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Instantaneous determination of chemical composition of *Festuca* sp. and *Dactylis* sp. at two different cut times using near infrared spectroscopy (NIRS)[1]

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SUMMARY – Near infrared spectroscopy (NIRS) was explored as a technique to predict crude protein, moisture, acid detergent fibre (ADF), neutral detergent fibre (NDF), acid detergent lignin (ADL) and digestibility. The sample set consisted of 123 samples of *Festuca arundinacea* Schreb and 111 of *Dactylis glomerata* L. The samples were measured by reflectance NIR in a 1100-2500 nm range. NIR models were developed based on a partial least square regression (PLSR) using different smoothing treatments like absorbance, first derivate and normalization. Calibration models established by near infrared spectroscopy (NIRS) were found to be suitable for the rapid, accurate and non-destructive quantification of *Dactylis glomerata* L. and *Festuca arundinacea* Schreb components. Crude protein, moisture and ADF were predicted with an excellent precision (R>0.90). NDF and digestibility calibrations were good because, in spite of having a good coefficient of determination (R>0.90), the errors in *Festuca arundinacea* Schreb, are slightly high. ADL had a poor prediction power due to the low coefficient of determination (R<0.75).

Keywords: Forage-quality, crude protein, digestibility, acid detergent fibre, neutral detergent fibre, moisture and acid detergent lignin.

RESUME – *"Détermination instantanée de la composition chimique de* Festuca *sp. et* Dactylis *sp. à deux périodes différentes de coupe en utilisant la spectroscopie dans le proche infrarouge (NIRS)". La spectroscopie dans le proche infrarouge (NIRS) a été exploitée comme une technique pour prédire la protéine totale, l'humidité, la fibre acide détergente (FAD), la fibre neutre détergente (FND), la lignine acide détergente (LAD) et la digestibilité. L'ensemble des échantillons était composé de 123 échantillons de* Festuca arundinacea *Schreb et 111 de* Dactylis glomerata *L. Les échantillons ont été mesurés par réflectance dans le proche infrarouge, en utilisant la gamme de longueurs d'onde entre 1100 et 2500 nm. Les modèles NIRS obtenus étaient basés sur la régression par moindres carrés partiaux, et différents traitements de lissage comme l'absorbance, la normalisation et la première dérivée, ont été utilisés. On peut considérer les modèles de calibration comme étant bons en ce qui concerne la rapidité, l'exactitude et la quantification non destructive des échantillons de* Dactylis glomerata *L. et* Festuca arundinacea *Schreb. Une excellente précision a été trouvée en protéine totale, humidité et FAD (R>0,90). La prédiction de FND et digestibilité a été considérée comme bonne parce que, malgré ses bons coefficients de détermination, les erreurs trouvées pour* Festuca arundinacea *Schreb ont été un peu élevées. Concernant LAD, la précision obtenue a été faible à cause d'un coefficient de détermination très petit (R<0,75).*

Mots-clés : *Qualité du fourrage, protéine brute, digestibilité, fibre acide détergente, fibre neutre détergente, humidité et lignine acide détergente.*

Introduction

Plant improvement for quality purposes depends on the ability to evaluate large number of individuals where the only way to deal with the increased variability is with more frequent analyses and sampling (Fassio and Cozzolino, 2004). Although the standard analytical techniques usually offer a high level of accuracy and precision, they also show some handicaps, such as high costs, high labour input and delay in reporting (Gáspár *et al*., 2005). The availability of rapid and non-destructive methods to evaluate seed quality traits is one of the most important factors that determine plant breeding projects success. Near infrared reflectance spectroscopy (NIRS) is a multitrait technique of large application in the analysis of quality characteristics in food and agricultural commodities (Shenk and Westerhaus, 1996).

[1] Work based on dry matter from PERMED project trials (WP3-T2), contract nº INCO-CT-2004-509140.

Based on the C-H, N-H, and O-H absorption frequencies by functional groups in the near-infrared (NIR) region (Jin and Chen, 2007), the rapid determination of chemical composition of grasses using near-infrared reflectance spectroscopy (NIRS) is reported in this work. This method is based on the construction of multivariate calibration models combining spectrometric data and traditional chemical composition results, obtained with standard laboratory methods. NIRS was used to develop calibration models for the chemical composition of gramineae to predict their quality. Reports were found in the literature that related to the determination of crude protein, moisture, fibre and digestibility in forages (García-Criado *et al.,* 1977).

