APPLICATION OF NEAR INFRARED REFLECTANCE SPECTROSCOPY (NIRS) FOR MACRONUTRIENTS ANALYSIS IN ALFALFA

(*Medicago sativa* **L.**)

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Abstract

Near infrared reflectance spectroscopy was used to assess the mineral composition of alfalfa (*Medicago sativa* L.) as a tool for nutritional diagnosis. One hundred and ninety four (n = 194) samples of alfalfa from different locations representing a wide range of soils were used. Samples were reflectance scanned in a NIRS 6500 (NIRSystems, USA) instrument. The coefficients of determination (R^2) of the regression estimate of the concentration of nitrogen, calcium, phosphorus, potassium, magnesium and sulphur and the errors in cross validation (SECV) were 0.93 (SECV: 1.6), 0.95 (SECV: 1.3), 0.93 (SECV: 1.9), 0.88 (SECV: 2.8), 0.82 (SECV: 1.9) and 0.75 (SECV: 4.7) respectively. The best NIRS predictions were obtained for calcium and nitrogen, meanwhile the poorest was obtained for sulphur.

Keywords: Nitrogen, phosphorus, calcium, potassium, magnesium, sulphur

Introduction

Alfalfa (Medicago sativa L.) is becoming more important in intensive animal production systems in Uruguay. The importance of alfalfa has been due to both high production and quality, combined with high growth during spring–summer. Also being a tolerant species for water deficiency during summer. One of the most important requirement to obtain high yields of alfalfa drymatter is an adequate supply of macro and micronutrients from the soil or fertilizer.

The analysis of the first 15 cm of soil has a great importance to assess the macro and micronutrients when the plant is in vegetative state for nutritional diagnosis (Cornforth, 1984; Mills & Jones, 1996; Pinkerton et al, 1997). Near infrared reflectance spectroscopy (NIRS) has been introduced as a rapid, inexpensive and accurate method to analyze grain, oilseeds and forages (Norris et al., 1976; Murray, 1993). The technique is based on the correlation between chemical properties, as determined by defined methods, and absorption of light at different wavelengths in the near infrared region, measured by reflectance. The objective of this paper is to explore the potential applications of NIRS as a diagnostic tool for macronutrients in alfalfa.

Material and Methods

Alfalfa samples were taken from commercial farms representing a wide range of soils during 1997. The samples came from two regions a) south region (Departments of Florida, San José and Canelones); b) north region (Departments of Río Negro and Paysandú). Alfalfa samples were mainly cv. E. Chaná, pure or in mixtures, with 1 or 2 years after planting. One hundred and ninety four (n = 194) composite samples were taken, at average sampling height was 27 cm (Coefficient of Variation, CV = 33). Each composite sample

was made from a minimum of six individual sub samples, cut to left about 15 cm (stems and leaves). Samples were dried in a forced-air drier at 60 ^{0}C. The samples were ground using a Willey Mill (1-mm sieve). Chemical analysis were:

- Nitrogen by sulphuric digestion following micro-Kjeldhal distillation and titration.

- Sulphur by nitroperchloric digestion following barium sulphate turbidimetry.

- Phosphorus by sulphuric digestion following vanadiumolibdate colorimetry

- Calcium, Magnesium: dry digestion (500°C, 6 hours) following determination by atomic absorption.

- Potassium: dry digestion (500°C, 6 hours) following determination by atomic emission.

Samples were scanned in reflectance mode (400 – 2500 nm) in a monocromator NIRS 6500 (NIRSystems, Silver Spring, MD, USA) in a small circular cup (50 mm diameter). Reflectance data was stored as log (1/R) at 2 nm intervals. Predictive equations were developed using modified partial least squares (MPLS) (Shenk and Westerhaus, 1993) regression with internal cross-validation (NIRS 2, 1995) and scatter correction using SNV (standard normal variate) and detrend (Barnes et al., 1989). Cross validation was used to avoid overfitting of the equations. The mathematical treatment applied was (1,4,4,1); where the first number indicates the order of derivative (one is first derivative of log 1/R), the second number is the gap in nm over which the derivative is calculated; the third number is the number of nm used in the first smoothing and the fourth number refers to the number of nm over which the second smoothing is applied. Calibration statistics calculated include the coefficient of determination in calibration (R^2) and the standard error of cross validation (SECV) (Shenk and Westerhaus, 1993). The optimum calibrations were selected on the basis of minimizing the standard error of cross validation (SECV). We calculated, also the

coefficient of variation to estimate the variability between NIRS analysis and chemical analysis as follows: CV = (SECV/ mean of chemical analysis).100 .

