps LC-20AT and a detector

UV-VisSPD-20th (Shimadzu, Kyoto, Japan). The samples and standard solutions of phenolic acids caffeic, vanillin acid, *p*-coumaric, ferulic acid, *m*-coumaric acid and *o*-coumaric acid were analyzed in a column Nucleodur100-5C18 250 x 3.0 mm, 5 μm (Macharey-Nagel).

The concentrations of phenolic acids studied are related to the heights of the individual peaks, obtained by the chromatographic analysis of each sample, with the chromatographic profile and the analytical curves of acids.

The analyses were made by elution of isocratic acetonitrile/0.1% phosphoric acid pH 2.0 (20:80) at room temperature and flow of 0.8 mL min^{-1}. The injection volume was 20 µL and detection at 313 and 250 nm simultaneously. The Solutions Lab software (Shimadzu) was used for data processing.

Since only *p*-coumaric and ferulic acids were found in significant amounts in the samples, only these two substances were measured. For calibration curve, it was used a stock solution containing 36 mg of *p*-coumaric acid (Merck ≥ 99%) and 10 mg of ferulic acid (Sigma ≥ 95%) prepared in 10 mL of acetonitrile/ 0.1% phosphoric acid (2: 8).

The standard solutions diluted (14 to 72 µg mL^{-1} of *p*-coumaric acid and 4 to 20 µg mL^{-1} ferulic acid) were used to construct the calibration curve. The presence of peaks in the samples that were not well separated from *p*-coumaric acid and ferulic acid led us to choose the dosage taken by the height of the peaks instead by area. The peak miscalculation by height is less than if calculated by area.

The experimental design was completely randomized in a factorial design (4 x 2 x 2 + 1), with 4 varieties in two processing forms (*in natura* and silage) and two extraction methods (neutral and ethanol solvent) + 1 (witness: no extraction). With three repetitions, for comparison between the treatments and the witness, it was used the Dunnett test at 5% probability, trough GLM procedure - SAS (2004). Once the effect of the treatments compared to the control was verified, the analysis of variance for the main factors was made. If their interactions and the test 'F' presented significance, the average treatment was compared by the Scott-test Knott, at 5% probability. The analyses were performed using the computer program Sisvar as described by Ferreira (2011).

After analyzing phenolic acids, degradability and data collection, a correlation analysis between the levels of phenolic acids and degradability parameters was performed using the Manova command line in SAS software, trough GLM procedure (SAS, 2004) to check the influence of phenolic acids on degradability and, for such, it was used the averages of least squares of each stage.

Results and Discussion

Silage variety SP 80-1816 presented DM values of 19.28; 19.08 and 42.13% higher ($p < 0.05$) than SP 80-1842, RB765418 and RB855536 varieties, respectively. In the fresh form (*in natura*), the SP 80-1816 varieties and RB 76-5418, did not differentiate between them ($p > 0.05$) for DM content, with an average of 28.74%, which was higher in the other varieties (Table 1).

Table 1. Averages of dry matter (%), neutral detergent fiber (%) and acid detergent fiber (%) of four varieties of sugarcane in two processing forms.

Varieties	Processing forms	
	Silage	*in natura*
	Dry matter (%)	
RB855536	17.07 Cb	24.30 Ba
RB765418	23.87 Bb	28.80 Aa
SP 80-1842	23.81 Bb	25.22 Ba
SP 80-1816	29.50 Aa	28.68 Aa
CV (%)	3.92	
	Neutral detergent fiber (NDF)	
RB 855536	59.88 Bb	66.94 Ba
RB 765418	65.34 Ba	64.15 Ba
SP 80-1842	77.54 Aa	72.83 Aa
SP 80-1816	82.47 Aa	75.11 Ab
CV (%)	6.1	
	Acid detergent fiber (ADF)	
RB 855536	38.25 Ba	46.11 Aa
RB 765418	47.29 Ba	41.29 Aa
SP 80-1842	55.09 Aa	45.68 Ab
SP 80-1816	45.04 Ba	45.82 Aa
CV (%)	13.74	

Means followed by different letters, capital in columns and lower case in lines, differ by Sott-Knott test and the F test ($p < 0.05$), respectively. CV - coefficient of variation.

Regarding the form of processing within each array, it appears that the RB varieties 855536, 765418 and SP 80-1842 RB, when handled *in natura,* they have the highest significance , while for the variety SP 80-1816, the average DM (29.09%) did not differ ($p > 0.05$) between processing forms. The importance of understanding the DM content of varieties is inferred, superficially, on the fermentation profile, when they are processed in the form of silage. The literature recommends that for silage quality, the DM content in fresh form must rely between 28-40% (Woolford, 1984). In this research, only RB765418 and SP 80-1816 varieties showed DM levels within the recommended for silage. It is observed in each variety, after ensiling, DM losses of 29.75; 17.11 and 5.60%, respectively, for the varieties RB 855536, RB 765418 and SP 80-1842.

