METH 19

Prediction of gas production kinetic parameters of forages by chemical composition and near infrared reflectance spectroscopy

Con formato: Español (España - alfab. internacional)

S. Andrés[1,4]*, A. Calleja[1,4], S. López[1,4], J.S. González[1,4], P.L. Rodríguez[2], F.J. Giráldez[3,4]

[1]Departamento de Producción Animal I, Universidad de León, E-24071 León, Spain

[2]Departamento de Zootecnia, Universidad de Extremadura, 10071 Cáceres, Spain

[3]Estación Agrícola Experimental, CSIC. Apartado 788, E-24080 León, Spain

[4]Unidad Asociada al CSIC Grupo de Nutrición-Praticultura del Departamento de Producción Animal I de la Universidad de León a través de la Estación Agrícola Experimental de León, Spain

*Corresponding author: Sonia Andrés Llorente
Tel.: +34 987 291 235; Fax +34 987 291 311.
E-mail addresses: dp1sal@unileon.es (S. Andrés)

Con formato: Inglés (Reino Unido)

METH 19

Abstract

This study was initiated to evaluate the potential of near infrared reflectance (NIR) spectroscopy to predict in vitro gas production parameters of botanically complex herbage samples. A total of 94 herbage samples harvested in natural meadows located in the mountains near León in Northwest Spain were analyzed to determine their chemical composition. In addition, all herbage samples were incubated in vitro in buffered rumen fluid to determine fermentation kinetics using a gas production technique, and scanned in a spectrophotometer to obtain NIR spectra. Prediction equations showed that NIR spectra could explain a high proportion of the variability ($R^2 > 0.94$) related to some in vitro gas production parameters (e.g., fractional rate of fermentation (c) and extent of degradation in the rumen at different passage rates (ED_{03} and ED_{06})) of the calibration set ($n = 62$). When these NIR equations were applied to the validation set ($n = 32$), most parameters were satisfactorily predicted with standard errors of prediction (SEP) of 3.88 ml for gas production at 24 h of incubation (GP24), 2.71 ml for asymptotic gas production (A), 0.0038 for c, 0.020 for ED_{03} and 0.018 for ED_{06}, accounting for less than 7% of the corresponding mean value. However, lag time (L) could not be predicted by NIR spectroscopy. The SEP was always lower when NIR spectra were used as predictors in comparison with chemical composition, perhaps because spectra contained information about feed constituents as well as physical properties of the samples. Nevertheless, results suggest the need for improved standardisation of this gas production procedure, to minimize the influence of sources of experimental error to increase repeatability and reproducibility, in order to obtain accurate NIR determination of feed fermentation kinetics and of parameter estimates.

Keywords: Gas production; NIR; chemical composition; herbage

Abbreviations: A, asymptotic gas production; ADF, acid detergent fibre; ADIN, acid detergent insoluble N; ADL, acid detergent lignin; c, fractional rate of fermentation; CP, crude protein; d 144, DM disappearance at the end of the fermentation time; ED_{03} and ED_{06}, effective ruminal degradability of DM at different rumen passage rates; GP24 and GP96, gas production at 24 and

96 h of incubation; L, lag time before degradation started; LIG, degree of lignification of the cell wall; MSPE, mean square prediction error; NDF, neutral detergent fibre; NIR, near infrared reflectance; RPD, ratio performance deviation; SEC, standard error of calibration; SE_{CV}, standard error of cross-validation; SEP, standard error of prediction; U^M, U^R and U^D, proportion of MSPE corresponding to the bias, regression and unexplained variance, respectively.

1. Introduction

In vivo digestibility predicts the animal response to a dietary treatment, but cannot describe dynamics of nutrient supply. Thus results are generally restricted to the experimental conditions under which the measurements were made (López et al., 2000). In addition, in vivo digestion studies are expensive and not readily applicable to large numbers of samples, or when small quantities of feedstuff are available. In order to avoid these problems, in vitro and in situ methods have been used extensively in ruminant nutrition studies (Givens and Deaville, 1999). These procedures provide information on fermentation kinetics, which can be incorporated to integrated compartmental models in order to predict events in the rumen (López et al., 2000). However, an in vitro gas production technique (Menke et al., 1979; Menke and Steingass, 1988; Theodorou et al., 1994) is even less animal dependent, and can be automated (Cone et al., 1996), thereby considerably reducing labour needs vs. in situ methods.

Eliminado:

Another technique used in feed evaluation is near infrared reflectance spectroscopy (NIRS), an analytical method that has proved useful in estimating chemical composition (Norris et al., 1976; García-Ciudad et al., 1993; Reeves III, 1997; Pram Nielsen et al., 2001) and in vivo digestibility (Guzmán et al., 1996; Gordon et al., 1998; Naydenova et al., 1998; Park et al., 1998) of a wide range of forages. Use of this procedure to predict gas production parameters of forages is of great interest (Murray, 1993), if it could provide information on ruminal fermentation kinetics in a simple, rapid and accurate method and, most importantly, obviate the necessity of using cannulated animals as donours of rumen liquid. There is, however, little information available examining the accuracy of NIRS prediction equations for gas production parameters.

METH 19

This study was initiated to assess and compare the ability of chemical composition data and NIR spectra to predict gas production parameters of botanically complex herbage samples obtained from natural meadows located in the mountains near León in Northwest Spain.

