

RESEARCH

USE OF NEAR INFRARED REFLECTANCE (NIR) SPECTROSCOPY TO PREDICT CHEMICAL COMPOSITION OF FORAGES IN BROAD-BASED CALIBRATION MODELS

Uso de la espectroscopía de reflectancia en el infrarrojo cercano (NIR) para predecir la composición química de forrajes en modelos de calibración amplia

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ABSTRACT

The objective of the study was to evaluate the potential of near infrared reflectance (NIR) spectroscopy as a rapid method to predict the chemical composition of forage in broad-based calibration models. In total, 650 samples representing a wide range of chemical characteristics, phenological states and origins were scanned in an NIR instrument. The coefficient of determination in calibration (R^2) and standard error in cross validation (SECV) for the NIR calibration models were as follows: dry matter 0.95 (SECV: 0.7%), crude protein 0.98 (SECV: 0.98%), ash 0.90 (SECV: 0.99%), *in vitro* organic matter digestibility 0.90 (SECV: 3.6%), acid detergent fiber 0.95 (SECV: 2.0%) and neutral detergent fiber 0.86 (SECV: 5.4%) on a dry matter basis. The results demonstrated the potential of NIR to predict the chemical composition of different forage plant species; however, it is suggested that the technique could be used as a routine procedure to apply in breeding programs only if calibration is done for each species, season and particular conditions.

Keywords: forage quality, NIR, chemical composition, near infrared analysis.

RESUMEN

El objetivo del trabajo fue evaluar el potencial de la espectroscopía de reflectancia en el infrarrojo cercano (NIR) como método rápido para la predicción de la composición química de forrajes en modelos de calibración amplia. Un total de 650 muestras de forrajes de un amplio rango de características químicas, origen y estados fenológicos, fueron analizadas en un instrumento NIR en reflectancia. Los coeficientes de determinación en calibración (R^2) y los errores estándares de la validación cruzada (SECV) para las calibraciones NIR fueron 0,95 (SECV: 0,7%), 0,98 (SECV: 0,98%), 0,90 (SECV: 0,99%), 0,95 (SECV: 2,0%), 0,86 (SECV: 5,4%) y 0,90 (SECV: 3,6%) para materia seca (MS), proteína cruda (PC), cenizas, fibra detergente ácido (FDA), fibra detergente neutro (FDN), y digestibilidad *in vitro* de la materia orgánica (DIVMO), base materia seca, respectivamente. Los resultados demostraron el potencial del NIR para predecir la composición química de forrajes; sin embargo, se sugiere que la técnica podría ser usada como un procedimiento de rutina en programas de mejoramiento sólo si se realiza calibración para cada especie, estación y condiciones particulares.

Palabras clave: composición química, NIR, calidad forrajera, espectroscopía de reflectancia en el infrarrojo cercano.

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INTRODUCTION

Improvements in crop and forage quality often entail screening of large numbers of samples, and this may limit the size of a plant breeding program. Near infrared reflectance (NIR) spectroscopy appears to be an attractive alternative to routine chemical analysis, resulting in substantial improvements in the efficiency of the breeding process for some aspects of quality (protein, digestibility).

The nutritive value of forage crops is related mainly to climatic conditions and stage of plant maturity, and the determination of the nutritive value is important in many pasture experiments, animal feeding trials and extension services (Givens and Deaville, 1999; Deaville and Flinn, 2000). Worldwide, the nutritive value of forages is often estimated by chemical or physical methods, and is expressed as the concentration of chemical constituents in the plant tissue. In Uruguay, as in many other countries, crude protein (CP), crude fiber (CF) and *in vitro* organic matter digestibility (IVOMD) have long been used for this purpose (Givens and Deaville, 1999; Deaville and Flinn, 2000). Since the 1970s, NIR spectroscopy has been used to analyze large batches of pasture and forage samples for CP, neutral detergent fiber (NDF), lignin and IVOMD (Norris *et al.*, 1976; Starr *et al.*, 1981; Ruano-Ramos *et al.*, 1999; Deaville and Flinn, 2000) and has been reported also as a technique to predict the chemical composition, dry-matter digestibility (DMD), dry-matter intake (DMI), organic matter digestibility (OMD) and digestible energy intake (DEI) in several grass and legume forages (Norris *et al.*, 1976; Roberts *et al.*, 2004).

