

**APPLICATION OF NEAR INFRARED REFLECTANCE SPECTROSCOPY (NIRS)
TO FORAGE EVALUATION IN URUGUAY**

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Abstract

Near infrared reflectance spectroscopy (NIRS) were used to assess the chemical composition of a wide range of forages. Nearly five hundred herbage samples covering a wide range of temperate pastures species and mixtures, previously analysed by conventional wet chemistry were scanned in reflectance with a NIRS 6500 (NIRSystems, Silver Spring, USA). The coefficients of determination in calibration (R^2) and the errors in cross validation (SECV) were 0.98 (5.8), 0.94 (22.2), 0.97 (5.6), 0.94 (6.9) and 0.89 (19.7) for crude protein, in vitro organic matter digestibility, dry matter, ash and acid detergent fiber, in g kg^{-1} on a dry weight respectively. The high correlation between NIRS and chemical analysis found in this study showed the potential use of NIRS for prediction forage quality.

Keywords: *Near infrared reflectance, NIRS, forages, quality, Uruguay*

Introduction

Forage quality can be assessed directly by feeding experiments, but is very costly and not possible with smaller quantities of forage from pasture evaluation and breeding trials. The two – stage *in vitro* rumen fermentation method used to determine *in vitro* organic matter digestibility (IVOMD) is the most suitable laboratory technique for predicting *in vivo* digestibility of forages. The method however, is laborious, time consuming and expensive. These disadvantages are particularly significant when the analysis is being conducted on a large number of samples associated with plant breeding programs. The use of near infrared reflectance spectroscopy (NIRS) to determine quality parameters in forages was reported since early seventies (Norris et al., 1976, Murray, 1986, 1993). The principle of NIRS is that light of wavelength 1100 – 2500 nm, reflected off powdered solids, contains compositional information which can be unravelled by a computer to report multiple analysis in under one minute. NIRS is based on a correlation between chemical properties, as determined by defined methods, and absorption light at different wavelengths in the near infrared region. In this paper the authors describe the use of NIRS for estimating crude protein, *in vitro* organic matter digestibility, dry matter, ash and acid detergent fiber in different forage species.

Material and Methods

Five hundred and thirty (n: 530) samples representing a wide range of species 286 grasses, 127 legumes and 53 mixtures of legumes and grasses. The samples were collected from two different years (1998, 1999), were dried in a forced-air drier at 60 °C for 48 hours and ground using a Willey Mill (1-mm sieve). Dry matter (DM), ash and crude protein (CP) were analysed according to AOAC (1990). Acid detergent fibre (ADF) was analyzed following the Goering and Van Soest (1970) method. *In vitro* organic matter digestibility

(IVOMD) were analyzed by two - stage in vitro technique (Tilley and Terry, 1963). All chemical analysis were performed in duplicate and expressed on a dry weight basis. Samples were scanned in reflectance mode (400 – 2500 nm) in a monochromator NIRS 6500 (NIRSystems, Silver Spring, MD, USA) in a small circular cup (50 mm diameter). Reflectance data were 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 standard error of calibration (SEC), the coefficient of determination in calibration (R^2), the standard error of cross validation (SECV) and the coefficient of determination in cross validation (1-VR) (Shenk and Westerhaus, 1993). The optimum calibrations were selected on the basis of minimizing the standard error of cross validation (SECV). The SECV/SD (standard error of cross validation /standard deviation of the constituent data) ratio was also calculated to evaluate the calibrations (Murray, 1993).

Results and Discussion

Table 1 shows the NIRS calibration statistics for all the chemical parameters in the pasture samples. The R^2 and SECV are high and consistent for DM, CP, IVOMD and Ash, but for ADF a lower R^2 was obtained. The relationships of NIRS data versus Chemical data for dry matter, in vitro organic matter digestibility, crude protein and ash, exhibited same

patterns; the ones for (a) dry matter and (b) in vitro organic matter digestibility are presented in figure 1. According to the ratio SECV/SD (less than 0.33) all the equations are acceptable for predicting the chemical parameters suggesting that the models built are very robust and not overfitted. The best equations were for dry matter and crude protein. It has been proposed for some authors that better calibration equations may be obtained by using individual species or groups of similar forage species instead (Marten et al., 1984) of the global broad-based calibrations (Barton, 1989; Shenk, 1989) as in this study. However, we have obtained high coefficients of determination and low SEC or SECV for all the components analysed suggesting global calibrations will detect less outlier samples and probably being more robust and efficient. The high correlation between NIRS and chemical analysis found in this study showed the potential use of NIRS for prediction of forage quality parameters in the range of temperate pastures species used in Uruguay.

