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Prediction of the nutritive value of annual forage clovers and serradella by near infrared spectroscopy (NIRS)

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Abstract. Near infrared reflectance spectroscopy (NIRS) was evaluated as a tool to estimate organic matter (OM), crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), water soluble carbohydrates (WSC), ether extract (EE) contents and in vitro organic matter digestibility (IVOMD) in samples of six annual legumes species (*Trifolium incarnatum*, *T. michelianum*, *T. resupinatum* –ssp. *resupinatum* and *majus*–, *T. vesiculosum* and *Ornithopus sativus*) grown as winter crops and harvested in different dates in spring. After selecting a collection of 316 samples, calibration equations were developed by modified partial least square regression of the reference values on the spectra records. The best coefficient of determination in cross validation (r^2) and range to error ratio (RER) values for OM, CP, ADF, NDF, WSC, EE and IVOMD were 0.71 and 11.8, 0.88 and 12.7, 0.91 and 15.3, 0.97 and 26.0, 0.91 and 20.6, 0.75 and 9.7 and 0.81 and 11.0, respectively. It is concluded that NIRS analysis is an useful tool for the prediction of the nutritive value of the annual forage legumes studied.

Keywords. Forage legumes – Chemical composition – Digestibility – NIRS.

Prédiction de la valeur nutritive des trèfles fourragères annuelles et de la serradelle par spectroscopie proche infrarouge (NIRS)

Résumé. *L'objectif de ce travail a été d'évaluer l'utilité de la technique de la spectrométrie dans le proche infrarouge (SPIR) pour la prédiction de la composition chimique et de la digestibilité de six espèces de légumineuses fourragères annuelles (*Trifolium incarnatum, T. michelianum, T. resupinatum –*ssp.* resupinatum *et* majus–, T. vesiculosum *et* Ornithopus sativus*) cultivées en cultures d'hiver et récoltées à des dates différentes au printemps. Des équations de calibrage SPIR ont été développées (méthode de «modified partial least squares») pour prédire des paramètres tels que : la matière organique (OM), protéines (CP), fibres (ADF, NDF), sucres solubles (WSC), lipides (EE) et la digestibilité in vitro de la matière organique (IVDMO), à partir d'une collection de 316 échantillons. Les meilleures valeurs de coefficients de détermination en validation croisée (r^2) et de rapports de la plage de valeurs à l'erreur de prédiction (RER) pour l'OM, CP, ADF, NDF, WSC, EE et IVOMD ont été 0,71 et 11,8, 0,88 et 12,7, 0,91 et 15,3, 0,97 et 26,0, 0,91 et 20,6, 0,75 et 9,7 et 0,81 et 11,0, respectivement. On en conclut que l'analyse SPIR constitue une méthode très utile pour la prédiction de la valeur nutritive des légumineuses fourragères annuelles étudiées.*

Mots-clés. *Légumineuses fourragères – Compositon chimique – Digestibilité – SPIR.*

I – Introduction

Galician dairy cow farms (located in the NW atlantic-humid corner of the Iberian peninsula) produce 2.2 million of tonnes of milk, accounting for about 40% of total dairy production and more than half of dairy cow producers in Spain (MARM, 2010). About half of dairy milk produced in Galicia comes from 2,000 farms (owning more than 300 tonnes of milk quota per holding) in

which the typical winter daily ration fed to the lactating cows (in a DM basis) includes 30-40% of maize silage and 15-25% of grass silage (Flores *et al*., 2011). Most of these farms follow an intensive forage rotation system of two crops per year in the arable land with maize and italian ryegrass as summer and winter silage crops, respectively, although more than one third of them practise the monoculture of maize, leaving the land uncultured during the winter between successive maize crops (Fernández-Lorenzo *et al*., 2009). The low protein content of the forage produced in this system, coupled with the increasing prices of the nitrogen and growing environmental concerns has led to a recent farmer's interest in the inclusion of legume forages in the rotation. Following some promising results arising from the evaluation of some new annual forage legume species as winter crops for silage (Resch-Zafra *et al*., 2010) a number of farms have began to grow annual *Trifolium* and *Serradela* species, arising the need of having reliable methods for the routine evaluation of its nutritional value for both research and advisory purposes.

NIRS is an alternative to standard analytical methods for determining nutritive value of forages and has become widely recognized as a valuable tool in the accurate determination of the chemical composition of a wide range of forages (Shenk and Westerhaus, 1985). The key to successful use of the NIRS technique is to develop a calibration model, based in a large calibration database which adequately represents the characteristics of the forage problem samples to be predicted.

In the present study, it is evaluated the potential use of NIRS to predict chemical composition and in vitro digestibility of species of new annual forage legumes grown for silage as the first step in providing a robust, fast and inexpensive laboratory method for estimating the nutritional value of these species.

II – Materials and methods

This work was carried out with samples of six annual forage legume species which were harvested at six different dates during the first and second spring growth (15 March-24 May and 26 April-5 July, respectively) in an experiment carried out at the Centro de Investigacións Agrarias de Mabegondo (A Coruña, Galicia, Spain) in year 2010. The species studied were Crimson clover (*Trifolium incarnatum* L. cv Viterbo), Balansa clover (*T. michelianum* Savi. cv. Bolta), Persian clover (*T. resupinatum* L. ssp. *resupinatum* cv. maral and ssp. *majus* cv. Kyambro), Arrowleaf clover (*T. vesiculosum* Savi. cv. Zulu) and French serradella (*Ornithopus sativus* Brot. cv. Margurita). Dry matter (DM) content of fresh samples was determined by oven-drying at 80ºC for 16 hours and the spectra of dry samples (n=545), ground in a Christy-Norris hammer mill to pass a 1 mm screen, were registered in a Foss NIR Systems 6500 monochromator (spectofotometric NIRSystems 6500 (FOSS NIRSystems, Inc., Silver Spring, Washington, USA).