The reduction of DM content of silages in some varieties can be linked with higher or lower yeast activity (Siqueira et al., 2007), besides the reduction of cell contents after processing, especially soluble

carbohydrates during the fermentation process (Woolford, 1984), resulting in DM losses through the waste and gases (McDonald, Henderson, & Heron, 1991).

Regarding the fiber content in neutral detergent fiber (NDF) there was no significant difference between the form of processing ($p < 0.05$), where the highest NDF values were observed in the varieties RB 855536 (66.94%) in the form *in natura* and SP 80-1816 (82.47%) when ensiled.

Within each processing form, both in silage as *in natura*, the variety SP 80-1842 and 80-1816 SP did not differ ($p > 0.05$) and had a higher average (80% NDF in silage and 73,97% NDF *in natura*) compared to other varieties in their respective forms of processing. However, analyzing the general average, 71.30% of NDF in silage and 69.75% of NDF in the sugarcane *in natura*, it is observed that these values are higher than what is normally found in researches, as in Oliveira et al. (2012), who evaluated varieties of sugarcane *in natura*, among these RB 855536 and found NDF of 50.08%. In addition, the authors reported general average of 49.29% of NDF, which is below the overall average for this research, of 69.75%. Individual characteristics of each variety associated with the maturation process, structural arrangement of the cell wall and changes in cell content may be related to variations verified in the NDF results (Van Soest, 1994).

There was no difference between the varieties for fiber in acid detergent (ADF) *in natura* ($p > 0.05$), with average of 44.72%. Moreover, the variety SP 80-1842, as silage presented ADF content 21% higher than the other varieties, with average of 43.52%.

For hemicellulose and cellulose contents, it was not verified significant interaction between varieties x processing forms. However, there were significant differences among varieties for the hemicellulose content. The SP 80-1816 variety presented hemicellulose content 33.90% higher than the other varieties that did not differ between each other ($p < 0.05$) (with an average of 22.05%). This increase was due to higher NDF content recorded in the variety SP 80-1816 (82.47%). For the cellulose, it was not verified difference ($p > 0.05$) between varieties, with an average of 38.29% (Table 2).

For lignin content, there was no significant interaction between varieties x processing types ($p < 0.05$). The varieties *in natura* showed average lignin values of 5.79%, not differing from each other ($p > 0.05$).

Table 2. Mean values of hemicellulose, cellulose and lignin, % of dry matter in four varieties of sugarcane in two processing forms.

Varieties	Means	
	Hemicellulose (%)	Cellulose (%)
RB 855536	21.23 B	35.72 A
RB 765418	20.14 B	38.46 A
SP 80-1842	24.80 B	40.55 A
SP 80-1816	33.37 A	38.45 A
CV (%)	24.26	24
	Lignin (%)	
	Processing form	
	Silage	in natura
RB 855536	6.06 Ba	5.74 Aa
RB 765418	7.08 Ba	6.61 Aa
SP 80-1842	9.96 Aa	5.12 Ab
SP 80-1816	7.43 Ba	5.71 Aa
CV (%)	17.72	

Means followed by different letters, capital in columns and lower case in lines, differ by Scott-Knott test and the F test ($p < 0.05$), respectively. CV - coefficient of variation.

Only in the silage variety SP 80-1842 was different (31.15% more) in lignin content compared to other varieties (average 6.85%). The highest levels of ADF checked in SP 80-1842, in the form of silage (55.09%) may be due to the higher concentration of lignin (9.96%), which affects the digestibility of this variety, as a result of the toxicity caused by lignin to rumen bacteria, especially the fibrolytic (Jung & Vogel, 1986).

Ruminal degradation of dry matter

Evaluating the ruminal parameters, significant differences were observed ($p < 0.05$) among the varieties studied in the degradation of soluble fraction (a) and effective degradability (ED) of DM forage (Table 3).

Table 3. Readily soluble fraction (A) and effective degradability (ED) of dry matter varieties of sugarcane.

