Alpine forage species analysed by near infrared and Fourier transform near infrared spectroscopy for quality parameters

F. Fornasier,[a] A. Tava,[a] A. Ursino,[a] M.C. Rosafio,[a] R. Riscassi,[b] I. Tradati,[b] M.S. Allegri[b] and M. Odoardi[a]

[a]*Istituto Sperimentale Colture Foraggere, 29 v.le Piacenza, I-26900 Lodi, Italy.*

[b]*Perkin-Elmer Italia S.p.A, 24 via Tiepolo, I-20052 Monza, Italy.*

Introduction

Near infrared (NIR) spectroscopy has been widely used for the evaluation of forage quality, due to its minimal sample preparation, reliability, speed of analysis and low cost. However, to obtain highly precise predictions, it is necessary to develop accurate calibrations for the specific product of interest, as the best results are usually obtained in this way. Continuous advances in research and technology offer new instruments, software and methodologies that can improve the accuracy of results.[1]

The aim of the present work was to compare the performances of two instruments, produced by two different companies, in predicting quality parameters relative to forage species grown and collected at a high altitude site in the Western Alps.

Materials and methods

Forage samples (196 samples in total) were collected from a bioagronomic field trial located at "Chiet", a high altitude (2002 m a.s.l.) summer farm, in the Condove district, in the Western Alps of Italy. In similar environments, forages are usually characterised by a low and peculiar fibre content and high protein levels,[2] and are mainly used as summer pastures for local-breed lactating cows for the production of typical, highly appreciated "malga" cheeses.

Twelve alpine populations and 25 commercial varieties belonging to 11 long-lived grass and leguminous species have been considered. The forage species grown were: *Agrostis tenuis, Dactylis glomerata, Festuca rubra, Festuca pratensis, Lolium perenne, Phalaris arundinacea, Phleum alpinum, Phleum pratense, Trifolium hybridum, Trifolium pratense* and *Trifolium repens*. Samples from replicated plots were collected twice a year, in July and September, from 1994 to 1998, dried to constant weight at 60°C, ground with a cyclone mill (1 mm sieve) and kept in plastic bottles until the chemical analyses were performed and the NIR spectra collected. Standard methods[3,4] were used for the chemical determinations of crude protein, NDF, ADF and Ash.

The entire set of samples, after wet analyses, were scanned for collecting spectra. NIR spectroscopic analyses were performed by using two instruments (*a* and *b*), with different technological solutions. The software for treatment of data were different as well.

(a) NIRSystems model 5000 monochromator (NIRSystems Inc., Silver Springs, MD, USA) and NIRS2 Software (ISI International, Port Matilda, PA, USA).

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Reflectance spectra (log 1/*R*) from 1100 to 2500 nm were recorded at 2 nm intervals, giving 700 datapoints per sample. Calibrations were obtained by means of the CALIBRATE option of ISI software. Equations were derived for each parameter using the MPLS (modified partial least squares) regression with first derivative as math option and *SNV* and detrend function to reduce interferences. The software then ramdomly separated samples into ten groups for cross-validation.[5]

(b) Perkin-Elmer FT-NIR Identicheck (Perkin-Elmer Limited, Beaconsfield, UK) with Perkin-Elmer Spectrum™ Software.

The spectra were collected in diffuse reflectance with ICRA (Identicheck Reflectance Accessory) from 4000 to 10000 cm^{-1} (1000–2500 nm) at 8 cm^{-1} resolution. Chemometric software Spectrum Quant+ was used to build up the calibrations, configured in first derivative and *SNV* function to remove the multiplicative interference of scatter and particle size. Validation was obtained by calculating a leave-one-out calibration, then calculating the error of prediction; the procedure was repeated for all samples and variance explained by the model calculated.

Results and discussion

The range of the chemical composition of samples used for this study is reported in Table 1. As expected, a wide and significant variation was found for all parameters, as a consequence of the high number of different botanical species involved, the seasonal changes and the agro-meteorological effects.[2]

Results obtained with the NIRSystems instrument and ISI software are presented in Table 2; those obtained with the Perkin-Elmer instrument and Spectrum Quant+ software are shown in Table 3.

