Near infrared spectroscopy estimation of feeding value of forage perennial grasses in breeding programmes by global and specific calibrations. Estimation of chemical composition and digestibility

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Near infrared (NIR) spectral analysis with a NIRSystems 6500 monochromator was applied to evaluate accuracy of predictive models for forage quality in clone breeding processes of the original Bulgarian varieties over different cuts and years. The varieties were the perennial grasses: *Dactylis glomerata* L., *Festuca arundinacea* Schreb. and *Bromus inermis* Leyss. Global calibrations for the 418 perennial grass samples and specific calibrations for each single grass species and internal cross-validations were performed by the PLS regression method. The effect of different spectral data pre-treatments was investigated on the residual standard errors of the NIR predictive models. Among 60 calibration equations, the model with the lowest *SECV* value was retained for each parameter in each database. No particular data pre-treatment was really better than the other ones. Generally, the best results of the global calibrations were obtained with SNVD and MSC. For the specific calibrations, SNVD and WMSC were the best treatments. In both cases, the first or second derivatives were needed after the first pre-treatment. Chemical composition and *in vitro* enzymatic digestibility of clones were predicted with accuracy similar to that of classical laboratory methods. For the cell wall component contents, the standard errors of cross-validation *SECV*(%DM) ranged from 0.49 for ADL (*Festuca*) to 2.02 for NDF (*Dactylis*). The digestibilities of dry and organic matter, IVDMD and IVOMD, were estimated with *SECV*s from 2.6 to 3.0%, the relative intake, from 0.06 to 0.09 rel% body weight and the relative feeding value, from 4.39 to 5.64 rel%. The global calibration models offer an acceptable accuracy for the estimation of the cell wall nutrient contents, the digestibility and the nutritive value. The standard errors of prediction of specific single species calibrations with smaller numbers of terms were lower in 60% of the cases than those obtained from the best global calibrations with higher numbers of terms. On average, *SECV*s from specific calibrations are better than those from global calibrations, but the differences are quite small, and for the prediction of totally new samples (new crops, another year), the global calibrations will detect less outlier samples. Even with very high variability between cuts and years, NIR spectroscopy is able via ANOVA GL Models to sort clones on their feeding value and to provide relevant information for the breeding programmes.

Keywords: NIR spectroscopy, PLS, global and specific calibration models, spectral data pre treatment, perennial grasses, clone breeding, cell wall, *in vitro* digestibility, ANOVA.

Introduction

Near infrared (NIR) reflectance spectroscopy is a rapid, reproducible, precise, non-destructive and low-cost method for analysis of the chemical and physical components in plant materials and for forage quality evaluation. [5,11,14,15,18,20,29] It is more and more frequently applied in plant breeding programmes, where large numbers of small quantities of the samples from several species, cuts and years are necessary to evaluate different forage quality parameters at early stages of the breeding process.[22] The principles of NIR reflectance spectroscopy in plant breeding have been described by Starr.[23] Genetic variability of cell wall constituents, measured by NIR reflectance spectroscopy, as a forage quality assessment for perennial grass and *in vitro* dry matter digestibility in plant breeding programmes were established by several authors.[1,13,14] NIR reflectance spectroscopy was applied to aid the breeding programme of more nutritious pasture plants[21] and specific calibrations were performed for a single species or plant part.

The purpose of this study was to develop linear global broad-based and specific calibration models by MPLS for analysis of cell wall components and digestibility to estimate the feeding value of clones from the three perennial grasses: *Dactylis glomerata* L., *Festuca arundinacea* Schreb., *Bromus inermis* Leyss. and to evaluate the accuracy of their prediction in breeding programmes.

Materials and methods

Plant material

Forage grass species—perennial grasses

The initial material for breeding on forage quality is the varieties of the three perennial grasses developed and recognised in Bulgaria.

