MONITORING KINETICS OF READILY AVAILABLE FERMENTABLE COMPONENTS BY MEANS OF *IN-VITRO* GAS PRODUCTION

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ABSTRACT
In vitro rumen incubations were used for measuring substrate disappearance and gas production dynamics. Samples were neutral detergent extracts of alfalfa and orchardgrass with or without added glucose. A logistic multi-pool model was used for describing feed fractions with distinct kinetic parameters, namely pools A, B1, B2 and C that represent fractions of increasing refractoriness. This technique based on gas measurement allowed to monitor early fermentation kinetics of readily available substrates thus overcoming the limitations encountered in traditional *in vitro* substrate disappearance. Pools A, B1 and B2 showed specific rates near 0.14 , 0.08 and 0.02 and lags of zero, 3 to 6 and 10 to 18 respectively. These results are very consistent with the expected nutritional behavior of the chemical entities included in each fraction.

KEYWORDS
Rumen, gas production, *in vitro*, forages, kinetics, fermentation rate

INTRODUCTION
Most forage evaluation techniques for assessing ruminal energy supply are based on *in vitro* or *in situ* measurements of substrate disappearance (Tilley & Terry 1963, Goering & Van Soest 1970 and Van Soest 1994) which can measure very efficiently the undigested residues at fairly long fermentation times, usually 48 hours. When used for rate studies the most significant data are those arising between 9 and 36 hours, which interpret most of the fermentable plant cell wall behavior. The values for the early fermentation times are quantitatively very difficult to assess on the basis of substrate disappearance, mainly due to analytical constraints for recovering soluble matters and the significant contribution of inoculant and microbial mass to such small residue disappearance (Mertens, 1993 and Pell & Schofield, 1993). However, in high producing animals the early energy supply may be the most limiting factor that controls nitrogen uptake for microbial protein synthesis at the time that ammonia peaks are observed. An alternative method that allows monitoring early fermentation is the rumen *in vitro* gas production measurement (Menke & Steingass, 1988) based on the balance of carbon metabolism and on the assumption that microbial growth is proportional to the amount of gas produced (Theodorou et al. 1994). This work was undertaken in order to assess the fermentation dynamics of purified plant cell walls alone or supplemented with readily fermentable carbohydrates by means of measuring *in vitro* cell wall disappearance and gas production.

MATERIALS AND METHODS
Purified cell walls of *Medicago sativa* and *Dactylis glomerata* either alone or supplemented with 20% glucose were incubated in *in-vitro* ruminal system (Goering &Van Soest, 1970) during 0, 2, 8, 16, 24, 36, 48, 72 and 96 hr. Gas was measured by the method of Theodorou et al.(1994) using the same media and five sterile bottles of 100ml per treatment. Gas pressure and volume were determined using a gas pressure transducer (Manufactured by IGER, UK) and recording was done every hour during the first 8 hours and then at increasing intervals until 120 hours. Gas production was adjusted by a dual or triple logistic model $V_i = Ý\ [V_p / (1+\exp(2+4S_p(L_p-t_i)))\ ]_n$ where V_i is the gas volume at time t_i, n is the number of pools, V is the maximum volume of a pool, S is the specific rate (S = maximum rate / maximum volume) of the same pool and L the lag time. Subscripts "p" correspond to either pool A, B1 or B2, which denote fractions of increasing refractoriness to rumen fermentation. The variance accounted for was calculated as VAF = [1 - (Residual Mean Square / Total Mean Square)] as described by Draper & Smith (1981). Analysis were performed in PROC NLIN Statistical Analysis System (SAS, 1996)

RESULTS AND DISCUSSION
In vitro cell wall disappearance obtained by the two systems was very similar. In alfalfa and orchardgrass they were 51.5% - 50.1% and 75.6% - 77.6% respectively. Results shown in figure 1 confirm that glucose contributes very significantly to gas production during early stages of fermentation. Actually most of the differences in cumulative gas production due to glucose addition are born in the first hours and maintained along the fermentation period.

The mathematical model to be used should represent the fractions with distinct nutritional behavior, namely soluble sugars in the cell content accounting for a very rapidly fermented fraction (Pool A), pectins, starch, phytoglycogen, fructosans, gluco and galactomannans and labil hemicellulosic and cellulosic components are considered as an insoluble but rapidly fermentable pool (Pool B1), the more refractory cell wall matrix polysaccharide fraction slowly fermented (Pool B2) and a pool of unavailable components made up mainly by lignified cell wall and refractory artifacts (Pool C) which must be subtracted from the fermentable matter for kinetics analysis (Waldo & Smith, 1971). Several models have been discussed by Beuvink & Kogut, (1993) and Schofield et al. (1994) and the non-linear logistic model was chosen in view of the possibility of describing a multi-pool kinetics situation. This model was fitted with two pools when pure cell wall substrate was incubated, and three pools when glucose was added in order to account for the contribution of this early fermenting fraction (table 1).