2. Materials and Methods

2.1. Feed Samples

This research was completed with 94 herbage samples harvested after the spring primary growth, and again after the summer/autumn secondary re-growth, of permanent meadows (i.e., plant communities classified within the vegetation type *Arrhenatheretalia* that according to Rodwell et al (2000) are defined as pastures on well-drained, relatively fertile mineral soils) located in the mountains near León in Northwest Spain at an altitude of 900 to 1450 m. Predominant forage species were *Alopecurus pratensis* L., *Anthoxanthum odoratum* L., *Arrhenatherum elatius* (L.) Beauv. ex J.& K. Presl., *Bromus hordeaceus* L., *Cynosurus cristatus* L., *Dactylis glomerata* L., *Festuca rubra* L., *Holcus lanatus* L., *Lolium perenne* L., *Poa pratensis* L., *Poa trivialis* L., *Trisetum flavescens* (L.) Beauv., *Trifolium pratense* L., *Trifolium repens* L., *Bellis perennis* L., *Carum carvi* L., *Centaurea nigra* L., *Cerastium fontanum* Baumg., *Plantago lanceolata* L., *Ranunculus bulbosus* L., *Rumex acetosa* L., *Taraxacum officinale* G.H. Weber ex Wiggers and *Veronica arvensis* L. The proportion of each species in the herbage samples was highly variable and, due to the three harvest seasons (i.e., spring, summer and autumn) and the various cutting dates within each season, plants were at very different stages of maturity. Samples were oven dried at 60ºC and ground to pass 1 mm screen for subsequent chemical analysis, in vitro gas production determination and NIR spectroscopy.

Eliminado: .

2.2. Chemical Composition

Dry matter (DM, method ID 934.01), ash (method ID 942.05) and crude protein (CP, method ID 984.13) contents were determined by procedures of the AOAC (1999). Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined with the

ANKOM fibre analyzer (Ammar et al., 1999) using the regents described by Van Soest et al. (1991). Sodium sulphite, but not α-amylase, was added to the solution for NDF determination. Acid detergent insoluble N (ADIN) was determined by measuring the N content of the ADF residue by the macro-Kjeldahl procedure noted above.

Eliminado: ..

NDF and CP contents were used to divide the herbage samples into two sets, to obtain similar means and standard deviations in both groups for these two parameters. The calibration set (n = 62) was used to develop the prediction equations and the validation set (n = 32) with samples not included in the calibration set used to validate the prediction equations.

2.3. In Vitro Gas Production Technique

Three sheep fitted with a rumen cannula and fed alfalfa hay (1 to 1.2 kg DM/d) twice daily were used to obtain rumen fluid prior to the morning meal. The rumen fluid was filtered through four layers of gauze, and diluted in the proportion 1:5 (v/v) with a culture medium containing buffer and mineral solutions and prepared anaerobically as described by Van Soest et al. (1966). Incubations were completed in 120 ml serum bottles, weighing 500 mg of each forage sample and dispensing 50 ml of the buffered rumen fluid. Bottles were sealed and placed in the incubator at 39ºC. Gas production was measured at times of 3, 6, 9, 12, 16, 21, 26, 31, 36, 48, 60, 72, 96, 120 and 144 h after inoculation, using a pressure transducer (Theodorou et al., 1994). Two incubation runs were completed in two weeks, the samples of the calibration set were incubated in the first run, and those of the validation set were incubated in the second run. Two serum bottles containing only buffered rumen fluid were incubated in each run as blanks and were used to compensate for gas production in the absence of substrate. Another two serum bottles containing the standard lucerne hay sample were incubated in each batch in order to correct the gas production data of the validation sample set. The correction of the gas volumes for a test sample incubated in a given run is calculated as:

$$GPc = \frac{GP_0 \times LH_C}{LH_V} \qquad (1)$$

METH 19

where: GP_c and GP_0 are the corrected and observed gas volumes for the test sample, respectively, and LH_c and LH_v are the gas volumes recorded for the standard Lucerne hay in the calibration and in the validation incubation runs, respectively. The correction used for gas volumes at each incubation time were based on calculations proposed by Menke and Steingass (1988).

Each forage sample was incubated in duplicate. After the last gas measurement at 144 h, the serum bottles were opened and their contents were filtered using sintered glass crucibles (coarse porosity No. 1, pore size 100 to 160 µm) under vacuum and oven dried at 100ºC for 48 h to estimate potential DM disappearance at d 144.

Gas production profiles were used to estimate fermentation parameters by fitting the exponential model proposed by France et al. (2000) to the cumulative gas production data as:

$$GP = A\left[1 - \exp^{-c(t-L)}\right] \qquad (2)$$

where: GP is the gas production (ml/500 mg DM) at each incubation time (t), A is the asymptotic gas production (ml), c is the fractional rate of fermentation (/h) and L is the lag time before fermentation started (h).

The extent of degradation of DM in the rumen at any defined rumen passage rate (ED_k) was estimated according to France et al. (2000) as:

$$ED_k = \frac{c \times d144}{(c+k)} \exp^{-kL} \qquad (3)$$

where: c and L are as defined in equation (2), k represents the rumen passage rate (/h) and d 144 is potential DM disappearance. The two rumen passage rates used were 0.02/h, representative of a low level of DM intake and 0.06/h, representative of a high level of DM intake.

