Tropical and Subtropical Agroecosystems

COMPARISON OF *IN SITU* AND *IN VITRO* DRY MATTER RUMEN DEGRADABILITY OF THREE DISTINCT QUALITY HAYS IN SHEEP

[COMPARACIÓN DE LA DEGRADABILIDAD RUMINAL DE LA MATERIA SECA *IN SITU* E *IN VITRO* DE HENOS DE TRES CALIDADES DISTINTAS EN OVINOS]

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SUMMARY

The objective of this work was to evaluate the dry matter rumen degradability of three distinct quality hays, using *in situ* and *in vitro* techniques. The animals used to incubate the bags in and as inoculum donors were six Santa Ines sheep, male, castrated, weighting 40±5.7 kg LW, all with rumen cannulas. Three forage hays were chosen for this experiment, based on their crude protein content. They were alfalfa (*Medicago sativa*) (ALF), signalgrass (*Brachiaria decumbens*) (SIG), and Tifton-85 (*Cynodon* sp.) (TIF). The *in situ* rumen degradability kinetics was determined using the nylon bag technique, and the *in vitro* degradability was the semi-automated gas production technique. There were differences between the three hays for CP ($P < 0.01$), ADIP ($P < 0.01$), DM, ($P < 0.05$) OM ($P < 0.05$), NDF ($P < 0.05$), hemicellulose ($P < 0.05$), and cellulose ($P < 0.05$) contents. Feeds SIG and TIF did not differ ($P > 0.05$) among them for ADF, ADL and silica contents, but both differed of feed ALF for the same constituents ($P < 0.05$). There were differences between *in situ* and *in vitro* degradabilities, mainly for readily soluble fraction and degradation rates. The *in situ* and *in vitro* techniques to estimate the degradation kinetics were consistent, except for data on the initial solubility and lag time. The *in vitro* technique showed strong dependence of the quality of inoculum; the inoculum from animals better fed presented the best results. When the focus was to determine the lag time, the first-order exponential model underestimated the observed results. The fermentative kinetics (gas production) described in greater fidelity the feed degradation due to microbial action.

Keywords: feed evaluation, gas production; rumen fermentation; ruminants.

RESUMEN

El objetivo fue evaluar la degradabilidad ruminal de materia seca de tres henos de diferentes calidades, utilizando las técnicas *in situ* e *in vitro*. Se emplearon seis ovinos Santa Inês, machos, castrados, de 40 ± 5,7 kg PV, todos con cánulas ruminales, para incubar las bolsas y como donantes de inóculo. Los henos fueron elegidos basados en su contenido de proteína cruda. Estos fueron alfalfa (*Medicago sativa*) (ALF), Brachiaria (*Brachiaria decumbens*) (SIG) y Tifton-85 (*Cynodon* sp.) (TIF). La degradabilidad ruminal *in situ* se determinó mediante la técnica de bolsas de nylon, y la degradabilidad *in vitro* se realizó por la técnica semi-automatizada de producción de gases. Hubo diferencia entre los tres henos para los contenidos de PC ($P < 0,01$), ADIP ($P < 0,01$), MS ($P < 0,05$) MO ($P < 0,05$), FDN ($P < 0,05$), hemicelulosa ($P < 0,05$), y celulosa ($P < 0,05$). SIG y TIF no difieren ($P > 0,05$) entre ellos para FDA, LDA y el contenido de sílice, pero ambos difieren de ALF para los mismos componentes ($P < 0,05$). Se encontró diferencias entre la degradabilidad *in situ* e *in vitro*, principalmente para la fracción rápidamente soluble y las tasas de degradación. Las técnicas *in situ* e *in vitro* para estimar la cinética de degradación fueron consistentes excepto para la solubilidad inicial y tiempo de retraso. La técnica *in vitro* mostró fuerte dependencia de la calidad del inóculo ya que el de los animales mejor alimentados presentó mejores resultados. Cuando la atención se centró en determinar el tiempo de retraso, el modelo exponencial de primer orden subestimó los resultados encontrados. La producción de gases describe con mayor fidelidad la degradación de los alimentos debido a la acción microbiana.

Palabras clave: evaluación de alimentos, producción de gas; fermentación rumial; rumiantes.

INTRODUCTION

Forages are a huge range of feeds that allow the animal production (meat, milk, wool, leather) with the lowest costs. However, as mentioned by Beever and Mould (2000), the great diversity of forages provides at the same time opportunities and challenges for using these feeds in ruminant diets. The diversity mentioned above not only refers to the huge number of species with forage potential but also the large variations found even for the same forage species.
Monogastric nutritionists may routinely make use of tables of feed composition to the diet balancing with some security, but the ruminant nutritionists should consider that at least one source should be a forage, and although there are many tables of composition, the variations found in these plants are huge, especially in the case of tropical forages.

In ruminant nutrition, it is a well known fact that the main source of protein for animal normally is not dietary protein but protein from microbial origin, synthesized in the rumen fermentation process of degradation from dietary protein, microbial protein recycling, nitrogen recycled via saliva or sources of non-protein nitrogen.

The objective of this work was to evaluate the dry matter rumen degradability of three distinct quality hays, using *in situ* and *in vitro* techniques, comparing the results from both techniques.

MATERIAL AND METHODS

The assays were carried out at the Laboratory of Animal Nutrition of the Centre for Nuclear Energy in Agriculture, University of São Paulo. The animals used were six Santa Ines sheep, male, castrated, weighting 40±5.7 kg LW, all with rumen cannulas.

The experiment comprised three periods, and each period was of 35 days (10 days for adaptation and change-over, 5 days for *in situ* and *in vitro* assays, and the remaining days for other studies as digesta flow, microbial synthesis and apparent digestibility). In the present article, *in situ* and *in vitro* dry matter rumen degradability data are detailed. Results from other studies of the same experiment are already published (Bueno et al., 2007; Bueno et al., in press).

