

COMPARISON OF LABORATORY METHODS FOR PREDICTING DIGESTIBILITY OF FEEDSTUFFS

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In this paper we compare laboratory methods for the prediction of *in vivo* digestibility: the TILLEY & TERRY (1963) method, modified by ALEXANDER & MCGOWAN (1966), the GOERING & VAN SOEST (1970) method, and the *in situ* and enzymatic methods. The best results in the prediction of the *in vivo* digestibility were obtained with the TILLEY & TERRY (1963) method, modified by ALEXANDER & MCGOWAN (1966) and by the *in situ* method, 48 hours of incubation + pepsin. There was a lower correlation between the results of the enzymatic methods and the *in vivo* digestibility, using *Aspergillus niger* and *Trichoderma viride* as sources of cellulase, though significant at a 5% level, for the prediction of the *in vivo* dry matter digestibility and D value. The *in situ* dry matter degradability can be used to predict the *in vivo* dry matter digestibility, at almost all the incubation times, at a 5% level of significance. The best results were obtained after 48 and 72 hours of incubation, $r = 0.83$ and $r = 0.86$, respectively. The results would indicate a good potentiality to predicting the dry matter digestibility from the rumen degradation characteristics of the forages.

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Neste trabalho fizemos a comparação entre os seguintes métodos laboratoriais utilizados para a previsão da digestibilidade *in vivo*: método de TILLEY & TERRY (1963), modificado por ALEXANDER & MCGOWAN (1966), método de GOERING & VAN SOEST (1970), métodos *in situ* e métodos enzimáticos. Os melhores resultados foram obtidos pelo método de TILLEY & TERRY (1963), modificado por ALEXANDER & MCGOWAN (1966) e pelo método *in situ*, 48 de incubação no rúmen + ataque com pepsina. Obtivemos uma baixa correlação entre os resultados dos métodos enzimáticos, com *Aspergillus niger* e *Trichoderma viride* como fontes de celulase, e a digestibilidade *in vivo*, ambos a um nível de 5% de significância, para a previsão da digestibilidade *in vivo* da matéria seca e do valor D. A degradabilidade da matéria seca *in situ* pode ser utilizada para a previsão da digestibilidade da matéria seca *in vivo*, para quase todos os tempos de incubação, para um nível de 5% de significância. Os melhores resultados são obtidos às 48 e 72 horas de incubação, respectivamente $r=0.83$ e $r=0.86$. A partir dos parâmetros da cinética da degradação da matéria seca podemos prever a sua digestibilidade, para um nível de significância de 5%.

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INTRODUCTION

The need to rapidly know the nutritive value of forages, with the objective of establishing appropriated animal production strategies, led us to determine which is the best laboratory method to predict the *in vivo* digestibility.

For the *in vitro* digestibility determination, we used three groups of methods. 1) Rumen microbial populations, and that included the TILLEY & TERRY (1963) method, modified by ALEXANDER & MCGOWAN (1966) that we can consider the best method for the laboratorial digestibility determination; the methods that have the purpose of saving time include the GOERING & VAN SOEST (1970) method, which is not more than a modification of the TILLEY & TERRY (1963) method, where the second stage is replaced by a neutral detergent hidrolisis; 2) enzymatic methods, in which the cellulolytic function of the rumen liquor is substituted by an enzymatic preparation, this methods obeyed two objectives: the diminution of the time expended and the avoiding of fistulated animals as rumen liquor donors; 3) within the *in situ* methods, that pretend to be more close to the animal metabolic processes, we have two type methods: the methods with variable incubation periods and the methods with stable incubation periods, followed or not by a pepsin incubation period.

MATERIAL AND METHODS

The 24 forages used are all gramineaes - oats, Italian ryegrass, perennial ryegrass and maize- in three stages of growth, green and ensiled, with a known chemical composition and *in vivo* digestibility. The forages were dried at 65°C and grounded in particles of 1 mm.

