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in

Delgado I. (ed.), Lloveras J. (ed.).
Quality in lucerne and medics for animal production

Zaragoza : CIHEAM
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 45

2001
pages 199-203

The prediction of the nutritive value of Mediterranean alfalfa forage by NIRS

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SUMMARY – The objective of the present work was to develop calibration equations for the prediction of chemical composition and forage digestibility in three different populations of Mediterranean alfalfa. For that purpose, samples from three previous research studies were used. NIRS calibration equations were developed for the prediction of ash content, crude protein (CP) and neutral detergent fibre (NDF) of hays (population 1) and green forage (population 2). The results obtained show that NIRS equations explain 77-95%, 93-99% and 92-90% of the variation in ash, CP and NDF existing in alfalfa hays and alfalfa green forage grown in Mediterranean locations. Calibration equations for *in vitro* (IVDMD) and *in vivo* (DMD, DMO) digestibility were also obtained for green forage (population 2) and hay and dehydrated alfalfa (population 3) respectively. The precision of the equations obtained for IVMD could be limited by the low repeatability of the Tilley and Terry analysis. The equations obtained for the prediction of *in vivo* DMD and OMD explain 71% and 87% of the variation existing in the reference data, which open great prospects for the use of NIRS in planning feeding strategies of Mediterranean livestock, based on alfalfa forage.

Key words: Chemical composition, digestibility, alfalfa forage, NIRS.

RESUME – *"La prédiction de la valeur nutritive du fourrage de luzerne méditerranéenne par SPIR". L'objectif de ce travail a été de développer des équations de calibration pour la prédiction de la composition chimique et la digestibilité de la luzerne méditerranéenne. On a utilisé des échantillons de trois études de recherche antérieures. Des équations de calibrage SPIR ont été développées pour la prédiction de la teneur en cendres, en matières azotées totales (MAT) et en fibres (NDF) de foins (population 1) et de fourrages verts (population 2). Les résultats obtenus montrent que les équations SPIR peuvent expliquer 77-95%, 93-99% et 92-90% de la variation de cendres MAT et NDF qui existe dans les foins de luzerne et dans le fourrage vert de luzernes cultivées dans la région méditerranéenne. Les équations de calibration pour la digestibilité* in vitro *(IVDMD) et* in vivo *ont été aussi obtenues respectivement pour les fourrages verts (population 2) et le foin et la luzerne déshydratée (population 3). La précision des équations obtenues pour IVDMD pourrait être limitée par la faible reproductibilité de l'analyse de Tilley et Terry. Les équations obtenues pour la prédiction de la digestibilité* in vivo *expliquent 71% et 87% de la variation existante dans les données de référence qui montrent de grandes perspectives pour l'utilisation de SPIR dans la planification des stratégies d'alimentation du bétail méditerranéen basées sur le fourrage de luzerne.*

Mots-clés : *Composition chimique, digestibilité, fourrage, SPIR.*

Introduction

Alfalfa is one of the most important forage plants cultivated in all the continents, and haymaking is the most important method of conservation of that forage. The nutritive value of alfalfa forage depends on the cultivar and many environmental factors (Alibés *et al.,* 1991).

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 turn 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 on 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 (eg. varieties, year of cultivation, harvest time, drying system, etc.) associated to a given forage.

The objective of the present work was to develop calibration equations for the prediction of chemical composition and digestibility in three different populations of Mediterranean alfalfa forage.

Material and methods

Population 1 (Hay). Samples and reference analyses

The fresh alfalfa samples were collected during 1994 to 1995 from an irrigated lucerne plot located at the Servicio de Investigación Agraria–Diputación General de Aragón (SIA-DGA, Spain) in the first and second years of growing. In order to get as much variability as possible, three replicates were taken for six cuts a year at four phenological states (vegetative, 10% bloom, 50% bloom and full bloom). Fresh samples were sun dried and then they were sent to the laboratory for reference analysis. Once the samples arrived at the laboratory, they were dried in a oven (48 h at 60ºC) and then they were ground on a Fritsch-Pulverisette mill (1 mm sieve). After milling, the samples were analysed for ash content and crude protein (CP) according to AOAC (1990), and neutral detergent fibre (NDF) according to Goering and Van Soest (1970).

