Quality evaluation of regional forage resources by means of near infrared reflectance spectroscopy

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Paper received January 14, 2004; accepted October 15, 2004

ABSTRACT

Quality parameters of grassland and pasture samples collected during a three-year period at two environmentally and geographically different areas were analysed by Near Infrared Reflectance Spectroscopy (NIRS). Chemical analysis for crude protein (CP), crude fibre (CF), neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL) and crude ash (ASH) carried out on two-thirds of the samples were used in calibration processes. The remaining one-third of the data was used to validate the best calibrations obtained. Samples selection is discussed. Different math pre-treatments (derivative, gap, primary smoothing and secondary smoothing), light scattering correction methods and calibration algorithms were tested to achieve the better predictive performances. We obtained the best results using different regression algorithms to correlate spectral information to chemical data. For CP ($R^2 = 0.94$, SEP=1.3), NDF ($R^2 = 0.95$, SEP=2.14) and ADF ($R^2 = 0.92$, SEP=2.06) Multiple Linear Regression (MLR) models fit chemical data better than Mean Partial Least Square (MPLS) regression. A molecular basis explanation of wavelengths selected was carried out. MPLS models worked well for CF ($R^2 = 0.93$, SEP=1.57), and ASH ($R^2 = 0.95$, SEP=1.17) while poor calibrations were obtained for ADL using both algorithms. To confirm the reliability of the models developed, uncertainties of predictions were compared with findings on nutritional variations and animal performances.

Key words: NIRS, Grasslands, Pastures, Forages quality.

RIASSUNTO

VALUTAZIONE DELLA QUALITÀ DI ERBAI E FORAGGI PASCOLIVI PER MEZZO DELLA SPETTROSCOPIA NEL VICINO INFRAROSSO (NIRS)

Su campioni di foraggio e pascolo, sono stati valutati i principali aspetti qualitativi mediante spettroscopia NIR. I campioni raccolti in aree geografiche differenti durante una campagna d’indagine triennale, sono stati analizzati relativamente al contenuto di protidi grezzi (CP), fibra grezza (CF), fibra neutro detergente (NDF), fibra acido detergente (ADF), lignina acido detergente (ADL) e ceneri grezze (ASH) con metodi convenzionali. Due terzi dei campioni sono stati impiegati per la procedura di calibrazione dello spettrometro NIR mentre i rimanenti sono stati utilizzati per valutare i migliori modelli di calibrazione ottenuti. La selezione dei campioni è stata effettuata in due modalità distinte e differenti metodi di pretrattamento degli spettri, di correzione degli effetti di scattering e algoritmi di calibrazione sono stati presi in considerazione al fine di cercare le migliori prestazioni strumentali per ogni costituente. I migliori risultati sono stati ottenuti ricorrendo a diversi algoritmi di regressione per correlare le informazioni spettrali dei campioni con i relativi dati analitici. Per i protidi grezzi (CP) ($R^2 = 0.94$, SEP=1.3), NDF ($R^2 = 0.95$, SEP=2.14) e ADF ($R^2 = 0.92$, SEP=2.06) i modelli lineari (MLR) sono apparsi più idonei che non i corrispondenti MPLS che invece risultano migliori per la quantificazione...
della fibra grezza (CF) ($R^2 = 0.93$, SEP=1.57) e delle ceneri (ASH) ($R^2 = 0.95$, SEP=1.17). La calibrazione per la lignina ha mostrato notevoli difficoltà ed i modelli ottenuti non sono da considerarsi idonei per la quantificazione di questo costituente nella tipologia di campioni presi in considerazione. Per le lunghezze d’onda selezionate (MLR) sono state discusse, su base molecolare, le evidenze sperimentali riportate in letteratura atte ad interpretare i risultati ottenuti. Per verificare l’affidabilità dei modelli selezionati, sono state comparative le incertezze della predizione con i risultati di sperimentazioni incentrate su variazioni nutrizionali e risposta animale.