Materials and methods

NIRS analyses were performed using a monochromatic spectrophotometer (InfraAlyzer 500, Bran+Luebbe GmbH) and milled grass samples were analysed. Spectra were recorded in reflectance mode. In this mode, a ceramic standard is placed in the radiant beam, and the diffusely reflected energy is measured. The scanning range from 1100 to 2500 nm and wavelength increments of 2 nm was used. Each sample was scanned twice and the average spectra were used for calibration. Samples used in the NIRS analyses, in the calibration and validation groups, were selected to represent the whole spectral and chemical variability in the population. A total of 234 forage samples belonging 123 to *Festuca arundinacea* Schreb and 111 to *Dactylis glomerata* L., derived from the harvest of 2 subsequent moments (winter and early spring) the years 2005/06 and 2006/07, and coming from a experimental field (PERMED E – *Improvement of Native Perennial Forage Plants for Sustainability of Mediterranean Farming Systems*) located in the Estação Nacional de Melhoramento de Plantas (Elvas, Portugal) were used for the NIRS analises. 108 Festuca samples and 96 Dactylis samples, were selected randomly, were used for calibration, and the remaining were used to validate the resulting calibration models.

Grass samples were analyzed for moisture by the constant weight method, drying at 105ºC; nitrogen content was determined by Kjendahl analysis, and a factor of 6.25 was used to estimate the protein content (PC) (AOAC, 1990); digestibility (D) was determined by Pepsina celulase method (Jones and Hayward, 1975); acid detergent fiber (ADF), neutral detergent fiber (NDF) and lignine were determined according to procedures described by Goering and Van Soest (1970). Calibrations were performed by using the "Sesame" software (version 3.01, Bran+Luebbe). Calibration equations were computed by using the raw optical data (log 1/R), or first derivate or normalization of the log 1/R data with several combination of segment (smoothing). The use of derivative spectra instead of the raw optical data to perform calibration is a way of solving problems associated with overlapping peaks and baseline correction. Partial Least Square Regression (PLSR) was used to construct models and the error between modelled (spectral data) and reference (chemical) values, the standard error of cross-validation (SECV), was reported. Model performance was measured by the multiple coefficient of determination (R^2), and the standard error of performance (SEP) and standard error of validation (SEV). Maximum coefficient of determination was also calculated (R^2max).

$$SECV = \sqrt{\frac{\sum (Cx - Ci)^2}{n-1}} \qquad SEP = \sqrt{\frac{\sum (Cx - Ci)^2}{m}} \qquad R^2 \max = 1 - \left(\frac{SEL}{SD}\right)^2$$

Were *Ci* is the reference measured values, *Cx* the NIRS modelled values, *n* the number of samples in the model and *m* is the number of samples in the validation.

An external validation procedure was carried out to determine the accuracy and precision of the equations obtained in the calibration for each component in each species. To evaluate this accuracy of the equations, different statistics were used, namely the coefficient of determination (r^2) (Williams, 1987).

Results and discussion

The means, ranges and standard deviations of *Festuca arundinacea* Schreb and *Dactylis glomerata* L. for chemical parameters and the number of outliers of grass parameters of calibration and validation are shown in Tables 1 and 2.

Table 1. Means, ranges, standard deviations (SD) and number of outliers of grass parameters in calibration and validation sets of NIRS analysis

Gramíneae	Atribute	n Outlier		n	Mean	SD	Range
Festuca	Protein (%)	6	Cal	108	15.64	3.32	10.1 – 22.3
			Val	15			
	Moisture (%)	16	Cal	108	5.77	1.72	3.02 – 8.68
			Val	15			
	ADF (%)	2	Cal	108	29.69	1.81	25.2 – 33.4
			Val	15			
	NDF (%)	4	Cal	108	49.34	5.67	39.3 – 63.8
			Val	15			
	ADL (%)	6	Cal	108	4.56	1.36	2.47 – 8.74
			Val	15			
	Digestibility	3	Cal	108	64.43	5.18	51.5 – 73.3
			Val	15			
Dactylis	Protein (%)	4	Cal	96	17.10	4.22	8.6 – 25.7
			Val	15			
	Moisture (%)	3	Cal	96	6.0	1.54	2.95 - 8.69
			Val	15			
	ADF (%)	7	Cal	96	30.59	3.03	22.0 – 37.8
			Val	15			
	NDF (%)	11	Cal	96	51.0	4.64	40.9 – 60.9
			Val	15			
	ADL (%)	5	Cal	96	5.16	1.61	2.44 – 9.35
			Val	15			
	Digestibility	7	Cal	96	66.13	7.86	46.8 – 81.8
			Val	15			

