Fermentation kinetics and *in vitro* degradation rates of grasses of the genus *Cynodon*

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ABSTRACT. The present study aimed to evaluate the fermentation kinetics and degradation rates of *Cynodon* grasses estimated by semi-automated technique of *in vitro* gas production. The forages were: *Coastcross*, Tifton 85 and Tifton 68. Pressure readings were taken at 0, 2, 4, 6, 8, 10, 12, 15, 19, 24, 30, 36, 48, 72 and 96 hours. Dry matter degradability (DMD) was obtained by the percentage of dry matter (DM) remaining after 0, 6, 12, 24, 48 and 96 hours of fermentation. Tifton 85 showed a higher total gas production ($p < 0.05$). Higher fermentation rates were found at the beginning of fermentation followed by subsequent reduction ($p < 0.05$) over time. Tifton 85 and Tifton 68 showed higher values ($p < 0.05$) for soluble fraction, potentially degradable insoluble fraction, insoluble fraction, potential and effective degradability of dry matter in relation to *Coastcross* grass. Higher gas production during *in vitro* incubation of dry matter was observed for Tifton 85 g.

Keywords: forage, gas production, semi-automated technique.

Cinética da fermentação e taxa de degradação *in vitro* de gramíneas do gênero *Cynodon*

RESUMO. Objetivou-se avaliar a cinética da fermentação e taxas de degradação de gramíneas do gênero *Cynodon* estimadas pela técnica semi automática de produção de gases *in vitro*. As forrageiras utilizadas foram: *Coastcross*, Tifton 85 e Tifton 68. As leituras de pressão foram realizadas nos tempos: 0, 2, 4, 6, 8, 10, 12, 15, 19, 24, 30, 36, 48, 72 e 96h. A degradabilidade da matéria seca (DMS) foi obtida pela porcentagem de matéria seca (MS) remanescente após 0, 6, 12, 24, 48 e 96h de fermentação. A gramínea Tifton 85 apresentou uma produção total de gases superior ($p < 0.05$). Maiores taxas de fermentação ocorreram no tempo inicial de fermentação decrescendo ($p < 0.05$) com o avançar do tempo. As gramíneas Tifton 85 e Tifton 68 apresentaram valores superiores ($p < 0.05$) de fração solúvel, insolúvel potencialmente degradável, insolúvel, degradabilidade potencial e efetiva da matéria seca em relação à gramínea *Coastcross*. Maior produção de gases durante a incubação *in vitro* da matéria seca foi observada para o capim Tifton 85.

Palavras-chaves: forragem, produção de gás, técnica semi automática.

Introduction

Tropical forage in Brazil remains one of the most economical food resources for livestock production (Moreira, Prado, Cecato, Wada, & Mizubuti, 2004; Sá et al., 2011). Thus, the search for forages with potential for high biomass production (above 10.000 kg DM ha^{-1} year^{-1}) associated with good nutritional value (over 10 crude protein, 60% degradable fiber) is a prerequisite for most ranchers (Oliveira et al., 2014b).

Among the forage available, some *Cynodon* hybrids exhibit these characteristics, i.e., rapid accumulation of dry matter, with high leaf: stem ratio, which can result in good nutritional value (Ferreira, Santos, Cecato, & Cruz, 2005; Monção et al., 2014).

However, quality of the forage depends on the structural arrangement and proportion of its constituents, which vary within a species, according to the age and part of the plant, soil fertility, among others (Van Soest, 1994). As the determination of the nutritional value of forage plants has high importance and complexity, it is normally used *in vivo* and *in situ* methods for evaluation, which is not always possible because as it requires considerable use of cannulated animals, food stocked, hand labor, time and high costs, thus limiting their applicability (Zeoula et al., 2000; 2002).

An alternative and interesting method that has been applied using fewer animals, limited to a single animal donor of rumen fluid, is the gas production

technique proposed by Mauricio et al. (1999), Pell and Schofield (1993).

This technique allows to estimate the ruminal degradation parameters through *in vitro* digestibility assay, due to the cumulative production of CO_2, NO_2, H_2 and CH_4, released during fermentation of the sample incubated in buffered rumen fluid (Pell & Schofield, 1993; Theodorou, Williams, Dhanoa, McAllan, & France, 1994).