2.4. Near Infrared Technology

Herbage samples were scanned at 2 nm intervals over the NIR spectral range of 1100 to 2500 nm using a spectrophotometer (Model InfraAlyzer 500, Bran+Luebbe GmbH, Norderstedt, Germany). Samples were scanned twice in duplicate re-packing, resulting in four spectra per sample, using two cells and recording absorbance data as log (1/R), with R being the reflectance.

The mean spectrum was used for each sample and, finally, different mathematical treatments of the spectra based on multiplicative scatter correction (The Unscrambler software version 8.0.5, Camo, Trondheim, Norway) with first or second order derivatives (SESAME software version 2.1, Bran+Luebbe, New York, USA).

2.5. Prediction Equations

When NIR spectra were used to predict gas production parameters, all equations were obtained using partial least square regression by means of SESAME software. A cross-validation was performed to avoid over-fitting of the partial least square regression equations. This procedure to estimate in vitro gas production data was referred to as the direct method.

For prediction of gas production parameters from chemical composition, independent variables were selected by stepwise multiple linear regression using SAS (1999). Once independent variables were selected, a cross-validation was performed using The Unscrambler program. Thus, the standard error of cross-validation (SE_{CV}) could be calculated and, hence, comparison with the NIR equations was possible.

Eliminado: the

Eliminado: it was possible the comparison with

Finally, the equations were tested using the validation set samples. The optimum model for each parameter was selected on the basis of minimising the standard error of prediction (SEP) obtained for the validation set. Moreover, the different components of the mean square prediction error (MSPE) were analyzed by means of Theil decomposition (Theil, 1966) as:

$$SEP^2 = MSPE = \frac{\sum (O-P)^2}{n} = (\bar{O}-\bar{P})^2 + (S_p - rS_O)^2 + (1-r^2)S_O^2$$

where: n represents the number of samples in the validation set, O the reference values, P the NIR predicted values, $\bar{O}$ and $\bar{P}$ the means of the reference and predicted values, S^2_o and S^2_p the variances of the reference and predicted values, and r the coefficient of correlation between reference and estimated values. These three terms are then standardised to derive proportional total MSPE due to bias (U^M), regression (U^R) and unexplained variance (U^D) as:

Eliminado: y

$$\frac{MSPE}{MSPE} = \frac{(\overline{O} - \overline{P})^2}{MSPE} + \frac{(S_P - rS_O)^2}{MSPE} + \frac{(1 - r^2)S_O^2}{MSPE} = U^M + U^R + U^D = 1$$

The concordance correlation coefficient (r_c) for the validation set was calculated as suggested by Dhanoa et al. (1999) as:

$$r_c = \frac{S_O^2 + S_P^2 - S_{(O-P)}^2}{S_O^2 + S_P^2 + (\overline{O} - \overline{P})^2}$$

In contrast to a Pearson correlation coefficient, r_c is a measure of both the linear relationship between the reference and the NIR predicted values as well as the agreement between both of them, indicating the strength of the relationship between both values lying along the equality line, assuming that the intercept is zero and the slope is 1 (i.e., the 45° line through the origin).

In addition, NIR prediction equations were developed to estimate gas volumes at 3, 6, 9, 12, 16, 21, 26, 31, 36, 48, 60, 72, 96, 120 and 144 h of incubation. The NIR spectra of the 32 validation samples were used to estimate incubation time gas volumes, and the exponential model proposed by France et al. (2000) was fitted to the new gas profiles in order to estimate the gas production parameters A, c and L. This procedure to estimate in vitro gas production data was referred to as the alternative method.

3. Results and discussion

Eliminado: ¶

3.1. Relationship between chemical composition and gas production kinetic parameters

The mean values, ranges and standard deviations of the chemical components CP, NDF, ADF, ADL and ADIN and the in vitro gas production parameters A, c, L, GP24, GP96, ED_{03}, ED_{06} and d144 are in Table 1. Chemical composition, fermentation kinetic parameters and extent of degradation in the rumen had considerable variation due to differences among samples in botanical composition (i.e., proportion of grasses and legumes) and maturity. However, mean values, ranges and standard deviations were similar for the calibration and validation sets, with differences that accounted for less than 10% (mean values) and 25% (standard deviations), showing that the sample sets were comparable (Moya et al., 1995).

METH 19

Table 2 shows the matrix of Pearson correlation coefficients between gas production parameters and chemical composition. The correlations were, in general, similar in sign and magnitude for both the calibration and validation sets. Thus, the c parameter had positive correlations with CP, ADIN and ADL contents, and a negative correlation with the NDF content, whereas parameter A had negative correlation coefficients with these chemical fractions.

All correlations could be attributed, in part, to the differing botanical composition of the herbage samples. It is well known that grasses, unlike legumes, have a higher proportion of cell wall, though less lignified, and a lower proportion of cell contents that are readily digestible (Ulyatt, 1981; Van Soest, 1994). In contrast, despite the higher lignification of legume cell walls, rate of fermentation is faster for legumes than grasses, owing to the surface properties of the cell wall of dicotyledons (Donefer, 1970; López et al., 1991). These factors could explain why herbage samples with a higher proportion of grasses, such as herbage from the first cut of the meadows, could have had slower rates of fermentation (c), but higher asymptotic gas production (A). On the contrary, herbage samples with a higher proportion of legumes (i.e., summer and autumn re-growths) showed higher rates of fermentation (c) but lower asymptotic gas production (A). The lower A value of legumes could be due to higher lignin contents and also to higher content of CP that contributes to a lesser extent than carbohydrates to total gas production (Menke and Steingass, 1988; López et al., 1998; Cone and Van Gelder, 2000). All results are consistent with those reported in other comparative studies examining rate and extent of ruminal degradation of temperate legumes and grasses using a gas production technique (Khazaal et al., 1993, 1995; Ammar et al., 1999).

