In Vitro Gas Production Technique to Predict DMD of Ensiled Forage Ruminant Based Diets

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IN VITRO GAS PRODUCTION TECHNIQUE TO PREDICT DMD OF ENSILED FORAGE RUMINANT BASED DIETS

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Abstract

The *in vitro* gas production technique was used as a tool to develop an improved prediction model of dry matter digestibility of ensiled forage based diets. Eleven diets were tested through conventional experiments of *in vivo* dry matter digestibility (DMD). The same diets were evaluated by the *in vitro* gas production technique using a gas pressure transducer . The parameters of the model $y = A - B\, Q^{t} Z^{\sqrt{t}}$ were calculated with data from the accumulated gas curves, i.e. y=cumulative gas production (ml), Q=e^{-b}, Z=e^{-c}, B=$e^{bT+c\sqrt{T}}$, being A the value for gas pool size (ml), plus *lag time*, *minimum lag time*, time for 50% gas production and time for 95% gas production. A stepwise linear regression procedure was used to obtain a model with the best fit. The higher ajusted R^2 (0.85) was obtained for a model with: 1. accumulated gas, 2. lag time, 3. Q, 4. B, 5. time for 50% gas production and 6. minimum lag time were included in the model.

Keywords: Gas production; Silage; *In vivo* Digestibility; *In vitro* technique

Introduction

Results obtained from *in vivo* trials for feed quality evaluation are usually required as a reference to develop and to validate less expensive and time consuming procedures. Appart from the *in vitro* DMD technique of Tilley and Terry (1963) and the detergent system of Goering and Van Soest (1970), few other methods had been generally accepted as a standard procedures for quality evaluation of forages.

After the results of Menke et al (1979), the evaluation of gas production *in vitro* was considered one of the most promising procedures, due to its potential relationship with the kinetics of feeds degradation. Summative equations, which includes data from proximal chemical analyses in addition to cumulative gas produced, has been proposed as a means of estimating the energy density of ceratin feeds (Menke and Steingass 1988). However, equations that are able to accurately predict fresh forages and silages *in vivo* DMD from *in vitro* gas produced have still to be developed. The objective of this study was to develop statistical models to estimate the *in vivo* DM digestibility of ruminants-silage-based diets using data from *in vitro* gas production.

Material and Methods

Experimental diets were: 1) CS (corn silage), 2) CS30 (corn silage + 30% concentrate), 3) CS60 (corn silage + 60% concentrate), 4) PS (alfalfa pasture based silage), 5) PSW30 (alfalfa pasture based silage + 30% wheat grain), 6) PSSr30 (alfalfa pasture based silage + 30% sorghum grain), 7) PSCP30 (alfalfa pasture based silage + 30% wet citrus pulp), 8) AS (alfalfa silage), 9) ASW30 (alfalfa silage + 30% wheat grain), 10) ASSr30 (alfalfa silage + 30% sorghum grain), 11) ASCP30 (alfalfa silage + 30% dry citrus pulp). Following procedures outlined by Schneider and Flatt (1975) and using a (3x3) Latin Square design, *in vivo* DMD values of corn silage diet and (4x4) for alfalfa pasture based silage and alfalfa silage diets were obtained. The kinetics of *in vitro* gas production of the diets were measured as suggested by Brooks and Theodorou (1997), using a gas pressure transducer (Theodorou et al 1994). Parameters describing kinetics of gas produced corresponds to those of the model of

France et al (1993). Linear regressions between *in vivo* DMD (***Y***) and accumulated gas production and estimated parameters from France et al (1993) model (***Xi***) were calculated with PROC GLM (SAS System for Windows v6.12, 1989-1996). Stepwise regression was used to select the model giving the best fit. In order to improve the accuracy of the regression analysis all outlier was deleted. The MLP program (Ross, 1987) was used to fit functions to the profiles of gas accumulation using the model of France et al (1993): $y = A - B\, Q^{t}\, Z^{\sqrt{t}}$, where Q=e^{b}, Z=e^{-c}, and B=$e^{bT+c\sqrt{T}}$. Here, y denotes the cumulative gas production (ml), t is the incubation time (h), A is the asymptotic value for gas pool size (ml), T is the lag-time, and b(h^{-1}) and c($h^{-0.5}$) are rate constants. Variables from the model of France et al (1993) are: X_1 (144 h cummulative gas produced), X_2 (Q), X_3 (Z), X_4 (B), X_5 (A), X_6 (*lag time*), X_7 (*minimum lag time*), X_8 (time for 50% gas production) and X_9 (time for 95% gas production).

Results and Discussion

A summary of the linear regression for each individual variable is shown in Table 1. The statistical results from the model including all variables in Table 1 are: SE= 2.797; R^2= 0.89 and R^2 adjusted for d.f. = 0.83, $P<0.001$). The analysis of colinearity showed that X_2 was highly correlated to X_9, X_5 to X_1, and X_2 to X_3 . Therefore, from each pair the variable with the lowest rank of entrance was deleted. After X3 and X5 were deleted, the statistics of the resulting model were SE = 2.626, R^2=0.88, and R^2 adjusted for d.f. = 0.85 (a value 2.4% higher than the 0.83 from the previous model) (Table 2). The variables selected for the best model were: 144 h cummulative gas produced, B, Q, time for 50% gas production, lag and minimum lag time. The entrance of variables in the multiple regression model given by the stepwise procedure is shown in Table 2. Until present time, no reasonably values of the correlation coefficients between total gas production and in vivo DMD of silage based diets are available. Moreover, in order to write down models like these ones, usually no more than the final volume of accumulated gas at end point and/or eventually information from classic chemical feed analysis (CP, NDF, ADF, Ash, etc) have been used. The parameters proposed by France et al (1993) to describe the kinetic of in vitro incubations has only been used to study ruminal microbial attachment process. Results of this research suggest that France´s parameters could be very useful to predict in vivo DMD of silage based diets. However, more than one variable should be entered into the model to obtain an acceptable level of prediction. For silage based diets 6 (out of 9) variables were used. Additional research is needed on the factors who modify the *lag time* and/or accumulated gas at differents times.

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Table 1 - Results of the linear regression for each individual variable.

Variable include in the model	R^2	R^2 adjusted	C(p) Mallows	MSE	SSE
Accumulated gas at 144h	0.55444	0.53588	44.300	21.615	518.8
A	0.55305	0.53442	44.507	21.682	520.4
B	0.55025	0.53151	44.923	21.818	523.6
Q	0.20670	0.17365	96.045	38.484	923.6
time for 95% gas production	0.19726	0.16381	97.450	38.942	934.6
time for 50% gas production	0.06061	0.02147	117.80	45.571	1093.7
Z	0.01558	-0.02544	124.50	47.756	1146.1
lag time	0.01331	-0.02780	124.80	47.866	1148.8
min. lag time	0.00127	-0.04034	126.60	48.450	1162.8

Table 2 - Summary of Stepwise procedure for dependent variable *y*.

Variable entered in the model	R^2 partial	R^2 model	C (p)	Prob > F
Accumulated gas	55.44	55.44	53.2069	0.0001
lag time	18.39	73.83	24.1685	0.0001
Q	8.00	81.84	12.6584	0.0001
B	2.86	84.70	9.8268	0.0001
time for 50% gas production	2.37	87.07	7.8240	0.0001
min. lag time	1.67	88.74	7.000	0.0001