In this context, this study evaluated the fermentation kinetics and degradation rates of *Cynodon* grasses estimated by semi-automated technique of *in vitro* gas production

Material and methods

The experiment was conducted at the Experimental Farm of the State University of Montes Claros – Unimontes, in the municipality of Janaúba, northern state of Minas Gerais and at the Food Science Laboratory, Department of Agricultural Sciences, Unimontes, *Campus* Janaúba. Forages (*Coastcross*, Tifton 85 and Tifton 68) were sampled in an area pre-installed in the Forage and Pasture Sector of this institution. Three consecutive cuts were made at 42 days of age, each cut was considered as the experimental unit. Samples were subjected to pre-drying in a forced air oven at 55ºC for 72 hours, ground in a Wiley knife mill, with 1 mm diameter sieve.

Each vial received 90 mL culture medium according to Theodorou, Williams, Dhanoa, McAllan, and France(1994). The vials were sealed with rubber stoppers (14 mm) and to prevent any fermentation were kept at 4°C overnight in a common refrigerator. On the next day, five hours before inoculation, vials were taken to the oven at 39°C until the moment of inoculation. Two male cattle equipped with rumen cannula were used to provide rumen fluid (inoculum). The collection was carried out manually in the ventral sac of the rumen with the aid of a cloth and subsequently inocula from two animals were homogenized and then packed in thermos bottle, previously heated (39°C) and immediately taken to the laboratory. The material was filtered through a double layer of cheesecloth with continuous injection of CO_2, kept in a water bath, and immediately inoculated. The inoculation was performed by injecting 10 mL inoculum per vial using a graduated plastic syringe. Immediately after inoculation, vial pressure was stabilized by inserting needles (25 x 7 mm) in the lids of the vials. The needles were posteriorly removed, vials were manually shaken and placed in an oven at 39°C. At this time, fermentation times

were started. Pressure readings were taken more frequently during the initial fermentation period and reduced later (0, 4, 6, 8, 10, 12, 15, 19, 24, 30, 36, 48, 72 and 96 hours); after the readings of 12, 24, 48, 72 and 96 hours, two vials were taken from each treatment to determine the degradability.

Gas pressure was obtained using the Universal Data logger, Logger AG100 equipment, a bench equipment with analog input, to which was connected the manipulo with a needle (0.7 mm) fixed at its tip to pierce the synthetic rubber stoppers of the vials. This equipment has a trigger button at the top of the manipulo to record pressure (psi).

Volume was measured by a graduated syringe (20 mL) at predetermined times (0, 6, 12, 24, 48, 72 and 96 hours) also with a coupled needle (0.6 mm). Immediately after inoculation, the initial reading was performed with the aim to standardize the pressure and discard the volume of gas in all tubes. From insertion of the needle into the synthetic rubber stopper, the pressure produced inside the vials was verified in the digital reader. After pressure reading, the volume of gases was determined by pulling the plunger until the transducer pressure returned to zero.

For measurement of gas production from the buffer and rumen fluid, two vials were incubated containing only these components.

For each reading time, the gas volume of the vials with the sample was subtracted from the volume obtained in the vials without samples. By obtaining the values of pressure and volume at each time, these were added to values of previous readings, thus enabling the construction of the curve corresponding to the soluble fraction for each incubation time.

The disappearance of dry matter was obtained for the relationship between the percentage of the material initially incubated and the residue after 0, 12, 24, 48, 72 and 96 hours of fermentation. The residue of degradation was obtained by filtering the contents of each vial in porosity 1 crucibles and further drying in an oven at 105°C for 12 hours.

Cumulative gas production data from the fermentation of each treatment were used in the model of France et al. (1993), according to Equation 1:

$$Y = A \left\{ 1 - \exp \left[(- b(t - L) - c) \times (\sqrt{t} - \sqrt{L}) \right] \right\} \quad (1)$$

where:
Y = cumulative gas production (mL);
A = asymptote or maximum potential gas production;
L = colonization time (lag time);

b (hour^{-1}) and c (hour$^{-0,5}$) = constant fractional rates.

Fractional rate (hour^{-1}) combined with gas production (μ) was calculated as Equation 2:

$$\mu = b + c/2\sqrt{t} \qquad (2)$$

where:
μ = gas production rate (hour^{-1});
b and c = parameters similar to the equation;
t = incubation time in hours.