Orchard Grass (*Dactylis glomerata* L.), variety Dabrava, 1978, grown under irrigation for a 4–5 year period in mixture with lucerne. The variety Pleven 1 yields on average 14,000–17,000 kg ha^{-1} dry matter and 2600–3000 kg ha^{-1} crude protein.[24]

Tall Fescue (*Festuca arundinacea* Schreb.), synthetic variety Albena, 1993, is suitable for artificial meadows and pastures on salted, overmoist and acidified soils. It is characterised by a high productivity of forage and seeds, intensive tillering, relatively tender vegetative mass and persistence of 8–9 years and is the standard variety of the country.

Smooth Brome (*Bromus inermis* Leyss.), variety Nika, 1993, is suitable for artificial meadows and pastures on sandy and stony soils with shallow soil layers. It is the standard variety for the country.

These three perennial grasses were studied because they are widely used as the country forage perennial grass cultures. The foreign varieties are not adapted for the Bulgarian agroclimatic conditions and display low quality and productivity.[25]

Crop breeding experiments

The plant material for this study came from breeding experiments of perennial grasses—large line breeding regrowths for each of the three perennial grasses in afour year period (1992, 1994, 1995 and 1996) at the experimental fields of the Forage Institute, Pleven.

Perennial grass populations are characterised by individual heterogeneity, which results in strongly expressed polymorphism in the different plants. This fact makes breeding evaluation of generative progenies of selected genotypes easier. This is the way recommended in crop breeding of perennial grasses for using the phenotypes of the investigated clones.[9,27] In the whole breeding process, they always take the same genotype. They keep their originality after studying their generative generations and they may be used as a generative starting point. Cloned generations express relatively good heritability of many biological characters, including chemical composition.[9]

In breeding nurseries of individual plants of the three grass species grown in the experimental fields of the Forage Institute, 50 elite genotypes in 1992 and 78 elite genotypes in 1994–1996 were selected. They were cloned in eight pieces and, respectively, 400 and 624 plants were obtained and planted in nurseries according to the established procedure.[24,26] During this period, a selection based on morphological and biological characteristics was carried out and the different clone numbers were selected for chemical and NIR reflectance spectroscopy analysis as follow: *Dactylis glomerata* L.—93, *Festuca arundinacea* Schreb.—352 and *Bromus*

inermis Leyss.—224. Plant material for chemical and NIR reflectance spectroscopy analyses represent the whole plant from regrowths of the three perennial grasses, cut in the spring (at early heading) and in the summer (42 days later).

Chemical methods for analysis of cell wall components and digestibility

Preparation of the plant material from the whole plants was performed by oven drying at 65°C and grinding to pass a 1.0 mm screen, Lab Mill QB-130, Labor Mim, Hungary.

Detergent analyses of Goering and Van Soest (1970)[8] were performed as the standard chemical analyses of the fibre components (NDF, ADF, ADL; hemicellulose was determined by the difference between NDF and ADF and cellulose by the difference between ADF and ADL) and were used as reference methods for NIR calibration development.[18]

The rate of lignification is presented as the ratio between ADL and NDF and multiplied by 100—relative units.

In vitro digestibility of dry (IVDMD) and organic (IVOMD) matter for each plant material were determined by the two-stage pepsin–cellulase method of Aufrere (1982).[3]

Relative feeding value and relative intake and digestible dry matter are presented following Linn and Martin (1991).[10] The published equations and coefficients according to the authors are based on the experimental data in the American System for forage feeding value and they are related for grasses, legumes and mixtures of these. The relative feeding value (RFV) is an index created for the evaluation at the same time of the factors intake and digestibility as effective methods for forage quality evaluation from ADF and NDF as follows:

Digestible Dry Matter (DDM) (%) =
88.9 – (0.779 × ADF%)
Dry Matter Intake (DMI) (% of body weight) =
120 / NDF%
Relative Feeding Value (RFV) =
(DDM × DMI) / 1.29

The application of these forage parameters is recommended only for the forage quality evaluation, together with values for cell wall constituents.

The characteristics of the databases under investigation are given in Table 3 for the three species together and in Table 4 for each individual species.