Two aliquots of each sample were scanned in a spinning circular cup with a quartz window of 37.5 mm diameter, at 2 nm intervals (1050 data points) in the wavelength range of 1100-2500 nm. The spectrum of each sample was the average of the two sub-samples. Initially, all spectral data were recorded as the log 1/reflectance (log 1/R values). Samples with extreme (i.e. outliers) or very similar spectra (Mahalanobis distance H of >3.0 and a minimum standardized distance NH <0.6, respectively) were excluded from the calibration data set, unless they were thought to provide relevant information (Shenk and Westerhaus, 1996). Data were processed using the software WinISI Version 1.5 (Infrasoft International, Port Matilda, USA, 2000).

Wet chemistry of selected samples (n = 316) was carried out by reference methods, which included the determination of organic matter (OM) by ignition in a muffle furnace overnight at 500ºC, crude protein (CP, as total Kjeldahl N x 6.25, being N the nitrogen content) following Castro *et al*., (1990), acid detergent fiber (ADF) and neutral detergent fiber (NDF) by procedure of Goering and Van Soest, (1970), water soluble carbohydrates (WSC) by colorimetry (Castro, 2000) and

ether extract (EE) by the Soxtec method (AOAC official method 2003.06). *In vitro* organic matter digestibility (IVOMD) was performed by the procedure described by Tilley and Terry (1963), modified by Alexander and McGowan (1966). All parameters were reported on a dry matter basis and analysed in duplicate.

Calibration equations were obtained using a Modified Partial Least Squares (MPLS) regression technique (Martens and Naes, 1987). This regression technique requires cross-validation to prevent overfitting, obtaining validation errors by partitioning the calibration set into several groups and pooling them into a standard error of cross-validation. MPLS of reference values on the second derivative of standard normal variate (SNV) and de-trended spectra (Barnes *et al*. 1989) was the best regression method for all the determinations. The statistics parameters used to test the performance of the calibration equations were the standard errors (SEC and SECV) and coefficients of determination (R^2 and r^2) obtained in the calibration and cross validation steps, respectively. The range error ratio (RER) of cross validation, as defined by the range of the population's reference values divided by the corresponding SECV was also considered since it is an useful statistic to test the accuracy of the calibration models (Williams and Sobering, 1996).

III – Results and discussion

The characteristics of the reference values of the calibration data set and the statistics to describe the quality of NIRS calibration and prediction equations selected are shown in Table 1. Both the number of samples and the variability in their chemical composition and *in vitro* digestibility were found to be adequate for developing initial NIRS calibrations. The values obtained for the coefficients of determination ranged from 0.85 (EE) to 0.98 (NDF) in the calibration step, and from 0.71 (OM) to 0.97 (NDF) in the cross validation step, showing a high proportion of variability in the reference data accounted for by the regression equation (except for OM and EE). These values, together with SEC and SECV are within the acceptable range cited by other authors which developed NIRS calibrations for estimating the nutritive value of temperate forages (Murray, 1993). On the other hand, the values of the RER statistic confirms the apparent good precision of the equations developed, according with Williams and Sobering (1996) who suggest a minimum RER value of 10 for an adequate estimation quality in NIRS calibrations. These results are also in agreement with those reported by Li *et al*. (2011) for the prediction of nutritive values of annual *Trifolium* species.

Table 1. NIRS statistics of the calibration equation used for the prediction of organic matter (OM), crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), water soluble carbohydrates (WSC), *in vitro* organic matter digestibility (IVOMD) and ether extract (EE) of the annual forage legume species

Variable	N	Mean	SD	Range	SEC	SECV	R^2	r^2	RER
Chemical composition (%DM)									
OM	316	88.24	1.77	82.05 - 93.32	0.47	0.95	0.91	0.71	11.83
CP	315	18.21	4.20	9.26 - 28.13	0.87	1.48	0.96	0.88	12.73
ADF	316	28.64	7.08	11.55 - 44.35	1.41	2.13	0.96	0.91	15.37
NDF	316	33.54	7.87	14.54 - 51.58	1.01	1.42	0.98	0.97	26.03
WSC	316	12.27	4.09	3.81 - 29.36	0.61	1.24	0.97	0.91	20.66
EE	242	1.75	0.42	0.91 - 2.95	0.17	0.21	0.85	0.75	9.71
In vitro OM digestibility (%)									
IVOMD (%)	308	75.20	6.50	54.69 - 86.05	1.98	2.84	0.90	0.81	11.05

N = number of samples, SD = standard deviation, SEC = standard error of calibration, SECV = standard error of cross validation, R^2 and r^2 = coefficient of determination in calibration and cross validation, RER = Range/SECV.

IV – Conclusions

This work demonstrates the promising potential of NIRS for assessing the nutritive value of annual forage legumes, showing a good performance in the prediction of crude protein, cell-wall components, water-soluble carbohydrates and *in vitro* OM digestibility. It is nevertheless advisable to check the equation in blind tests on open sets into the future and include new samples from different years and environments to increase the robustness of the predictions.

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