To adjust the degradability data at 0, 6, 12, 24, 48, 72 and 96 hours, we used the mathematical models proposed by Mehrez, Ørskov, and McDonald(1977) and Ørskov and McDonald (1979), which allowed to estimate the potential (PD) and effective (ED) degradability, according to Equation 3 and 4:

$$PD = A \to t \leq L \qquad (3)$$
$$PD = a + b\,(1\text{-}e^{-c\,\times\,t}) \to t > L \qquad (4)$$

where:
DP = degradability of food (%) at time t (hours);
A = readily soluble fraction (%);
a and b = model parameters, the sum (a + b) numerically corresponds to the potential degradability of food; and
c = degradation rate (%hour^{-1}).

It was also calculated the potentially fermentable soluble fraction of food (B), according to Equation 5:

$$B = (a + b) - A \text{ or } 100 - (A+C), \qquad (5)$$

where:
C = non-degradable fraction (calculated as 100-PD).

Effective degradability (ED) of food was calculated as equation 6:

$$ED= (a+b)/[c/(c+kp)] \qquad (6)$$

where:
kp = food passage rate (%hour^{-1}) through the rumen, considered as 5%hour^{-1}.

Estimates of the parameters described in the mathematical model were calculated using non-linear interactive methods. These results fitted by least squares estimates were obtained from the use of the Gauss - Newton method using a NLIN procedure, with the aid of Statistical Analysis System (SAS, 2004).

To obtain the statistical models that measure the ratio between the volume of gas produced during fermentation and pressure (psi), we used the correlation analysis by means of the Manova command line of GLM procedure of SAS. Once detected satisfactory correlation (above 85%) and significant between the volume and pressure, we used the option Selection = Stepwise in the Model command line of GLM procedure of SAS for automated selection of the best model among the tested models (linear, quadratic and cubic).

Values of cumulative gas volume, degradation rate, colonization time, effective and potential degradability, readily soluble fraction, potentially fermentable soluble fraction and non-degradable fraction were subjected to analysis of variance using the Analysis of Variance System (Sisvar) for balanced data, described by Ferreira (2011) and whenever significant, the means of treatments (g) were compared by Scott-Knott test at 5% probability by Sisvar (Ferreira, 2011).

Results anddiscussion

Data of pressure varies from 0 to 7.8 psi (pound per square inch) and volume data ranged between 0 and 42 mL. The reading times allowed to obtain pressure values higher than 7 psi, which can cause instability in the correlation between the variables and changes in microbial growth (Theodorou et al., 1994). Although the values obtained were higher than 7 psi, it was found a high correlation (Figure 1).
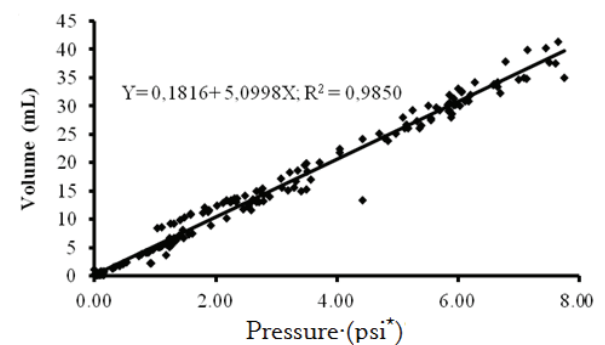

Figure 1. Data of pressure and volume obtained during gas production test using grasses of the genus *Cynodon* (*pound per square inch) as substrate.

The predicted regression equation in this experiment was V$_{(mL)}$ = 5.0998X + 0.1816, (R^2 = 0.98), where V = volume (mL), and P = pressure (psi). The high correlation (R^2 = 0.9850) reflected the accuracy in estimating the gas volume produced from the values of pressure generated during the fermentation process. The equation estimated showed a linear behavior, similar to that found by Marques et al. (2013), who evaluated the ruminal fermentation kinetics of silages made of different shoot fractions of four

varieties of cassava, but with predicted volume of 4.96 mL for each psi. It differed from the equation obtained by Mauricio et al. (2001), in which $V_{(ml)} = -0.004 + 4.43 P + 0.051 P2$, ($R^2 = 0.99$) in a study conducted at the University of Reading, England. The equation relating volume and pressure found in this study shows that the implementation of semi-automated technique requires specific equations for each site and respective altitude.

Gas production parameters estimated by France et al. (1993), total gas production (A) colonization time (L), and fermentation rate (μ) after 96 hours of incubation are listed in Table 1.

Table 1. Total gas production (A), in mLg^{-1}DM, colonization time (L) in hours and fermentation rate (μ) in mLg^{-1}DMhour^{-1}, in 96 incubation hours of three grasses of the genus *Cynodon*.