Spectral databases for NIR reflectance spectroscopy

Spectral databases

All the selected samples were scanned twice and the spectra were collected as log(1/*R*) over the visible and near infrared region in segments both from 400 to 1098 and 1100 to 2498 nm with a 2 nm step on a monochromator model 6500 NIRSystems Inc., Silver Spring, MD, USA. The spectral and mathematical treatments of the data were performed using the ISI NIRS 3 ver. 4. software (Infrasoft International, Port Matilda, PA, USA) on a reduced wavelength range (708–1092,8 and 1108-2492,8).

After averaging the duplicates, the spectral boundaries for calibrations were defined by a PCA method.[18] The spectra were sorted according their Mahalanobis distances (*H* statistic). All the samples displayed distances less than 3.6. The *H* statistic distribution shows that the population is very homogenous and than all the spectra were retained for further calculations.

A PCA analysis has been performed to estimate the spectral distances between the three species. The PCA was performed after SNVD treatment and a first derivative (1-5-5) using the segments 708–1092,8 and 1108–2492,8.

Spectral data pretreatment

The NIR spectra of the perennial grasses are affected by particle geometry and size, by scatter coefficients and by pathlength variations. Then, calibrations are improved with pre-treatment of the spectral data. The full spectrum is corrected in the following ways.[18,19]

Detrend (DET).[4] The dominant feature of NIR diffuse reflectance spectra is the increasing level of the log (1/*R*) reflectance values over the range 1100 to 2500 nm. This trend is generally linear but it becomes curvilinear for the spectra of samples packed with different pressures. This trend can be removed by considering the deviates from a second-degree polynomial function.

Standard normal variate (SNV).[4] The mathematical transformation of the log(1/*R*) spectrum is per-

formed by calculating the mean and the standard normal variate of this spectrum. Each new corrected value is the original absorbance from which the mean of the whole spectrum is subtracted and divided by the standard deviation of the spectrum.

Standard normal variate and detrend (SNVD).[18,19] This treatment of the spectra combines the detrending and the standard normal variate corrections.

Multiplicative scatter correction (MSC).[7,18,19] Multiplicative scatter correction was proposed by Geladi *et al.* in 1985[7] to eliminate or reduce the difference in light scatter between samples before calibration. This correction consists of computing a simple linear regression between the spectral values of each sample and the average spectrum of the database and in correcting each of the log(1/*R*) values by subtracting the intercept and dividing by the slope of the estimated regression equation. The corrected spectra, with minor variability due to differences in light scatter, are then used for the calibration.

Weighted multiplicative scatter correction (WMSC).[18,19] This correction is similar to the previous one. To compute the simple linear regression, the absorbances are weighted according to their standard deviation.

The spectra are also corrected using derivatives. Derivatives of a spectrum can be calculated in several ways, but the most common is the segment-gap method.[18] In general, it is impossible to say that a spectral pre-treatment or a particular derivative will work better than other treatments for any constituent in any product. Usually, trial and error are the only way to optimise the best spectral treatment by searching for the lowest prediction error.[18]

Global calibrations

Global calibrations were obtained by using a Modified Partial Least Squares (MPLS) Regression method, available in ISI NIRS 3, which is the classical PLS algorithm with a standardisation of the X residuals at each iteration. This regression method requires cross-validation to prevent overfitting. Cross-validation estimates calibration performances by partitioning the calibration set into several groups (between four and six in our study). PLS equations from 1 to 14 terms are computed with five-sixths of the samples and the prediction errors are obtained on the last group. The procedure is repeated six times until every sample has been predicted once and the group prediction errors are combined into a standard error of cross-validation (*SECV*). The calibration equations were optimised by testing six mathematical pre-treatments of the raw optical data. The derivatives were optimised by changing segments and gaps.[17] The different derivatives are coded by a triplet: the first figure represents the degree of the derivative (0 for raw log(1/*R*), 1 for the first derivative, 2 for the second derivative), the second and the third figures give a subtraction gap and the smoothing segment expressed in data points, respectively. In the ISI software, we used the option "Teach automatic sequence" to create a macro command able to compute and store 60 models per constituent. Among the 60 equations, the model with the lowest *SECV* value is retained for each parameter in each database. In total 660 calibrations were tested and compared. The flow chart of the global calibration trials is presented in Figure 1.