Forage analysis by NIR spectroscopy might be undertaken either for plant selection in breeding programs, for commodity trading, for investigating individual farm production problems or for least-cost ration formulation (Murray, 1993). Spectroscopy in the NIR region will provide information about the relative proportions of C-H, N-H and O-H bonds, which are the primary constituents of the organic molecules of forage, and relies on calibrations, which utilize absorbance at many wavelengths to predict the composition of a sample (Murray, 1993). In all these applications NIR spectroscopy has a role in reducing costs, time requirements and the number of samples required

for testing, together with an increase in the number of samples that may be analyzed (Murray, 1993).

Although NIR spectroscopy is used extensively in the USA and Europe for forage analysis, few reports were found in the literature about the application of this technology in South America for routine analysis. The objective of this study was to explore the use of broad-based NIR spectroscopy calibration models as a rapid method to predict chemical composition and biological parameters in different forage species.

MATERIALS AND METHODS

Samples (n = 650) representing a wide range of forages (species, origin, growth stages) were collected from field experiments located at INIA (National Agricultural Research Institute), La Estanzuela experimental research station (34°19' S lat; 57°43' W long), Colonia, Uruguay, during 1997-2001. Plant material represents the whole plant cut, thus including both leaves and stems. Both grasses and legumes were cut on several cutting dates from March to December, to show changes in composition with increasing plant maturity throughout the growing season. The green crops (wheat *Triticum aestivum* L., oats *Avena sativa* L., triticale *Triticosecale* Wittmack) were harvested during July, August, September and October, and included different growth stages such as boot, early heading, full heading and pre-milk stage (Table 1).

Samples were dried in an air forced oven at 60°C to constant weight for 48 h, and ground in a Wiley forage mill to pass a 1 mm screen (Arthur H. Thomas, Philadelphia, Pennsylvania, USA). Nitrogen was determined using a semi-micro automated Kjeldahl instrument (Tecator, Sweden) and converted to CP using the factor 6.25 (AOAC, 1990). Neutral-detergent fiber (NDF) was estimated following the procedure of Van Soest *et al.* (1991) and acid-detergent fiber (ADF) was estimated using the procedures of Goering and Van Soest (1970). IVOMD was estimated using the two-stage rumen fluid-pepsin technique, with rumen fluid (48 h) followed by HCl-pepsin digestion (48 h) (Tilley and Terry, 1963). Analytical DM was determined by drying the sample at 105°C for 24 h and ash by incinerating the dry sample at 500°C for 4 h. All chemical analysis was performed in duplicate and the results expressed on a dry weight basis.

Table 1. Species and number of samples used to build the near infrared reflectance (NIR) calibration models.
Cuadro 1. Número de especies y muestras usadas en la construcción de los modelos de calibración en reflectancia en el infrarrojo cercano (NIR).

Species	Scientific name	Number of samples
Tall fescue	<i>Festuca arundinacea</i> L.	200
Oat (whole crop)	<i>Avena sativa</i> L.	32
Wheat (whole crop)	<i>Triticum aestivum</i> L.	32
Triticale (whole crop)	<i>Triticosecale</i> W.	32
Italian ryegrass	<i>Lolium multiflorum</i> Lam.	50
Lucerne	<i>Medicago sativa</i> L.	40
Red clover	<i>Trifolium pratense</i> L.	40
White clover	<i>Trifolium repens</i> L.	50
Birdsfoot trefoil	<i>Lotus corniculatus</i> L.	40
Moha	<i>Setaria italica</i> L.	24
Corn (whole plant)	<i>Zea mays</i> L.	20
Orchard grass	<i>Dactylis glomerata</i> L.	20
Sorghum (whole plant)	<i>Sorghum bicolor</i> (L.) Moench	25
Other species and mixtures of grasses and legumes		45
TOTAL		650

Samples were scanned dry (approx. 10 g), in the visible (VIS) and NIR region (400 - 2500 nm) in a scanning monochromator instrument NIRS 6500 (NIR Systems, Silver Spring, Maryland, USA) in a small circular quartz cup (50 mm diameter) sealed with disposal paper. Spectral data were stored as the reciprocal logarithm of reflectance (Log 1/R) at 2 nm intervals. Samples were not rotated during spectral collection. Two pairs of lead sulfide detectors collected the reflectance spectra which were referenced to corresponding readings from a ceramic disk. The spectrum of each sample was the average of 32 successive scans (16-32-16). Calibration equations were developed using modified partial least squares regression (MPLS) (Shenk and Westerhaus, 1993) with internal cross-validation and scatter correction using standard normal variate transformation (SNV) and detrending (Barnes *et al.*, 1989).