Parole chiave: Spettroscopia nel vicino infrarosso (NIR), Erbai, Pascoli, Qualità dei foraggi

Introduction

Forage quality can be evaluated using Near Infrared Reflectance Spectroscopy (NIRS), a non-destructive, rapid and cost-effective investigation technique (Shenk, 1991). The NIRS method is based on the absorptions of C-H, N-H, and O-H groups present in organic constituents (Reeves, 2000). These absorptions, falling in the 700-2500 nm spectral region (Wheeler, 1960, Reeves, 2000), are essentially the overtones and combination bands of the stronger ones found in mid-infrared (2500-25000 nm) (Murray and Williams, 1987). The application of NIRS in animal nutrition has been discussed by several authors (Murray, 1986; Shenk and Westerhaus, 1991; Buxton and Martens, 1991; Cosgrove et al., 1994). Different studies have dealt with NIRS calibration for crude proteins (Marten et al., 1983; Barton, et al., 1990; Shenk et al. 1999; Fornasier et al., 1999), fibre fractions (ADF and NDF) (Norris et al., 1976; Buxton, 1991; Shenk et al. 1999), crude fibre (Derdenne, 1996), lignin (Reeves, 1988) and ash (Fornasier et al., 1999), but little (e.g., Derdenne, 1996) regarding feedstuffs has been published taking into account all of these constituents at the same time. Moreover, it seems that only limited effort (Garcia-Criado et al. 1987, 1989; Rabotnikof et al., 1995) has been spent on studies involving forages with different botanical composition, heterogeneous stage of maturity and diverse geographical provenance.

The aim of our research was to investigate the best NIRS calibrations for an heterogeneous collection of grassland and pasture samples representing the most important forage resources available for the dairy sheep production system in Lazio (Central Italy). In particular, we were interested in evaluating whether NIRS can satisfactorily meet a reliable level of accuracy, using different algorithms and data treatments, in predicting the main nutritional parameters of heterogeneously close populations of forages and in developing good models to employ in future predictions by providing, when possible, a plausible molecular interpretation of the regression terms selected.

Material and methods

Forage sample sets

A set of 173 forage samples, collected during a three-year period, was used in this study. Of these, 127 samples were harvested in dairy sheep farms located in Viterbo province (Italy), and were representative of forage sources like grassland, mixed forage crops and mixed hay fields available for sheep feeding. The remaining 46 samples were harvested in a high area (1200 - 2000 m altitude above sea level) in Frosinone province (Italy) during the grazing season (from April to November). Agronomic-botanical classification and maturity stages of forage samples are shown in Table 1 and Table 2.

Sample preparation

Forage samples were dried in a forced air oven at 65°C over 48 h; then they were ground through a mill (Retsch Müller, Germany) to pass 1 mm screen. To maintain optimal preservation conditions, sealed polyethylene containers were used to store prepared samples.

Chemical analysis

Milled samples were analysed for dry matter (DM) and crude ash (ASH) following the ISO-5894 method (ISO, 2002). Crude protein was determined by the distillation Kjeldal method according to the CEE-ASPA protocol (Martillotti et al., 1987).
Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were analysed following methods reported by Van Soest and Robertson (1980) and adapted to ANKOM Fibre Analyser (Ankom Technology Corp. Fairport, NY, USA). Also, crude fibre (CF) was measured using the ANKOM Fibre Analyser. All data are reported as percentages on a dry matter basis (Table 3) and were already available at the moment of NIRS analysis. The distributions of forage samples used for NIR calibration are reported in Table 3 and Figure 1.

**NIR spectral data acquisition**
After 24 h of dehydration in a forced air oven at 105°C, 2-3 g aliquots of each sample were submitted to the scanning process using a NIRSysterm Scanning Spectrophotometers model 5000 (Silver...
Table 3. Statistics of forage samples used for NIR calibration.