Population 2 (Green plants). Samples and reference analyses

The samples came from a previous study (Alibés *et al*., 1991). They were samples of alfalfa forage (cultivar Aragón) grown under irrigation during 1986 and 1987 in six Mediterranean countries (France, Italy, Morocco, Portugal, Spain and Turkey). Samples were taken, harvested weekly in three cuts (second spring cut, full summer cut, and last cut or autumn cut). In the laboratory, samples were dried in an oven (48 h at 65ºC) and then they were sent to only one laboratory which ground the samples on a Fritsch Pulverisette mill (1 mm sieve) and carried out the analysis: ash content, CP, NDF and *in vitro* dry matter digestibility (IVDMD) according to Tilley and Terry (1963).

Population 3 (Hay and dehidrated). Samples and reference analyses

Twenty five samples of alfalfa hays and dehydrated lucerne from different agronomical studies were used. Samples were from different cultivars (Aragón, San Isidro and Victoria), differents cuts, years and locations around Aragón (Spain). The phenological stage was also considered because samples came from early bloom and full bloom. The samples were tested for *in vivo* digestibility using six four-five year old castrated rams with an average weight of 45 kg. The rams were allocated in metabolic cages. The adaptation period took longer than 7 days and there were 7 days of data collection. The offered diet consisted of hay or dehydrated lucerne, chopped at a length of 5-7 cm and offered at maintenance level twice a day, at 8 h and 14 h. During the assay the animals had water and mineral blocks *ad libitum*.

NIRS Analyses

NIRS Analyses were carried out using a FossNIRSystems 6500 monochromator (FossNIRSystems, 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 alfalfa 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 derivatisation. 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, 1995; Shenk and Westerhaus, 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 calibration sets of population 1 and 2 (Tables 1 and 2) cover a great variability as demonstrated by the broad ranges observed in the reference data for ash, CP, NDF and IVDMD values. Population 2, shows a bigger variability than population 1 because it contains samples grown in many different agro-climatic conditions.

Table 1. NIR calibration and cross validation statistics for ash, crude protein (CP) and neutral detergent fibre (NDF) of alfalfa hays (Population 1)[†]

	N	Mean	Range	SEC	R^2	SECV	r^2	RER
Ash	102	9.99	7.06-11.85	0.38	0.82	0.44	0.77	10.88
CP	109	21.42	14.85-32.78	0.80	0.95	0.93	0.93	19.27
NDF	108	39.00	29.37-53.65	1.21	0.95	1.56	0.92	15.56

[†]Abbreviations: SEC = standard error of calibration, SECV = standard error of cross validation, R^2 and r^2 are the coefficient of determination in calibration and cross validation, N = number of samples.

Table 2. NIR calibration and cross validation statistics for ash, crude protein (CP), neutral detergent fibre (NDF) and *in vitro* dry matter digestibility (IVDMD) of green alfalfa samples from Mediterranean countries (Population 2)[†]

	N	Mean	Range	SEC	R^2	SECV	r^2	RER
Ash	278	11.14	6.12-15.55	0.37	0.96	0.41	0.95	23.00
CP	285	20.81	8.15-39.47	0.69	0.99	0.76	0.99	41.21
NDF	139	38.18	16.43-66.97	1.63	0.98	1.82	0.97	27.76
IVDMD	222	69.16	45.68-87.35	2.75	0.92	3.15	0.90	13.22

[†]Abbreviations: SEC = standard error of calibration, SECV = standard error of cross validation, R^2 and r^2 are the coefficient of determination in calibration and cross validation, N = number of samples.

Table 1 and Table 2 summarise the statistical values for the best calibration equations obtained for population 1 and 2. The high R^2 and r^2 values obtained for the CP, NDF and IVDMD equations indicate that NIRS have an excellent precision for the prediction of these parameters (Shenk and Westerhaus, 1996). For ash content, while a r^2 of 0.77 was obtained on the population 1, that statistic reaches a value of 0.95 when using population 2 as calibration set. The important increase gained in the precision of the equation obtained for the prediction of ash content, could be explained by the increase in the number of samples (102 versus 278) and the existence of a broader range of the reference values (7.06-11.85 versus 6.12-15.55).