We used the TILLEY & TERRY (1963) method modified by ALEXANDER & MCGOWAN (1966). 0.5 g of samples were weighed in triplicate and incubated for 48 hours with a mixture of 40 ml of MCDUGALL (1948) buffer solution and 10 ml of rumen liquor saturated with CO₂. The anaerobiose conditions in the tubes were maintained by a Busen's valve. In the end of the

first fermentation stage we stopped the microbial activity with 2.2N HCl at a pH of 1.2. Followed by a 48-hour incubation period with 50 ml of a solution of acid pepsin, at 39°C. The residue obtained after filtration in a G2 crucible was dried at 105°C and then ashed at 500°C. The method uses blank test and standard forages to control the rumen liquor activity.

For the GOERING & VAN SOEST (1970) method, we used the TILLEY & TERRY (1963) method, modified by ALEXANDER & MCGOWAN (1966), as base, but after the acid-pepsin treatment was replaced with a 1-hour extraction with neutral detergent solution.

For rumen liquor donors, we used three permanently fistulated sheep, adult Romney-Marsh males, that were fed with a standard diet, ad libitum medium quality hay, 200g concentrate and a mineral supplement.

The enzymatic method used was the one reported by BARTIAUX-THILL et al. (1980), which is a three-stage method. 250 mg of sample was weighed in triplicate: 1 - Pre-treatment with 20 ml 1N HCl and boiled 30 minutes; 2 - Pre-treatment: 20 ml acid-pepsin solution 0.3N (2 g/l in HCl) 24 hours at 40°C; and 3 - Treatment with cellulase: 20 ml of cellulase, 6.25 in a phosphate-citric acid buffer at pH 4.6, 24 hours at 40°C. The residue obtained after filtration in a G2 crucible was dried at 105°C and then ashed at 550°C. We used two sources of cellulase, *Aspergillus niger* and *Trichoderma viride*.

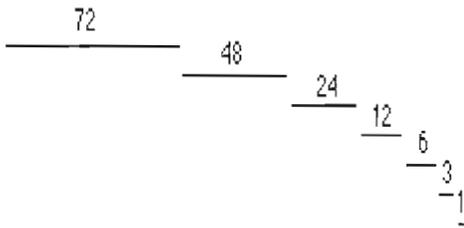
The *in situ* method uses nylon bags made of precision woven nylon cloth (Hydro-Bios- NY55 HC) measuring 17 x 9 cm and with a pore size of 55µm. For each bag about 1 g of sample dry matter was weighed in triplicate. The bags were attached to a PVC structure. These were attached to the fistula cap with a 25 cm long nylon string. The bags were then immersed completely in water to acquire weight and were incubated in the rumen for 48 hours. Four bags were incubated simultaneously.

When taken out of the rumen, the bags were washed under running water for 5 minutes and incubated with 50 ml of a pepsin acid solution (2 g pepsin 1:10000 with 900 ml of H₂O and made

up to 1 liter of solution with 100 ml of 1N HCl), and incubated 48 hours at 39°C. At the end of the incubation period the bags were washed and dried at 105°C, and the residue ashed at 550°C.

For the prediction of the digestibility from the *in situ* degradability we weighed 5 g of the sample, in triplicate, into nylon bags of the dimension and pore size given above. The bags were attached in the same PVC structure mentioned above and following the same procedure. We incubated 6 bags in each sheep. The bags were incubated by 0, 1, 3, 6, 12, 24, 48 and 72 hours. The zero-hours bags were only washed in running water for 15 minutes. At the end of each period of incubation, the bags were washed in running water, until the rinsing water was colorless and dried at 65°C, in a ventilated oven.

In standard methods of incubation, samples are inserted together and removed at intervals (PAINE et al. 1982). We used a method in which the bags were inserted at different times. At each sampling time, all bags were removed and replaced with a new set. None of the incubation times ran concurrently. In our opinion, this procedure simplifies the technique:



Three adult Romney-Marsh males, fitted with a permanent 40 mm rumen cannula were used. They were fed the same diet as the rumen liquor donors.