Parameters	Grasses			SEM	p value
	Tifton 85	Tifton 68	*Coastcross*		
A	246.08a	192.55c	209.82b	4.0531	0.000
L	1.064a	0.871b	0.222c	0.0516	0.001
μ	0.015a	0.011b	0.013a	0.0008	0.039

Different lowercase letters in the same column are significantly different (p <0.05) by Scott-Knott test. SEM- Standard Error of the Mean.

Tifton 85 grass showed the highest total gas production (p <0.05) compared to the other grasses, demonstrating greater degradation in the rumen. These values are close to those reported by Velásquez et al. (2009), who examined Tifton 85 g and Tanzania grass and registered 246.15 and 247.61 mL g^{-1}, respectively, for total gas production. Monção et al. (2014), Oliveira, Monção, Gabriel, Lempp, and Moura (2014a) analyzed the ruminal degradation of different grasses, including Tifton 85, Tifton 68 and *Coastcross*, and pointed out that the structural arrangement of the cell wall components (cellulose, hemicellulose and lignin) influence the extent of degradation and the degradation rate of the slowly soluble fraction, that is, a greater or lesser gas production will depend on the chemical composition of each grass. The formation ester bond between hemicellulose and lignin reduces ruminal degradation, which, in turn, leads to a lower gas production (Van Soest, 1994).

The gas production rates obtained herein were similar (p> 0.05) for the Tifton 85 and *Coastcross* (0.015 and 0.013mLg^{-1} DMhour^{-1}, respectively). Moreira et al. (2010) investigated the cumulative gas production and ruminal parameters using the model of France et al. (1993), through semi-automated *in vitro* technique, of carbohydrate sources for ruminants. The authors found that the treatments containing sugarcane (high content of readily soluble sugar, sucrose) tended to have numerically higher values of gas accumulation.

Colonization time (lag time) is an important parameter that is related to the degradation of the fiber (Mertens & Loften, 1980). The shorter time for the colonization onset (p <0.05) was observed to *Coastcross*, followed by Tifton 68 (0.222 and 0.871 hours, respectively). Tifton 85 presented longer Lag Time, that is, it took longer for the rumen microorganisms initiate the colonization and degradation of substrates compared to other grasses. Reduction in colonization time is favored by readily fermentable substrates and by physical and chemical characteristics of the sample cell wall. Nevertheless, Faria et al. (2008) stated that the correlation between the cumulative gas production and total carbohydrate degradation is high, but some differences in the magnitude of this correlation can be detected depending on the technique used.

Curves of cumulative gas production of DM of grasses studied are illustrated in Figure 2.
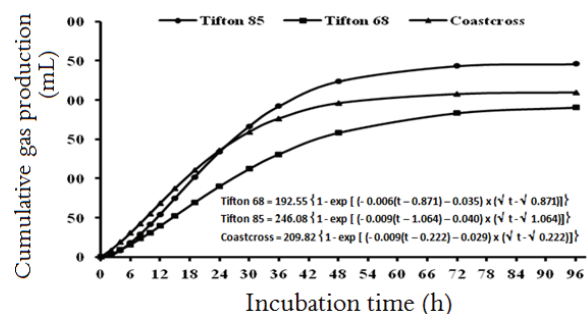

Figure 2. Cumulative gas production (PCG) (in mLg^{-1}DM) x fermentation time of three grasses of the genus *Cynodon*.

In the early incubation times (up to 6 hours), the grasses showed similar cumulative gas production rates, however, from the 30 fermentation hours, Tifton 85 had a higher cumulative gas production compared to other grasses. Sá et al. (2011) studied the ruminal degradation kinetics parameters of *Brachiariabrizantha* cv. Marandu, cut at 54 days, by the *in vitro* gas production technique and reported 160.0 mLg^{-1} DM for the cumulative gas production in the fermentation period of 96 hours. This value was lower than those found in this experiment for the different evaluated grasses.

For the fermentation rate (μ) of grasses, after 6 incubation hours, mean values were 0.017; 0.015 and 0.013% per hour, respectively for Tifton 85, Tifton 68 and *Coastcross* (Figure 3).