Feeds and animal management

Three forage hays were chosen for this experiment, based on their crude protein content. The studied hays were alfalfa (*Medicago sativa*), signalgrass (*Brachiaria decumbens*), and Tifton-85 (*Cynodon* sp.). Henceforth, these feeds will be nominated as ALF, SIG and TIF, respectively. All hays were bought from local commerce.

At the beginning of each experimental period, all the hays were chopped in 2-3 cm size particles. At this moment, sampling of feeds were taken, dried at 60 ºC and ground in Wiley mil, with 2 mm screen sieve, for *in situ* assay, and with 1 mm screen sieve, for *in vitro* assay and chemical analysis.

Feeds were characterized chemically (Table 1) according to AOAC (1995) (DM - dry matter; MM - mineral matter, OM - organic matter and CP - crude protein) and Van Soest and Wine (1967) (NDF – neutral detergent fibre; ADF - acid detergent fibre; ADL - acid detergent lignin). There also were determination of acid detergent insoluble protein, here called ADIP, and estimate of hemicellulose, cellulose and silica, as described by Van Soest and Wine (1967).

The animals which bags were incubated in were the same which supplied inocula for the *in vitro* technique. During the experiment, animals were fed exclusively the respective hays, supplemented with minerals and they had free access to water.

In situ rumen degradability assay

In situ rumen degradability kinetics was determined using the technique described by Ørskov and McDonald (1979).

Nylon bags (35 µm porosity), containing around 3 g of the same hay offered to the animals, were incubated directly into the rumen of the animals, in duplicates. Bags were withdrawn after 3, 8, 16, 24, 48, 72, and 96 h of incubation.

Bags were also prepared to determine the washing loss of readily soluble material (A), as suggested by McDonald (1981), which characterizes the initial degradability.

Data were fitted by the Ørskov and McDonald (1979) model, modified by McDonald (1981), which is given as:

$$p = a + b.(1 - \exp^{-c.t}); \qquad \text{for } t \geq t_0$$

$$p = A; \qquad \text{for } t < t_0$$

Where:
p represents the degradability at the time t; a and b are mathematical constants of the model; c is the fractional rate of degradation, t, the time of incubation; t_0, a discrete lag time, and A, the washing loss, or the readily soluble fraction.

As (a+b) corresponds numerically to the asymptotic value (potential degradability), B, given as (a+b) – A, biologically represents the insoluble fraction which is potentially degradable.

For the *in situ* incubation, which is physically limited by the animal rumen volume, only bags containing the hay correspondent to the animal diet were incubated.

In vitro rumen degradability and gas production assay

The gas production technique was carried out according to Mauricio et al. (1999), using rumen liquor from the same experimental animals as microbial source for the inoculum. The rumen liquor (solid and liquid phases) was sampled in the morning before the first meal, and after an overnight fastening. The inoculum was prepared using the same proportions of solid and liquid phases of the rumen digesta (Bueno et al., 2005). These fractions were homogenized in a blender for just a few seconds and filtered through nylon cloth (35 µm porosity).

Inocula were prepared separately from animals fed different hays. As in each period there were six animals fed three hays (two for each), rumen liquor from two animals were pooled to make one inoculum. All feeds (substrates) were inoculated with all inocula, to test the specificity of the inoculum.

The following times were used to measure gas production volumes: 0, 3, 6, 9, 12, 16, 20, 24, 30, 36, 48, 60, 72 and 96 h after inoculation, and the *in vitro* degradation of feeds were determined in the following times: 0, 3, 9, 16, 24, 48, 72 and 96 h after inoculation.

Dry matter rumen degradability was calculated as the difference between the total amount of sample placed in each bottle to be fermented and amount of residue recovered by filtration through sintered crucible (n.1) after incubation.

Data of gas production were fitted by the France et al. (1993) model, given as:

$$V = V_f\,(1 - \exp^{-b\,(t - t0) - c\,(\sqrt{t} - \sqrt{t0})}); \qquad \text{for } t \geq t_0$$

$$V = 0; \qquad \text{for } t < t_0$$

Where:
V is the volume of gases produced after t hours of incubation; V_f, the final volume, or potential gas production, or the asymptotic value; b and c are mathematical constants; t is the time of incubation; and t_0, the lag time.

Data of rumen degradation were fitted by the Ørskov and McDonald (1979) model, modified by McDonald (1981) as briefly described above.

Table 1. Chemical characterization, as g/kg DM, of the alfalfa (ALF), signalgrass (SIG) and Tifton-85 (TIF) hays.

constituents	feeds			sed*
	ALF	SIG	TIF	
Dry matter**	841.7 [c]	851.9 [a]	848.5 [b]	1.25
Organic matter	900.3 [c]	925.5 [a]	907.1 [b]	2.92
Neutral detergent fibre	520.8 [c]	777.9 [b]	803.5 [a]	11.02
Acid detergent fibre	417.7 [b]	470.2 [a]	460.7 [a]	8.32
Acid detergent lignin	105.7 [a]	60.7 [b]	65.6 [b]	3.96
Hemicellulose	103.1 [c]	307.7 [b]	342.8 [a]	6.55
Cellulose	307.7 [c]	382.3 [a]	361.3 [b]	4.90
Silica	4.3 [b]	27.2 [a]	33.9 [a]	3.46
Acid detergent insoluble protein	21.4 [a]	8.2 [c]	15.6 [b]	0.68
Crude protein	190.8 [a]	29.0 [c]	75.1 [b]	2.89
ADIP/CP***	11.2 [c]	28.3 [a]	20.7 [b]	1.79