|                  | CP   | CF   | NDF  | ADF  | ADL  | Ash  |
|------------------|------|------|------|------|------|------|
| **Grasslands**   |      |      |      |      |      |      |
| N.               | 127  |      |      |      |      |      |
| Mean*            | 20.57| 20.05| 45.88| 29.69| 5.77 | 12.29|
| SD*              | 5.62 | 5.89 | 7.44 | 6.21 | 2.43 | 3.60 |
| VC%              | 27.31| 29.36| 16.22| 20.93| 42.15| 29.26|
| Min*             | 8.16 | 10.85| 32.64| 18.88| 0.32 | 6.63 |
| Max*             | 37.23| 39.86| 69.09| 46.72| 13.34| 26.10|
| Range*           | 29.07| 29.01| 36.45| 27.84| 13.02| 19.46|
| **Pastures**     |      |      |      |      |      |      |
| N.               | 46   |      |      |      |      |      |
| Mean*            | 14.71| 23.35| 56.87| 34.03| 8.40 | 7.76 |
| SD*              | 4.65 | 5.41 | 7.88 | 7.42 | 3.66 | 1.63 |
| VC%              | 31.61| 23.15| 13.85| 21.81| 43.57| 21.05|
| Min*             | 8.30 | 8.82 | 36.52| 16.40| 1.48 | 4.77 |
| Max*             | 31.17| 33.13| 67.26| 46.72| 14.57| 11.90|
| Range*           | 22.87| 24.31| 30.74| 30.31| 13.08| 7.13 |
| **All samples**  |      |      |      |      |      |      |
| N.               | 173  |      |      |      |      |      |
| Mean*            | 19.01| 20.93| 48.82| 30.85| 6.33 | 11.15|
| SD*              | 5.96 | 5.93 | 8.98 | 6.82 | 2.93 | 3.77 |
| VC%              | 31.35| 28.34| 18.39| 22.09| 46.28| 33.82|
| Min*             | 8.16 | 8.82 | 32.64| 16.40| 0.32 | 4.77 |
| Max*             | 37.23| 39.86| 69.09| 46.72| 14.57| 26.10|
| Range*           | 29.07| 31.05| 36.45| 30.32| 14.25| 21.33|

N. = number of samples
* Data are expressed as per cent on dry matter.
** Including mixed grasslands and mixed hay fields
NIR calibration

All samples were grouped on the basis of their provenance and showed significant differences in chemical composition (as assessed by Mann-Whitney U Test for each constituent: P ≤ 0.011). They were therefore pooled in sets of single samples that were then submitted to following selection steps. After selection, two new subsets were obtained from the 173 samples. The first one, including approximately two-third of the samples, was used as the calibration set. The second one, approximately one-third of the samples, was used to perform the equations validation. The selection was carried out randomly using the suitable MANAGE program option (ISI4 – ISI, Port Matilda, PS, USA). Starting math pre-treatments (derivative, gap, primary smoothing and secondary smoothing) 1,2,2,1, and 1,2,2,2, were used allowing gap and primary smoothing to vary by the increments 4,8,12,16,20,24,28,32. A second mode for data pre-treatment was also adopted, starting with 1,5,5,1 and 1,5,5,2 and was variable in gap by the same values and in primary smoothing by increments 10,15,20,25,30,35,40, in agreement with Buxton and Mertens (1991). Still, the derivative was varied from 1 to 4 concurring with the experience of Shenk and Westerhaus (1991).

Two calibration algorithms were used: Multiple Linear Regression (MLR) with Stepwise selection of wavelengths and Modified Partial Least Square (MPLS) regression (Geladi and Kowalski, 1986). Light scattering phenomena were corrected following methods reported by Sinnaeve et al. (1994), using Multiplicative Scattering Correction (MSC) and its weighted version (WMSC) (Isacsoo and Naes, 1988).
Detrended and Standard Normal Variate (DSNV) (Barnes et al., 1989) algorithms were used. To minimise over-fitting we had to set up F statistic for each regression coefficient > 10 and to accept (only for MLR algorithm) a maximum number of wavelength terms below seven. During the calibration process, the elimination of samples that did not fit in the developing regression models was carried out. A critical “T” value (predicted – observed analytical data/ SEC) of 2.5 was used as the cut off for identifying and removing outliers (Gansworthy et al. 2000). The equations developed during the cal-

**Figure 2.** Categorized histograms of all analytical data after validation set selection: a) random; b) ranked.
Table 4. Statistics of both calibration and validation sample sets obtained with two different methods of selection.