Higher fermentation rates were observed in the initial fermentation time, decreasing over time, which is due to the higher concentration cell content of components (proteins, starch, ether extract, soluble carbohydrates), highly digestible by rumen microorganisms resulting in increased

fermentation rate (Van Soest, 1994; Monção et al., 2014). Velásquez et al. (2009) worked with fermentation rate, and after 6 hour incubation, observed higher values for Tifton 85 at 42 days and Tanzania grass at 28 days (0.052% and 0.050% hour^{-1}).
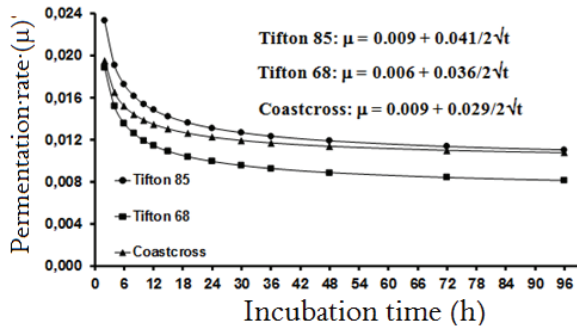

Figure 3. Fermentation rate (μ) of dry matter according to incubation time of three grasses of the genus *Cynodon*.

There was effect (p<0.05) for the soluble fraction (a), potentially degradable insoluble fraction (b) insoluble fraction (FI), potential degradability (PD) and effective degradability of dry matter at passage rate of 5% hour^{-1} (Table 2).

Table 2. Soluble fraction (A), potentially fermentable insoluble fraction (B), degradation rate (c), non-degradable fraction (FI), potential degradability (PD) and effective degradability (ED) of dry matter of three grasses of the genus *Cynodon*.

Grasses	Parameters					
	A	B	c	FI	DP	DE
Tifton 85	10.37a	61.69a	0.026a	27.98a	72.02a	31.25a
Tifton 68	11.76a	63.90a	0.023a	24.34a	75.66a	30.71a
Coastcross	8.03b	50.62b	0.031a	41.35b	58.65b	26.40b
SEM	0.7214	2.2435	0.0024	2.6648	2.6648	0.4534
p value	0.004	0.000	0.100	0.000	0.000	0.000

Different lowercase letters in the same column are significantly different (p < 0.05) by Scott-Knott test. SEM-Standard Error of the Mean.

Tifton 85 and Tifton 68 showed higher values (p<0.05) for these variables in relation to *Coastcross*. No difference (p> 0.05) was detected for degradation rate 'c' of grasses, with a mean of 0.026% hour^{-1}. Regarding degradation parameters, the fraction 'a' is nutritionally represented by soluble carbohydrates of rapid ruminal degradation. The fraction 'b' is represented by cell wall components, of slow ruminal degradation, due to structural arrangements of these components with lignin. Monção et al., (2014) also studied the *in situ* degradability of dry matter of five *Cynodon* grasses (Tifton 85, Jiggs, Tifton 68, Russel and Vaquero) and registered 21.3 and 21.9% for potentially degradable fraction in the rumen for Tifton 85 and Tifton 68, respectively. Moreover, for potentially degradable insoluble fraction (b), the authors found 46.21 and 48%, respectively, for Tifton 85 and Tifton 68. These values are not similar to those

observed in this study, which is normal because of soil and climate variations that, combined or individually, acts on the plant, causing morphophysiological changes.

Regarding potential (PD) and effective (ED) degradability of dry matter, *Coastcross* presented poorer results when compared to the other treatments and higher values for the non-degradable fraction (41.3%). It is noteworthy that genotypes Tifton 85 and Tifton 68 have high potential use in diets for ruminants in relation to *Coastcross* that presented PD values 18.6 and 22.48% lower than Tifton 85 and Tifton 68, respectively. This reduction was also verified in ED, 15.52 and 14%, respectively, which is not desirable, mainly dealing with animals with high nutritional requirements. High ED of substrate indicates higher availability of nutrients in the rumen for synthesis of short chain fatty acids and microbial protein (Van Soest, 1994). Lower PD and ED of *Coastcross* may be associated with the lower content of soluble carbohydrates, fraction (a) and components available for degradation of fiber, fraction (b), corroborated by the highest value of non-degradable fraction (FI), which was 58.06% higher than the mean value (26.16%) of Tifton 85 and Tifton 68.

Conclusion

Tifton 85 had higher values of fermentation kinetics and fermentation rates by the semi-automated technique of gas production.

Tifton 85 and Tifton 68 g showed better potential and effective degradability of dry matter.

Acknowledgements

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