Eliminado: had

3.2. Prediction of gas production parameters

Near infrared spectra and chemical composition data, selected by stepwise multi-linear regression, were used as independent variables to predict gas production kinetic parameters. The accuracy of prediction achieved in each case can be assessed from the statistics in Table 3. With the exception of d 144 and lag time (L), all gas production kinetic parameters, and extent of degradation in the rumen (ED), could be satisfactorily predicted from NIR spectra, with robust calibration equations (R^2 >0.80) and acceptable validation (RPD > 2.5 for GP24, GP96, A and c; RPD > 2.0 for ED). Williams and Sobering (1993) suggested that RPD should be higher than 2.5 in any equation with an acceptable predictability. For these variables, the standard error of calibration (SEC) accounted for less than 5.1%, whereas SE_{CV} and SEP amounted to less than 7% of the mean value of the corresponding parameter.

Cumulative gas production volumes (GP24 and GP96), fractional fermentation rate (c) and extent of DM degradation in the rumen at different assumed passage rates (ED_{03} and ED_{06}) had better calibration statistics when NIR spectra were used as independent variables (Table 3), despite strong correlations between these parameters and chemical composition (Table 2). This could be due to NIR spectra containing information about not only the chemical components (i.e., chemical bonds and functional groups of the compounds), but also about other chemical and physical properties of the samples. NIR spectra can explain a high proportion of the variability related to NDF and CP contents of botanically complex samples (García-Ciudad et al., 1993; Andrés et al., 2005), and are related to other attributes of the herbage material scanned, such as the particle size of the sample and/or the chemical bonds between lignin and hemicelluloses, factors that may have an effect on fermentation kinetics parameters estimated by the in vitro gas production technique. With regard to the asymptotic gas production (A), the statistics were very similar to those for the GP96, and in both cases the best equation of prediction using NIR spectra was that obtained from raw absorbance data without mathematical treatment, probably as a result of the strong correlation between both parameters (r = 0.98; P < 0.0001). However, the lag time (L) could not be predicted by either chemical composition data or the NIR spectra (Table 3).

Eliminado: an

As expected, in comparison to the prediction of the chemical composition of similar forages using NIR spectroscopy technology (García-Ciudad et al., 1993; Andrés et al., 2005), statistics assessing the performance of the calibration equations were not as good when spectra were used to predict some nutritional attributes, such as the rumen fermentation kinetics, estimated by the gas production technique. Parameters measured by biological methods are subject to higher uncontrolled variability due to a multiplicity of sources of experimental and sampling error (e.g., differences between animals, days, incubation runs, replicates) that can affect predictive capability of NIR equations that are highly dependent upon the error of the reference method. Accuracy in the determination of reference values for use in development of NIR prediction equations is critical, as the accuracy of the NIR prediction are only as good as the reference values used for calibration (Coates, 2002). Hence, a higher level of tolerance might be applied when evaluating prediction statistics of rumen fermentation parameters using NIR spectral data. Even so, the lag time (L) could not be successfully predicted by NIR spectra, with poor calibration and validation statistics. The lag phenomenon is not fully understood biologically, and thus the actual definition of lag time is mainly mathematical.

In the present study, samples for calibration and validation were incubated in different batches, and a standard feed (i.e., Lucerne hay) was used to account for possible variation in rumen fluid with time, given the large differences among incubation runs that are usually higher than 10% of the mean, and can be up to 50% (Van Laar et al., 2000). It is possible that the reproducibility of reference values could be improved by incubating feeds in various, rather than single, batches or by using rumen fluid from individual animals so that experimental error, and not just analytical error, is considered.

There are not many results reported that examine the ability of NIR spectra to predict fermentation kinetic parameters estimated by an in vitro gas production technique. Consistent with our results, Goodchild et al. (1998) observed that gas production produced when some forage legumes were incubated in vitro could be predicted accurately ($R^2 > 0.95$; $SE_{CV} = 7.2$ ml/g) by NIR spectra. However, Herrero et al. (1996) found low coefficients of determination ($R^2 = 0.60$ to 0.78) for the cross validation of volumes of gas produced at 6, 12, 24, 36 and 48 h when incubating kikuyu grass in vitro. The calibration and cross-validation of equations using

METH 19

NIR spectra were even poorer for shorter or longer incubation times, whereas the kinetic parameters A, c and L could not be successfully calibrated (Herrero et al., 1996). In a later study, Herrero et al. (1997) considerably improved the prediction of gas volumes at all incubation times by using a spectrally structured sample population for calibration. However, in this study the gas kinetic parameters estimated using various exponential models could not be calibrated and validated using NIR spectra, and so attributed lack of fit of the NIRS equations to the nonlinear nature of some of these parameters (Herrero et al., 1997). However, Fakhri et al. (1999) reported that NIR could accurately predict asymptotic gas production for a variety of concentrate feeds, and Lovett et al. (2004) reported an acceptable ability ($R^2 = 0.60$ to 0.80) to predict kinetics of gas production for maize silage. The discrepancies in all of these studies may be attributed to the different feeds used in each case for development of prediction equations, the number of samples used for calibration and validation and variation in reference values. The number of samples used and the variation in the reference values were higher in the present study than in previous reports, explaining in part the better performance of our NIR prediction equations. In fact, Herrero and Jessop (1998) observed that all gas volumes were more accurately predicted using NIRS when the range of values was extended by using more forages.