| Method          | Calibration | Validation |
|-----------------|-------------|------------|
|                 | CP          | CF         | NDF    | ADF    | ADL    | Ash |
| Ranked selection| 19.26       | 20.54      | 48.76  | 30.97  | 6.33   | 11.32|
|                 | 5.99        | 6.07       | 8.98   | 6.65   | 2.99   | 4.17 |
|                 | 31.12       | 29.57      | 18.42  | 21.48  | 47.21  | 36.82|
|                 | 8.16        | 8.82       | 32.64  | 16.40  | 0.32   | 4.83 |
|                 | 37.23       | 39.86      | 67.45  | 46.72  | 14.57  | 26.10|
|                 | 29.07       | 31.04      | 34.81  | 30.32  | 14.25  | 21.27|
| Random selection| 19.57       | 20.62      | 49.19  | 30.56  | 6.12   | 11.62|
|                 | 5.90        | 5.90       | 8.93   | 6.77   | 2.98   | 4.58 |
|                 | 30.11       | 28.62      | 18.16  | 22.14  | 48.69  | 39.44|
|                 | 8.16        | 8.82       | 32.64  | 16.40  | 0.32   | 4.77 |
|                 | 37.23       | 39.86      | 67.45  | 46.72  | 14.57  | 26.10|
|                 | 29.07       | 31.04      | 34.62  | 30.32  | 14.25  | 21.33|

* Expressed as percent on dry matter basis

Grasslands and mixed hay fields were ranked and selected using either of the following rules: i) the smallest standard error of calibration (SEC) and, only for MPLS algorithm, the smallest error of cross-validation (SECV), ii) the largest coefficient of multiple determination $R^2$, iii) minimum number of outliers discarded. The best final equation was selected on the basis of its prediction performance assessed as the smallest standard error of prediction (SEP) on the validation set.

Results

Chemical analysis

Chemical composition of the forage samples showed a wide range of values (Table 3). As far as protein content is concerned, our samples had a high mean value mainly due to the contribution of grasslands from Viterbo province. Lower mean protein content characterised pasture samples, although a higher variability degree, expressed as variation coefficient (VC%), may be observed. Fibrous fractions (NDF, ADF and ADL) and CF were on the average higher for pastures than for grasslands and mixed hay fields. As regards CF and NDF contents, samples from Viterbo province appeared more variable when compared to pastures. Other fractions ADF and ADL showed similar data dispersion in both sample groups. Grasslands and mixed hay fields were higher in ashes than pastures and a higher degree of variability was observed.

Near Infrared Reflectance analysis

A preliminary evaluation of NIRS performance suggested that we take into account only...
the organized selection of samples. Ranking selection led us to obtain the best results in most cases. As Figure 3 synthetically shows, calibration/validation general parameters seems better using sub-sample sets selected in an ordinate manner rather than in a random one. Only as far as SEC and Bias values are concerned (8 cases out of 12 and 10 cases out of 12, respectively), selecting random calibration/validation sample set seems to give the best results. Nevertheless, other parameters (Slope, Standard Deviation Ratio, SEP and SEP/SEC ratio) indicate organized selection as the best approach. Thus, the rest of the paper will deal only with calibrations developed on sample sets selected in an organized way. Table 5 shows the calibration statistics obtained using MLR. The table also reports the wavelengths selected during the calibration. In Figure 4, spectral variance and spectral line selected by regression are plotted together. Amplitude and sign of the latter are proportional to the regression coefficient values themselves. Table 6 reports the results obtained working with MPLS, while summary of validation statistics for both MLR and MPLS algorithms are presented in Table 7. Figure 5 shows scatter grams of observed versus NIRS predicted validation data for CP, CP, NDF, ADF and ASH.

Table 5. Calibration results using MLR algorithm and scatter correction and math pre-treatment parameters adopted.

| Wavelength (nm) | I | II | III | IV | V | VI | SEC* | R^2 | Scatter Correction | Math Treatment |
|-----------------|---|----|-----|----|---|----|------|-----|--------------------|----------------|
| Crude proteins  | 1616 | 1704 | 2052 | 2196 | 2412 | -   | 1.18 | 0.95 | DSNV              | 4,10,5,2        |
| Crude fibre     | 1468 | 1580 | 1776 | 2056 | 2412 | -   | 1.56 | 0.91 | DSNV              | 3,5,5,2         |
| NDF             | 1600 | 1644 | 1736 | 1828 | 2184 | 2300| 1.64 | 0.97 | MSC               | 1,16,12,1       |
| ADF             | 1388 | 1576 | 1680 | 2004 | 2312 | -   | 1.43 | 0.95 | DSNV              | 3,5,1,1         |
| ADL             | 1660 | 1684 | 1700 | 2272 | -   | -   | 1.73 | 0.63 | NMSC              | 1,5,1,1         |
| Ash             | 1420 | 1628 | 1844 | 1940 | 2149 | -   | 0.67 | 0.94 | DSNV              | 3,8,2,1         |