References

- AOAC.** (1990). Official methods of analysis of the Association of Official Analytical Chemists. Edited by K. Helrich. Published by the Association of Official Analytical Chemists, Inc., 15th edition, Arlington, Virginia; USA. 1298 pp.
- Barnes, R.J.; Dhanoa, M.S. and Lister, S.J.** (1989). Standard Normal variate transformation and detrending of near infrared diffuse reflectance spectra. *Appl. Spectrosc.* **43**:772 - 777.
- Barton.II, F.E.** (1989). .NIR spectroscopy for forage analysis XVI International Grassland Congress, Nice, France. 899 – 891.
- Goering, H.K and Van Soest, P.J.** (1970). In: Forage fiber analysis. Agriculture Handbook N^o 379. US. Dep. Agric. Washington, DC.
- Marten, G.C.; Brink, G.E.; Buxton, D.R.; Halgerson, J.L.; Hornstein, J.S.** (1984). Near Infrared reflectance spectroscopy analysis of forage quality in four legume species. *Crop. Sci.* **24**. 1179 – 1182.
- Murray, I.** (1986). Near Infrared analysis of forages. In. Recent Advances in Animal Nutrition. Edited by W. Haresign and D.J.A. Cole. Studies in the Agricultural and Food Sciences. Butterworths. 141 -156.

Murray, I. (1993). Forage analysis by near infrared spectroscopy. In. Chapter 14. Sward Herbage Measurement Handbook. Edited by A. Davies; Baker, R.D.; Grant, S.A. and Laidlaw, A.S. British Grassland Society. 285 - 312.

Norris, K.H., Barnes, R.F., Moore, J.E., Shenk, J.S. (1976). Predicting forage quality by near infrared reflectance spectroscopy. *J. Anim. Sci.* **43**: 889 – 897.

Shenk, J.S. (1989). NIRS as an analytical tool for world agriculture. XVI International Grassland Congress, Nice, France. 883 – 884.

Shenk, J.S. and Westerhaus, M.O. (1993). Analysis of Agriculture and Food Products by Near Infrared Reflectance Spectroscopy. Monograph. Infracsoft International. Port Matilda, P.A. USA.

Tilley, J.M.A. and Terry, R.A. (1963). A two stage technique for the in vitro digestion of forage crops. *J. Br. Grassland Soc.* **18**: 104 – 111.

Table 1 - NIRS calibration statistics for chemical parameters in pasture samples in g kg⁻¹ of a dry weight basis.

	n	Mean	R²	SECV	SECV/SD
DM	505	916.9	0.97	5.60	0.15
<i>CP</i>	480	162.6	0.98	5.80	0.15
<i>IVOMD</i>	450	667.7	0.94	22.2	0.29
<i>Ash</i>	500	124.4	0.94	6.90	0.28
<i>ADF</i>	150	337.1	0.89	19.7	0.34

n: number of samples in calibration, SECV: standard error in cross validation, SD: standard deviation, DM: Dry matter, CP: crude protein, IVOMD: in vitro organic matter digestibility, ADF: acid detergent fiber.

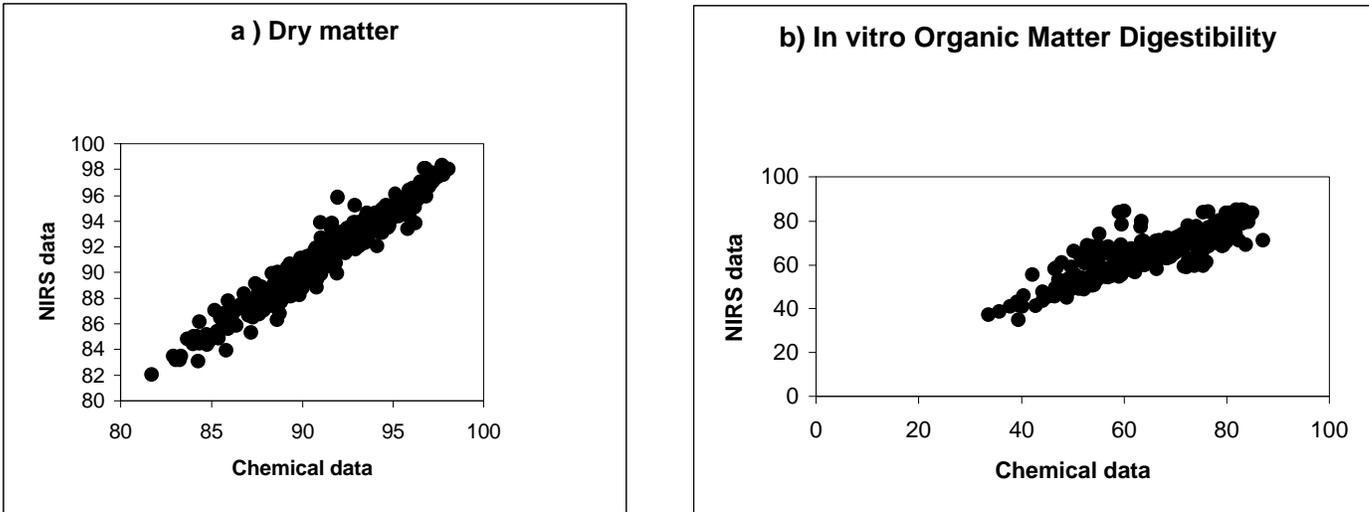


Figure 1 - NIRS data vs. dry matter contents and in vitro organic matter digestibility.