* sed: standard error of difference between means
** dry matter expressed as g/kg fresh matter
*** ratio between acid detergent insoluble protein (ADIP) and crude protein (CP), as %
[a,b,c] means with different superscripts, within rows, are significantly different ($P < 0.05$)

Statistical analysis

This experiment had two statistical designs. The *in situ* assays were carried out in double Latin square design (3 diets, 3 periods, 6 animals) resulting in a 3×6 rectangle (n = 18) (Mead et al., 1993). For this design, the data were submitted to analysis of variance according to the model:

$$Y_{ijk} = \mu + D_i + A_j + P_k + e_{ijk'}$$

where Y_{ijk} represents the dependent variable; μ, the overall mean; D_i, the diet effect (i = 1 to 3); A_j, the animal effect (j = 1 to 6); P_k, the period effect (k = 1 to 3); and e_{ijk}, the residual error.

The *in vitro* gas production assays were carried out according to a complete factorial design (3 inocula x 3 substrates), repeated three times (3 periods) (n = 27). That was done due to many reasons, mainly because the *in vitro* assays do not consider the animal effect as a source of variation. Thus, considering the inoculum source as a variation source, the analysis of variance was done according to the statistical model:

$$Y_{ijk} = \mu + S_i + I_j + P_k + S_i \times I_j + e_{ijk},$$

where Y_{ijk} represents the dependent variable; μ , the overall mean; S_i, the effect of substrate (incubated feed) (i = 1 to 3); I_j, the effect of inoculum (j = 1 to 3); P_k, the effect of period (k = 1 to 3); $S_i \times I_j$, the effect of substrate*inoculum interaction; and e_{ijk}, the residual error.

For statistical analysis we used the PROC GLM of the statistical program SAS (SAS, 2000). The level of probability for acceptance or rejection of the hypothesis test was 5%. Means were adjusted (least square means) and compared by the standard errors of differences between means (sed) and the minimum significant differences obtained using the Student t test at probability level of 5%. Data were also compared by Pearson correlation coefficient (r).

RESULTS AND DISCUSSION

Chemical composition

There was difference between the three hays for CP ($P < 0.01$), ADIP ($P < 0.01$), DM, ($P < 0.05$) OM ($P < 0.05$), NDF ($P < 0.05$), hemicellulose ($P < 0.05$), and cellulose ($P < 0.05$) contents (Table 1). Feeds SIG and TIF did not differ ($P > 0.05$) among them for ADF, ADL and silica contents, but both differed of feed ALF for the same constituents ($P < 0.05$) (Table 1).

In a similar work, Korndörfer (1999) reported contents of DM, OM, NDF and ADF very close to those found in this work for ALF and SIG. However, for CP content, her results were 61 and 140 g/kg DM, respectively for SIG and ALF, differing, therefore, of those reported in Table 1.

The CP contents varied practically 2.5 times, between the tested hays. It is important to highlight that a great part of CP was represented by CP insoluble in acid detergent (ADIP), and, therefore of low availability to the rumen microbiota.

The composition of hays is quite characteristic, not differing from values found in literature, except for protein contents of feeds SIG and TIF. These contents are below of expected values for commercial hays, however they were ideal for this experiment.

According to Van Soest (1994), the minimum CP content in ruminant diet to supply N to rumen microbial activities and to not compromise the intake and digestibility should be around 60 – 80 g/kg DM. Thus, the feeds are placed exactly below (29), above (191) and within (75) this range, respectively for feeds SIG, ALF and TIF. It is important to notice that the values cited by Van Soest (1994) are not for animal protein requirement. NRC (1985) recommends, for sheep weighting 40 kg LW, a diet with 116 g CP/kg DM.

In the literature, the few data about *Brachiaria decumbens* as a feed for sheep refer to grazing systems. Data about signalgrass hay are very scarce.

The ALF composition presented results very similar to that related to high quality alfalfa hays (Alexandrov, 1998; Ferret et al., 1999; Moreira et al., 2001a; 2001b).

The feed TIF presented chemical characteristics similar to those found by Ribeiro et al. (2001) for Tifton-85 hay with 42 – 56 days of growth, characterising it as medium quality hay. Ataíde Júnior et al. (2001), however, found a higher protein quality composition for Tifton-85 hays with 35, 42, and 56 days of growth (respectively, 171, 146 and 122 g CP/kg DM).

***In situ* dry matter rumen degradability**

The *in situ* degradation profiles of tested feeds are presented graphically in Figure 1. It is possible to observe that the data fitting by the Ørskov & McDonald (1979) model was satisfactory ($R^2 > 0.95$), for the three hays. The biological (A, B, $A+B$, t_0, p_{effect} and c) and mathematical (constants a and b) parameters of the model used to fit the data are presented in Table 2.

The model of Ørskov & McDonald (1979) is represented by a first order exponential equation, $p = a + b\ (1 - e^{-c \times t})$. The curvature degree of the profiles generated by it is given by *c*. When the degradation rate, *c*, tends to zero (or is very small, such as in the case for SIG, *c* = 0.014/h), the equation is close to a line, as shown in Figure 1. This causes an overestimation of the potential degradability (*A*+*B*), what may produce unrealistic values as degradabilities higher than 1000 g/kg (Table 2).

The fractional rate of degradation (*c*) for SIG and TIF is in accordance to Sampaio (1990), Bueno (1998), Korndörfer (1999), Machado et al. (2001) and Cabral Filho (2007) who estimated values of c for tropical forages ranging from 0.02 to 0.05/h. The rate of degradation for ALF, however, was higher than those mentioned by Alexandrov (1998), Korndörfer (1999) and Machado et al. (2001) (respectively, 0.077, 0.090 and 0.081/h).