Results and Discussion

Table 1 shows the mean concentrations of N, P, Ca, Mg, K and S in alfalfa samples and NIRS calibration statistics for each of these macronutrients. The NIRS coefficients of determination were high for calcium and nitrogen, 0.95 y 0.93, respectively and low for sulphur 0.75. This difference in precision to predict individual mineral concentration from NIRS analysis is visualized in figure 1 which shows the relationship between chemical and NIRS data for calcium and sulphur. The calibration showed a lower number of outliers (difference n total vs. n cal.). In decreasing ranking the coefficient of determination in calibration were 0.93, 0.88, 0.82 and 0.75 for potassium, phosphorus, magnesium and sulphur, respectively. Although potassium showed high coefficient of determination, the number of outliers increased in relation to nitrogen and calcium (Table 1 and Figure 1). NIRS measures absorption by molecular bonds, so pure minerals have no NIR absorption bands. However, NIRS have the capability to predict macronutrient concentration. According to some authors (Shenk and Westerhaus, 1993) NIRS may utilise naturally occurring correlations between mineral concentrations and concentrations of organic constituents that NIR can measure such as protein, fiber and specular characteristics. In relation to sulphur, the poor calibration statistics may be explained because this element is present in different organic compounds and NIRS measures minerals indirectly (Clark et al., 1989). NIRS successfully predicted nitrogen and calcium in the alfalfa samples. Phosphorus, potassium and magnesium have acceptable prediction when high accuracy is not needed. Sulphur needs to improve prediction for future use. Finally, the information

generated in this work not only is useful for diagnosis of nutritional status for fertilizer requirements, but also to assess quality forage as feed for animal nutrition purposes.

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Table 1 - Chemical analysis and NIRS calibration statistics for macronutrients in alfalfa samples.

	N	P	K	Ca	Mg	S
Mean concentration g /kg	43.8	36.6	24.5	19.2	2.9	3.9
SD g /kg	5.1	6.1	5.4	4.7	0.6	0.8
R^2	0.93	0.88	0.93	0.95	0.82	0.75
SECV	1.6	2.8	1.9	1.3	1.9	4.7
n total	194	194	194	194	194	194
n Cal.	192	186	172	191	192	172
CV	4	8	8	7	66	121

Mean: correspond to chemical analysis, SD: standard deviation (chemical analysis); R^2: coefficient of determination of calibration; n total: number of samples in the total set, n cal.: number of samples in calibration set, SECV: standard error in cross validation; CV: coefficient of variation.
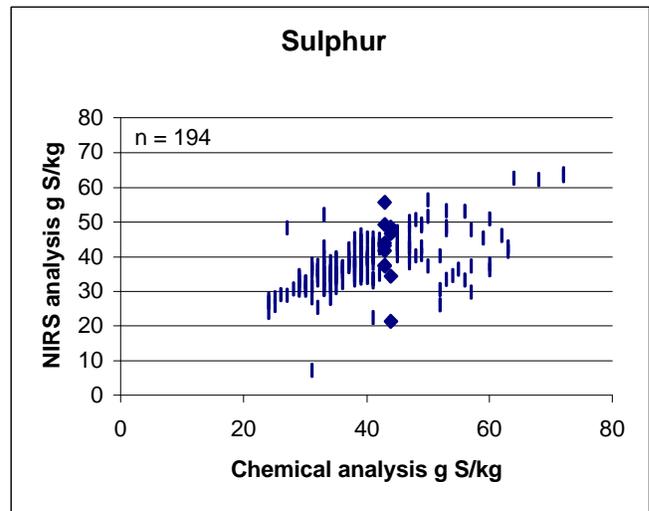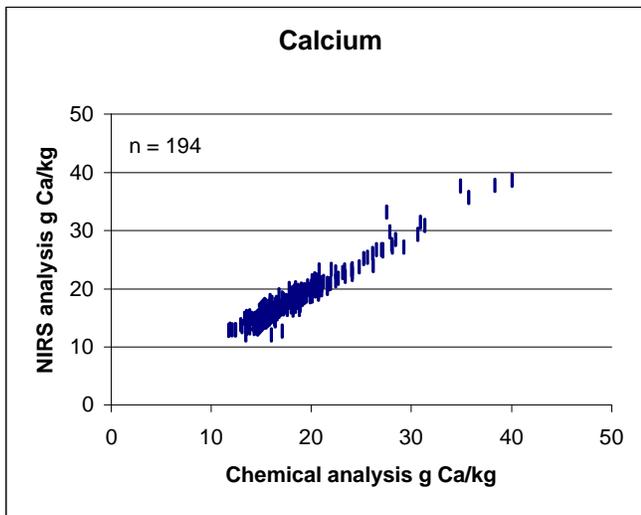

Figure 1- Relationship between chemical and NIRS data for calcium and sulphur in alfalfa samples