SEC= Standard Error of Calibration; SEC= \[\left(\sum (X_{NIR} - X_{LAB})^2 / (N-1)\right)^{1/2}\]
R^2 = Squared multiple correlation coefficient
SECV = Standard Error of Cross-Validation
* data expressed as percent on DM basis
Figure 4. Wavelength selected by Linear Regression models plotted against the overall standard deviation of spectra.

### Discussion

The better performance obtained by selecting validation samples in a ranked manner is in agreement with the findings of other researchers regarding the poorer calibrations afforded by random selection among a closed population of samples (Barnes and Dhanoa, 1987; Garcia-Criado et al., 1990).

#### Crude Protein

Wavelengths selected by the Stepwise selection process for protein content (Figure 4) were in agreement with those reported by other authors. Workman (1996) indicated the main region associated with protein content (as Kjeldhal nitrogen) to be the wavelength range 2148-2200 nm for combination bands (N-H bend 2\textsuperscript{nd} overtone, C-H...

### Table 6. Calibration results using MPLS algorithm and scatter correction and math pre-treatment parameters adopted.

| Constituent   | SEC* | R**  | SECV* | R\(^2\)(CV) | FPLS | \(\lambda\) | Scatter Correction | Math Treatment |
|---------------|------|------|-------|-------------|------|-------------|--------------------|---------------|
| Crude proteins| 1.02 | 0.97 | 1.62  | 0.92        | 4    | 347         | DSNV               | 3,4,2,2       |
| Crude fibre   | 1.07 | 0.96 | 1.45  | 0.934       | 9    | 334         | WMSC               | 4,8,4,1       |
| NDF           | 1.91 | 0.95 | 2.17  | 0.94        | 7    | 346         | MSC                | 1,8,4,1       |
| ADF           | 1.10 | 0.97 | 1.32  | 0.95        | 8    | 294         | MSC                | 4,24,20,2     |
| ADL           | 1.63 | 0.58 | 1.89  | 0.44        | 4    | 334         | DSNV               | 3,12,2,2      |
| Ash           | 0.257| 0.99 | 0.72  | 0.91        | 11   | 344         | DSNV               | 3,4,4,2       |

\(\lambda\) = number of spectral data used
SEC = Standard Error of Calibration; SEC = \[S \ (X_{NIR} - X_{LAB})^2 \ / \ (N-1)\]\(^{1/2}\)
R\(^2\) = Squared multiple correlation coefficient
SECV = Standard Error of Cross-Validation
R\(^2\)(CV) = Squared multiple correlation coefficient for cross validation
* data expressed as percent on DM basis
stretch/C=O stretch and C=O stretch/N-H stretch). Our best MLR calibration for proteins includes a 2196 nm wavelength. Also, this is in agreement with Murray (1986) who reported that the most relevant segment for nitrogen was centred at 2166 nm. Furthermore, other selected wavelengths at 2052 nm, 1704 nm and 1616 nm fall within or around two known bands associated with crude proteins: 2030-2050 nm (C=O stretch 2nd overtone of primary amide) and a 1620-1700 nm (Ar. C-H 1st overtone region) (Workman, 1996). On grasses, Garcia-Criado et al. (1990), selecting validation samples both in a structured and random manner, found a 2060 nm significant spectral line. As far as the statistical indexes are concerned (Table 5 and Table 6), our protein calibration errors are quite a bit higher than those published by others (Parnell and White, 1983; Martin et al., 1985; Barton et al., 1990; Buxton and Mertens, 1991; Fornasier et al., 1999). As a matter of fact, SECs normally varied between 0.4 % and 0.8%, but in most cases, sample sets were not as heterogeneous as those we had to deal with. Also, our samples showed a wider range than those reported by other authors (e.g., Garcia-Criado et al., 1990; Fornasier et al. 1999). Indeed, in similar circumstances, Shenk and Westerhaus (1991) found a good calibration (SEC=1.05%) fitting data as much as our MLR model. Validation statistics showed a lower over-fitting risk for MLR than for MPLS. The latter model for predicting protein content had an SEP value slightly higher than the 20 percent of relative SEC indicated by Windham et al. (1989) as rule of thumb. This is satisfied for the MLR model. However, both calibrations were obtained at best using high derivative order (4th and 3rd for MLR and MPLS, respectively).