The estimated total gas production during fermentation was underestimated by 3 to 4% with respect to the observed values. Gas production (G) as a function of cell wall digested (DCW) along time fitted the linear regression G (ml) = -7.48 + 425 (g) DCW with r^2=0.97 and $s_{y.x}$=15.6 . The treatments with pure cell wall as substrate yielded slightly over 50% of the gas under pool B1 with a specific rate of 0.07 to 0.08 . The rate of pool B2 was significantly lower and also very similar in the legume and grass cell wall fermentation. Lag times in orchardgrass were greater than in alfalfa, particularly for pool B2, which is consistent with the known lower fermentation rate of grasses as compared to legumes (Van Soest, 1994). However, lag time of pools B2 were much higher than expected, but it might be explained by a delayed enzymatic hydrolysis that takes place once the more available remaining substrate becomes limiting.

The effect of adding glucose was well partitioned in the model by recognizing a third distinct fraction, called pool A. It contributed near 20% to total gas production and its specific rate was about 0.14 being very similar in the two substrates. Lag of this pool was deliberately set as zero in order to be consistent with the observed gas collection data. From digestibility values and assuming a complete fermentation of the glucose by 96 hr, it can be calculated that glucose inclusion explained 28.5 and 20.5% of the fementable matter in alfalfa and orchardgrass respectively. The amount of

glucose added was closely accounted for by the gas production of pool A in orchardgrass but not so in alfalfa where one third of the gas theoretically derived from glucose did not appear in such pool. It was also observed that treatments with glucose had lower gas production than without glucose in pools B1 and B2 probably due to substrate competition in the *in-vitro* rumen or to other factors that may be limiting the fermentation. In this regard the incubation media was sufficiently buffered for handling 500mg samples without dropping pH. However, addition of glucose had a positive effect on fermentation rates of pools B1 and B2 as well as a reduced lag time in pool B1. These may be due to the beneficial effects of glucose on microbial activity by means of improving the reduction of the incubation media. Kinetic parameters of pool B2 suggest for this fraction a high probability of passage to post-ruminal compartments prior to achieving completing digestion.

The overall view of the results show a remarkable consistency of the data, being the pools clearly differentiated in terms of each parameter. Also, the addition of glucose was satisfactorily represented in size and rate under the strategy of fixing lag time as zero for pool A. Statistical analysis showed very good curve fitting with the models, with an optimum VAF in all of them.

It is concluded that gas production measurement is an effective tool for monitoring early fermentation kinetics in *in vitro* rumen fermentations. Mathematical treatment of data can generate widely different outputs, suggesting that the nutritional meaning of the models should be stressed. In this regard, the question remains open with respect to the shift from an enzyme limited rate situation in early fermentation to a substrate limiting one in later stages, which is particularly relevant to *in vitro* ruminal systems.

REFERENCES

Beuvink, J.M.W. and J. Kogut. 1993. Modelling gas production kinetics of grass silage incubated with buffered ruminal fluid. J. Anim. Sci. **71** : 1041-1046.

Draper, N.R. and H. Smith. 1981. Applied regression analysis. 2nd Ed. J.Wiley & Son, Inc. N.Y.

Goering H. K. and P.J. Van Soest. 1970. Forage Fiber Analysis. ARS-USDA Agric. Hanbook N° 379.

Menke, K.H. and H. Steingass. 1988 Estimation of the energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid. Animal Res.**28**:7.

Mertens, D. R. 1993. Rate and extent of digestion. *In* Quantitative aspects of ruminant digestion and metabolism. Eds. Forbes J.M. and J. France. CAB International. University Press. Cambridge.

Pell A. N. and P. Schofield. 1993. Computerized monitoring of gas production to measure forage digestion *in vitro*. J. Dairy Sci. **76**:1063-1073.

Schofield, P., R.E., Pitt, and A.N. Pell. 1994. Kinetics of fiber digestion from *in vitro* gas production. J. Anim. Sci. 1994. **72**: 2980-2991.

Theodorou M., B. Williams, M. Dhanoa, A. McAllan and J. France. 1994. A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. Animal Feed Scince and Tecnology , (**48**):185-197.

Tilley, J. M. A. , and Terry. 1963. A two-stage technique for the in vitro digestion of forage crops. J. Br. Grassl. Soc. **18**:104-111.

Van Soest, P.J. 1994. Nutritional Ecology of the Ruminant. 2nd.Ed. Comstock Publishing Associates. Cornell University Press.

Waldo, D., L.W.Smith and L. Cox. 1971. Model of cellulose disappearance from the rumen. J. Dairy Sci.. **55** : 125

Table 1
In vitro rumen gas production and fermentation kinetics parameters.

	Alfalfa	Alfalfa + Glucose	Orchardgrass	Orchardgrass + Glucose
OBSERVED GAS PRODUCTION				
(ml/g fermented matter)	406	481	366	385
ESTIMATED GAS PRODUCTION				
Pool A	-	88	-	72
Pool B1	212	203	182	141
Pool B2	183	173	169	156
Total	395	464	351	369
LAG TIME (hr)				
Pool A	-	0	-	0
Pool B1	5.5	3.6	6.6	4.7
Pool B2	10.5	10.9	18.6	14.2
SPECIFIC RATE				
Pool A	-	0.145	-	0.138
Pool B1	0.082	0.095	0.071	0.086
Pool B2	0.023	0.026	0.023	0.026
AF*	0.99	0.99	0.99	0.99

* Variance accounted for.

Figure 1
Cumulative Gas production curve during in-vitro rumen fermentation.