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The prediction of chemical composition and *in vitro* digestibility of samples of *Atriplex halimus* by NIR spectroscopy

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SUMMARY – *Atriplex halimus* is a perennial shrub widely expanded under Mediterranean conditions, which provides a notable amount of forage during times when food scarce is remarkable. To assess its nutritive value it is necessary to know their changes over time. Conventional analytical methods have been used to evaluate feed and forage materials. However the cost, labour and time-consumption are very high. Near Infrared Spectroscopy (NIRS) is a physical technique with the potential of allowing rapid and accurate determination of the chemical composition and nutritive value of different feeds. As part of a research study of the seasonal variation in composition of the *Atriplex halimus* in the Aragon region of Spain, 318 plant samples were obtained and analysed for the chemical and *in vitro* composition: dry matter (DM), ash, crude protein (CP), neutral and acid detergent fibre (NDF, ADF), acid detergent lignin (ADL), *in vitro* dry matter digestibility (IVDMD), potassium (K), chloride (Cl) and calcium (Ca). Accuracy of determination by NIRS was evaluated by the coefficient of determination in calibration and cross validation (R^2 and r^2), standard error of calibration (SEC), standard error of cross validation (SECV). The results obtained showed that NIRS could explain 81, 92, 92, 89, 85, 92, 63, 70 and 90%, of the variation in ash, CP, NDF, ADF, ADL, IVDMD, Na, K and Cl respectively. NIR spectra of these samples gave useful calibration models for all of these attributes except calcium (r^2 = 0.04).

Key-words: Nutritive value, chemical composition, *Atriplex*, seasonal variation, NIRS.

RESUME – *"Prédiction de la composition chimique et de la digestibilité* in vitro *de l'*Atriplex halimus *par spectroscopie proche infrarouge (NIRS)". L'*Atriplex halimus *est un arbrisseau à feuilles persistantes, largement répandu le long des plages méditerranéennes, à cause des conditions climatiques. Il fournit une quantité considérable de fourrage dans les périodes où la nourriture se fait spécialement rare. Pour en calculer la valeur nutritionnelle il faut connaître ses changements dans le temps. Nous avons appliqué les méthodes analytiques conventionnelles pour évaluer les valeurs alimentaires et fourragères. Le coût, la main-d'œuvre et le temps nécessaire pour appliquer ces méthodes sont très élevés. Le NIRS est une technique physique permettant de définir promptement et avec exactitude la composition chimique et la valeur nutritionnelle de plusieurs aliments. Au cours d'une investigation entreprise dans la région espagnole de l'Aragon sur les variations saisonnières de la composition de l'*Atriplex halimus*, 318 échantillons ont été prélevés et analysés pour en déterminer la composition chimique et la digestibilité* in vitro *: matière sèche (DM), cendres, matières azotées totales (CP), constituants pariétaux (NDF), lignocellulose (ADF), lignine (ADL), digestibilité de la matière sèche* in vitro *(IVDMD), sodium (Na), potassium (K), chlorure (Cl) et calcium (Ca). L'exactitude de la composition définie par le NIRS a été évaluée par le coefficient de définition dans le calibrage et la validation croisée (R^2 et r^2), l'erreur standard du calibrage (SEC), et l'erreur standard de la validation croisée (SECV). Les résultats obtenus ont affiché que le NIRS peut expliquer 81, 92, 92, 89, 85, 92, 63, 70 et 90%, de la variation de la valeur des cendres, CP, NDF, ADF, ADL, IVDMD, Na, K et Cl, respectivement. Les spectres NIRS de ces échantillons ont donné des modèles utiles de calibrage pour tous les éléments mentionnés, sauf pour le calcium (r^2 = 0,04).*

Mots-clés : *Valeur nutritionnelle, composition chimique,* Atriplex*, variation saisonnière, NIRS.*

Introduction

In arid and semi-arid areas, the herbaceous vegetation withers during dry periods and becomes lethargic during cold periods, leading to a considerable scarcity of forage during large part of the year. *Atriplex halimus* is a spontaneous shrub-like species in Aragon, accustomed to dry conditions, such as water scarcity, high temperatures in summer and high salinity of the soil (Osmond *et al*., 1980, cited by Le Houérou, 1992); it is a perennial shrub producing considerable biomass for browsing, which may cover part of the cattle's food needs during the periods when there is a lack of forage (Wilson, 1977). In addition, this shrub species creates a micro-habitat around it, which favours the

development of herbaceous vegetation as the leaves provide shade and organic matter (Wills *et al*., 1990). These characteristics converts the *Atriplex halimus* into an attractive species for the dry area of Aragon. However, *Atriplex halimus* is characterised by high mineral and ash contents (Muñoz *et al.*, 1994) which must be taken into account when the nutritive value is determined.

Near Infrared Reflectance Spectroscopy (NIRS) is a technology which due to its features is of great value for an instantaneous prediction of the nutritive value of feeds and forages (Garrido, 1997).

NIRS technology is based on the fact that each of the major chemical components of a sample has near infrared absorption properties in the region 700-2500 nm. The summation of these absorption properties, combined with the radiation-scattering properties of the sample, determines the diffuse reflectance spectra which in turns provides information about chemical, physical and sensorial properties (Shenk and Westerhaus, 1995). NIRS has been used by forage researchers engaged in plant breeding, crop management, plant physiology and animal nutrition. However, the accuracy of the NIRS equations obtained depended of the adherence to critical imperatives outlined by Shenk and Westerhaus (1995), one of the critical factors being the ensuring that the calibration samples adequately represent all the variation sources (e.g. varieties, year of cultivation, harvest time, drying system, etc.) associated to a given forage.