The digestibility parameters determined in the different methods are the dry matter digestibility, the organic matter digestibility and the organic matter in dry matter digestibility (D value). The *in situ* dry matter degradability is only used for the prediction of the *in vivo* dry matter digestibility.

A simple linear regression and paired *t*-tests were used to compare the relationship between *in vitro* and *in vivo* digestibilities. Based on the results of the organic matter digestibility, we made the variance analysis, for the three stages of growth, the two forms of conservation and tested the interaction between species and growth, species and conservation, and growth and conservation. The multiple comparison of the average means were made using the *t* test (STEEL & TORRIE 1980).

RESULTS AND DISCUSSION

In Table 1, we can observe the regression equation between the *in vivo* digestibility results and the *in vitro* results for dry matter digestibility. Tables 2 and 3 present the same equations for organic matter digestibility and D value respectively.

In the prediction of the *in vivo* digestibility by biological methods, we verified that the TILLEY & TERRY (1963) method, modified by ALEXANDER & MCGOWAN (1966) and the *in situ* method, have given the best results. Some authors, namely GASA et al. (1989) and MATHISON et al. (1988) reported that the *in situ* method gave better results than the *in vitro* method, for prediction of the *in vivo* digestibility. RIVIÉRE et al. (1989) found a correlation between the *in vivo* digestibility and the *in situ* method of 0.77 and with the TILLEY & TERRY (1963) method of 0.76. However, other authors, such as SCALES et al. (1974), reported that the nylon bag technique was not a valid method for the prediction of the *in vivo* digestibility because the values were 23% above the *in vivo*. MÍKA et al. (1982), verified a similar relationship. CABRERA & VAN DER MEER (1987), when comparing the *in vivo* digestibility with the *in situ*, concluded that this method gave results significantly higher than the *in vitro* methods. After 12 hours of incubation, the *in situ* degradation is more intensive than the *in vitro* (NORDKVIST et al. 1987). In WANAPAT & TOPARK-NGARM (1985) both methods underestimated the digestibility values.

Table 1

Prediction of *in vivo* dry matter digestibility from the results obtained by the *in vitro* and *in situ* methods.

Method	Constant	Variable	n	r	r.s.d.
TILLEY & TERRY	25.481	0.547	24	0.860*	3.353
VAN SOEST	31.275	0.417	24	0.501*	5.696
<i>In situ</i>	22.867	0.538	24	0.865*	3.304
<i>Aspergillus niger</i>	37.868	0.398	24	0.476*	5.790
<i>Trichoderma viride</i>	32.222	0.498	24	0.575*	5.385

* ≥ 0.05

Table 2

Prediction of *in vivo* organic matter digestibility from the results obtained by the *in vitro* and *in situ* methods.

Method	Constant	Variable	n	r	r.s.d.
TILLEY & TERRY	28.210	0.580	24	0.890*	3.380
VAN SOEST	12.290	0.710	24	0.780*	4.670
<i>In situ</i>	23.950	0.590	24	0.910*	3.050
<i>Aspergillus niger</i>	51.420	0.230	24	0.290	7.060
<i>Trichoderma viride</i>	44.030	0.350	24	0.370	6.850

* ≥ 0.05

Table 3

Prediction of *in vivo* D value from the results obtained by the *in vitro* and *in situ* methods.