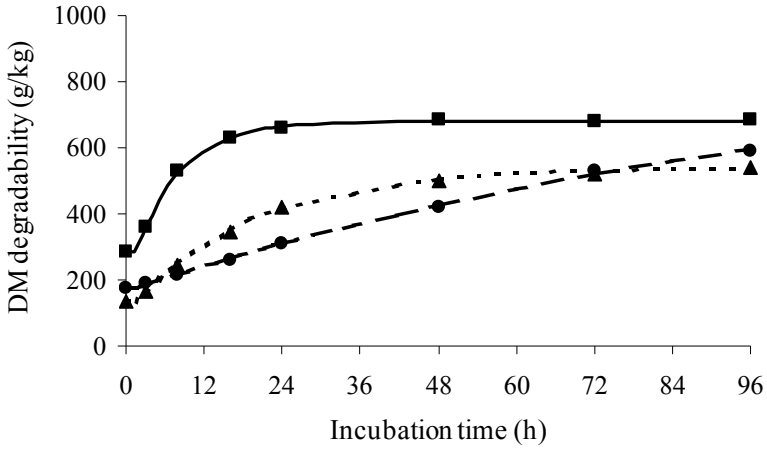

Figure 1. *In situ* degradation kinetics profiles for alfalfa (■ and solid line), signalgrass (● and dashed line) and Tifton-85 (▲ and dotted line) hays in sheep fed exclusively the correspondent hay (marks refer to the mean value of six observed data and lines, to the Ørskov & McDonald (1979) model fitted for each feed).

Table 2. Parameters of the Ørskov & McDonald (1979) model for *in situ* dry matter degradation of alfalfa (ALF), signalgrass (SIG) and Tifton-95 (TIF) hays (n = 18).

parameters*	Feeds			sed**
	ALF	SIG	TIF	
mathematical				
a	172.8	115.3	113.2	33.59
b	505.5 ^{ab}	997.1 ^{a}	427.5 ^{b}	251.75
biological				
A	288.3 ^{a}	177.3 ^{b}	137.0 ^{c}	0.68
B	389.2 ^{b}	935.0 ^{a}	403.8 ^{b}	254.03
A+*B*	677.9 ^{ab}	1112.2 ^{a}	540.8 ^{ab}	253.39
c	0.145 ^{a}	0.014 ^{c}	0.051 ^{b}	0.0062
t_0	1.57	3.78	0.93	1.949
p_{effect} (k_e = 0.02/h)	617.7 ^{a}	389.4 ^{c}	418.8 ^{b}	14.33
p_{effect} (specific k_e)	592.6 ^{a}	386.2 ^{b}	400.8 ^{b}	14.59

* *a* and *b*: model mathematical constants; *A*: readily soluble fraction (g/kg); *B*: potentially fermentable insoluble fraction (g/kg); *A*+*B*: potential degradability (g/kg); *c*: degradation rate of fraction *B* (/h); t_0: lag time (h); p_{effect}: effective degradability (g/kg); k_e: rumen escape rate; specific k_e: real k_e obtained by digesta markers (0.0301; 0.0204 and 0.0244/h, respectively for ALF, SIG and TIF)

** sed: standard error of the difference between means

^{a,b,c} means with different superscripts, within rows, are significantly different (P < 0.05)

The values of effective degradability (p_{effect}) (with k_e = 0.02/h) for ALF, SIG and TIF were, respectively, 618, 389 and 419 g/kg (Table 2). Results for ALF were lower than those presented by Machado (2001) (698 g/kg) for fresh alfalfa, which usually has a higher degradability when compared to hay. Alexandrov (1998) also found effective degradability for alfalfa hay a little bit higher (659 g/kg).

The effective degradabilities of signalgrass hays found by Korndörfer (1999) (537 and 542 g/kg, respectively for hays with 28 and 56 days of growth) also were higher than that found for SIG. The reason could be the particle size that was smaller for Korndörfer (1999) who used 1 mm screen sieve, while in this experiment the screen sieve was 2 mm. The effective degradability of TIF was similar to those reported by Machado et al. (2001) and Cabral Filho (2007) for Tifton hays.

In vitro dry matter rumen degradability

The *in vitro* degradation profiles of tested feeds (ALF, SIG and TIF) using, as inoculum, rumen liquor from sheep fed exclusively the correspondent hay are graphically represented in Figure 2.

It is possible to notice that, also for this technique, the data fitting by the model of Ørskov & McDonald (1979) was satisfactory ($R^2 > 0.95$), for the three feeds (Figure 2), however it is possible to notice a sigmoid trend stronger than that one observed for *in situ* data (Figure 1). The biological (A, B, $A+B$, t_0, p_{efet} and c) and mathematical (constants a and b) parameters of model used to fit the observed data are presented in Table 3.

Figures 1 and 2 are represented at the same scale, what make possible to observe that the material disappearance occurs faster for the *in situ* than for the *in vitro* technique.

Data of Figures 2 and Table 3 are related to the *in vitro* degradation kinetics of a feed (substrate) using as inoculum the rumen liquor from sheep fed exclusively the same feed. However, in this assay, as mentioned previously, the specificity of inoculum in relation to the substrate was evaluated, testing, for this, all substrates with all inocula.

In Table 4, mean data of degradation kinetics of the three hays for each one of the tested inocula are presented.

On the general data analysis, the constants a and b and the biological parameters A, B, c, $A+B$, t_0 and p_{effect} were significantly influenced ($P < 0.001$) by the substrate. The same variables, except a, also were influenced ($P < 0.05$) by the inoculum source. The effects of substrate*inoculum interaction were observed for the variables b, B, $A+B$ e p_{effect} ($P < 0.05$).

