Using of NIRS to estimate the leaf:stem ratio and different fractions of stem in a alfalfa crop

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Introduction

The nutritive value of the alfalfa is highly dependent of the stage of maturity.[1] Forage at different phenological stages varies concerning digestibility because of differences in ontogeny[2] and chemical composition.[3] Furthermore, leaf:stem ratio is an important factor determining diet selection and forage intake in alfalfa.[4] On the other hand, although the digestibility of leaves is in practice constant along the cycle,[5] the nutritive value of stem diminishes along the time, and this decrease starts from the bottom to the top. As a consequence, the digestibility of the bottom strata of the stem is lower than the one of top strata. In order to explain the variation of the nutritive value of alfalfa it is necessary to consider the variation of leaf:stem ratio of the crop, and the stratification canopies. However, manual determination of these fractions is a tedious, time consuming and costly process. Leaf:stem ratio has been predicted successfully by NIRS in rye grass sward,[6] but research has not been conducted using NIRS to predict the percentage of the different stem fractions in forage. The objective of this study was to evaluate the suitability of NIRS for predicting the dry proportion of these morphological fractions in alfalfa.

Material and methods

Hand collected samples of alfalfa were taken from a trial designed to obtain the most appropriate cultivars to be grown in the Medium Ebro Valley. The design was complete randomised blocks with three replications. For our experiment, five cultivars (Ampurdan, Aragon, Artal, Baraka and Giulia) were used as representative of the most important variability of the trial. The samples were taken in 2000 at the Agriculture Research Centre of Zaragoza during the growth and next four regrowths (between April and October) at two phenological states. A total of 112 samples was collected.

Alfalfa were cut at 2-3 cm level in plots of 12 m^2 and a sample of approximately 2 kg fresh weight were taken. It was taken to the laboratory and 25 plants were chosen at random and manually separated into leaf and stem fractions. The stem fraction included stems, petioles and inflorescences. Further, each stem was proportionally divided into three fractions according to its length (bottom, medium and top). Whole plant samples, leaves and each fraction of stems were dried separately in a forced air oven at 60 ºC afterwards, they were weighted to determine the leaf:stem ratio and the percentage of different fraction of the stems. Whole plant samples were ground in a Fritsch Pulverisette mill (1mm sieve). After that, they were stored in plastic bags at room temperature prior to NIRS analysis.

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NIRS analysis

Ground forage samples were scanned twice between 1100 and 2500 nm (in 2 nm steps) using a monochromator Foss NIRSystems 6500 and a quartz window ring cup placing 5 g of sample approximately. All spectra and reference data were recorded with the ISI software

Calibration models were developed for the proportion (dry matter basis) of leaves, bottom and middle stem fractions using the Modified Partial Least Square (MPLS) technique. The standard normal variate and detrend (SNVD) scatter correction procedure was applied to the spectral data. The spectra were transformed through a mathematical first order derivatisation. The statistics used for equation development and evaluation were, the standard error of calibration (SEC) and cross-validation (SECV), the determination coefficient in calibration (R^2c) and in cross-validation (R^2cv), the ratio of the range in the reference data to the SECV (RER) and the ratio of the standard deviation to the SECV (RPD).[8]

Results and discussion

The statistical parameters of the MPLS calibrations are described in Table 1. The comparison between the NIR predicted values with the reference ones for the leaves percentage, and bottom and medium fractions of the stems is given in figures 1, 2 and 3 respectively.

Table1. NIR calibration and cross-validation statistics for morphological composition of alfalfa

	N	Mean	Range	SEC	R^2c	SECV	R^2cv	RPD	RER
Leaves	112	48.77	36.12-76.01	2.01	0.94	2.32	0.92	3.52	17.19
Bottom strata	110	22.06	8.39-30.59	1.76	0.87	2.10	0.82	2.34	10.62
Middle strata	110	17.36	9.81-25.19	1.47	0.83	1.7	0.78	2.10	9.05

N: Number of samples; SEC: Standard error of calibration; R^2c: Coefficient of determination in calibration; SECV; Standard error of cross-validation; R^2cv: Coefficient of determination in cross-validation; RPD: Ratio of the standard deviation to the SECV; RER: Ratio range to the SECV.

The variability covered by the calibration set, demonstrated by the broad range, (Table 1) for the percentage of leaves is similar to the one that can be found in a alfalfa crop.[9] The determination coefficients in calibration and validation obtained for the percentage of leaves and the percentage of bottom and medium strata show a good quantitative information.[10] On the other hand, the calibration and cross validation errors for the percentage of leaves are of the same order or lower than the errors reported by others authors.[11,6] RER and RPD values indicate that the values predicted by the equation for the percentage of leaves are bigger than the minimum values recommended.[12] However, for the percentage of bottom and middle strata would be desirable to increase the collective of calibration in order to improve the RPD ratio.

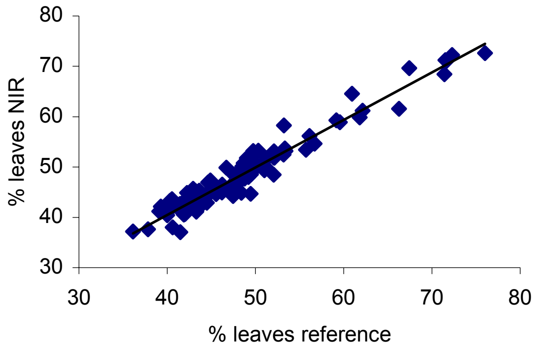

Figure 1. NIR predicted *v.* reference values for leaves dry matter percentage

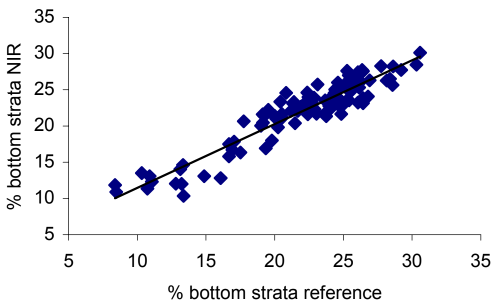

Figure 2. NIR predicted v. reference values for bottom strata dry matter percentage

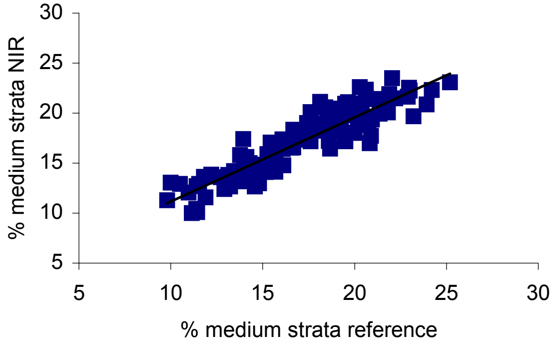

Figure 3. NIR predicted v. reference values for medium strata dry matter percentage

Conclusion

NIRS could be used effectively to predict the leaf:stem ratio andd thee percentage of bottom and middle strata of alfalfa. The use of NIRS could reduce the number of samples requiring separation by hand.

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