Method	Constant	Variable	n	r	r.s.d.
TILLEY & TERRY	27.050	0.540	24	0.890*	2.750
VAN SOEST	15.200	0.640	24	0.770*	3.790
<i>In situ</i>	22.370	0.570	24	0.880*	2.830
<i>Aspergillus niger</i>	41.280	0.310	24	0.520*	5.090
<i>Trichoderma viride</i>	37.640	0.370	24	0.550*	4.970

* ≥ 0.05

A significant correlation between the results of the TILLEY & TERRY (1963) method and the *in vivo* digestibility is given by different authors: SCALES et al. (1974) reported an r value = 0.93, KAMASTRA et al. (1973) an r value = 0.95 and NASTIS & MALECHEK (1988) an r value = 0.97. MOLONEY & FLYNN (1992) found an r value = 0.95 for the correlation between the *in vivo* and *in vitro* measurement of the dry matter digestibility. In our study, this method gave a high correlation with a standard deviation lower than the *in situ* method (Table 4) and was thus the most precise estimate.

The GOERING & VAN SOEST (1970) method, gave results correlated, at the 5% level, with the *in vivo* dry matter digestibility; this correlation is lower, especially with the results of organic matter digestibility, in contrast to that reported by different authors. NASTIS & MALECHEK (1988) found an $r^2 = 0.76$ and ALMEIDA et al. (1982) reported that the *in vitro* VAN SOEST digestibility method was the best suited for the prediction of digestibility when the forages have a fiber content higher than 40%.

Table 4

Variance analysis of the results of organic matter digestibility by *in vivo*, *in vitro* and *in situ* methods, for the three stages of growth and two forms of conservation

	n	<i>in vivo</i> (%)	n	TILLEY & TERRY (%)	VAN SOEST (%)	<i>in situ</i> (%)	<i>A. niger</i> (%)	<i>T. viride</i> (%)
Species								
Oat	36	66.67b	18	64.24	75.62	69.89	53.33 a	53.15 a
Italian ryegrass	36	66.69b	18	64.10	73.77	73.33	57.36 b	57.70 b
Perennial ryegrass	36	65.34b	18	65.11	74.23	71.89	61.91 c	62.22 c
Maize	36	62.43a	18	64.21	75.58	67.37	67.37 d	66.26 d
Growth								
Young	48	69.69b	24	71.91 c	76.99 b	79.35 b	60.92 b	60.88 b
Normal	48	67.75b	24	68.20 b	80.08 c	73.93 b	61.60 b	62.04 b
Later	48	58.41a	24	52.98 c	67.32 a	58.58 a	57.47 a	56.58 a
Conservation								
Green	72	67.60b	36	67.57 b	44.57 b	72.59 b	65.41 b	63.23 b
Ensiled	72	62.96a	36	61.15 a	40.38 a	68.65 a	54.58 a	56.43 a
SD		4.746		2.799	3.417	9.262	4.339	3.704
S Specie (Sp.)		*		NS	NS	NS	*	*
Growth (Grow.)		*		*	*	*	*	*
Conservation (Con.)		*		*	*	NS	*	*
Sp. x Grow.		*		*	*	*	*	*
Sp. x Con.		NS		*	*	NS	*	NS
Grow. x Con.		*		NS	*	NS	NS	NS

Note. n - Number of observations; SD - Standard Deviation; S - level of significance ≥ 0.05 ; a, b and c - Within each column, means with the same letter are not significantly different.

The enzymatic method that we used gave results that were significantly correlated, at the 5% level, with the *in vivo* dry matter digestibility, but the correlation is lower. In the prediction of the *in vivo* organic matter digestibility, we did not obtain a significant correlation. MIKA et al. (1982) reported that the pepsin+cellulase method is the one that gave the worst results in the prediction of the *in vivo* digestibility; however, this method gave better results than the chemical methods. These results are in contrast to the reports of different authors. For BUGHRARA et al. (1986), the correlation

between the different cellulase methods and the *in vivo* and *in vitro* methods were more highly significant and had a range between 0.89 and 0.97. PORTUGAL MELO (1983), in a study in which they compared different sources of enzymes, concluded that the *Trichoderma viride* cellulase is the most effective, and gave an r value = 0.996, with the pepsin+cellulase method.