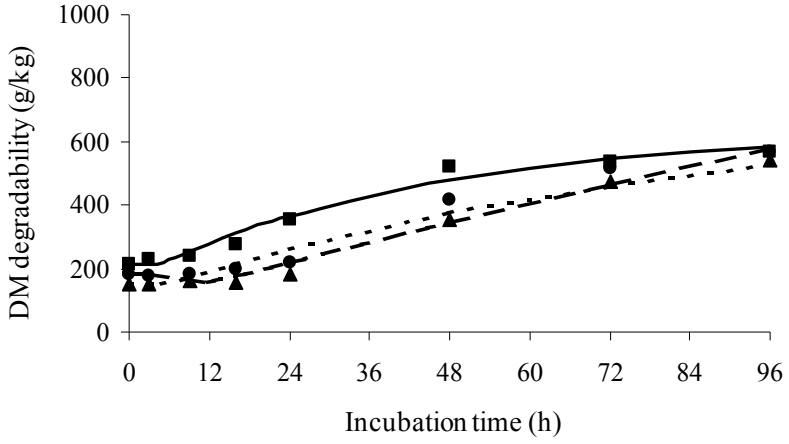

Figure 2. *In vitro* degradation kinetics profiles for alfalfa (■ and solid line), signalgrass (● and dashed line) and Tifton-85 (▲ and dotted line) hays using as inoculum rumen liquor from sheep fed exclusively the same feed (marks refer to the mean value of three observed data and lines, to the Ørskov & McDonald (1979) model fitted for each feed)

Table 3**.** Parameters of the Ørskov & McDonald (1979) model for *in vitro* dry matter degradation of alfalfa (ALF), signalgrass (SIG) and Tifton-95 (TIF) hays, using as inoculum source rumen liquor from sheep fed exclusively the correspondent hay.

parameters*	Substrates			sed**
	ALF	SIG	TIF	
mathematical				
a	167.5 [a]	94.5 [b]	110.0 [b]	8.12
b	467.7 [b]	5293.2 [a]	620.7 [b]	865.00
biological				
A	215.3 [a]	158.3 [b]	146.3 [c]	5.32
B	420.3 [b]	5229.7 [a]	584.3 [b]	865.01
A+B	635.0 [b]	5387.7 [a]	730.7 [b]	863.70
c	0.023 [a]	0.001 [c]	0.011 [b]	0.0022
t_0	4.27 [b]	11.91 [a]	4.93 [b]	0.893
p_{effect} (k_e = 0.02/h)	414.8 [a]	342.0 [b]	334.9 [b]	5.16
p_{effect} (specific k_e)	334.5 [b]	343.5 [a]	334.3 [b]	1.82

* *a* and *b*: model constants; *A*: readily soluble fraction (g/kg); *B*: potentially fermentable insoluble fraction (g/kg); *A+B*: potential degradability (g/kg); *c*: degradation rate of fraction *B* (/h); t_0: lag time (h); p_{effect}: effective degradability (g/kg); k_e: rumen escape rate; specific k_e: real k_e obtained by digesta markers (0.0301; 0.0204 and 0.0244/h, respectively for ALF, SIG and TIF)

** sed: standard error of the difference between means

[a,b,c] means with different superscripts, within rows, are significantly different (P < 0.05)

Table 4. Means of parameters of the Ørskov & McDonald (1979) model for *in vitro* dry matter degradation of substrates, using as inoculum the rumen liquor from sheep exclusively fed alfalfa (ALF) signalgrass (SIG) or Tifton-85 (TIF) hays.

parameters*	Inoculum source			sed**
	ALF	SIG	TIF	
mathematical				
a	121.6	124.6	127.7	4.71
b	969.1 [b]	2329.1 [a]	845.5 [b]	498.87
biological				
A	175.5 [a]	177.8 [a]	167.9 [b]	3.08
B	915.2 [b]	2297.8 [a]	805.3 [b]	499.41
A+B	1090.7 [b]	2475.2 [a]	973.1 [b]	498.66
c	0.012 [a]	0.006 [b]	0.013 [a]	0.0013
t_0	7.99 [a]	8.65 [a]	5.27 [b]	0.517
p_{efet} (k_e = 0.02/h)	348.4 [a]	361.5 [b]	375.4 [b]	2.98

* *a* and *b*: model constants; *A*: readily soluble fraction (g/kg); *B*: potentially fermentable insoluble fraction (g/kg); *A+B*: potential degradability (g/kg); *c*: degradation rate of fraction *B* (/h); t_0: lag time (h); p_{effect}: effective degradability (g/kg); k_e: rumen escape rate

** sed: standard error of the difference between means

[a,b] means with different superscripts, within rows, are significantly different (P < 0.05)

In situ vs. *in vitro* dry matter rumen degradability

Data related to the *in vitro* degradation kinetics, like those presented here, are very scarce in the literature, and they are even fewer for tropical forages. A summary of correlations obtained between *in situ* and *in vitro* techniques to estimate rumen dry matter degradability (respectively from Tables 2 and 3) is presented in Table 5.

By data from Tables 2 and 3, however there is a significant correlation between them (r = 0.054; P = 0.03), the readily soluble fractions (*A*) obtained by *in vitro* technique are a little lower than those obtained by *in situ* technique. The reason for this could be the pore size of bags which are greater (35 μm) than the pores of sintered crucibles used in the *in vitro* technique.

The pore size of bags used in *in situ* technique allows the escape of particles (particularly small and high density particles). This can be seen mainly for

substrate ALF, which presented values of *A* of 288 and 215 g/kg, respectively for *in situ* and *in vitro* techniques. As legume forage, the particles generated by ALF milling are more compact and dense, thus, they escape from the bags easily. This ease is not observed at the same proportion when sintered crucibles are used to measure the solubility. For grasses, the particles generated by milling are longer, less compact and less dense, what is mainly due to the morphological differences of plants and to the higher fibre contents (Huntington and Givens, 1995).

