Italian Journal of Animal Science

ISSN: (Print) 1828-051X (Online) Journal homepage: http://www.tandfonline.com/loi/tjas20

The use of near-infrared reflectance spectroscopy (NIRS) in the prediction of chemical composition and in vitro neutral detergent fiber (NDF) digestibility of Italian alfalfa hay

Nico Brogna, Maria Teresa Pacchioli, Alessandra Immovilli, Fabrizio Ruozzi, Ralph Ward & Andrea Formigoni

To cite this article: Nico Brogna, Maria Teresa Pacchioli, Alessandra Immovilli, Fabrizio Ruozzi, Ralph Ward & Andrea Formigoni (2009) The use of near-infrared reflectance spectroscopy (NIRS) in the prediction of chemical composition and in vitro neutral detergent fiber (NDF) digestibility of Italian alfalfa hay, Italian Journal of Animal Science, 8:sup2, 271-273, DOI: 10.4081/ijas.2009.s2.271

To link to this article: http://dx.doi.org/10.4081/ijas.2009.s2.271

The use of near-infrared reflectance spectroscopy (NIRS) in the prediction of chemical composition and *in vitro* neutral detergent fiber (NDF) digestibility of Italian alfalfa hay

Nico Brogna[1], Maria Teresa Pacchioli[2], Alessandra Immovilli[2],
Fabrizio Ruozzi[2], Ralph Ward[3], Andrea Formigoni[1]

[1]Dipartimento di Morfofisiologia Veterinaria e Produzioni Animali, Università di Bologna, Italy

[2]CRPA S.p.A., Centro di Ricerca delle Produzioni Animali, Reggio Emilia, Italy

[3]Cumberland Valley Analytical Services, Inc. Hagerstown, MD, USA

Corresponding author: Nico Brogna. Dipartimento di Morfofisiologia Veterinaria e Produzioni Animali, Facolta' di Medicina Veterinaria. Via Tolara di Sopra 50, 40064 Ozzano dell'Emilia (BO), Italy - Tel: +39 051 2097361 – Fax: +39 051 2097373 Email: nico.brogna@unibo.it

ABSTRACT - The objective of the present work was to develop calibration equations for the prediction of chemical composition and forage digestibility from different populations of alfalfa hay harvested in Parmigiano-Reggiano cheese production area of Northern Italy. Due to annual climatic variations that affect soil and growing conditions and consequently cause high variability in chemical and physical composition, 319 hay samples from four years were used to build robust calibration. NIRS calibration equations were developed for the prediction of *in vitro* NDF digestibility (IVNDFd) and 20 chemical parameters (dry matter, starch, fat, sugar, fiber fractions, crude protein, nitrogen fractions and some minerals) of Italian hay. The results obtained show that NIRS equations greatly explain the variation in the composition existing in alfalfa hays grown in Northern Italy, except for a few parameters characterized by low variability range value. The equations obtained for the prediction of biological and chemical parameters explain the major part of the variation existing in the reference data, which open great prospects for the use of NIRS in planning feeding strategies of livestock, based on alfalfa forage.

Key words: NIRS, Alfalfa hay quality, NDFd (Neutral Detergent Fiber digestibility), Chemichal parameters.

Introduction – Alfalfa is one of the most important forage plants cultivated in all the continents, and haymaking is the most frequent method of preservation. Moreover, it has a significant economic position in the animal feed market and creates interest in the Parmigiano-Reggiano cheese production area of Northern Italy. Particularly for these productions the feeding strategy based on alfalfa forage needs a rapid determination of its nutritive value, which depends on the cultivar and many environmental factors. Traditionally wet chemical analyses have been used to characterize forages, and to predict their nutritive value. However, these are time-consuming, costly and in some cases hazardous chemicals are involved. Near infrared reflectance spectroscopy (NIRS) 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), and in the prediction of digestibility parameters (Norris *et al.*, 1976; Park *et al.*, 1997). NIRS technology is based on the opportunity that 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). However, the accuracy of the NIRS equations developed depends on the guarantee that the calibration samples adequately represent the sources of variability (eg. varieties, year of cultivation, growing condition, harvest time, drying system, etc.) associated to a given forage (Shenk and Westerhaus, 1995). The aim of the present work was to develop prediction equations that confirm the ability of NIR spectroscopy for a rapid estimation of quality parameters of alfalfa hay samples from Northern Italy with a wide range of maturity and growth stages.