Specific calibrations

The specific calibration trials were performed using the mathematical and the spectral treatments found in the global calibration experiment for the clones of *Dactylis glomerata* L., *Festuca arundinacea* Schreb. and *Bromus inermis* Leyss. They are developed by the same manner and the same criteria as the global calibrations. The number

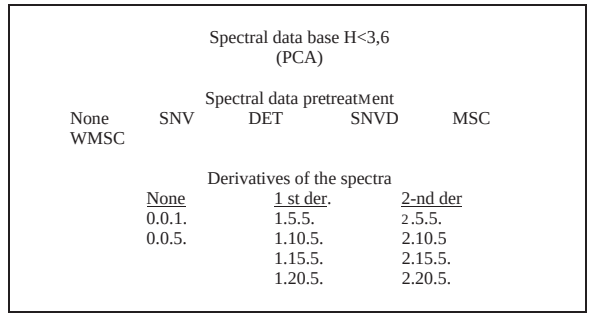

Figure 1. Flow chart for the global and specific calibration trials. Scatter correction (Standard Normal Variate, Detrend, Multiplicative Scatter Correction, Weighted Multiplicative Scatter Correction); Mathematical treatments (derivative, gap in data points, segment in data points).

Table 1. Mahalanobis distances (*H* statistic) of cross projection between the three populations using 20 PCs with the individual loadings.

	Individual loading bases								
	Bromus			*Dactylis*			*Festuca*		
Projected bases	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max
Bromus	0.26	1	4.98	2.13	5.24	14.78	3.83	7.58	13.27
Dactylis	1.41	22.83	80.89	0.32	1	2.62	1.72	6.71	25.28
Festuca	3.66	40.42	109.3	2.79	8.69	20.42	0.40	1	2.93

of cross-validation groups is six for *Dactylis*, four for *Festuca* and five for *Bromus*.

The statistics of the most interest were the determination coefficient on the calibration and validation sets (*R2C* and *R2CV*), standard error of calibration (*SEC*), standard error of cross-validation (*SECV*) and the ratio of the standard deviation of original data by the *SECV* (*SD/SECV*). This ratio is independent of the units and allows a comparison of the different equations.

Results and discussion

The results of the PCA analysis are reported in Table 1. The number of principal components chosen was 20 which express 99.8% of the total variance. The distances are computed on the basis on the specific loadings: a new PCA is performed from each population, leading to a non-symmetric distance matrix. The distances show that the three populations are different and thus specific calibrations can be justified. The two wider populations consist in the *Festuca* and *Dactylis* samples with the lowest *H* distances when the other samples are projected. The smaller population in term of spectral variance is coming from the *Bromus* samples. When projecting the *Dactylis* and *Festuca* samples on its spectral space, we observe average *H* values of 22.8 and 40.4 respectively. Figure 2 represents the three populations is the space of the two first components. However, if the distances are computed with the same loadings coming from the three populations, the cross distances are largely reduced (Table 2): the gravity centres of each group are located at distances ranging from 0.85 to 1.45. Then, the use of global calibrations can be justified as well.

Table 2. Mahalanobis distances of cross projection between the three populations using 20 PCs with the common loadings.

	Common loading base								
	Bromus			*Dactylis*			*Festuca*		
Projected bases	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max
Bromus	0.26	0.96	4.97	0.66	1.45	3.47	0.51	1.08	3.04
Dactylis	0.73	1.38	4.90	0.41	1.04	2.80	0.59	1.21	3.40
Festuca	0.51	1.20	5.25	0.59	1.40	3.41	0.27	0.85	2.79