In both techniques, there was an overestimation of potential degradability (*A+B*) for the substrate SIG, due to an unsatisfactory data fitting, caused by the low degradation rate of fraction *B* (*c*) (0.014 and 0.001/h, respectively for *in situ* and *in vitro* techniques). Although the data of *A+B* have presented correlation between the techniques (r = 0.76; P < 0.001), the overestimation of this parameter was surprisingly high for the *in vitro* technique.

Gas production

In Table 6, the data presented are related to the parameters of the fermentative kinetics of hays when incubated with inoculum from animals fed exclusively the same feed (hay). Graphically, the profiles of gas production from feed *in vitro* fermentation are presented on Figure 3.

The lag times (t_0) estimated for ALF and SIG are similar to those observed by Bueno et al. (2005) for alfalfa hay (6.1 to 7.1 h) and for signalgrass (7.0 to 8.3 h).

The fermentation rate (μ) of this model, differently from fractional degradation rate (*c*) from Ørskov & McDonald (1979) model, varies according to the elapse time. This variation of μ is presented in Figure 4. While the degradation rate of Ørskov & McDonald (1979) is a constant, i.e., is the same from the beginning to the end of feed degradation, the fermentative rate represents the differences of fermentation (and for instance the degradation) rate during the process. Different fractions of the feed are fermented in different speeds. The non structural carbohydrates, for example, are degraded more rapidly than the fibres. The time-dependent fermentation rate of France et al. (1993) model represents more faithfully the process. As shown in Figure 4, feeds with more cellular content (easily fermentable energy) have initially higher μ. With the elapsed time, these components become scarce and other sources of energy to be fermented are fermentable with less speed.

Table 5. Correlation coefficients (r) for mathematical (*a* and *b*) and biological (*A*, *B*, *A+B*, *c*, t_0 and p_{effect}) parameters (n=18) obtained by the model of Ørskov & McDonald (1979) for data fitting.

in situ	*in vitro*							
	a	*b*	*A*	*B*	*A+B*	*c*	t_0	p_{efet}
A	r=0.33 ns							
B		r=0.76 ***						
A			r=0.54 *					
B				r=0.78 ***				
A+B					r=0.76 ***			
C						r=0.63 **		
t_0							r=0.35 ns	
p_{efet}								r=0.60 **

ns: not significant (P > 0.05); * P < 0.05; ** P < 0.01; *** P < 0.001

Table 6. Parameters of the France et al. (1993) model and relative gas productions for alfalfa (ALF), signalgrass (SIG) and Tifton-85 (TIF) hays, using as inoculum source the rumen liquor of sheep fed the same hay.

parameters*	Substrates			sed**
	ALF	SIG	TIF	
V_f (ml/g DM)	137.8 [b]	256.0 [a]	165.6 [b]	24.71
b (/h)	0.063 [a]	0.018 [c]	0.031 [b]	0.0050
c (/h^{½})	-0.297 [a]	-0.101 [b]	-0.142 [b]	0.0303
t_0 (h)	6.35	7.52	5.34	1.038
R1	0.960 [a]	0.566 [c]	0.817 [b]	0.0545
R2	0.778 [a]	0.452 [c]	0.610 [b]	0.0221

* V_f: final volume or potential gas production; b and c: model constants; t_0: lag time; *R1*: ratio between gas production after 96 h and V_f; *R2*: ratio between gas productions after 48 and 96 h

** sed: standard error of the difference between means

[a,b,c] means with different superscripts, within rows, are significantly different ($P < 0.05$)

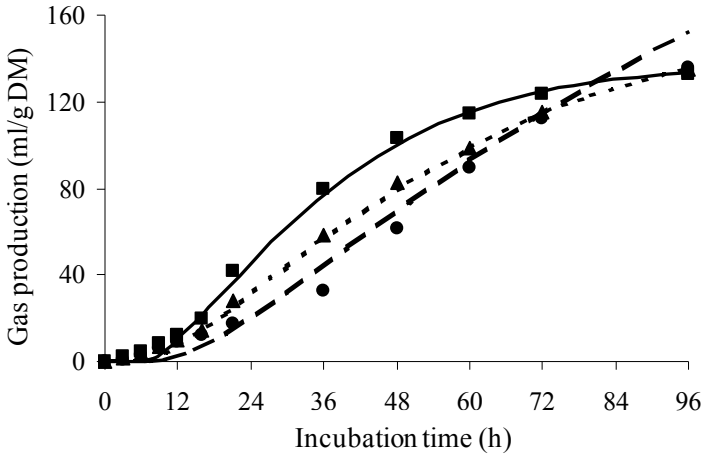

Figure 3. *In vitro* fermentation kinetics profiles for alfalfa (■ and solid line), signalgrass (● and dashed line) and Tifton -85 (▲ dotted line) hays using as inoculum the rumen liquor from sheep fed exclusively the same tested feed (marks refers to mean observed data and lines, to data fitted by model of France at al. (1993))

Feed SIG, during all incubation time, presented the lowest fermentation rates (μ), indicating a lower quality of fermentable compounds. Nogueira Filho et al. (2000) found μ for *Brachiaria humidicola* and *Cynodon dactylon*, after 6 h of incubation, of 0.016 and 0.022/h, and after 48 h, of 0.020 and 0.028/h, respectively. Data presented in Figure 4 point μ after 6 h of 0.016 and 0.022/h and after 48 h of 0.006 and 0.008/h, respectively for SIG and TIF. The rapid decrease, compared to data from Nogueira Filho et al. (2000), are related to low quality of grass hay tested here, for the reserves of easily fermentable carbohydrates reserves were quickly exhausted.