Material and methods – The 319 alfalfa hay samples were collected during 2005 to 2008 from all the provinces of Parmigiano-Reggiano cheese production area, with intent that cover a wide range of chemical and spectra variation. The hay samples also embraced a range of sward types, harvesting dates and growing conditions. Each sample was dried following the two step process (Abrams, 1984; Mertens, 1993), grounded and chemically analyzed for: crude protein (CP) with combustion methods AOAC (990.03), NDIN, ADIN and SolP (Goering and Van Soest, 1970; Licitra *et al.*, 1996), NDF (Van Soest *et al.*, 1991), ADF and ADL (AOAC, 973.18), fat (AOAC, 2003.05), starch (Holm *et al.*, 1986), sugar (Dubois, 1956) and IVNDFd, (Tilley and Terry, 1963). Were also determined ash (AOAC, 942.05), metals and other elements (Ca, P, Mg, K, S, Cl, Na and Fe) as raccomended by AOAC (985.01). The samples were scanned on Foss NIRSystem 5000 monochromator in the 1098-2500 nm spectral region, using the spinning ring cup cell as sample transport module. Spectra recorded as log 1/R, where R is the reflectance, were taken at 8 nm intervals using 173 wavelenghts for calibration equation. Mathematical treatments of spectral data were performed with the WinISI II V1.5 software (Infrasoft International, Port Matilda, PA, USA), using first order derivative with gaps and smoothing each 4 data point and standard normal variate and detrend procedure (SNV-D) was applied for scatter correction. The Modified Partial Least Squares (MPLS) regression technique was used to develop the NIRS calibrations. The statistics used for the accuracy assesment of prediction equation were the standard error of calibration (SEC) and cross validation (SECV), the coefficient of determination for the calibration (R^2) and cross validation (1-Vr) and the RER value.

Table 1. Chemical composition and in vitro NDF digestibility, NIR calibration and cross-validation

Constituent	N	Mean	Min	Max	SD	N1	SEC	R^2	SECV	1-VR	RER
DM (%)	319	93.48	87.95	96.82	1.26	305	0.62	0.73	0.70	0.67	12.74
CP (%DM)	319	14.77	5.21	26.66	4.69	302	1.04	0.95	1.15	0.94	18.65
SolP (%DM)	319	5.16	1.30	10.75	2.13	305	0.70	0.89	0.80	0.86	11.87
NDIN (%DM)	316	3.85	1.31	10.81	1.86	294	0.75	0.78	0.82	0.74	11.64
ADIN (%DM)	317	1.53	0.56	4.63	0.51	297	0.18	0.73	0.20	0.68	20.32
IVNDFd (%NDF)	316	39.42	16.39	61.06	8.35	299	2.81	0.87	3.10	0.85	14.39
NDF (%DM)	318	54.59	29.94	75.87	8.30	300	2.22	0.93	2.45	0.91	18.75
ADF (%DM)	319	37.86	22.93	50.85	4.58	302	1.37	0.91	1.56	0.88	17.85
ADL (%DM)	319	7.20	3.43	12.79	1.64	303	0.51	0.89	0.59	0.86	15.95
Fat (%DM)	297	1.81	0.60	3.73	0.54	282	0.27	0.72	0.30	0.67	10.56
Starch (%DM)	307	1.74	0.42	5.87	0.94	283	0.51	0.47	0.58	0.33	9.41
Sugar (%DM)	307	6.54	1.00	17.10	2.53	290	1.08	0.79	1.19	0.74	13.57
Ash (%DM)	317	10.44	4.76	21.69	2.71	297	0.75	0.90	0.87	0.86	19.39
Ca (%DM)	316	1.23	0.29	3.15	0.56	296	0.15	0.91	0.17	0.89	16.39
P (%DM)	316	0.27	0.07	0.50	0.06	300	0.03	0.70	0.04	0.55	11.20
Mg (%DM)	316	0.22	0.09	0.60	0.09	295	0.03	0.81	0.03	0.76	14.91
K (%DM)	316	2.42	0.94	5.03	0.62	299	0.19	0.88	0.23	0.83	17.50
S (%DM)	314	0.22	0.08	0.44	0.06	300	0.03	0.76	0.03	0.66	10.59
Cl (%DM)	310	0.55	0.06	2.14	0.37	293	0.08	0.93	0.10	0.91	21.60
Na (%DM)	316	0.08	0.01	0.48	0.08	290	0.03	0.43	0.04	0.32	12.88
Fe (ppm)	316	766	52	5265	930	291	196	0.90	227	0.87	22.99

SEC = standard error of calibration, SECV = standard error of cross validation, R^2 and 1-Vr are the coefficient of determination in calibration and cross validation, N and N1 are respectively the number of samples in calibration and in cross-validation, RER = range/SECV.