Although the ash and mineral content of forage and food for cattle have been successfully predicted several times by NIRS (Shenk and Westerhaus, 1985; Ruano *et al*., 1999), other authors (Valdes *et al*., 1985; Clark *et al.*, 1989; Reeves, 2001) have not achieved good calibrations between the content of different minerals and the NIRS spectra, probably due to their lack of association with hydrated inorganic molecules or organic molecules (Clark *et al*., 1986).

The objective of the present work was to estimate the potential of the NIRS technique to predict the chemical composition, mineral content and *in vitro* dry matter digestibility of *Atriplex halimus* browse part.

Materials and methods

Twelve populations of *Atriplex halimus* were collected, 11 autochthonous from Aragon, each one from a different area, and 1 from Tunisia. These populations were planted in 1990 in a plot of land of the city of Zaragoza (Spain). Fifteen shrubs per population were planted, distributed into three random blocks with a plantation framework of 4×2 m^2.

In 1992, the plants were sampled every two months in order to study their evolution throughout the year. The sampling was conducted by picking shoots of approximately 15 cm, which were considered as the part that can be browsed by the animals.

The samples obtained were dried at 60°C in an oven with forced ventilation and later ground to a size of 1 mm. The chemical analyses performed were: ash and crude protein (CP) (AOAC, 1990), neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL) (Goering and van Soest, 1970); the sodium was determined by atomic absorption spectrophotometer, the chloride content by chloride-meter. The *in vitro* dry matter digestibility (IVDMD) was determined by the Tilley and Terry (1963) method.

NIRS analyses were carried out using a Foss NIR Systems 6500 monochromator (Foss NIR Systems, Silver Spring, MD, USA) which scans the spectral range 400-2498 nm. The analysis was carried out using the spinning small ring cup cell. Two sub-samples of each *Atriplex* sample were scanned and the average spectra of both sub-samples was used for calculations. All spectra and reference data were recorded and managed with the software ISI-NIRS2 Version 4.0 (Infrasoft International, Port Matilda, PA, USA).

The Modified Partial Least Squares (MPLS) regression technique was used to develop the NIRS calibrations. The standard normal variate and detrend (SNVD) scatter correction procedure was applied to the spectral data. Then the spectra were transformed through a mathematical first order derivation. The statistics used for equation development and evaluation were: the standard error of

calibration (SEC) and cross validation (SECV), the coefficient of determination for the calibration (R^2) and for the cross validation (r^2), and the RER value.

The R^2 and r^2 statistics give the percentage of the variance explained by the regression model and for the calibration and validation sets respectively. The SEC and SECV values are the standard errors of the estimate and both are a measure of the error between the lab (reference) analysis and the NIRS value. The SECV is used to determine the optimum number of PLS factors that can be supported in the prediction model and it is preferred to the SEC for assessing the accuracy of a NIRS equation (Shenk and Westerhaus, 1995a,b, 1996). The RER value is defined as the ratio of the range in the reference data for the samples to the SECV values (Williams and Sobering, 1996).

Results and discussion

The statistical coefficients of the best calibration equations obtained are shown in Table 1. The samples obtained are characterised by a wide range in the concentration of the different chemical composition components and IVDMD except for the determination of calcium (1.04-2.71%). No references have been found in literature that specify the variation range of this mineral in the *Atriplex halimus* species, so we do not know if this is the real range of calcium or if we have not collected samples with sufficient variability. Thus, we think that it is necessary to continue working on this issue.

Table 1. Performance of NIR calibrations for the *Atriplex halimus*†

	n	Range	SEC	R^2	SECV	1-VR
CP	295	8.34 - 31.03	1.35	0.93	1.43	0.92
Ash	308	11.42 - 29.28	1.47	0.87	1.80	0.81
NDF	141	29.71 - 52.72	1.80	0.94	2.07	0.92
ADF	139	13.82 - 37.48	1.53	0.91	1.74	0.89
ADL	145	5.75 - 21.56	0.85	0.87	0.91	0.85
IVDMD	142	45.87 - 90.09	2.90	0.93	3.05	0.92
Ca	152	1.04 - 2.71	0.73	0.02	0.76	0.04
Na	115	1.00 - 7.06	0.68	0.71	0.76	0.63
K	112	0.99 - 5.37	0.49	0.82	0.62	0.70
Cl	192	0.24 - 10.38	0.59	0.93	0.69	0.90

†SEC: standard error of calibration; R^2: coefficient of determination in calibration; SECV: standard error of cross-validation; 1-VR: coefficient of determination in cross-validation; n: number of samples.

The standard errors obtained for the different determinations are similar to those obtained by other authors who worked with Mediterranean tree foliage (Waelput *et al*., 1990). The crossed validation determination coefficients obtained can be considered as acceptable, varying between 0.63 and 0.93, except for the determination of calcium (0.04). Several authors have successfully obtained calibration equations to analyse calcium in different forages (Ruano *et al*., 1999). The limited variation range of the calibration population may be the cause of the low value of 1-VR obtained for this mineral.

Conclusions

The NIRS technique can be considered as acceptable to predict the chemical composition, IVDMD and Na, Cl and K content of *Atriplex halimus*. Results indicated that this technique was not suitable for the determination of Ca in *Atriplex halimus*.

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