The equation obtained for the prediction of the IVDMD, explains 90% of the variation existing in digestibility, however the standard error of the estimate (SECV = 3.15%) is high. Dardenne *et al.* (1996) reported a SECV value of 1.38% for the prediction of enzymatic organic matter digestibility (OMD) in a population of 287 samples of alfalfa hay ranging from a minimum OMD value of 50% to a maximum of 83%. The high SECV obtained in the present study could be explained by the variability in the data existing within and between all the batches of *in vitro* analysis done to obtain reference data for the 222 samples analysed by NIRS.

A useful statistic to evaluate the robustness of an equation for routine analysis is the RER value. In the present study the RER value obtained for ash (10.88, 23), CP (19.27, 41.21), NDF (15.56, 27.76) and IVDMD (13.22) and for both populations indicate that the equations should be capable of predicting the required values with an accuracy bigger than the minimum value recommended, that is, one tenth of the range as stated by Williams and Sobering (1996).

Table 3 shows preliminary results obtained for the prediction of the *in vivo* digestibility data. The low number of samples available for the equation development makes it difficult to conclude about the stability of the statistics obtained, once the calibration set could be extended with further samples. However, taking into account that the range covered by the calibration set is wide enough to cover the most practical situations of the nutritive value of alfalfa hays (Alibés and Tisserand, 1990), it can be said that NIRS could explain 71% and 87% of the variation existing in the dry matter digestibility (DMD) and OMD values of the alfalfa hays with an even better accuracy (1.65% and 2.64%) than that reported for many equations traditionally used by nutritionists in assessing the quality of forages, based on chemical or *in vitro* analyses (Garrido *et al*., 1994).

Table 3. NIR calibration and cross validation statistics for dry matter digestibility (DMD) and organic matter digestibility (OMD) of hays and dehydrated alfalfa samples (Population 3)[†]

	N	Mean	Range	SEC	R^2	SECV	r^2	RER
DMD	22	60.96	55.50-68.00	1.07	0.87	1.65	0.71	7.57
OMD	20	59.88	44.80-70.60	2.05	0.92	2.64	0.87	9.77

[†]Abbreviations: SEC = standard error of calibration, SECV = standard error of cross validation, R^2 and r^2 are the coefficient of determination in calibration and cross validation, N = number of samples.

Conclusions

Near Infrared Spectroscopy is a useful technology for the rapid determination of the nutritional value of Mediterranean alfalfa forage.

The errors associated to the prediction of IVDMD are higher than those reported for the prediction of *enzymatic* digestibility. A better strategy in obtaining equations for the prediction of IVDMD or *enzymatic* digestibility could be to adjust analytical values using a linear regression (derived with every batch of unknown samples) based on similar samples of known *in vivo* digestibility and then to use the predicted corrected digestibility values to obtain the NIRS calibrations.

The paper shows clearly the potential of NIRS for the prediction of the *in vivo* digestibility of Mediterranean alfalfa forage, however an important limitation is to accumulate enough samples with known *in vivo* values (obtained from a standard protocol) for obtaining robust NIRS calibrations.

In obtaining NIRS calibrations for the prediction of *in vivo* digestibility it could also be possible to use reference *in vivo* data coming from different laboratories (without a standard protocol) and to include a mathematical correction for laboratory differences in the NIRS equations. It is of paramount importance in planning feeding strategies of Mediterranean livestock based on alfalfa forage, to evaluate that strategy in the framework of a collaborative research project.

Acknowledgements

The authors would like to thank to all those who provided samples for the present study. The study was undertaken in the framework of a research programme co-ordinated by the IAMZ entitled *"Mediterranean Network for the Calibration of Nutritional Parameters of Alfalfa Hay using NIRS".* Thanks are also given to the IAMZ.

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