Prediction of protein in fresh leaves of alfalfa by NIRS with an interactance-reflectance probe

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Abstract

The objective of this work was to estimate the protein content of fresh leaves of alfalfa (*Medicago sativa* L.), using near infrared reflectance spectroscopy (NIRS) technology. The sample set included 33 varieties grown under irrigation in the province of Salamanca (Spain). Two cutting dates were considered, and samples of leaves were taken from three plant positions (apical, middle and basal). A total of 190 samples were obtained. The protein content ranged between 9.92-45.32 % of dry matter. NIRS calibrations were developed by two regression methods (multiple linear regression-MLR and partial least squares regression-PLSR) and three mathematical treatments (log 1/R, first and second derivatives). The best regression model reached a coefficient of multiple determination (r^2) of 0.68 and a standard error of prediction (SEP) of 3.27 % in validation. This study found that NIRS calibrations based on spectra of fresh leaves have potential for the rapid screening of crude protein in forage breeding programs.

Keywords: protein, NIRS, fresh alfalfa leaves.

Introduction

Forage quality of alfalfa (*Medicago sativa* L.) is a prime consideration in the development of a profitable harvest management program. Nutritional parameters, such as protein content, are important to decide when to harvest, for the selection of varieties, or to detect quality changes between harvests. On the other hand, in plant physiology studies, the diagnosis of nutrient status is based on the chemical analysis of plant material, mainly leaves. Depending on the degree of the nutrient migration, younger or older leaves should be used for analysis. Near infrared reflectance spectroscopy (NIRS) has emerged in the last 30 years as a rapid method for testing the quality, and characterise the composition of forages. Typically, forages analysed using this technique are dried and ground prior to scanning. However, relatively few studies have been carried out to evaluate the potential of NIRS in undried forages (Park *et al.*, 1999). The aim of this study was the development of NIR calibrations for protein content estimation using a spectral reading system based on a reflectance probe and fresh leaves. Fibre-optic offers considerable opportunities to work directly with intact material as a first step toward the non-destructive and rapid evaluation of plant samples.

Material and methods

A total of 190 samples of fresh leaves of alfalfa were obtained from 33 varieties grown under irrigation in the province of Salamanca (western Spain). In two different harvests done at anthesis (August and September, 2003), leaves were sampled from three positions: basal, medium, and apical. The NIR spectra of the central leaflets were recorded using a 1.5 m optical fibre probe connected to a FT-NIR InfraProver II (Bran+Luebbe, Norderstedt, Germany). The reflectance probe is a bi-directional optical fibre bundle for diffuse reflectance measurements from 1100 to 2200 nm. Protein content (% DM) was estimated by the reference Kjeldahl method. NIR calibrations were developed by two methods: multiple linear regression (MLR), and partial least squares regression (PLSR), using in both cases three data transformations: log 1/R, first and second derivative (1D, 2D). Other details of the experiments and calibration development can be read in Petisco *et al.* (2004).

Results and discussion

The statistical data of the samples included in the calibration and validation sets, determined by the reference method, are summarized in Table 1. A wide range of variation was obtained as a result of sampling leaves of 33 different varieties in three plant positions, and including two cutting dates.

Tabla 1. Chemical analysis of alfalfa leaves (% DM).

Component	Set	N [1]	Range	Mean	SD [2]
Protein (%)	Calibration	120	9.92-45.32	26.88	9.86
	Validation	70	13.44-43.85	28.69	8.24

[1] Number of samples; [2] Standard deviation.

NIR spectra of central leaflets of three fresh leaves taken from different plant positions (apical, middle and basal) are shown in Figure 1. The hydrogen bonds in water absorb significant amounts of NIR radiation and result in broad peaks that obscure spectral information derived from other compounds (Abrams *et al.*, 1988). Thus, the spectra in Figure 1 are dominated by two IR absorption maximums, peaking around 1450 and 1940 nm and due to the water content.