Eliminado: w

Herrero et al. (1997) reported better predictions of gas volumes, vs. kinetic parameters, estimated by fitting nonlinear models to gas production data. Therefore they suggested that instead of developing equations to predict those kinetic parameters from NIR spectra, it would be more suitable to calibrate static gas volumes, and then to use predicted values to fit appropriate models to estimate fermentation kinetic parameters. To investigate this approach, NIR prediction equations were developed using our calibration set of samples for cumulative gas volumes at 3, 6, 9, 12, 16, 21, 26, 31, 36, 48, 60, 72, 96, 120 and 144 h of incubation. Gas volumes at these incubation times were then predicted for the 32 samples of the validation set from their NIR spectra, and the exponential model was fitted to the new gas profiles in order to estimate the gas production parameters A, c and L. The statistics resulting from the comparison between the kinetic parameters estimated by fitting either the observed (i.e., actual) or the NIR predicted gas volumes are in Table 4 (i.e., alternative method). Validation statistics and concordance coefficients were satisfactory for all parameters, except L. Comparing results from Tables 3 (direct method) and 4 (alternative method), it seems that the later method resulted in worse predictions of GP24, kinetic parameters A and c, and extent of degradation (ED). This could be due to errors inherent to the two stage process required (i.e., estimation by NIRS of gas volumes with subsequent application of the model proposed by France et al. (2000)). Lag time (L) could not be satisfactorily predicted by any approach, indicating the likely lack of precision in its reference values.

Eliminado:

Eliminado: Both stages

Eliminado:

4. Conclusions

Gas production parameters were more accurately predicted using NIR spectroscopy than chemical composition data, perhaps due to NIR spectra containing information about chemical components as well as the physical properties of the sample. Accuracy of prediction of gas production parameters was not improved when gas volumes at each incubation time were predicted. In general, it seems that standardisation of this gas production procedure to minimise the influence of some sources of experimental error, and increase the repeatability and reproducibility of the measurements and parameter estimates, is critical to improving the prediction of gas production parameters from NIR spectra.

Acknowledgments

S. Andrés is grateful to the “Junta de Castilla y León”, Spain, for financial assistance under a predoctoral grant. The project was funded by the Spanish Ministry of Science and Technology (Project 1FD97-0776).

References

AOAC., 1999. Official Methods of Analysis of the Association of Official Agricultural Chemists. AOAC International, Arlington, VA, USA.

Ammar, H., López, S., Bochi, O., García, R., Ranilla, M.J., 1999. Composition and in vitro digestibility of leaves and stems of grasses and legumes harvested from permanent mountain meadows at different maturity stages. J. Anim. Feed Sci. 8, 599-610.

Andrés, S., Giráldez, F.J., López, S., Mantecón, A.R., Calleja, A., 2005. Nutritive evaluation of herbage from permanent meadows by near infrared reflectance spectroscopy 1.- Prediction of chemical composition and *in vitro* digestibility. J. Sci. Food Agric. [In press].

Coates D.B., 2002. Is near infrared spectroscopy only as good as the laboratory reference values? An empirical approach. Spectroscopy Europe 14/4, 24-26.

Cone, J.W., Van Gelder, A.H., Visscher, G.J.W., Oudshoorn, L., 1996. Influence of rumen fluid and substrate concentration on fermentation kinetics measured with a fully automated time released gas production apparatus. Anim. Feed Sci. Technol. 61, 113-128.

Dhanoa, M.S., Lister, S.J., France, J., Barnes, R.J., 1999. Use of mean square prediction error analysis and reproducibility measures to study near infrared calibration equation performance. J. Near Infrared Spectrosc. 7, 133-143.

Donefer, E., 1970. Considerations in determining the digestibility of harvested forages. In: Barnes, R.F. (Ed), Proceedings of the National Conference on Forage Quality Evaluation and Utilization, Nebraska, USA, pp.Q1-Q6.

Fakhri, S., Moss, A.R., Givens, D.I., Owen, E., 1999. Prediction of in vitro gas production parameters of concentrate feeds by near infrared reflectance spectroscopy (NIRS). Proc. Br. Soc. Anim. Sci. p. 115.

France, J., Dijkstra, J., Dhanoa, M.S., López, S., Bannink, A., 2000. Estimating the extent of degradation of ruminant feeds in vivo from a description of their gas production profiles observed in vitro. 1. Derivation of models and other mathematical considerations. Brit. J. Nutr. 83, 143-150.

García-Ciudad, A., García-Criado, B., Pérez-Corona, M.E., Vázquez de Aldana, B.R., Ruano-Ramos, A., 1993. Application of near infrared reflectance spectroscopy to chemical analysis of heterogeneous and botanically complex grassland samples. J. Sci. Food Agric. 63, 419-426.

Givens, D.I., Deaville, E.R., 1999. The current and future role of near infrared reflectance spectroscopy in animal nutrition: a review. Aust. J. Agric. Res. 50, 1131-1146.