Parameters	Processing form		CV (%)
	Silage	*in natura*	
A	18.51 B	31.94 A	-
DE	30.86 B	43.09 A	-
CV (%)	5.39	7.21	

Parameters	Varieties				CV (%)
	RB855536	RB765418	SP80-1842	SP80-1816	
A	26.64 a	22.94 b	28.74 a	22.58 b	5.39
DE	38.27 a	32.71 b	42.23 a	34.70 b	7.21

Means followed by different letters in lines, differ by Scott-Knott test and the F test ($p < 0.05$), respectively. CV - coefficient of variation.

For the fraction (a), the lowest means were observed in SP 80-1816 and RB 765418 varieties, not differing from each other ($p > 0.05$), with average of 22.76%. The RB 855536 and SP 80-1842 varieties did not differ from each other ($p > 0.05$), with average 17.80% higher than the varieties SP 80-1816 and RB 765418. However, for effective degradability (ED), lower values were found in varieties SP 80-1842 and RB 855536, not differing from each other ($p > 0.05$), with average of 33.70%.

Ribeiro, Pires, Carvalho, and Chagas (2009) studied the degradation parameters of dry matter

(DM) and cell wall constituents of sugarcane treated with sodium hydroxide (NaOH) or calcium oxide (CaO) by the *in situ* technique, and they found the values of 38.5% of fraction (a) in sugarcane, similar to that found in this study (22.58 to 28.74%). These values are high, but justifiable since it is a massive food with a high concentration of soluble sugars such as sucrose, fructose and galactose (Ribeiro, Pires, Carvalho, & Chagas, 2009), monomers of high solubilization were not quantified in this study, but represented by fraction (a).

In the study of the processing form it was found that the fraction (a) *in natura*, was 42.04% greater than fraction (a) of the silage ($p < 0.05$). For ED, independently of the varieties studied, for the *in natura* case the average was 28.38% higher than the silage (30.86%).

These results are justifiable by homofermentative and heterofermentative bacteria present in silage. These bacteria classes use simple sugars for fermentation of organic matter and the substances produced by this reaction, the organic acids such as lactic and acetic acid, are responsible for reducing the fraction levels of ensiled sugarcane (Woolford, 1984). Since ED is influenced by the readily soluble fraction, the results for this variable were proven by the fraction behavior in both forms of processing.

About the insoluble fraction but potentially degradable (b), the degradation rate (c), the undegradable fraction (UF) and the potential degradability (PD) of sugarcane DM, it was not observed significant effects ($p > 0.05$) in both of processing forms (Table 4).

Table 4. Means values for potentially degradable insoluble fraction (B), degradation rate (C), undegradable fraction (UF) and potential degradability (PD) of sugarcane dry mass (*in natura* and ensiled).

Parameters	Means values	CV%
B (%)	32,65	24,56
C (h^{-1})	0,03	20,75
UF (%)	42,13	18,12
PD (%)	57,87	13,19

CV - coefficient of variation.

It is important to point that the fraction (b), degradation rate (c) and undegradable fraction (UF) are parameters related to cell wall components. Notably, the ensiling process does not interfere positively or negatively in the cell wall degradation of plants. However, the lignin rate in the cell wall, represented by UF, might probably act negatively in the degradation of cellulose and hemicellulose, represented generally by the fraction (b). Lignin acts negatively on the cell wall degradation, due to two main factors: its toxicity to ruminal microflora caused by the presence of phenolic acids, and its connection with the hemicellulose, through ester bonds, hindering microbial action on the substrate (Van Soest, 1994).

Fiber degradation in neutral detergent (NDF)

In the insoluble fraction, but potentially degradable standardized (Bp) of NDF, the varieties RB 855536 and SP 80-1842 did not differ between each other ($p > 0.05$) (with an average of 53.70%) and presented a higher average compared to other varieties (Table 5).

Table 5. Means values for potentially degradable insoluble fraction standardized (Bp); undegradable fraction standardized (Ip); potential degradability (DP) and effective degradability (DE) of fiber degradation in neutral detergent of four sugarcane varieties.

Parameters	Varieties				CV%
	RB855536	RB765418	SP80-1842	SP80-1816	
Bp (%)	51.87 a	22.93 b	55.54 a	37.10 b	25.34
Ip (%)	48.13 b	77.08 a	44.46 b	62.90 a	18.24
DE (%)	18.07 a	9.86 b	17.75 a	13.57 b	18.58

Means followed by different letters in lines differ by the 'F' test. CV - coefficient of variation.

It is interesting to note that the variety RB 765418 presented average Bp fraction of 22.93%, which is lower ($p < 0.05$) than the average of varieties RB 855536 and SP80-1842. This result of 22.93% for variety RB 765418 indicates that in the rumen this variety has a higher microbial degradation response. This was justified by the results of the degradation rate, which was 6% $hour^{-1}$, a higher value ($p < 0.05$) than the other varieties, averaging 3.5% $hour^{-1}$. Researches with grasses show mixed results in fraction content (Bb) of sugarcane, such as the ones reported by Romão et al. (2013), with an average of 38.4%.

