A comparison of near infrared spectroscopy with neutral detergent cellulase techniques to predict the *in vivo* digestibility of grass silages

Begoña De La Roza,[1] Adela Martínez,[1] Sagrario Modroño,[1] Gonzalo Flores[2] and Alejandro Argamentería[1]

[1]*Servicio Regional de Investigación y Desarrollo Agroalimentario, Apdo. 13, 33300 Villaviciosa, Spain. E-mail: begonard@princast.es.*

[2]*Centro de Investigaciones Agrarias de Mabegondo, Apdo 10, 15080 Coruña, Spain.*

Introduction

During the last two decades, all studies using near infrared (NIR) reflectance spectroscopy to predict animal response have demonstrated the usefulness of this technique. NIR predictions would be limitated more by the accuracy of the animal data than from the capability of extracting spectral information.[1–3]

Grass silage is the recommended method of forage conservation for winter and summer feeding in wet Spanish regions. Some silages may have a good analytical and enzymatic organic matter digestibility but fail to perform due to unpredictable problems[4] (botanical compost composition, different fermentation patterns, etc.) that have influence on *in vivo* organic matter digestibility (OMD) and, consequently, metabolisable energy.

Most laboratory methods (chemical and enzymatic) for estimating the OMD[3] have a lower predictive ability than NIR, normaly adjusting analytical values using a linear regression based on similar samples of known OMD. It is desirable for NIR to be used to directly predict OMD in order to improve animal feeding strategies.

The purpose of this study was to compare directly predicted OMD results using NIR techniques with those results obtained from estimated regression equations for in vivo and neutral detergent cellulase organic matter digestibility (EOMD), using grass silages from the northwest of Spain, to enable us to offer a good advisory service.

Material and methods

Silages studied

A total of 204 grass silages samples ensiled with different additives, with or without wilting and using diverse machinarie with know *in vivo* digestibillity, originating from feeding trials, using rams and cows how experimental animals, at the center in the course of the last decade and from exchange with CIAM-Mabegondo, were selected to develop calibration and validation sets.

Laboratory methods

The enzymatic digestibility (EOMD)[7] comprises two steps: pretreatment with neutral detergent solution and incubation with cellulase for 24 h at 40ºC. It was conducted in duplicate on the freeze-dried or oven-dried subsamples and milled to 0.75 mm.

NIR scanning and calibration procedures

For NIR measurements, the ground samples were scanned, using an NIRSystems 6500 scanning monochromator (NIRSystems, Inc., Silver Spring, MD, USA) over a wavelength range from 1100 to 2500 nm. Spectra were collected as log 1/*R*. Population boundaries were established with a maximum standardised *H* distance from the average spectrum of 3.0.[8] Four of the samples were identified from NIR spectra as outliers: a grass silage from natural pasture and another three grass silages from gramineae pasture. These outliers were discarded. Calibration equations were obtained by WINISI II software (Infrasoft International, Port Matilda, PA, USA), using a full wavelength range every 6th wavelength using modified partial least squares as the regression method.[9]

First, global calibrations with total samples (n = 200) were obtained and each equation selected was evaluated according to the lowest standard errors [standard error of calibration (*SEC*) and standard error of cross-validataion (*SECV*), respectively] and the highest coefficients of determination (R^2 and CVR^2).

Second, a procedure was used, based on neighbourhood distances,[10] to establish the calibration (n = 150) and validation (n = 50) populations. The best combination was selected on the basis of the lowest standard error of prediction (*SEP*).

Results and discussion

The EOMD and OMD values of the silages on total population are given in Table 1. The results show that the grass silage population was very variable and this distribution of data showed the different nutritive quality of silage.The NIR calibration equations were developed for OMD and EOMD. In both cases, the second derivative transformation of the spectral data produced the most acceptable equations. Calibration statistics associated with the selected equations are presented in Table 1. The coefficient of determination (R^2) between *in vivo* values and NIR spectra was 0.86 and between laboratory values and NIR spectra 0.94. The standard errors of cross-validataion (*SECV*) were consistenly better for the EOMD. These results showed the variation associated with animal measurements.

The relationship between OMD and EOMD to estimate grass silage digestibility was developed by simple linear regression (Table 4). The regression analysis indicated a low adjusted R^2 (0.51), although the use of a cellulase-based method offers a good alternative.[11] The results agree with those reported by

Table 1. Range of reference values and NIRS calibration statistics (n = 200) of *in vivo* organic matter digestibility (OMD) and enzymatic organic matter digestibility (EOMD).

Parameters	Range	*SD*	*SEC*	R^2	*SECV*	CVr^2	Range/*SECV*	*SD*/*SECV*
OMD	46.9–78.4	6.9	2.57	0.86	2.82	0.84	11.18	2.47
EOMD	31.3–81.0	8.9	2.16	0.94	2.49	0.92	19.94	3.59

SD: Standard deviation
SEC: Standard error of calibration
R^2 and CVr^2: Determination coefficients on the calibration and the validation sets respectively
SECV: Standard error of cross validation
range/*SECV* and *SD*/*SECV*: ratio of the *SECV* to the range and *SD* of the reference datas respectively

other observations,[11,12] one reason being that *in vivo* measurements of OMD were obtained using sheep in some cases and cattle in others. The validity of applying digestibility predictions based on sheep data to cattle diets is often debated,[13] although the problem is more serious with cereal grains than with forages.[13] The mean of OMD values estimated was higher than the reference values. It was noted that these differences were greater between the higher and lower grass silage digestibility.