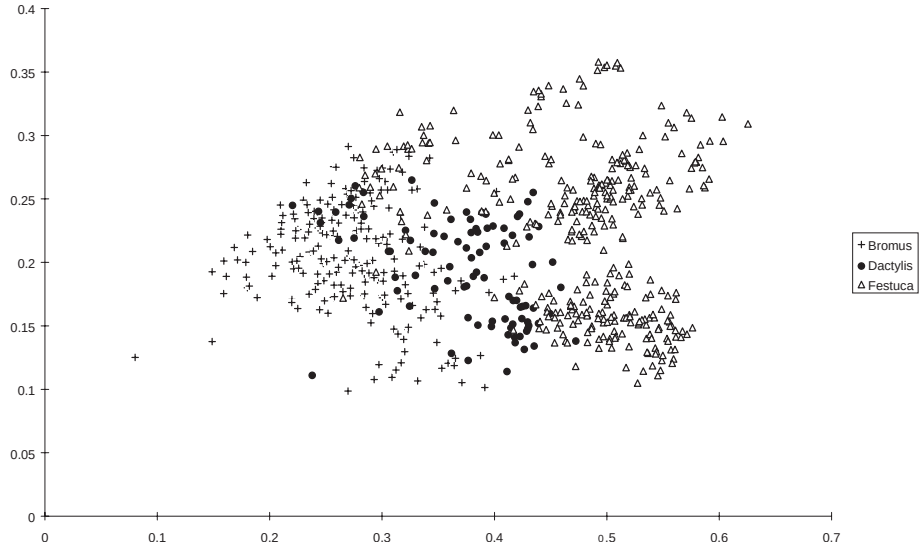

Figure 2. Scatter plot of the three populations in the first two components (common loadings).

The best global calibrations were searched out by using the scheme described in Figure 1. For each parameter 60 calibrations were built up. The best predictive models obtained are summarised in Table 3. The relation between NIR reflectance spectroscopy predicted and reference values are shown in Figure 4. The best calibrations for each parameter in a specific clone population for *Dactylis*, *Festuca* and

Table 3. Characteristics of the clone forage data bases and performance of the best predictive model among 60 global Modified PLS equations tested for each parameter for the clones of the three perennial grasses.

Parameter	*N*	Mean	Min	Max	*SD*	T	*R2C*	*SEC*	*SECV*	*R2CV*	*SD/SECV*	Scatter	Math
NDF (%DM)	327	55.77	39.83	66.32	5.44	10	0.92	1.56	1.68	0.90	3.2	WMSC	2.10.5
ADF (%DM)	327	33.24	22.98	43.16	4.38	9	0.93	1.14	1.29	0.91	3.4	SNVD	2.5.5
ADL (%DM)	327	3.19	1.12	6.23	0.88	6	0.76	0.48	0.52	0.72	1.9	None	2.5.5
HEMI (%DM)	327	24.15	13.21	30.18	3.48	13	0.84	1.40	1.52	0.81	2.3	SNVD	1.10.5
CELLU%DM)	327	30.14	20.65	37.79	3.75	8	0.92	1.07	1.18	0.90	3.2	MSC	2.5.5
LIGNIF(rel%)	327	5.67	2.70	10.00	1.49	10	0.74	0.77	0.88	0.65	1.7	MSC	2.10.5
IVDMD (%)	418	64.58	46.17	84.06	7.11	14	0.87	2.60	2.89	0.84	2.5	SNVD	1.10.5
IVOMD (%)	418	62.21	43.94	81.61	7.15	14	0.87	2.60	2.85	0.84	2.5	SNVD	1.10.5
DDM (%)	327	62.94	55.28	71.00	3.42	9	0.93	0.92	1.00	0.91	3.4	SNV	2.10.5
DMI (%bw)	327	2.17	1.18	3.01	0.23	11	0.92	0.07	0.07	0.90	3.3	SNV	2.10.5
RFV (rel%)	327	106.51	79.60	165.16	16.38	11	0.93	4.25	4.71	0.92	3.5	MSC	2.15.5

Table 4. Characteristics of the clone data bases and performance of the best predictive model among 60 global Modified PLS equations tested for each parameter for the clones for each of the three perennial grasses.