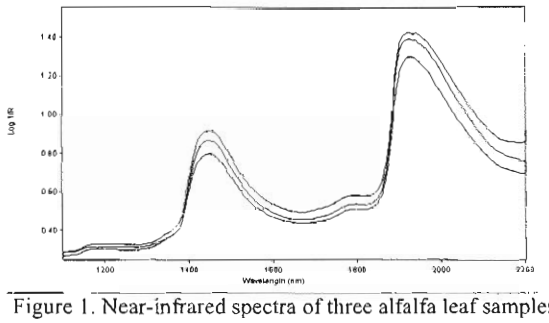

Figure 1. Near-infrared spectra of three alfalfa leaf samples.

Tabla 2. Calibration and validation statistics using multiple linear regression (MLR) and partial least squares regression (PLSR) methods.

	MLR				PLSR		
	Log 1/R	1D	2D		Log 1/R	1D	2D
				Protein (%)			
				Calibration set			
No. terms	7	7	7	No. factors	13	6	7
R^2 [1]	0.80	0.84	0.78	R^2 [1]	0.94	0.91	0.96
SEC [2]	4.43	4.08	4.59	SEC [2]	2.45	3.10	2.01
				SECV [3]	4.81	4.48	5.42
				Validation set			
r^2 [4]	0.59	0.62	0.67	r^2 [4]	0.66	0.68	0.57
SEP [5]	3.13	3.68	3.39	SEP [5]	3.63	3.27	4.57

[1] Coefficient of multiple determination; [2] Standard error of calibration; [3] Standard error of cross validation; [4] Coefficient of determination; [5] Standard error of prediction

Statistics reported for NIRS calibrations using MLR and PLSR are listed in Table 2. Figure 2 shows the relationship between NIRS and reference data for protein content in the external validation set. The

accuracy of the statistics of calibration was better with PLSR, however, there were no large differences between the statistics of external validation obtained with both regression methods. Thus, we obtained a coefficient of determination (r^2) and a standard error of prediction (SEP) of 0.67 and 3.39% (MLR, 2D) and 0.68 and 3.27% (PLSR, 1D), respectively. Coefficients of multiple determination obtained were similar to those reported by Dardenne *et al.* (1996) and Gatius *et al.* (2003) for fresh alfalfa; however SEP values were higher than those achieved by the aforementioned authors. These results could be due to the different approach of our experiment, using a reflectance probe, and central leaflets. According to Reeves and Van Kessel (2000) fibre-optic bundles are at least partially responsible for the reduced accuracy found with fibre-optic systems when compared to other methods of sample presentation. However, this study was designed to anticipate agronomic requirements and to this end spectra were collected using a fibre optic interactance probe. Further efforts are needed for developing accurate and robust calibrations for chemical constituents using fibre-optic based spectrometers. NIRS calibrations based on spectra of fresh leaves may have potential for the rapid screening of crude protein in forage breeding programs and agronomical studies, once sampling presentation and calibration and data set size and structure are optimised.

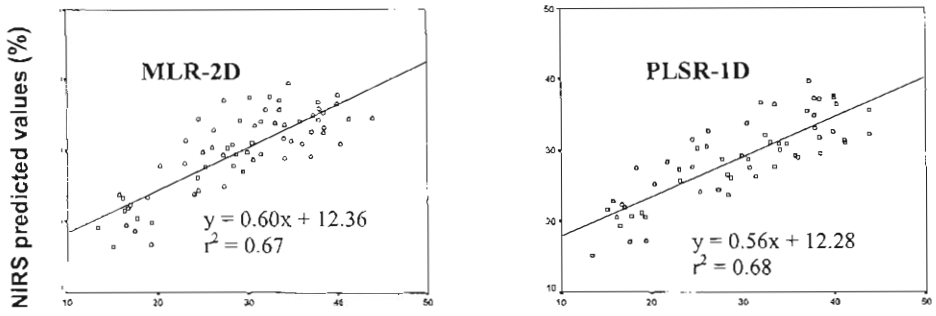

Figure 2. Relationship between protein content determined by Kjeldahl and NIRS methods in the external validation set.

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