The two series of calibrations showed very good indices of performance, R^2CV and % Var *CV*, always higher than 0.9 or 90% respectively, with the best value for crude protein (0.98–98.0) and the

Table 1. Range of the chemical composition (% DM) of the set of alpine forage species used for calibration.[a]

Variable	*n*	Mean	Range	*SD*
CP	196	12.50	3.36 – 24.13	5.13
Ash	121	7.80	3.79 – 11.24	1.52
NDF	196	46.55	26.6 – 60.10	7.70
ADF	196	25.82	17.77 – 32.56	3.26

[a]n, number of samples; CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre

Table 2. Calibration statistics of the selected set of alpine forage samples, including standard error of calibration (*SEC*), coefficient of determination (R^2), standard error of cross-validation (*SECV*) and coefficient of determination of cross-validation (R^2CV) using the CALIBRATE option of ISI software.

Variable	*n*	*SEC*	R^2	*SECV*	R^2CV
CP	188	0.63	0.98	0.76	0.98
Ash	108	0.29	0.96	0.44	0.92
NDF	187	1.04	0.98	1.27	0.97
ADF	182	0.87	0.93	0.99	0.91

Table 3. Calibration statistics using PCR+ algorithm of Spectrum Quant+ software.[a]

Variable	*SEE*	% Variance	*SEP*	% Variance *CV*
CP	0.757	98.2	0.794	98.0
Ash	0.481	91.8	0.533	91.3
NDF	1.672	96.6	1.864	96.3
ADF	1.054	91.0	1.080	90.9

[a] *SEE*: Standard error of estimate;
% Variance: percentage of variance explained by the mathematical model
SEP: Standard error of prediction
% Variance *CV*: percentage of variance explained after validation leave-one-out-at-a-time

lowest for ADF (0,91–90.1). These good results can be explained by the chemical characteristics of the samples analysed: the wide range of variation in chemical composition seems adequate to cover the whole variation usually found in these materials, with many samples distributed at the extremes, as shown by the scatter plots of predicted vs specified values of the four DM components (Figures 1, 2, 3 and 4).

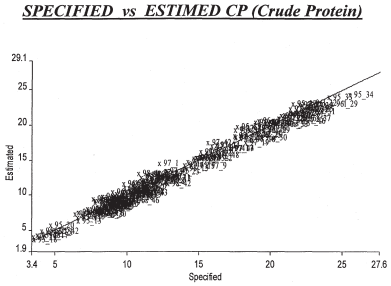

Figure 1. Scatter plot of the predicted *v.* specified values of CP content in the set of samples analysed.

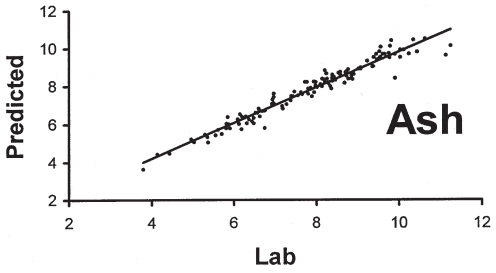

Figure 2. Scatter plot of the predicted *v.* specified values of Ash content in the set of samples analysed.

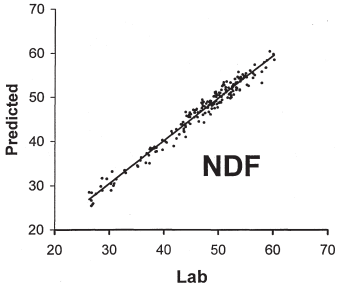

Figure 3. Scatter plot of the predicted *v.* specified values of NDF content in the set of samples analysed.

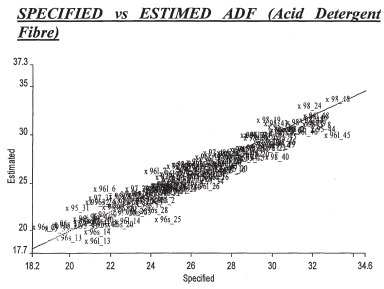

Figure 4. Scatter plot of the predicted *v.* specified values of ADF content in the set of samples analysed.

The results obtained by the two instruments are in practice superimposable, with very good performances of cross-validation with both procedures: Calibrate option of ISI software and Spectrum Quant+.

Conclusions

From the 11 different species of perennial forages grown at high altitude, accurate and precise calibrations for the main quality components of dry matter have been obtained. Therefore these NIR calibrations seem to be sufficiently strong and powerful to give rapid, simultaneous determinations of several qualitative factors of forages obtained in similar environments, in a fast and reliable way, with minimal costs.

The two instruments used, although different in technological solutions and softwares, both gave very good performances.

References

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