Prediction of fiber fractions, ash and organic matter digestibility in untreated maize stover by near infrared reflectance spectroscopy

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ABSTRACT

Near infrared reflectance (NIR) spectroscopy was used to predict acid and neutral detergent fibre (ADF and NDF), ash, and organic matter digestibility (OMD) in maize stover samples. Samples were analyzed by reference laboratory methods and spectra collected using a NIR spectrophotometer in reflectance (1100 – 2500 nm). Predictive equations were developed using partial least squares (PLS) with full cross validation using second derivative. The coefficient of determination in calibration ($R^2$) and the standard error in cross validation (SECV) were for ADF 0.85 (SECV: 16.5 g kg$^{-1}$ DM), for NDF 0.92 (SECV: 58.4 g kg$^{-1}$ DM), for ash 0.90 (SECV: 1.9 g kg$^{-1}$ DM) and for OMD 0.85 (SECV: 10.4 g kg$^{-1}$ DM). The results from this study suggested that maize stover samples might be analyzed by NIR spectroscopy in order to determine ADF, NDF and ash. However, the prediction accuracy is less than desirable to be used for routine analytical purposes.

RESUMEN

El uso de la reflectancia en el infrarrojo cercano (NIR) fue evaluada para la determinación de fibra detergente neutro (FDN), fibra detergente ácida (FDA), cenizas y digestibilidad de la materia orgánica (DMO) en rastrojo de maíz. Las muestras se analizaron usando métodos de referencia y los espectros se obtuvieron usando un instrumento NIR (1100 – 2500 nm). Las ecuaciones de predicción fueron desarrolladas usando el método de mínimos cuadrados (PLS), validación cruzada y segunda derivada. Los coeficientes de determinación ($R^2$) en calibración y el error standard de la validación cruzada (SECV) fueron para FDA 0.85 (SECV: 16.5 g kg$^{-1}$ DM), para FDN 0.92 (SECV: 58.4 g kg$^{-1}$ DM), para cenizas 0.90 (SECV: 1.9 g kg$^{-1}$ DM) y para DMO 0.85 (SECV: 10.4 g kg$^{-1}$ DM). Los resultados sugieren que la técnica NIRS puede determinar FDA, FDN, y cenizas. Sin embargo la precisión no es buena para ser usada en análisis de rutina.

Palabras clave: rastrojo maíz, NIR, valor nutritivo.

INTRODUCTION

Forages provide the basis for most of the ruminant production systems, where extensive production from grassland is the major land use for animal production (Church, 1991; Arengheore, 2000; Wilkins, 2000). Shortages of feed resources often impose major constraints on the improvement of animal production systems in developing countries and it is well known that the plant species, phenology and condition at harvest dictate the potential quality of the forage for animal end use (Arengheore, 2000; Wilkins, 2000). Annual forage crops must have particular characteristics in order to warrant their use in animal production systems, in particular related to factors such as seasonality of yield and product quality, providing with both high nutritive value and high yields per unit of land (Church, 1991).

Maize (Zea mays L.) is one of the most important cereal crops in the world, primarily grown for its grain that is utilised as human food. This crop also domina-
iates the world’s total production of crop residues (Bwire and Wiktorsson, 2002; NRC, 1983; Tolera et al., 1998). The proportion of stover in the maize plant represents about 50% or more of the whole plant biomass and most of the fiber constituents, are much less digestible than the fiber contained in the grain (Wei et al., 2009). It has been reported that both yield and quality traits of maize stover are all very important when maize is used as forage (Wei et al., 2009). However, due to lack of technical know-how maize crop residues such as stover are often wasted or underutilised by the farmers in Uruguay (NRC, 1983).

Near infrared reflectance (NIR) spectroscopy is being widely adopted for routine analysis of forages, but requires consistent sample handling and a calibration method that relates spectral data (NIR absorption) with laboratory reference method information through regression models (Shenk and Westerhaus, 1993). Calibration is the key to successful use of the NIR technique and there are a number of essential steps required to develop a calibration including sample selection, acquisition of spectra and reference data, pre-treatment of spectral data, derivation of the regression model and validation of the model. However, both the processing of the sample (e.g. grinding, drying) and presentation of the sample to the instrument are important factors in the robustness and accuracy of NIR as analytical techniques (Deaville and Flinn, 2000; Murray and Cowe, 2004). NIR has been widely used for composition analysis of several forages and crops where the low cost of analysis in comparison with traditional chemical analysis has been one of the most important advantages of this technique (Deaville and Flinn, 2000; Murray and Cowe, 2004). For example, it has been reported that the cost of commercial analysis of grain materials using NIR is about 25% lower than that using routine chemical feed analysis (Tao et al., 2013; Xu et al., 2013).