In our analytical routine for fresh and preserved forages we use an equation to obtain metabolisable energy (ME) value in MJ kg^{-1} DM.[14] It is predicted from EOMD on the basis of specific regressions from each forage population.[7] The EOMD values were obtained by NIR measurement.

To summarise, although the calibrations statistics associated with OMD are worse than the stadistics associated with EOMD, the total error to predict OMD was much lower if the prediction was made directly using NIR equations based on animal dates. A standard procedure for estimating ME incorporated the standard error of NIR measurements for EOMD, the standard error of linear regression to estimate OMD and the standard error of the model.

To see the real differences between OMD values estimate by NIR or by linear regression in base on EOMD, the total population was divided into two sets, a calibration set of 150 samples and the remaining 50 samples were reserved for validation purposes. This has not been usual for traditional methods because of the shortage of *in vivo* data. The range in OMD and EOMD are listed in Table 2. The results obtained for the NIR equations are show in Tables 2 and 3. Again, the second derivative transformation of the spectral data produced the most acceptable equations and the enzymatic method gave the highest R^2 and lowest *SECV* and *SEP*. Figure 1 shows the relationship, for the validation set, between OMD predicted and *in vivo* OMD and Figure 2 shows the relationship between EOMD predicted and laboratory EOMD.

Table 2. Range of reference values and NIRS calibration statistics (*n* = 150) of *in vivo* organic matter digestibility (OMD) and enzymatic organic matter digestibility (EOMD).

Parameters	Range	*SD*	*SEC*	R^2	*SECV*	CVr^2	Range/*SECV*	*SD*/*SECV*
OMD	46.9–78.4	7.2	2.58	0.87	3.45	0.78	9.14	2.10
EOMD	31.3–81.0	9.7	2.46	0.94	2.79	0.92	17.82	3.48

SD: Standard desviation
SEC: Standard error of calibration
R^2 and CVr^2: Determination coefficients on the calibration and the validation sets respectively
SECV: Standard error of cross validation
Range/*SECV SD/SECV:* Ratio of the SECV to the Range and SD of the reference datas respectively

Table 3. Range of validation set and validation statistics (*n* = 50) for in vivo (OMD) and enzymatic (EOMD) organic matter digestibility of grass silages.

Parameters	Range	*SD*	*SEP*	Bias	R^2
OMD	46.9–76.1	6.3	3.01	-0.57	0.79
EOMD	48.4–77.4	6.8	2.29	-0.02	0.89

SD: Standard desviation
SEP: Standard error of prediction
R^2: Determination coefficient for prediction set;

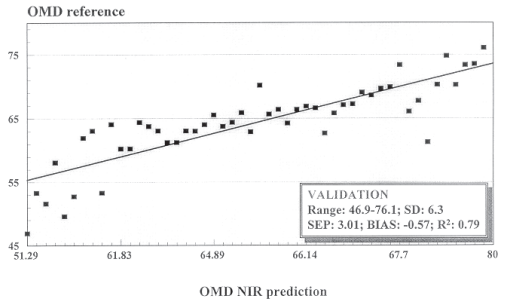

Figure 1. NIR validation statistics (*n* = 50) between *in vivo* and NIR prediction organic matter digesibility (OMD) of grass silages.

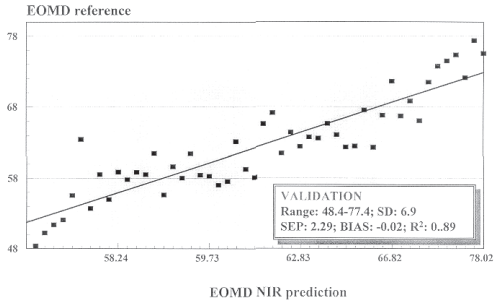

Figure 2. NIR validation statistics (*n* = 50) between *in vivo* and NIR prediction enzymatic organic matter digesibility (EOMD) of grass silages.

Table 4. Relationship between *in vivo* organic matter digestibility (OMD) and enzymatic organic matter digestibility (EOMD).

Digestibility procedures	Mean	Range	samples n°	Prediction equation	*RMSE*	R^2
OMD	65.4	46.9-78.4	200	OMD = 31.58 + 0.56 × EOMD	4.96	0.51
EOMD	60.9	31.3-81.0				
OMD	65.8	46.8-78.4	150	OMD = 31.92 + 0.56 × EOMD	4.89	0.56
EOMD	60.3	31.3-81.0				

Furthermore, a simple linear regression (Table 4) between OMD and EOMD on the 150 grass silages was developed. The results are similar to those reported using 200 samples, low adjusted R^2 (0.56).

Conclusions

Considering the opportunities to obtain representative *in vivo* OMD values, NIR can develop robust prediction equations with low *SECV* or *SEP* values, to use for routine advisory application on feed evaluation. For grass silages evaluation, the use of laboratory methods (EOMD in this case) can be less accurate than NIR procedures. In terms of accuracy and speed, the NIR technique have replaced EOMD because it can provide more precise information because it is directly linked to specific sample attributes.

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