The uncertainty of prediction using our best model for CP content (SEP =1.3%, that is 6.7% of the mean sample set CP content) seems acceptable if compared with the findings of other authors. In an isoenergetic ration trial, Moloney (1998) observed non significant differences among treatments with an intake variability of CP up to 6% in sheep growth and carcass weight.

**Crude Fibre**

For crude fibre calibration, 5 wavelengths were selected using MLR. Most notably, a significant absorption was observed at 2412 nm as for CP, and as in that case, we could not find a fully satisfying explanation. Only wavelengths at 1468 nm and 1776 nm could be expected according to what is described by Workman (1996). Indeed, he reported among the bands associated with fibre as cellulose, O-H stretch 1st and 2nd overtones, respectively, localised at 1490 and 1780 nm. We obtained better performances in CF prediction with MPLS than

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**Table 7. Validation results of best equation developed both using MLR and MPLS algorithms.**

|          | MLR       |        |        |        | MPLS       |        |        |        |
|----------|-----------|--------|--------|--------|------------|--------|--------|--------|
|          | SEP       | R²     | BIAS   | SLOPE  | SDR        | SEP    | R²     | BIAS   | SLOPE  | SDR        |
| Crude proteins | 1.30 | 0.94 | 0.07 | 0.98 | 4.22 | 1.33 | 0.94 | 0.30 | 1.01 | 4.18 |
| Crude fibre | 1.83 | 0.91 | -0.02 | 0.97 | 3.22 | 1.57 | 0.93 | 0.21 | 1.00 | 3.75 |
| NDF      | 2.14 | 0.95 | 0.19 | 0.95 | 4.34 | 2.21 | 0.94 | -0.08 | 1.02 | 4.19 |
| ADF      | 2.06 | 0.92 | 0.37 | 1.00 | 3.38 | 2.35 | 0.91 | 0.74 | 1.04 | 2.84 |
| ADL      | 2.45 | 0.58 | 0.56 | 0.95 | 1.2 | 2.23 | 0.56 | 0.52 | 0.90 | 1.21 |
| Ash      | 1.40 | 0.88 | 0.29 | 1.01 | 2.61 | 1.17 | 0.95 | 0.14 | 1.00 | 2.11 |

SEP = Standard Error of the Prediction; SEP = [Σ (XNIR - XLAB)² / (N-1)]¹/２
R² = Multiple Coefficient of Determination
Bias = difference between laboratory and predicted mean values; Bias = XNIR – XLAB
SDR = Standard Deviation of validation sample set and SEP ratio; SDR = SDLAB / SEP
MLR both in terms of standard errors and SDR (SDR = SDval/SEP where SDval is the standard deviation of the validation sample set used) (Chang et al., 1998). The bias value, although not as good as for MLR (0.2% the former, –0.02% the latter) also respects the 0.6*SEC limit suggested by Shenk et al. (1993). Yet, high derivative orders gave the best results.

Van Soest's fibrous fractions

Our Neutral Detergent Fibre and Acid Detergent Fibre calibrations seem good enough. Their uncertainties match on average that which has been published by others. For forages, Shenk et al. (1985) and Reeves (1988) reported SECs (1.9% and 2.5%, respectively) equal or higher than ours but SEPs that are comparable. Yet the MLR model shows better calibration performances than MPLS. This is particularly true for SEC, SEP and SDR. MLR based calibration selected 6 wavelength for NDF (1600 nm, 1644 nm, 1736 nm, 1828 nm, 2184 nm, 2300nm) and 5 for ADF (1388 nm, 1576 nm, 1680 nm, 2004 nm, 2312 nm). In each case, from three to four spectral lines match quite well to those described elsewhere. Workman (1996) reports absorbance at 1820 nm for a combination of O-H stretch, 2nd overtone as band associated with fibre as cellulose. Also, he reported the 2300-2360 nm interval frequently occurring in wavelength selection for the forages fibre multivariate calibration. For NDF calibration, Buxton and Mertens (1991) observed a 1726 nm band close to the 1736 nm selected by our model. Notably, their work shows that even using a different calibration program, this absorbance was not selected as well. Also, these authors reported for ADF absorbance (1560-1564 nm and 2030 nm) close to the 1576 nm and 2004 nm which we observed. The C-H stretch, 1st overtone band associated with fibre as lignin at 1685 nm reported by Workman (1996) match quite well with the 1682 nm one selected in our ADF calibration process. Absorbance at 2312 nm seems similar to that observed for NDF (2300 nm) and drops in the same series of cases. Both algorithms MLR and MPLS worked better when spectra were not submitted to a derivative math treatment for NDF calibration while a high derivative order is required for a good calibration in the ADF case.