The mathematical treatment applied was (1,4,4), where the first number indicates the order of the derivative (1 is first derivative of log 1/R), the second number is the gap in data points over which the derivative is calculated, and the third number indicates the number of data points used in the first smoothing (NIRS 2, 1995).

Calibration models developed were evaluated using the coefficient of determination in calibration (R^2) and the standard error in cross validation (SECV) (Shenk and Westerhaus, 1993).

The optimum calibration model was selected on the basis of minimizing the SECV and the highest R^2 (closest to 1). The ratio performance deviation (RPD) calculated as standard deviation (SD)/SECV was used to evaluate the performance of the calibrations (Murray, 1993). 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. Where the error exceeds one-third of the SD of the population, regression can be misleading ($RPD > 3$ are considered good for screening purposes, and $RPD > 5$ good for analytical applications) (Williams, 2001). An independent set of samples was used to validate the NIR calibration models ($n=250$). The ranges of chemical composition in the validation set were for DM 88.6 - 97.5 (SD: 1.9%); for CP 4.2 - 38 (SD: 9.8%); for ash 6.9 - 38.7 (SD: 6.2%); for ADF 16.1 - 48.9 (SD: 7.4%); for NDF 45.4 - 87.5 (SD: 9.2%) and 46 - 88 (SD: 1%) for IVOMD in percentage dry matter. Statistical parameters such as standard error of prediction (SEP), slope, bias and simple correlation coefficient were calculated for the prediction (NIRS 2, 1995).

RESULTS AND DISCUSSION

Table 2 shows the descriptive statistics and the broad-based NIR calibration results for DM, CP, ADF, NDF, ash and IVOMD in the forage samples. A wide variation in the chemical composition was expected due to the large number of species, years

Table 2. Chemical composition and near infrared reflectance (NIR) calibration statistics for forage samples (% dry weight basis).**Cuadro 2. Composición química y calibraciones en reflectancia en el infrarrojo cercano (NIR) para muestras de forraje (% base seca).**

	N	Mean	Min	Max	SD	R ²	SECV	SEP	RPD
DM	600	91.7	81.7	98.1	2.9	0.95	0.70	0.96	4.1
CP	610	16.5	2.4	37.1	6.1	0.98	0.98	1.8	6.2
Ash	581	12.3	6.2	29.7	2.9	0.90	0.99	2.2	2.9
ADF	200	32.8	22.7	48.4	11.1	0.95	2.0	3.8	5.5
NDF	151	67.4	44.7	75.6	9.7	0.86	5.4	8.2	1.8
IVOMD	542	67.0	44.2	95.6	12.5	0.90	3.0	5.7	4.2

N: number of samples in calibration; Min: minimum; Max: maximum; SD: standard deviation; R²: coefficient of determination in calibration; SECV: standard error of cross-validation; SEP: standard error of prediction; RPD: SD/SECV; DM: dry matter; CP: crude protein; ADF: acid detergent fibre; NDF: neutral detergent fibre; IVOMD: *in vitro* organic matter digestibility.

and growth stages used to develop the NIR calibration models. In the forage samples the IVOMD value varied the most, followed by CP, ADF and ash concentration, respectively.

The broad-based NIR calibration models obtained indicated good correlations with all the chemical variables analyzed (R² > 0.90), although the lowest R² coefficients were obtained for NDF (R²: 0.86). These results were similar with those reported by other authors (Jones *et al.*, 1987; Brown *et al.*, 1990; Pires and Prates, 1998; Roberts *et al.*, 2004). The poor calibration model results for NDF (SECV: 5.4% DM) might reflect the variability of the wet chemistry method utilized as well as the effect of sample characteristics such as high starch or nitrogen content. It is important to note that amylase was not added during the determination of NDF. For CP, the SECV (SECV: 0.98% DM) were similar to those reported by other authors in temperate forages (Abrams *et al.*, 1987; Roberts *et al.*, 2004).