The equations for the utilization of the *in situ* dry matter degradability results for the prediction of *in vivo* dry matter digestibility are given in table 5. The dry matter degradability was determined by the equation:

$$\text{Dry matter degradability (\%)} = \frac{\text{Initial dry matter} - \text{residual dry matter}}{\text{initial dry matter}} \times 100$$

The best results are at the incubation times of 48 and 72 hours. It was with these last two methods, that we found the higher correlation, at the 5% level. GASA et al. (1989) reported an r

value = 0.97 for the prediction of the *in vivo* digestibility, with an incubation time of 48 hours.

Table 5

Prediction equations for the *in vivo* dry matter digestibility from the *in situ* dry matter degradability.

Incubation time (hours)	Constant	Variable	n	r	r.s.d.
0	45.612	0.498	24	0.398*	6.039
1	60.395	0.014	24	0.130	6.526
3	43.912	0.510	24	0.488*	5.744
6	45.971	0.428	24	0.462*	5.839
12	44.730	0.383	24	0.534*	5.563
24	41.321	0.375	24	0.683*	4.808
48	35.228	0.450	24	0.829*	3.680
72	30.086	0.455	24	0.836*	3.614

* ≥ 0.05

Reporting the study of the relationship between some rumen degradation characteristics and the dry matter digestibility. The dry matter degradability (p) at each of a series of incubation times (t) is exponential and can be described, following ØRSKOV et al. (1980), by the equation:

$$p = a + b(1 - e^{-ct})$$

where a , b and c are constants particular to each forage, a is the measure of the rapidly soluble dry matter or protein fraction, b the fraction which is subjected to degradation and c the constant rate of disappearance of fraction b and $(a+b)$ was the potential protein

degradability. The constant a , b and c was estimated by the method of McDONALD (1981).

As report ØRSKOV (1984) we verify a reasonably precise estimate of feed digestibility using the rumen degradation characteristics (Tables 6 and 7). Some authors report better results that we have founded, ØRSKOV (1989) reported a r value = 0.77 from the correlation between $(a + b)$ and digestibility and a r value = 0.85 from the correlation between $(a + b) + c$ and digestibility. KHAZAAL et al. (1993) also have founded highest accuracy for predicting apparent digestibility from nylon bag degradability ($R^2 = 0.89$).

Table 6

Correlation coefficients between degradation characteristics of forages, and dry matter digestibility.

Factors used in regression analysis	Equation			
	n		r	r.s.d
a	24	58.68 + 0.9 a	0.10	6.63
b	24	50.14 + 0.26 b	0.60*	5.32
c	24	58.74 + 72.96 c	0.18	6.55
(a + b)	24	39.53 + 0.3 (a + b)	0.68*	4.92

* ≥ 0.05

Table 7

Multiple correlation coefficients between degradation characteristics of forages, and dry matter digestibility.

Factors used in regression analysis	n	Equation	r	r.s.d.
a + b	24	40.15 + 0.28 a + 0.30 b	0.68*	5.03
(a + b) + c	24	30.74 + 0.35 (a + b) + 160.12 c	0.78*	4.28
a + b + c	24	31.70 + 0.31 a + 0.36 b + 161.24 c	0.78*	4.37

* ≥ 0.05

CONCLUSION

We can assume that the TILLEY & TERRY (1963) method, modified by ALEXANDER & MCGOWAN (1966) and the *in situ* method were equally good for the prediction of the *in vivo* digestibility, although the GOERING & VAN SOEST (1970) method and the enzymatic methods also gave results significantly correlated with *in vivo* digestibility. The TILLEY & TERRY (1963) method, modified by ALEXANDER & MCGOWAN (1966), is a simple method that permits a great number of samples to be tested at the same time.

The *in situ* dry matter degradability also gave a good correlation with the *in vivo* dry matter digestibility; the best incubation times were 48 and 72 hours.

The results would indicate a good potential for predicting the dry matter digestibility from the rumen degradation characteristics of the forages.

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