Once again the obtained accuracy for NDF content in forage (SEP = 2.14%DM, that is 4.4 percent of the mean value of the validation sample set)
may be sufficient for a good estimation of animal diet. Indeed, for sheep, Molony (1998) reports no significant bodyweight gain in a trial allowing NDF content to vary as much as 17 % among different treatments under isoenergetic dietary conditions. These findings confirm the reliability of our model, as the uncertainty of prediction is one order of magnitude smaller than the diet NDF variability tested.

ADL calibration shows poorer results when compared with the previous ones. A low SDR ratio accounts for a lower robustness. SECs were about twice the values found in references for forages (Reeves, 1987) and maize (Dardenne, 1996), although our sample sets were very different from a compositional point of view. On the other hand, wavelengths used by the MLR model match quite well with bands reported by Workmann (1996) for lignin. Different derivative order leads to the best results using the two different algorithms: 1st for MLR and 3rd for MPLS, respectively. However, lignin analysis is generally problematic. Therefore, it is not surprising that the accuracy of ADL estimation by NIR is questionable.

**Crude Ash**

Ash calibration results are similar to, if not better, than, those reported by other authors (Reeves, 1987; Dardenne, 1996) as far as SEC values are concerned (0.32 % and 0.69%, respectively, for MPLS). However, the SEPs obtained are slightly higher than Whindham’s et al. (1989) criterion. It seems worth emphasizing that in our MLR model for ASH prediction, which includes 5 wavelengths in total, two of them (1940 nm and 2148 nm) match perfectly to the findings of Vasquez de Aldana et al. (1995) for Mg content in their investigation of grasslands.

**Conclusions**

Near infrared reflectance may permit the reliable evaluation of grassland and pasture quality. In particular, protein content, crude fibre, neutral and acid detergent fibrous materials and ashes, in our condition, are well predictable. The equations developed account for the high variability of forages, reflecting agronomic, botanical, seasonal and geographical differences. Accuracy of prediction, evaluated as bias and SEP, satisfies the expectations in this field of research. Regarding methodological aspects, our work shows that the best results were obtained using different regression algorithms to correlate spectral data to chemical values: in some cases (CP, NDF, ADF) Multiple Linear Regression worked as well as or better than Modified Partial Least Squares, with the advantage that most of the wavelengths selected were explainable on an interpretative molecular basis. Moreover, different data pre-treatment and scatter correction methods led to the best calibration conditions. This demonstrates a strong computational effort. But nowadays, thanks to Information Technology advances, this is becoming less of a problem. From a merely practical point of view on a regional scale, the NIRS method allows more rapid and less expensive determinations than those which are possible using wet chemical methods.

The encouraging results obtained in this study suggest that by expanding sample calibration collection a higher reliability, quality and predictive capability of models in the field of forages can be achieved. This constitutes an important step toward a widespread quality assessment program of grasslands and pastures on a regional scale with a positive effect on sheep and cow breeding systems.

The authors wish to thank the Regional Breeders Association (ARAL, Roma, Italy) for the technical and instrumental support; Mr. Corrado Bruti for his precious help in sample processing and chemical analysis of forages and Dr. Domenico Giontella for the formulation and storage of chemical data.

This work was supported by a research grant from Lazio Region – Assessorato allo Sviluppo del Sistema Agricolo e del Mondo Rurale (DOCUP 5b, contracts n. 2/97 and n. 4/97).
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