The calibration statistics for CP suggested that NIR spectroscopy could predict this parameter in a wide range of forages with high accuracy (broad-based calibration). It is widely accepted that IVOMD is not a particularly good predictor of *in vivo* organic matter digestibility, while the pepsin-cellulase method is more accurate, although it is very dependant on both the type of forage and laboratory procedures (Deaville and Flinn, 2000; Roberts *et al.*, 2004). Several authors have suggested that *in vivo* OMD is the best reference method for developing NIR equations for OMD (Murray, 1993; Deaville and Flinn, 2000).

In this study, broad-based NIR calibration models for IVOMD indicated excellent correlations (R²: 0.90; SECV: 3% DM). These results suggested that broad-based NIR calibration models could be acceptable to estimate IVOMD in a broad range of forages. However, in relation to the use of broad-based NIR calibration models as tools for rapid screening in breeding programs, the main concern arises when we apply this calibration to detect minor changes in digestibility in a single species (e.g. narrow range in IVOMD) (Table 3). For example, this was observed in this study for tall

Table 3. Near infrared reflectance (NIR) calibration statistics for chemical parameters of single grass species and legumes (% dry matter).**Cuadro 3. Calibraciones en reflectancia en el infrarrojo cercano (NIR) para especies puras de gramíneas y leguminosas (% materia seca).**

		N	SD	R ²	SECV	RPD
Tall fescue	DM	199	0.95	0.87	0.4	2.5
	CP	200	3.8	0.99	0.5	7.4
	IVOMD	198	5.3	0.92	2.0	2.7
	Ash	200	1.8	0.84	0.8	2.3
Wheat whole crop	DM	32	0.76	0.98	0.3	3.0
	CP	30	10.1	0.98	1.0	10.1
	IVOMD	31	11.0	0.98	2.0	5.5
	Ash	32	1.9	0.80	1.1	1.8
Legumes*	DM	130	1.4	0.92	0.74	1.9
	CP	130	3.2	0.93	0.95	3.4
	IVOMD	125	10.2	0.91	5.6	1.8
	Ash	130	1.6	0.73	1.3	1.2

DM: dry matter; CP: crude protein; IVOMD: *in vitro* organic matter digestibility; R²: coefficient of determination in calibration; SECV: standard error of cross-validation; N: number of samples in calibration; SD: standard deviation, RPD = SD/SECV, * Legumes: include lucerne, red and white clover.

fescue (*Festuca arundinacea* Schreb.). In view of these results, increased accuracy could be expected if separate calibrations for each grass or legume species were developed for breeding purposes.

In a different group of samples, NIR analysis improved when calibration sets were developed from samples representing the same range of management and environmental conditions as the samples that were analyzed. However, in some cases this improvement has not been as great as that observed with homogenous calibrations or single products (Brown *et al.*, 1990). The use of groups of species having a wide range of chemical, botanical, and physical characteristics tends to reduce the standard error and biases of the calibration models developed (Blosser *et al.*, 1988). In our results this was verified in the prediction of DM content and IVOMD. This might explain the changes in chemical composition that produce variations in the predictions when we used a calibration based on the previous growing seasons, a critical point when we applied calibrations developed in the prior season to detect differences in a breeding program (Table 4).

Several authors have indicated that broad-based calibration models for forage chemical constituents have the potential to offer accuracy comparable to more local (specific) equations (Abrams *et al.*, 1987). Such an application offers the advantage of either reducing or eliminating the rather big effort that is now required for each NIR laboratory to assemble a

calibration set and perform conventional chemical analysis in order to develop calibration equations for individual samples.

Broad-based calibrations have demonstrated acceptable accuracy for parameters such as DM content, CP and ash concentration for all the forage species analyzed. One important point was noted by Blosser *et al.* (1988), that at the beginning of a growing season, the only available equations are those established at some other time (e.g. the previous growing year) until sufficient numbers of samples have been analyzed during the current growing season. In this study, this was verified in the prediction of both DM content and IVOMD for a particular year. This is a critical point when we apply NIR calibration models developed in the last season to detect differences in a new season in a breeding program. Parameters such as CP and ash concentration apparently did not show this seasonal effect. The RPD obtained in this study were considered excellent and suggested that DM content (4.1), IVOMD (4.2), ADF (5.5) and CP (6.2) NIR calibrations models might be used for routine analysis of these parameters. Intermediate and poor NIR calibration models were found for ash (2.9) and NDF (1.5), respectively.