Table 2. Statistic treatment, errors (SEP, SECV and SEV). Coefficient of correlation in calibration (R), coefficients of determination in calibration (R^2) and validation (r^2) and maximium coefficient of determination (R^2_{max})

Specie	Atribute	Statistic treatment	SEP	SECV	SEV	R	R^2	R^2max	r^2
Festuca	Protein (%)	Normalization	0.61	0.65	0.79	0.98	0.96	0.97	0.96
	Moisture (%)	Normalization	0.43	0.65	0.77	0.94	0.88	0.94	0.95
	ADF (%)	1st Derivate	0.75	0.83	0.93	0.91	0.83	0.83	0.85
	NDF (%)	1st Derivate	1.00	2.17	3.36	0.94	0.88	0.97	0.91
	ADL (%)	Normalization	1.1	1.21	1.35	0.57	0.33	0.34	0.12
	Digestibility	Normalization	1.55	2.26	2.27	0.93	0.86	0.91	0.91
Dactylis	Protein (%)	Absorbance	0.61	0.85	0.88	0.98	0.96	0.98	0.93
	Moisture (%)	1st Derivate	0.43	0.76	0.79	0.94	0.88	0.92	0.72
	ADF (%)	1st Derivate	0.75	0.86	0.98	0.93	0.87	0.94	0.93
	NDF (%)	Normalization	1.00	1.97	2.02	0.93	0.86	0.92	0.87
	ADL (%)	1st Derivate	1.1	1.40	1.40	0.58	0.34	0.54	0.04
	Digestibility	Normalization	1.55	1.65	1.88	0.98	0.96	0.96	0.94

Crude protein, moisture, ADF, NDF and digestibility were predicted by NIRS with a high accuracy (Table 2). The coefficients of correlation (R) are very high (> 0.90) reaching values close to one. Shenk and Westerhaus (1996) indicate that NIR equations with coefficient of correlation values higher than 0.90 have an excellent accuracy.

The comparison of the values obtained at the laboratory with the ones predicted by NIR by cross-validation, reveals the goodness of the predictions obtained for each parameter; this goodness is

proved by the errors, because the SECV values are slightly higher than the SEP values (Table 2). The difference between SECV and SEP in humidity is high due to the residual humidity content. This residual content humidity content is variable in time and it is affected by the conservation time in laboratory. Time between chemical analisys and NIRS analisys is really important to establish good models, and it could explain the differences between SECV and SEP in this work, just as Cozzolino (2003) explained. Furthermore, the use of the constant weight method is not the more recommended way to get the humidity, when used as reference chemical method in NIRS calibrations.

Fibre is a really interesting component in *Festuca arundinacea* Schreb and *Dactylis glomerata* L. because it is a principal component. Bad coefficients of correlation were observed in ADL calibrations, while the differences between SECV and SEP are increasing from ADF < NDF < ADL (Table 2). As Cozzolino (2003) showed, NIRS prediction power depends on the anayized parameter complexity; this precision decreased in a slithly way in NDF and widely in ADL case. This drecrease in NIRS prediction is due to the difficulty of determining complex constituents in the cellular wall, as NDF or ADL, by near infrared region (Abrams *et al.,* 1987).

In digestibility, the comparison of the values obtained in laboratory with those predicted by NIR by cross-validation, reveals the goodness of the predictions obtained for *Dactylis*, having very similar SECV and SEP values, 1.65 against 1.55. In *Festuca* the differences are higher (2.26 against 1.55) (Table 2). When external validation was applied to samples not belonging to the same group the coefficients of determination in validation (r^2) are fully acceptable (Table 2). All this confirms the high accuracy of the developed equations.

Conclusions

To sum up, calibration models established by near infrared spectroscopy (NIRS) were found to be suitable for the rapid, accurate and non-destructive quantification of *Dactylis glomerata* L. and *Festuca arundinacea* Schreb components, including protein, moisture, ADF, NDF and digestibility.

Having good calibrations, protein, moisture and ADF were predicted more successfully than NDF or digestibility. On the other hand ADL was poorly predicted by NIRS.

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