Another important fact about μ for SIG is that its small rates during the incubation period make impossible a good data fitting by the model.

As the potential gas production (V_f) is an estimated asymptotic value, not always it is reached during this period. Bueno et al. (2005) introduced two parameters, one is the relation between the gas production at 96 h incubation and the V_f(*R1*), and this relation indicates if the incubation was long enough to describe the potential gas production; and other is the relation between the gas production volumes at 48 and 96 h incubation (R_2), and with this relation is possible to compare different feeds.

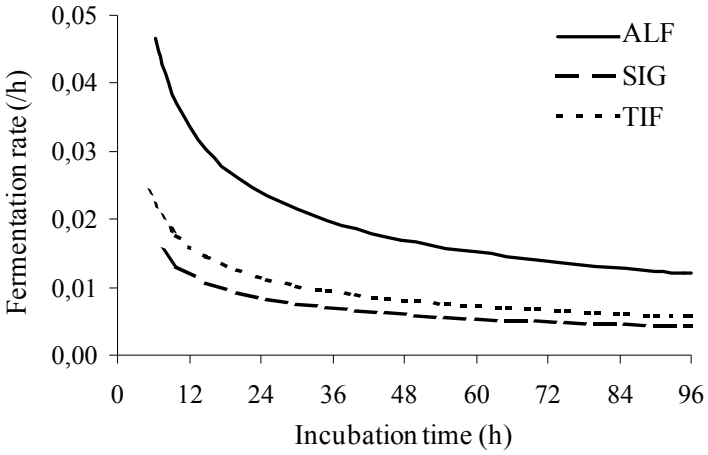

Figure 4. Fermentation rate (μ), derived from the France et al. (1993) model, for alfalfa (ALF), signalgrass (SIG) and Tifton-85 (TIF) hays.

The *R1* for SIG, however, was much lower than for the other feeds, even with a relatively long time for the test (96 h), indicating that the model did not fit so well. ALF showed the highest *R1* (0.960), i.e., 96% of its potential was expressed during the test.

Another parameter introduced to understand and compare the feeds is *R2* (Table 6). *R2* represents proportionally how much of the total gas production determined in the test (96 h) was produced until 48 h of incubation. Assuming a theoretical escape rate (k_e) of 0.0208/h, the mean rumen retention time would be approximately 48 h. Therefore, it is desirable that most of the fermentation occurs in this period, i.e., *R2* should be as close to 1 for the feed be considered of good quality, from the fermentation point of view.

The highest value of *R2* (Table 6) was obtained for ALF, followed by TIF and SIG (0.78, 0.61 and 0.45, respectively), which reflects the different fermentation rates (μ) (Figure 3).

In the gas production assay, besides evaluating the substrates, inocula from animals fed exclusively tested feeds was tested, as well as the interaction inoculum*substrate.

Table 7 presents the fermentative kinetics parameters for the tested inocula.

The inocula from animals fed ALF and TIF were consistent in most of the parameters and they distinguished from that fed SIG, the worst among them. The main parameters for this comment are the values of *R1* and *R2*.

There was no interaction inoculum*substrate ($P > 0.05$) for paramenters V_f, *b*, *c*, t_0, *R1* e *R2*. This indicates as independency for the use of these inocula to evaluate those forages.

Table 7. Means of parameters of the model of France et al. (1993) and relative gas productions of substrates, using as inoculum the rumen liquor of sheep fed exclusively the tested hays.

parameters*	Inoculums source			sed**
	ALF	SIG	TIF	
V_f (ml/g DM)	161.4 [b]	195.4 [a]	168.2 [ab]	14.27
b (/h)	0.037 [a]	0.030 [b]	0.042 [a]	0.0029
c (/h^{½})	-0.171 [b]	-0.164 [b]	-0.208 [a]	0.0303
t_0 (h)	5.44	7.99	6.84	0.599
R1	0.819 [a]	0.704 [b]	0.855 [a]	0.0315
R2	0.643 [a]	0.562 [b]	0.647 [a]	0.0128

* V_f: final volume or potential gas production; *b* and *c*: model constants; t_0: lag time; *R1*: ratio between gas production after 96 h and V_f; *R2*: ratio between gas productions after 48 and 96 h

** sed: standard error of the difference between means

[a,b] means with different superscripts, within rows, are significantly different ($P < 0.05$)

CONCLUSION

The *in situ* and *in vitro* techniques to estimate the degradation kinetics were consistent, except for data on the initial solubility and lag time. When the focus was to determine the lag time, the first-order exponential model underestimated the observed results. The fermentative kinetics (gas production) described in greater fidelity the feed degradation due to microbial action.

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REFERENCES

Alexandrov, A.N. 1998. Effect of ruminal exposure and subsequent microbial contamination on dry matter and protein degradability of various feedstuffs. Animal Feed Science and Technology, 71: 99-107.

Association of Official Agricultural Chemists**.** 1995. Official methods of analysis of the AOAC. 16.ed. Arlington: AOAC International, 1: 4/1-4/30.

Ataíde Júnior, J.R.; Pereira, O.G.; Valadares Filho, S.C.; Garcia, R.; Cecon, P.R.; Alves, M.J. Moreira, A.L. 2001. Consumo, digestibilidade e desempenho de novilhos alimentados com rações à base de feno de capim-Tifton 85, em diferentes idades de rebrota. Revista Brasileira de Zootecnia, 30: 215-222.

Beever, D.E.; Mould, F.L. 2000. Forage evaluation for efficient ruminant livestock production. In: Givens, D.I.; Owen, E.; Axford, R.F.E.; Omed, H.M. (Eds.). Forage evaluation in ruminant nutrition. Wallingford: CAB Publishing, cap.2, p.15-42.