Goodchild, A.V., El Haramein, F.J., Abd El Moneim A., Makkar, H.P.S., Williams, P.C., 1998. Prediction of phenolics and tannins in forage legumes by near infrared reflectance. J. Near Infrared Spectrosc. 6, 175–181.

Gordon, F.J., Cooper, K.M., Park, R.S., Steen, R.W.J., 1998. The prediction of intake potential and organic matter digestibility of grass silages by near infrared spectroscopy analysis of undried samples. Anim. Feed Sci. Technol. 70, 339-351.

Guzmán, J.L., Garrido, A., Gómez-Cabrera, A., Guerrero, J.E., Murray, I., 1996. Use of NIRS to estimate the improvement in digestibility after ammonia treatment in cereal straws. Anim. Feed Sci. Technol. 57, 149-157.

Herrero, M., Jessop, N.S., 1998. Broad-based calibrations of in vitro gas production of forages by near infrared reflectance spectroscopy. In: Deaville, E.R., Owen, E., Adesogan, A.T., Rymer, C., Huntington, J.A., Lawrence, T.L.J. (Eds), In Vitro Techniques for Measuring Nutrient Supply to Ruminants. Occasional Publication No. 22. British Society of Animal Science, Midlothian, UK, pp.234-237.

Herrero, M., Murray, I., Fawcett, R.H., Dent, J.B., 1996. Prediction of the in vitro gas production and chemical composition of kikuyu grass by near infrared reflectance spectroscopy. Anim. Feed Sci. Technol. 60, 51-67.

Herrero, M., Jessop, N.S., Fawcett, R.H., Murray, I., Dent, J.B., 1997. Prediction of the in vitro gas production dynamics of kikuyu grass by near infrared reflectance spectroscopy using spectrally structured sample populations. Anim. Feed Sci. Technol. 69, 281-287.

Khazaal, K., Dentinho, M.T., Ribeiro, J.M., Ørskov, E.R., 1993. A comparison of gas production during incubation with rumen contents in vitro and nylon bag degradability as predictors of apparent digestibility in vivo and the voluntary intake of hays. Anim. Prod. 57, 105-112.

Khazaal, K., Dentinho, M.T., Ribeiro, J.M., Ørskov, E.R., 1995. Prediction of apparent digestibility and voluntary intake of hays fed to sheep: comparison between using fibre components, in vitro digestibility of characteristics of gas production or nylon bag degradation. Anim. Prod. 61, 527-538.

López, S., Carro, M.D., González, J.S., Ovejero, F.J., 1998. Comparison of different in vitro and *in situ* methods to estimate the extent and rate of degradation of hays in the rumen. Anim. Feed Sci. Technol. 73, 99-113.

López, S., Carro, M.D., González, J.S., Ovejero, F.J., 1991. Rumen degradation of the main forage species harvested from permanent mountain meadows in North-western Spain. J. Agric Sci. (Cambridge) 117, 363-369.

López, S., Dijkstra, J., France, J., 2000. Prediction of energy supply in ruminants, with emphasis on forages. In: Givens, D.I., Owen, E., Axford, R.F.E., Omed H.M. (Eds), Forage Evaluation in Ruminant Nutrition. CAB International, Wallinford, pp.63-94.

Lovett, D.K., Deaville, E.R., Mould, F., Givens, D.I., Owen, E., 2004. Using near infrared reflectance spectroscopy (NIRS) to predict the biological parameters of maize silage. Anim. Feed Sci. Technol. 115, 179-187.

Menke, K.H., Steingass, H., 1988. The estimation of the energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid. Anim. Res. Dev. 28, 7-55.

Menke, K.H., Raab, L., Salewski, A., Steingass, H., Fritz, D., Schneider, W., 1979. The estimation of the digestibility and metabolizable energy content of ruminant feedstuffs from gas production when they are incubated with rumen liquor in vitro. J. Agric. Sci. (Camb.) 93, 217-222.

Moya, L., Garrido, A., Guerrero, J.E., Lizaso, J., Gómez, A., 1995. Quality control of raw materials in the compound feed industry. In: Batten, G., Flinn, P.C., Welsh, L.A., Blakeney, A.B. (Eds), Leaping Ahead with Near Infrared Spectroscopy. Royal Australian Chemical Institute, Melbourne, Australia. pp.111-116.

Murray, I., 1993. Forage analysis by near infrared reflectance spectroscopy. In: Davies, A., Baker, R.D., Grant, S.A., Laidlaw, A.S. (Ed), Sward Management Handbook. British Grassland Society, Reading, UK. pp.285-312.

Naydenova, Y., Tomov, P., Dardenne, P., 1998. Near infrared spectroscopy estimation of feeding value of forage perennial grasses in breeding programmes by global and specific calibrations. Estimation of chemical composition and digestibility. J. Near Infrared Spectrosc. 6, 153-165.

Norris, H.K., Barnes, R.F., Moore, J.E., Shenk, J.S., 1976. Predicting forage quality by near infrared reflectance spectroscopy. J. Anim. Sci. 43, 889-897.

Park, R.S., Agnew, R.E., Gordon, F.J., Steen, R.W.J., 1998. The use of near infrared reflectance spectroscopy (NIRS) on undried samples of grass silage to predict chemical composition and digestibility parameters. Anim. Feed Sci. Technol. 72, 155-167.