The objective of this study was to evaluate the potential use of NIR spectroscopy as rapid and low cost tool for the routine analysis of the nutritive value of maize stover samples sourced from farms in Uruguay.

MATERIALS AND METHODS

Plant materials used were composed of maize stover samples (n = 61), collected from commercial farms in Uruguay, during two consecutive harvests, representing different maize plant materials (hybrids, varieties, as well as different maturities). Uruguay (34°S, 57°W) (South West) is located entirely within the temperate zone where average high and low temperatures in summer (January) are 28 °C and 17 °C, and in winter (July) are 14 °C and 6 °C, respectively. Rainfall is relatively evenly distributed throughout the year with mean annual precipitation of 950 mm. Samples were collected directly from the farms, placed in plastic bags and delivered to the laboratory for chemical and NIR analysis.

Samples were dried in oven at 60 °C for 48 hours and ground in a Wiley forage mill to pass a 1 mm screen (Arthur H. Thomas, Philadelphia, PA, USA). Acid detergent fibre (ADF) and neutral detergent fibre (NDF) were estimated using the procedures reported elsewhere (Van Soest et al., 1991; Robertson and Van Soest, 1981). Organic matter digestibility (OMD) was estimated in vitro by the two-stage rumen fluid – pepsin technique, with rumen fluid incubation (48 hours) followed by HCl-pepsin digestion (48 hours) (Tilley and Terry, 1963). Ash was determined by incinerating the dry sample at 500 °C for 4 hours (AOAC, 1990). All chemical analysis were expressed on a dry weight basis and analysed in duplicate.

Spectra were collected in the near infrared (NIR) region in reflectance (1100 – 2500 nm) at 2 nm intervals using a scanning monochromator NIRSystems 6500 (NIRSystems, Silver Spring, MD, USA). Samples were scanned dry in a circular cell cup and reflectance data were stored as logarithm of the reciprocal reflectance (log 1/R). Samples were not rotated when spectra collection was made. The spectrum of each sample was the average of 32 successive scans (1050 data points per scan). Spectral collection was performed using Infrasoft International software (ISI, version 3.01, Infrasoft International, NIRSystems, Silver Spring, MD, USA).

Spectra were exported in NSAS format into The Unscrambler X (CAMO, Norway) for multivariate analysis. Principal Component Analysis (PCA) was performed before partial least squares regression (PLS) models were developed and was used to derive the first principal components from the spectral data. PCA is a mathematical procedure for resolving sets of data into orthogonal components whose linear combinations approximate the original data to any desired degree of accuracy (Naes et al., 2002). These were used in further analysis to examine the relevant and interpretable structure in the data as well as outlier detection. The regression method used to build the calibration equations was PLS, with full cross validation. Due to the limited number of samples available, calibration models were developed and evaluated using full cross validation (Dardenne et al., 2000; Cozzolino et al., 2006). Full cross validation was also used to estimate the optimal number of terms in the calibration to avoid overfitting (Naes et al., 2002). The maximum number of terms used to develop the calibration equation was 10 in all cases, where the optimal number of terms to be included in the calibration models was determined as having the lowest standard error of cross validation (SECV). Pre-processing was achieved using second derivative (2nd order polynomial, 20 data points smoothing) (Shenk and Westerhaus, 1993). The resulting calibration equations between the chemical reference values
and the NIR data were evaluated based on the coefficient of determination in calibration ($R^2_{cal}$) and SECV. The ratio of standard deviation (SD) and SECV namely residual predictive value (RPD) were used to test the accuracy of the calibration models (Williams, 2001). The RPD for the NIR calibration for the parameters evaluated demonstrated how well the calibration models performed in predicting the reference data. If a product shows a narrow range in composition, or if the error in estimation is large compared with the spread (as SD) in composition, then regression finds increasing difficulty in finding stable NIR calibrations. An RPD value greater than three was considered adequate for analytical purposes in most of the NIR applications for agricultural products (Williams, 2001).

**RESULTS AND DISCUSSION**

Table 1 shows the descriptive statistics for ADF, NDF, OMD, and ash in the maize stover samples analysed.

The chemical composition of the maize stover samples showed a wide range in composition due to the different varieties and farms sampled. The variability in chemical composition was considered suitable to develop NIR calibrations for these chemical parameters in maize stover (Cozzolino *et al.*, 2006).

Table 2 summarises the cross validation statistics for the prediction of chemical composition in maize stover samples by NIR using second derivative as pre-processing technique.