Parameter	*N*	Mean	Min	Max	*SD*	T	*R2C*	*SEC*	*SECV*	*R2CV*	*SD/SECV*	Scatter	Math
Dactylis glomerata L.													
NDF (%DM)	58	56.96	43.27	66.32	6.10	6	0.96	1.27	2.02	0.89	3.0	DET	2.5.5
ADF (%DM)	58	34.20	22.98	43.16	5.59	6	0.97	0.96	1.22	0.95	4.6	WMSC	0.0.1
ADL (%DM)	58	3.94	2.23	5.88	0.99	2	0.57	0.65	0.68	0.53	1.4	WMSC	0.0.1
HEMI (%DM)	58	22.76	14.99	28.90	3.13	8	0.92	0.98	1.44	0.82	2.4	DET	2.20.5
CELLU%DM)	58	30.26	20.65	37.37	4.89	9	0.99	0.42	0.91	0.96	5.4	SNV	2.5.5
LIGNIF(rel%)	58	6.90	4.00	10.00	1.50	2	0.55	0.98	1.03	0.52	1.4	SNVD	2.5.5
IVDMD (%)	66	66.40	53.20	84.06	7.52	4	0.92	2.07	2.60	0.87	2.9	WMSC	2.5.5
IVOMD (%)	66	64.24	49.94	81.61	7.18	6	0.92	2.01	3.02	0.82	2.3	None	2.5.5
DDM (%)	58	62.26	55.28	71.00	4.36	8	0.98	0.69	0.91	0.96	4.8	MSC	0.0.1
DMI (%bw)	58	2.13	1.81	2.77	0.25	8	0.94	0.06	0.09	0.87	2.8	None	1.10.5
RFV (rel%)	58	103.66	80.62	152.63	19.38	7	0.95	4.28	5.64	0.91	3.4	MSC	0.0.5
Festuca arundinacea Schreb.													
NDF (%DM)	179	53.81	39.83	64.88	4.97	8	0.91	1.46	1.62	0.89	3.0	SNVD	2.20.5
ADF (%DM)	179	32.96	23.34	41.52	4.18	5	0.93	1.10	1.61	0.92	3.4	SNVD	2.5.5
ADL (%DM)	179	2.81	1.12	5.76	0.78	7	0.70	0.43	0.49	0.61	1.6	SNVD	1.10.5
HEMI (%DM)	179	20.86	13.21	27.43	2.91	9	0.77	1.41	1.60	0.70	1.8	DET	2.15.5
CELLU%DM)	179	30.15	22.22	37.79	3.64	5	0.92	1.02	1.15	0.92	2.2	SNVD	2.5.5
LIGNIF(rel%)	179	5.19	3.00	10.00	1.30	4	0.62	0.80	0.89	0.54	1.5	DET	2.5.5
IVDMD (%)	228	62.24	46.47	81.51	6.97	6	0.86	2.63	2.96	0.82	2.4	WMSC	2.5.5
IVOMD (%)	228	59.87	43.94	77.68	7.02	6	0.87	2.55	2.86	0.83	2.4	WMSC	2.5.5
DDM (%)	179	63.23	56.56	70.72	3.26	5	0.93	0.86	0.94	0.92	3.5	SNVD	2.5.5
DMI (%bw)	179	2.25	1.85	3.01	0.22	8	0.92	0.06	0.07	0.90	3.1	WMSC	2.15.5
RFV (rel%)	179	110.75	83.96	165.16	16.30	8	0.91	3.96	4.39	0.93	3.7	MSC	2.20.5
Bromus inermis Leyss.													
NDF (%DM)	90	58.91	49.45	66.07	4.06	4	0.87	1.48	1.65	0.84	2.5	SNVD	2.20.5
ADF (%DM)	90	33.49	28.89	42.03	3.83	4	0.85	1.48	1.68	0.82	2.2	SNVD	2.20.5
ADL (%DM)	90	3.46	2.02	6.23	0.97	5	0.82	0.41	0.51	0.73	1.9	MSC	2.5.5
HEMI (%DM)	90	25.41	19.99	30.18	2.40	4	0.81	1.04	1.20	0.75	2.0	SNVD	2.10.5

Table 4 (continued). Characteristics of the clone data bases and performance of the best predictive model among 60 global Modified PLS equations tested for each parameter for the clones for each of the three perennial grasses.