Figures 1 and 2 plot the relationship between NIR predicted data and reference values for CP content and IVOMD in calibration. Similar trends were observed for the other parameters (figures not included).

Table 4. Effect of two cutting times on the near infrared reflectance (NIR) calibration statistics for chemical parameters in tall fescue (% dry matter).

Cuadro 4. Efecto de dos momentos de corte sobre las calibraciones en reflectancia en el infrarrojo cercano (NIR) para parámetros químicos en festuca (% materia seca).

		N	SD	R ²	SECV	RPD
Cut 1	DM	45	0.75	0.68	0.54	1.4
	CP	45	1.5	0.90	0.55	2.7
	IVOMD	45	1.6	0.58	1.4	1.1
Cut 2	DM	66	1.6	0.85	0.80	2.0
	CP	66	4.2	0.91	1.72	2.5
	IVOMD	66	1.8	0.30	1.45	1.2
Cut 1+Cut 2	DM	111	1.2	0.76	0.77	1.6
	CP	111	3.8	0.90	1.70	2.2
	IVOMD	111	5.4	0.86	2.84	2.0

DM: dry matter; CP: crude protein; IVOMD: *in vitro* organic matter digestibility; R²: coefficient of determination in calibration; SECV: standard error of cross-validation; N: number of samples in calibration; SD: standard deviation, RPD = SD/SECV.

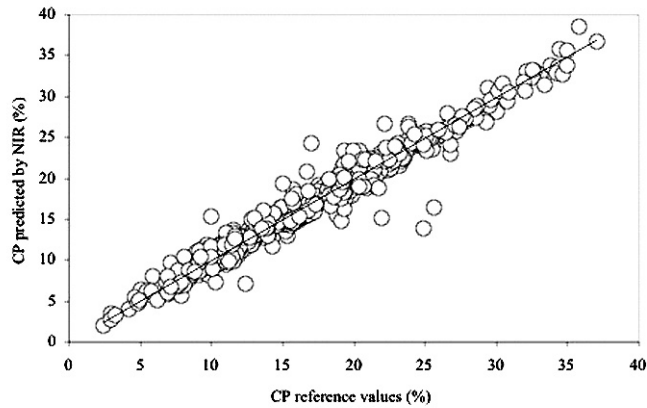


Figure 1. Near infrared reflectance (NIR) spectroscopy predicted values versus reference values for crude protein (CP) (% DM).

Figura 1. Valores estimados de reflectancia en el infrarrojo cercano (NIR) versus valores de referencia para proteína cruda (CP) (% DM).

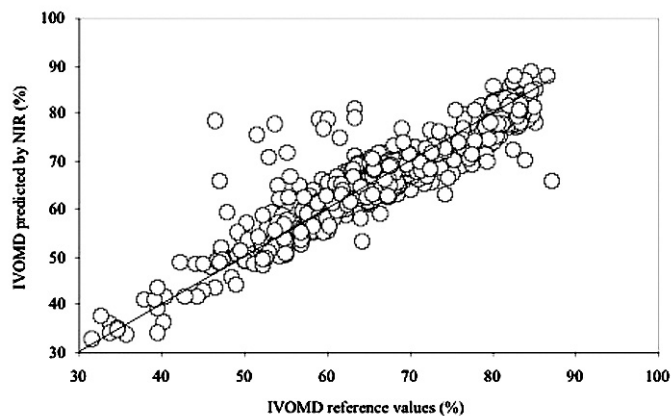


Figure 2. Near infrared reflectance (NIR) spectroscopy predicted values versus reference values for *in vitro* organic matter digestibility (IVOMD) (%).

Figura 2. Valores estimados de reflectancia en el infrarrojo cercano (NIR) versus valores de referencia para digestibilidad *in vivo* de la materia orgánica (IVOMD) (%)

CONCLUSIONS

Overall, the broad-based NIR calibrations for DM, CP, ADF, and IVOMD resulted in relatively high correlation coefficients and low SECV. Further work needs to be done to build robust NIR calibration models for a single species (e.g., tall fescue), to be able to detect minor changes in parameters such as IVOMD, with the objective to select new plant materials. On the other hand, a new calibration for each season or year might be necessary until

acceptable accuracy is obtained. Calibration models for chemical parameters such as CP and ash apparently did not show seasonal effects.

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