NON-DESTRUCTIVE METHOD FOR DETERMINING ASH CONTENT IN PASTURE SAMPLES: APPLICATION OF NEAR INFRARED REFLECTANCE SPECTROSCOPY

B. R. Vázquez de Aldana, B. García-Criado, A. García-Ciudad, and M. E. Pérez-Corona

Instituto de Recursos Naturales y Agrobiología, CSIC. Apdo. 257, 37071 Salamanca, Spain

ABSTRACT: Ash content is a useful parameter in forage and grass quality studies, but the traditional methods used to quantify this parameter are tedious and sample processing time-consuming. Near infrared reflectance spectroscopy (NIRS) overcomes some drawbacks of the traditional methods. Measurement of a sample's diffuse reflectance is rapid and non-destructive and chemical reagents are not necessary, which is environmentally friendly. We predicted the ash content of herbage samples from semiarid grassland communities by NIRS. The samples were collected on different sites, over four consecutive years. The calibration equations generated from log 1/reflectance to predict ash content had a standard error of calibration (SEC) of 4.6 g kg^{-1} and R^2 of 0.88. The standard error of prediction (SEP) was 5.1 g kg^{-1} and r was 0.94.

INTRODUCTION

Ash content refers to the total amount of the inorganic content in any sample. This parameter is very useful in forage quality studies, but is rarely included. Conventional methods for determining ash content need muffle furnaces for combustion of the samples. These furnaces have limited capacity and require extensive periods of heating and cooling. The tediousness of the method and the time

required for sample processing are the main reasons why ash content is not included as parameter in complex experiments. Near infrared reflectance spectroscopy (NIRS) overcomes some of the drawbacks of traditional methods. Measurement of a sample's diffuse reflectance is rapid and non-destructive and chemical reagents are not necessary, which is environmentally friendly.

Norris et al. (1976) published initial results of forage quality evaluations using NIRS. Since then, the technique has been successfully employed to predict different parameters such a protein, neutral detergent fibre, acid detergent fibre and lignin in forage crops.

The ability of NIRS to predict inorganic constituents in plant samples initially seemed remote since there are no direct bonds between inorganic elements and hydrogen. However, NIRS can predict some macronutrients with acceptable accuracy (Clark et al., 1987; Vázquez de Aldana et al., 1995).

We were interested in finding the suitability of NIRS for predicting the ash content of herbage samples from semiarid grassland communities. The quality of the grassland communities studied is of interest as regards ruminant feeding in "Dehesa" ecosystems (Central-Western Spain). These are land-use systems with multiple silvopastoral uses.

MATERIAL AND METHODS

Herbage samples were collected from semi-natural grassland communities on several sites in the "Dehesa" zone (Central-West part of Spain). Samples were taken from different community types according to variations in the topographic gradient, at different maturity stages, over a period of four consecutive years (Table 1). The botanical composition of the samples was complex. Considerable variability was observed in the weight proportions of the main botanical components: grasses ranged between 0-100%; legumes between 0-73%, and forbs between 0-91%. More information about sampling can be found in Vázquez de Aldana et al. (1995).

TABLE 1. Summary of pasture samples included in the study.

Year	Sampling date	Slope position	Number of samples
1986	June (one harvest)	lower	22
1987	June (one harvest)	lower	28
		upper	26
1988	June (one harvest)	lower	26
		upper	25
1989	June/July (three harvests fortnightly)	lower	15
		medium	14
		upper	14

The samples were dried and ground and passed through a 0.5-mm sieve. Ash content was determined by combustion of the samples in a muffle furnace at 450°C for 12 hours.

NIRS spectra were obtained on the ground and dried plant material using a Technicon InfraAlyzer 500, scanning from 1100 to 2500 nm. In all samples (170) the

spectral data were recorded as $\log 1/R$ (R =reflectance). A set of 95 samples was selected with the PICKS program (InfraAlyzer Data Analysis System, Technicon Instrument Corporation, NY) for the calibration process. Modified stepwise multiple linear regression between ash content data and spectral data was used to develop the calibration equations. The validation set included the remaining samples (75). The calibration equation was validated by means of simple linear regression between NIRS-predicted values and those obtained by the conventional method. More details about data processing and manipulation can be found in Garcia-Ciudad et al. (1993).

RESULTS AND DISCUSSION

The ash content of herbage samples determined by combustion are shown in Table 2. The ash content of the calibration set ranged from 41.8 to 100.4 g kg⁻¹, close to the validation set (from 47.5 to 94.7 g kg⁻¹). The mean value was 69.4 for the calibration and 68.3 for the validation set.

Calibration statistics and the wavelengths selected for predicting ash content are shown in Table 3. The mathematic treatment used to develop the calibration equations was $\log 1/R$. According to Windham et al. (1991), equations generated from $\log 1/R$ to predict ash content have lower calibration and validation errors than equations generated with derivative mathematical treatment of $\log 1/R$.

The standard error of calibration (SEC) was 4.6 and the coefficient of multiple determination (R^2) was 0.88. The plot of the NIRS predicted versus the combustion data of the validation set revealed good correlation (Figure 1). These results are similar to those reported by Berardo et al. (1991) and Abreu et al. (1992). The number of wavelengths used for the calibration equation was seven. Redshaw et al. (1986) and Berardo et al. (1991) used four wavelengths to predict ash content in different forage species. The higher number of wavelengths required in our calibration is due to the greater heterogeneity of the samples. The wavelength data at 1940 nm (Table 3) support part of the chemical information. Watson et al. (1976)

TABLE 2. Statistical data for ash content (g Kg^{-1}) determined by combustion (conventional method).

Set	N ^a	Range	Mean	SD
Calibration	95	41.8-100.4	69.4	13.0
Validation	75	47.5-94.7	68.3	12.5

a: number of samples

TABLE 3. NIRS calibration statistics for measuring ash content in pasture samples.

N	R ²	SEC	Wavelength (nm)
95	0.88	4.6	1896, 1940, 1956, 2028, 2320, 2336, 2440

N: number of samples

R²: coefficient of multiple determination

SEC: standard error of calibration

reported that the prediction of ash content by NIRS is related to correlations between ash and either protein or oil content but not to orthophosphate, the main component of flour ash. This contention agrees with Halack et al. (1992) who used the wavelength at 1940 nm to predict both the ash and protein contents of hay samples. Consequently, this might explain why the inorganic content is predicted by NIRS.

One constituent of ash is silicon dioxide, which ranged between 0.5 and 2.0% in grassland herbage samples (data not shown). It also represents the part of the

FIGURE 1. Plot of NIRS predicted and conventional analysis ash contents for pasture samples

inorganic content non-soluble in acids. When the sample scanned has high soil contamination, the silica content increases and the calibration equation for predicting ash content may include characteristic wavelengths of the reflectance spectrum of silicon dioxide (Windham et al., 1991). The silicon dioxide content in our sample set was not high enough and hence none of the wavelengths we used (Table 3) is related to silicon dioxide. Soil contamination is considered to be specially detrimental in grass silage because it reduces the nutrient concentration and unfavourably affects the fermentation process. Soil contamination in grass samples is easily detected by NIRS because it strongly affects the baseline of the spectrum in the 1100-1300 zone (Paul, 1988). Consistent with the foregoing, we avoided including samples with signs of high soil contamination.

CONCLUSIONS

We conclude that NIRS can be used as a rapid and accurate method for predicting the ash content of pasture samples with different characteristics of botanical composition, slope position, maturity stage and sampling year.

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