It was not reported significant interaction between varieties x processing forms for the degradation rate of insoluble fraction, but potentially degradable standard. However, there were differences ($p < 0.05$) among the varieties studied (Table 6) and no effect ($p > 0.05$) in the form of processing varieties.

For the degradation rate (c) in the form *in natura*, variety RB 765418 presented an average 55.55% higher ($p < 0.05$) compared to other varieties which did not differ between each other ($p > 0.05$) (with an average of 4% $hour^{-1}$). This means that in approximately 11 hours of ruminal degradation of Bp fraction from variety RB 765418, 100% of substrate will be available for microbial protein synthesis, volatile short chain fatty acids or as a source of energy for rumen microorganisms. At the same time, in silage treatment the lowest

degradation rates were observed in varieties RB 855536 and RB 765418, with average of 2 to 3% $hour^{-1}$ respectively. In other words, in 24 hours of ruminal degradation, probably 48 and 72% of the Bp fraction will be degraded, respectively.

Table 6. Degradation rate (c) of potentially degradable insoluble fraction standardized of neutral detergent fiber of four sugarcane varieties in two processing forms.

Varieties	Processing form	
	Silage	*in natura*
RB 855536	0.02 B	0.04 B
RB 765418	0.03 B	0.09 A
SP 80-1842	0.04 A	0.04 B
SP 80-1816	0.04 A	0.04 B
CV%	14.20	

Means followed by different letters in columns differ by Scott-Knott test ($p < 0.05$).

The potentially degradable insoluble fraction standardized (Bp) of NDF had value and significance effects as mentioned for the fraction (b). In this study, it was found Bp values ranging from 22.93 to 55.54%. Research with grasses show mixed results of the NDF Bp fraction as those reported by Ribeiro et al. (2009) of 38.9 and Romão et al. (2013) of 38.4%. These variations happened by the reason of the individual treatments made in each study, and the varieties characteristics. The undegradable fraction (UF) follows the same results as standardized undegradable fraction (Ip).

The RB 855536 and SP 80-1842 varieties presented higher values of effective degradability (ED) than others ($p < 0.05$), however, variety RB 765418 presented the lowest value.

Evaluation p-coumaric acid and ferulic acid

There was no interaction between varieties x form processing x extraction method ($p > 0.05$). Interaction was observed ($p < 0.05$) in varieties x processing form and processing form x extraction methods, with values of *p*-cumaric acids in sugarcane (Table 7).

For silage, the highest levels of *p*-coumaric acid were found in the variety RB 855536 which was 24.64% higher than the varieties RB 765418 and SP 80-1842 (with average of 12.75%) and 52.60% higher than the variety SP 80-1816 (with average of 8.02%). For the *in natura* form, varieties did not differ between each other ($p > 0.05$), with average of 8.86%. Between the two processing forms, silage varieties RB 855536, RB 765418 and SP 80-1842 showed higher levels of *p*-coumaric acid compared to *in natura*. When combining the results of *p*-coumaric acid, as a monomer of lignin and its effect on the degradation of fiber component, it is noted that the variety SP 80-1816, ensiled, showed lower concentration of *p*-coumaric acid, 8.2%, compared to other varieties. These results allow us to infer that in this variety (SP 80-1816) the *p*-coumaric acid probably has a little negative interference in the rumen, a fact confirmed by the results of DP and Bp of NDF previously discussed. Thus, the highest result of Bp fraction of the variety RB 855536 of 51.87% is justified by the high concentration of *p*-coumaric acid, 16.92%. Deschamps (1999) compared the concentration of *p*-coumaric acid detected in sugarcane and elephant grass, and recorded the highest concentration in sugarcane, as an indication to explain its low digestibility when compared to the elephant grass.

Table 7. Means values of *p*-coumaric acid (%) and ferulic acid (%) and extraction methods (%) in diferent varieties and processing forms of sugarcane.