Pram Nielsen, J., Bertrand, D., Micklander, E., Courcoux, P., Munck, L., 2001. Study of NIR spectra, particle size distributions and chemical parameters of wheat flours: a multiway approach. J. Near Infrared Spectrosc. 9, 275-285.

Reeves III, J.B., 1997. The use of mid infrared diffuse reflectance spectroscopy to determine quantitatively the chemical composition of tall fescue. J. Dairy Sci. 80, 2454-2465.

Eliminado: w

Rodwell, J.S., Dring, J.C., Averis, A.B.G., Proctor, M.C.F., Malloch, A.J.C., Schaminée, J.H.J., Dargie, T.C.D., 2000. Review of coverage of the National Vegetation Classification. JNCC Report, No. 302. Joint Nature Conservation Committee, Peterborough.

SAS, 1999. SAS/STAT® User's Guide (Version 8). SAS Publishing, Cary, NC, USA.

Theil, H., 1966. Applied Economic Forecasting. North Holland Publishing Company, Amsterdam, Netherlands.

Theodorou, M.K., Williams, B.A., Dhanoa, M.S., McAllan, A.B., France, J., 1994. A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. Anim. Feed Sci. Technol. 48, 185-197.

Ulyatt, M.J., 1981. The feeding value of temperate pastures. In: Morley, F.H.W. (Ed), Grazing Animals. World Animal Science, B-1. Elsevier Scientific Publishing Company, Amsterdam, Netherlands, pp.125-141.

Van Gelder, A.H., Cone, J.W., 2000. Influence of protein fermentation on gas production profiles. In: Gas Production: Fermentation kinetics for feed evaluation and to assess microbial activity. Occasional Publication of the British Society of Anim. Science, Edinburgh, pp.23-24.

Van Laar, H., Van Straalen, W., Cone, J., Van Gelder, T., de Boever, J., D'heer, B., Vedder, H., de Bot, P., Van Hees, J., 2000. Repeatability and reproducibility of the automated gas production test. In: Gas Production: Fermentation kinetics for feed evaluation and to assess microbial activity. Occasional Publication of the British Society of Animal Science, Edinburgh, UK**.** pp.31-32.

Van Soest, P.J., 1994. Nutritional Ecology of the Ruminant. Cornell University Press, Ithaca, New York, USA.

Van Soest, P.J., Wine, R.J., Moore, L.A., 1966. Estimation of the true digestibility of forages by the in vitro digestion of cell walls. Proceedings of the 10th International Grassland Congress, Helsinki, Finland. pp.438-441.

Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583-3597.

Williams, P.C., Sobering, D.C., 1993. Comparison of commercial near infrared transmittance and reflectance instruments for analysis of whole grains and seeds. J. Near Infrared Spectrosc. 1, 25-32.

Table 1

Range, mean and standard deviation of the chemical data (ash, CP, NDF, ADF, ADL and ADIN) and gas production parameters of the calibration and validation sets

	Calibration set (n=62)			Validation set (n=32)		
	Range	Mean	SD	Range	Mean	SD
Chemical parameters (g/kg DM)						
Ash	61 – 201	93	20.5	65 – 149	98	20.3
CP	52 – 179	118	28.1	58 – 168	121	33.4
NDF	359 – 684	518	77.9	392 – 673	484	92.9
ADF	210 – 383	290	40.6	222 – 422	276	52.0
ADL	11.2 – 55.4	31.5	10.16	16.4 – 63.5	43.8	13.69
ADIN	0.49 – 4.45	2.23	1.113	0.4 – 5.5	3.13	1.580
Gas parameters						
GP24 (ml)	78 – 128	104	11.0	84 – 121	109	10.4
GP96 (ml)	125 – 160	146	7.4	127 – 160	145	7.1
d144	0.68 – 0.82	0.74	0.031	0.63 – 0.77	0.73	0.028
A (ml)	125 – 163	147	7.9	129 – 161	146	7.6
c (/h)	0.030 – 0.080	0.055	0.0118	0.037 – 0.079	0.063	0.0134
L (h)	0.09 – 1.77	1.06	0.357	0.14 – 1.46	0.81	0.309
ED_{03}	0.36 – 0.58	0.46	0.047	0.37 – 0.52	0.48	0.041
ED_{06}	0.23 – 0.44	0.33	0.045	0.25 – 0.39	0.35	0.040

GP24 and GP96: gas production (ml/500 mg DM) at 24 and 96 h of incubation; d 144: potential DM disappearance at the end of the fermentation time; A: asymptotic gas production; c: fractional rate of fermentation; L: lag time before degradation started; ED_{03} and ED_{06}: effective ruminal degradability of DM at different rumen passage rates (0.03 and 0.06/h)

Table 2

Coefficients of Pearson correlation between some chemical data (CP, NDF, ADL and ADIN) and the gas production parameters and the

	Calibration set (n=62)				Validation set (n=32)			
	CP	NDF	ADL	ADIN	CP	NDF	ADL	ADIN
GP24	0.578	-0.798	0.136	0.468	0.607	-0.843	0.554	0.604
GP96	-0.365	0.273	-0.645	-0.566	-0.508	0.292	-0.568	-0.574
D 144	0.410	-0.297	-0.435	-0.048	0.160	-0.128	-0.392	-0.201
A	-0.503	0.436	-0.685	-0.670	-0.616	0.431	-0.657	-0.677
c	0.783	-0.952	0.456	0.774	0.817	-0.951	0.805	0.866
L	0.212	0.022	-0.046	0.095	0.496	-0.384	0.489	0.577
ED_{03}	0.772	-0.875	0.178	0.572	0.784	-0.878	0.507	0.637
ED_{06}	0.769	-0.909	0.255	0.625	0.800	-0.914	0.588	0.695