Results and conclusions –A total of 21 parameters were examined in the calibrations.

The hay samples selected for this study varied widely in their chemical composition and digestibility parameter as shown by the mean, minimum, maximum and standard deviation. The performance statistics of the MPLS regressions for each parameters are presented in Table 1. Looking at the main chemical parameters, the results show that the standard error of calibration (SEC) is very low for all parameters, in relation to the range within the population. The correlation coefficient (R^2) is high, ranging from 0.70 for P to 0.95 for CP except for starch (0.47) and Na (0.43). Comparison of the correlation coefficients for cross validation (1-Vr), also shows that all calibrations are robust in nearly every cases, but less for DM, ADIN, fat and scarce for starch. Statistics regarding ash, metals and other elements show high coefficient of determination in calibration (R^2) and in cross validation (1-Vr), unless for P, S and Na. This is surprising as minerals do not absorb energy in the near infrared region of the electromagnetic spectrum although others have also found that NIRS is capable of predicting with good accuracy. It has been suggested that some of the wavelengths selected for these minerals are similar to those selected for the measurement of chlorophyll and organic-acid salts of Ca, K and Mg. Therefore it would appear that NIRS is indirectly measuring the minerals which are chelated, or closely bound to organic compounds. The range error ratio (RER) is defined as ratio of the range in the reference data for the samples to the SECV values. Williams and Sobering (1996) consider that the RER should ideally be at least 10, as happens in all the parameters exept for starch (RER=9.41). The results of this work have shown that NIRS analysis can provide accurate prediction of a wide range of chemical components of hay, including *in vitro* NDF digestibility. This offers considerable opportunities for the use of this technique in the support of ruminant feeding systems.

REFERENCES – Abrams, S. M., 1984. Laboratory procedures for determining dry matter, crude protein and acid detergent fiber. Proceedings of National Alfalfa Hay Quality Testing Workshop. Chicago, IL. **AOAC**, 2000. (990.03) Protein (Crude) in Animal Feed: Combustion Method. (973.18) Fiber (Acid Detergent) and Lignin in Animal Feed. (942.05) Ash of Animal Feed. (985.01) Metals and Other Elements in Plants. Official Methods of Analysis, 17th edition. Association of Official Analytical Chemists. **AOAC**, 2006. (2003.05) Crude Fat in Feeds, Cereal Grains and Forages. Official Methods of Analysis, 18th edition. Association of Official Analytical Chemists. **Dubois**, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28:350. **Goering**, H.K., Van Soest, P.J., 1970. Forage fiber analyses (apparatus, reagents, procedures, and some applications). ARS/USDA Handbook N° 379. **Holm**, J., Bjorck, I., Drews, A., Asp, N.G., 1986. A rapid method for the analysis of starch. Starch/die starke 7:224-226. **Licitra,** G., Hernandez, T.M., Van Soest, P.J., 1996. Standardization of procedure for nitrogen fractionation of ruminant feeds. Anim. Feed Sci. Technol. 57:347-358. **Mertens**, D. R., 1993. Determining dry matter in diverse types of feeds. Proc. NFTA Forage Analysis Workshop, Denver, CO. pp B1-B10. **Norris**, K.H., Barnes, R.F., Moore, J.E., Shenk, J.S., 1976. Predicting forage quality by infrared reflectance spectroscopy. J. Anim. Sci. 43:889-897. **Park**, R.S., Gordon, F.J., Agnew, R.E., Barnes, R.J., Steen., R.W.J., 1997. The use of near infrared reflectance spectroscopy on dried samples to predict biological parameters of grass silage. Anim. Feed Sci. Technol. 115:169-178. **Shenk**, J.S., Westerhaus, M.O., 1985. Accuracy of NIRS instruments to analyse forage and grain. Crop Sci. 25, 1120-1122. **Shenk**, J.S., Westerhaus, M.O., 1995. Analysis of Agricultural and Food Products by Near Infrared Reflectance Spectroscopy. Monograph, Foss-NIRSystems. Silver Spring, USA. **Tilley**, J.M.A., Terry, R.A., 1963. A two stage technique for the in vitro digestion of forage crops. Journal of the British Grassland Society. 18:104-111. **Van Soest**, P.J, Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583-3597. **Williams**, Ph., Sobering, D.C., 1996.In: A.M.C. Davies, P. Williams (eds), Near Infrared Spectroscopy: The Future Waves. NIR Publications, Chichester, UK. 185 – 188.