Varieties	Processing form	
	Silage	*In natura*
	p-coumaric acid (%)	
RB 855536	16.92 Aa	7.63Ab
RB 765418	13.48 Ba	10.46Ab
SP 80-1842	12.02 Ba	9.10 Ab
SP 80-1816	8.02 Ca	8.26 Aa
CV (%)	32.83	
	ferulic acid (%)	
RB 855536	4.17 Bb	6.74 Aa
RB 765418	4.79 Ba	5.33 Ba
SP 80-1842	4.94 Ba	5.35 Ba
SP 80-1816	7.83 Aa	7.18 Aa
CV (%)	22.9	
	Extraction methods (%)	
No extraction	9.89 Aa	9.02 Ab
Ethanol 80%	14.39 Aa	6.54 Bb
NDF	13.55 Aa	11.02 Aa
CV (%)	32,83	

Means followed by different letters, capital in columns and lower case in lines, differ between each other by Scott-Knott test and the F test ($p < 0.05$), respectively. CV - coefficient of variation.

There was interaction between varieties x processing form ($p > 0.05$) on ferulic acid content in sugarcane varieties. The SP 80-1816 variety showed higher ($p < 0.05$) concentration of ferulic acid in both forms of processing, with no differences between the forms ($p > 0.05$), averaging 7.50%.

The silage variety SP 80-1816 presented ferulic acid content 40.82% higher than the other varieties, with an average of 4.63%. *In natura*, variety RB 855536 and SP 80-1816 did not differ between each other ($p > 0.05$), with an average of 6.96 which is 23.27% higher than the average (5.34%) of varieties RB 765418 and SP 80-1842 which showed no difference ($p > 0.05$). Comparing the results of the phenolic acids mentioned, the concentration of ferulic acid in RB 855536 variety had lower negative influence on ruminal fiber degradation. It stands out that only in this variety (RB 855536) there was significant difference of processing forms, being observed a ferulic acid concentration of 38.13% higher when this variety was processed *in natura*.

There was a significant interaction for processing methods x processing forms. There was no significant difference between the processing methods when treatments were processed as silage, averaging 12.61%. The i*n natura* processing form and the NDF processing method was 22.17 and 68.50% higher than the methods without extraction, and ethanol was 80%, respectively.

The processing methods without extraction and with 80% of ethanol quantified higher concentration of *p*-coumaric acid when the treatments were processed as silage (9.89 and 14.39%, respectively). There was no effect ($p > 0.05$) of processing forms in quantifying the concentration of *p*-coumaric acid when NDF processing method was used.

According to Deschamps and Ramos (2002), because of the phenolic acids restriction on the cell wall, with low chance of being detected in the fraction (a) of sugarcane, the extractor's efficiency has been taken into consideration. Thus, it is possible to solubilize a part of these acids (*p*-coumaric acid) presented in the cell wall, and this solubilization was observed in this study. The phenolic acids *p*-coumaric and ferulic, the most detected in forage plants, interconnect to the lignin linking to the cell wall components (cellulose and hemicellulose) by ester or ether linkages, and this may influence the degradation of these polysaccharides, forming barriers for the microorganisms action and hydrolysis of the molecule, thereby interfering with carbohydrate digestibilty (Jung & Vogel, 1986).

Correlations between degradability and phenolic acids

There was no significant correlation ($p > 0.05$) between the parameters of ruminal degradation of dry matter and neutral detergent fiber with the concentration of *p*-coumaric acid and ferulic acid. This behavior shows that the levels of *p*-coumaric acid and ferulic acid did not affect the sugarcane degradation, and this suggests that other factors or phenolic acids were limiting the rumen degradation level. Gomes et al. (2011) evaluated the correlations of lignin content with ruminal parameters of the fiber from grasses and legumes, and also found no correlations. These results show that only the isolated content of *p*-coumaric acid and ferulic acid are insufficient to justify the degradability results, and also that other factors are involved, such as structural arrangements and the proportions of the types of ester and ether bonds of lignin. Jung and Vogel (1986) suggested that the inhibitory mechanisms of lignin on the cell wall are complex, requiring more analytical procedures to understand the interactions. Singh et al. (2015) evaluated the phytochemical characteristics of sugarcane, and found the presence of new phenolic acids such as chlorogenic acid, hydroxycinnamic acid and sinapic acid. The authors also argue that the presence of several flavonoid compounds (apigenin, luteolin, and tricine) also represents the structural arrangement of phenolic acids, increasing the complexity of the ruminal degradation of the cell wall (Duarte-Almeida, Salatino, Genovese, & Lajolo, 2011; Feng, Luo, Tao, & Chen, 2015).

Conclusion

Considering the ruminal degradation parameters of dry matter and neutral detergent fiber, the RB 855536 and SP 80-1842 varieties, in the form of processing *in natura* and neutral detergent processing method, presented better results.

The verification of the concentrations of phenolic acids *p*-coumaric and ferulic are not sufficient to justify the effect of microbial action on the varieties studied in different forms of processing. Thus, more research is needed.

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