The $R^2_{cal}$ and SECV using the second derivative were for ADF 0.85 (SECV: 16.5 g kg$^{-1}$ DM), for NDF 0.92 (SECV: 58.4 g kg$^{-1}$ DM), for ash 0.90 (SECV: 1.9 g kg$^{-1}$ DM) and for OMD 0.85 (SECV: 10.4 g kg$^{-1}$ DM). The RPD values obtained were 2.5, 2.4, 2.5 and 3.5 for ADF, OMD, NDF, and ash, respectively. The NIR calibration models obtained indicated that good correlations were achieved for ash ($R^2 > 0.90$ and RPD higher than 3) while intermediate calibrations were achieved for the other chemical parameters measured (ADF, NDF and OMD) ($R^2 = 0.85$). The poor predictive ability of the NIR calibration models evaluated using the RPD values (RPD lower than 3) might reflect the variability in the reference method utilized, the characteristic of the sample (e.g. differences in cell wall composition, nitrogen content) as well as in the number of samples used to develop the calibrations.

It is widely accepted that *in vitro* OMD is of a particularly good predictor of *in vivo* organic matter digestibility, while the pepsin-cellulase method is considered more accurate, although it is very dependent on both the type of forage and laboratory procedures (Deaville and Flinn, 2000; Roberts *et al.*, 2004). Several authors have also suggested that *in vivo* OMD might be more adequate as reference method for developing NIR equations for OMD (Deaville and Flinn, 2000; Roberts *et al.*, 2004). This might explain the low calibrations statistics and poor predictive ability obtained for OMD in the set of samples analysed.

The coefficients of determination obtained for ADF and NDF explain more than 80% of the explained variance ($R^2 > 0.85$) in the calibration models, however the standard errors indicated (RPD values lower than 3) that they cannot be used to predict these parameters for routine analysis. According to Alomar and co-workers (2009) this reinforces the idea that the NIR spectra can recover signals from chemical bonds that in some way are related to empirical entities, such as those mentioned above (Alomar *et al.*, 2009). Therefore, NIR calibrations models might be only used to monitor overall quality (e.g. low, medium and high) rather to be used for the routine analysis of these chemical parameters in maize stover samples. Figures 1A and B show the NIR predicted values versus the reference values for ADF and NDF using the second derivative as pre-processing method. The data presented in the fi-
Figures reinforced the good agreement between the NIR predicted values and the reference method.

Figure 2 shows the coefficients of regression or loadings derived from the PLS calibrations were examined as they indicate specific NIR regions related with the parameter measured (Naes et al., 2002).

The regression coefficients for ADF were found around 1192 nm, at 1456 nm associated with O-H absorption bands (second overtone), at 1928 nm with O-H first overtone, at 2234 nm and at 2312 nm and 2342 nm related to C-H and C=C tones, respectively (Workman and Weyer, 2008; Xu et al., 2013). These spectral regions are characteristic of both water absorption (O-H overtones) and fibre fractions (cellulose) (Workman and Weyer, 2008; Xu et al., 2013). Additionally it was observed that wavelengths around 1666 nm and 1724 nm both related to C-H first overtones and at 2248 nm related with C-H and C=O stretch combinations and

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**Figure 1.** Near infrared predicted ADF (Figure 1A) and NDF (Figure 1B) versus reference data for maize stover samples.

**Figura 1.** Relación entre los valores de referencia y valores de predicción NIR obtenidos para la determinación de FDA (panel A) y FDN (panel B) en rastrojos de maíz.

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**Figure 2.** Regression coefficients for the optimal calibrations for the prediction of chemical parameters in maize stover samples using NIR spectroscopy.

**Figura 2.** Coeficientes de regresión derivados de las calibraciones NIR obtenidas para la predicción de los parámetros químicos en los rastrojos de maíz.
deformations (Workman and Weyer, 2008; Xu et al., 2013). The coefficients of regression for ash were observed in the wavelength regions associated with O-H (dry matter). The coefficients of regression for OMD showed an inverted but similar pattern in the same wavelength regions as described for ADF. For NDF, no relevant information was observed in the NIR region.

CONCLUSIONS

The results from this study suggested that maize stover samples might be monitored (low, medium and high) by NIR spectroscopy for parameters such as ADF, NDF and OMD. Only the NIR calibrations for ash can be used for routine analysis. Overall, the prediction accuracies are less than desirable to be used for routine analytical purposes. It is suggested that more samples and harvests are needed in order to improve and validate the proposed NIR method before its use for routine analysis.

REFERENCES