Bueno, I.C.S. 1998. Comparação entre técnicas *in vitro* e *in situ* de avaliação de braquiária para ruminantes. Dissertation of the University of São Paulo, Piracicaba, Brazil. 133p.

Bueno, I.C.S.; Cabral Filho, S.L.S.; Abdalla, A.L. 2010. Rumen microbial growth in sheep fed three distinct quality hays. Tropical and Subtropical Agroecosystems, 12: (in press).

Bueno, I.C.S.; Cabral Filho, S.L.S.; Gobbo, S.P.; Louvandini, H.; Vitti, D.M.S.S.; Abdalla, A.L. 2005. Influence of inoculum source in a gas production method. Animal Feed Science and Technology, 123–124: 95-105.

Bueno, I.C.S.; Vitti, D.M.S.S.; Abdalla, A.L.; Louvandini, H. 2007. Consumo voluntário, digestibilidade aparente e cinética digestiva de três forrageiras em ovinos. Ciência Animal Brasileira, 8: 713-722.

Cabral Filho, S.L.S.; Bueno, I.C.S.; Abdalla, A.L. 2007. Substituição do feno de Tifton pelo resíduo úmido de cervejaria em dietas de ovinos em mantença. Ciência Animal Brasileira, 8: 75-83.

Ferret, A.; Plaixats, J.; Caja, G.; Gasa, J.; Prió, P. 1999. Using markers to estimate apparent dry matter digestibility, faecal output and dry matter intake in dairy ewes fed Italian ryegrass hay or alfalfa hay. Small Ruminant Research, 33: 145-152.

France, J.; Dhanoa, M.S.; Theodorou, M.K.; Lister, S.J.; Davies, S.J.; Isac, D. 1993. A model to interpret gas accumulation profiles with *in vitro* degradation of ruminant feeds. Journal of Theoretical Biology, 163: 99-111.

Huntington, J.A.; Givens, D.I. 1995. The *in situ* technique for studying the rumen degradation of feeds: a review of the procedure. Nutrition Abstracts and Reviews (Series B), 65: 63-93.

Korndörfer, C.M. 1999. Eficiência de utilização do feno de *Brachiaria decumbens* na alimentação de ovinos Santa Inês. Thesis of the University of São Paulo, Piracicaba, Brazil. 110p.

Machado, M.C.; Bueno, I.C.S.; Abdalla, A.L.; Castilho, L.A.; Cabral Filho, S.L.S. 2001. Degradabilidade ruminal *in situ* de alimentos para ovinos da raça Santa Inês. (Compact disc) In: Reunião Anual da Sociedade Brasileira de Zootecnia, 38. Piracicaba, Brazil.

Mauricio, R.M.; Mould, F.L.; Dhanoa, M.S.; Owen, E.; Channa, K.S.; Theodorou, M.K. 1999. Semi automated *in vitro* gas production technique for ruminant feedstuff evaluation. Animal Feed Science and Technology, 79: 321-330.

McDonald, I. 1981. A revised model for estimation of protein degradability in the rumen. Journal of Agricultural Science, 96: 251-252.

Mead, R.; Curnow, R.N.; Hasted, A.M. 1993. Statistical methods in agriculture and

experimental biology. London: Chapman & Hall, cap.5, p.59-88: Control of random variation by blocking.

Moreira, A.L.; Pereira, O.G.; Garcia, R.; Valadares Filho, S.C.; Campos, J.M.S.; Moraes, S.A.; Zervoudakis, J.T. 2001a. Consumo e digestibilidade aparente dos nutrientes da silagem de milho e dos fenos de alfafa e de capim-coastcross, em ovinos. Revista Brasileira de Zootecnia, 30: 1099-1105.

Moreira, A.L.; Pereira, O.G.; Garcia, R.; Valadares Filho, S.C.; Campos, J.M.S.; Moraes, S.A.; Zervoudakis, J.T. 2001b. Produção de leite, consumo e digestibilidade aparente dos nutrientes, pH e concentração de amônia ruminal em vacas lactantes recebendo rações contendo silagem de milho e fenos de alfafa e de capim-coastcross. Revista Brasileira de Zootecnia, 30: 1089-1098.

National Research Council**.** 1985. Nutrient requirements of sheep. 6.ed. Washington: National Academic Press, 99p.

Nogueira Filho, J.C.M.; Fondevila, M.; Urdaneta, A.B.; Ronquillo, M.G. 2000. *In vitro* microbial fermentation of tropical grasses at an advanced maturity stage. Animal Feed Science and Technology, 83: 145-157.

Ørskov, E.R.; Mcdonald, I. 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. Journal of Agricultural Science, 92: 449-453.

Ribeiro, K.G.; Garcia, R.; Pereira, O.G.; Valadares Filho, S.C.; Cecon, P.R. 2001. Eficiência microbiana, fluxo de compostos nitrogenados no abomaso, amônia e pH ruminais, em bovinos recebendo dietas contendo feno de capim-Tifton 85 de diferentes idades de rebrota. Revista Brasileira de Zootecnia, 30: 581-588.

Sampaio, I.B.M. 1990. Seleção de pontos experimentais de colheita de material para o estudo de degradação de matéria seca no rúmen. In: Reunião da Sociedade Brasileira de Zootecnia, 27, Campinas, Brazil, p.13.

SAS Institute. 2000. The SAS system for windows. Release 8.01. Cary.

Van Soest, P.J. 1994. Nutricional ecology of the ruminant. 2ed. New York: Cornell University Press, 476p.

Van Soest, P.J.; Wine, R.H. 1967. Use of detergent in the analysis of farmers feeds. IV. Determination of plant cell wall constituents. Journal of AOAC, 50: 50-55.

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