GP24 and GP96: gas production at 24 and 96 h of incubation; d 144: potential DM disappearance at the end of the fermentation time; A: asymptotic gas production; c: fractional rate of fermentation; L: lag time before degradation started; ED_{03} and ED_{06}: effective ruminal degradability of DM at different rumen passage rates (0.03 and 0.06/h)

Calibration set: $P < 0.05$ if $|r| > 0.250$; $P < 0.01$ if $|r| > 0.325$; $P < 0.001$ if $|r| > 0.408$
Validation set: $P < 0.05$ if $|r| > 0.349$; $P < 0.01$ if $|r| > 0.448$; $P < 0.001$ if $|r| > 0.553$

METH 19

Table 3

Prediction of gas production parameters by chemical data and NIRS technology (direct method)

Y	X variables		R^2	SEC	SE_{CV}	SEP	U^M	U^R	U^D	RPD	r_c
GP24	Chemical data	NDF, Ash, ADL	0.789	5.022	5.579	6.757	0.393	0.135	0.472	1.539	0.823
	NIRS technology	NIR spectra (MSC+2D), p = 3	0.889	3.648	4.319	3.877	0.000	0.223	0.777	2.673	0.936
GP96	Chemical data	Ash, ADL, LIG, CP	0.767	3.542	3.851	3.910	0.386	0.090	0.524	1.816	0.861
	NIRS technology	NIR spectra (log(1/R)), p = 6	0.827	3.052	3.761	2.416	0.032	0.002	0.967	2.898	0.934
d144	Chemical data	ADL, LIG	0.607	0.019	0.020	0.022	0.011	0.091	0.899	1.296	0.665
	NIRS technology	NIR spectra (2,10,5), p = 6	0.667	0.019	0.023	0.019	0.045	0.041	0.914	1.485	0.743
A	Chemical data	Ash, ADL, LIG, CP	0.819	3.328	3.624	3.716	0.483	0.092	0.426	2.045	0.895
	NIRS technology	NIR spectra (log(1/R)), p = 5	0.801	3.492	4.012	2.711	0.098	0.027	0.875	2.797	0.926
c	Chemical data	NDF	0.906	0.0036	0.0037	0.0043	0.111	0.094	0.796	3.116	0.945
	NIRS technology	NIR spectra (2,4,4), p = 4	0.941	0.0028	0.0038	0.0038	0.000	0.256	0.744	3.351	0.959
L	Chemical data	-	-	-	-	-	-	-	-	-	-
	NIRS technology	NIR spectra (MSC+2D), p = 3	0.597	0.225	0.267	0.288	0.528	0.000	0.472	1.074	0.569
ED_{03}	Chemical data	NDF, ADL	0.848	0.019	0.019	0.021	0.224	0.125	0.651	1.964	0.882
	NIRS technology	NIR spectra (2,6,4), p =6	0.941	0.012	0.016	0.020	0.009	0.357	0.634	2.015	0.898
ED_{06}	Chemical data	NDF, ADL	0.875	0.016	0.017	0.020	0.288	0.154	0.558	2.006	0.891
	NIRS technology	NIR spectra (2,6,4), p = 6	0.953	0.010	0.014	0.018	0.000	0.368	0.632	2.275	0.919

GP24 and GP96: gas production at 24 and 96 h of incubation; d 144: potential DM disappearance at the end of the fermentation time; A: asymptotic gas production; c: fractional rate of fermentation; L: lag time before degradation started; ED_{03} and ED_{06}: effective ruminal degradability of DM at different rumen passage rates (0.03 and 0.06/h); NIR spectra (-, -, -) = pre-treatment of the NIR spectra, where the first number is the derivative order, the second number is the gap between points used to calculate the difference and the last one is the number of data points used to smooth the data; MSC = multiplicative scatter correction; 2D = second order derivative; R = reflectance; p = number of terms in the equation; LIG = degree of lignification of the cell wall calculated as ADL/NDF; R^2 = coefficient of determination; SEC = standard error of calibration; SE_{CV} = standard error of cross-validation; SEP = standard error of prediction; U^M, U^R and U^D = proportion of MSPE corresponding to the bias, regression and unexplained variance, respectively; RPD = ratio performance deviation (SDref data/SEP); r_c = concordance correlation coefficient

METH 19

Table 4

Gas production parameters obtained when the model proposed by France et al. (2000) was fitted to the gas production volumes estimated by NIRS (alternative method) for the validation set (n = 32)

Y	R^2	SEP	U^M	U^R	U^D	RPD	r_c
GP24	0.866	5.161	0.061	0.416	0.523	2.016	0.901
A	0.821	3.183	0.001	0.013	0.986	2.389	0.895
c	0.880	0.0051	0.001	0.273	0.727	2.643	0.929
L	0.459	0.376	0.513	0.132	0.355	0.822	0.499
ED03	0.918	0.016	0.121	0.375	0.505	2.010	0.934
ED06	0.912	0.016	0.181	0.311	0.507	2.068	0.929