Parameter	*N*	Mean	Min	Max	*SD*	T	*R2C*	*SEC*	*SECV*	*R2CV*	*SD/SECV*	Scatter	Math
CELLU%DM)	90	30.03	21.28	36.29	3.11	8	0.85	1.19	1.42	0.80	2.2	SNVD	0.0.1
LIGNIF(rel%)	90	5.81	4.00	9.00	1.40	5	0.78	0.65	0.80	0.68	1.8	MSC	2.5.5
IVDMD (%)	124	67.91	53.74	83.51	5.56	7	0.83	2.31	3.00	0.71	1.8	MSC	2.5.5
IVOMD (%)	124	65.43	51.14	81.36	5.68	6	0.82	2.40	2.81	0.76	2.0	SNVD	2.5.5
DDM (%)	90	62.81	56.16	70.29	2.98	4	0.85	1.15	1.30	0.82	2.3	SNVD	2.20.5
DMI (%bw)	90	2.05	1.82	2.43	0.14	7	0.87	0.05	0.06	0.84	2.3	SNVD	0.0.1
RFV (rel%)	90	99.92	79.60	132.23	11.32	7	0.86	4.17	4.73	0.83	2.4	WMSC	0.0.1

Bromus and the best predictive models overall are presented in Table 4. including the same statistical parameters as for the global calibration models.

From these results we make the following comments.

1) The variations of the *R2C* values in the global calibration models are rather large: 0.74 for ADL to 0.93 for ADF, DDM and RFV. However, the *SEC* for the cell wall contents (NDF, ADF and ADL) are very good and lower than many values observed in the literature[15] and demonstrate the quality of the reference values. The IVOMD and IVDMD values are similar to those found in the literature.[15] The range for these parameters are especially large with a range of ±40%.

2) The differences between *SEC* and *SECV* show that the models built with 327 and 418 samples are very robust and not overfitted. It is interesting to notice that no sample has been removed for the calibrations avoiding the problem of the outlier limit detection. It is well known that removing too many outliers makes the calibration results better than they actually are. Keeping all the data (spectra and reference values) is again a good sign of the quality of the data set.

3) The best results are obtained with the following scatter corrections: SNVD was retained for the analysis of ADF, hemicellulose, IVDMD and IVOMD; MSC for analysis of cellulose, lignification and RFV; SNV for DDM and DMI. Only one parameter ADF was found with WMSC and one ADL without scatter correction. Except for ADL, all the parameters work better with a scatter correction method. The differences in the *SECV*s between SNV, SNVD, MSC and WMSC are very small and we could use any one of them. As an example, Figure 3 represents the distribution of the 60 *SECV*s for ADF in global calibration: the best *SECV* is 1.29 and the worst is 1.43. Anyway, it seems that SNVD is one of the best ways to evaluate ADF and *in vitro* digestibility of dry and organic matter.

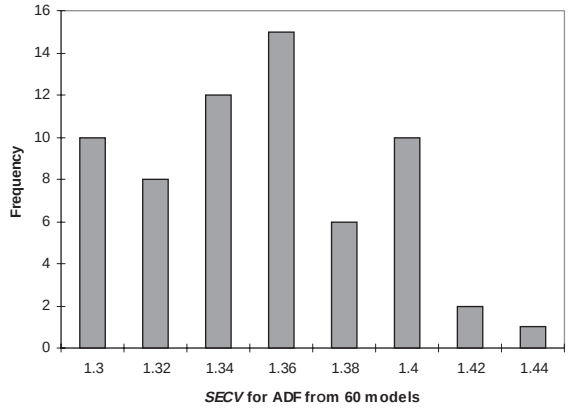

Figure 3. Histogram of the 60 SECV for ADF in global calibrations.

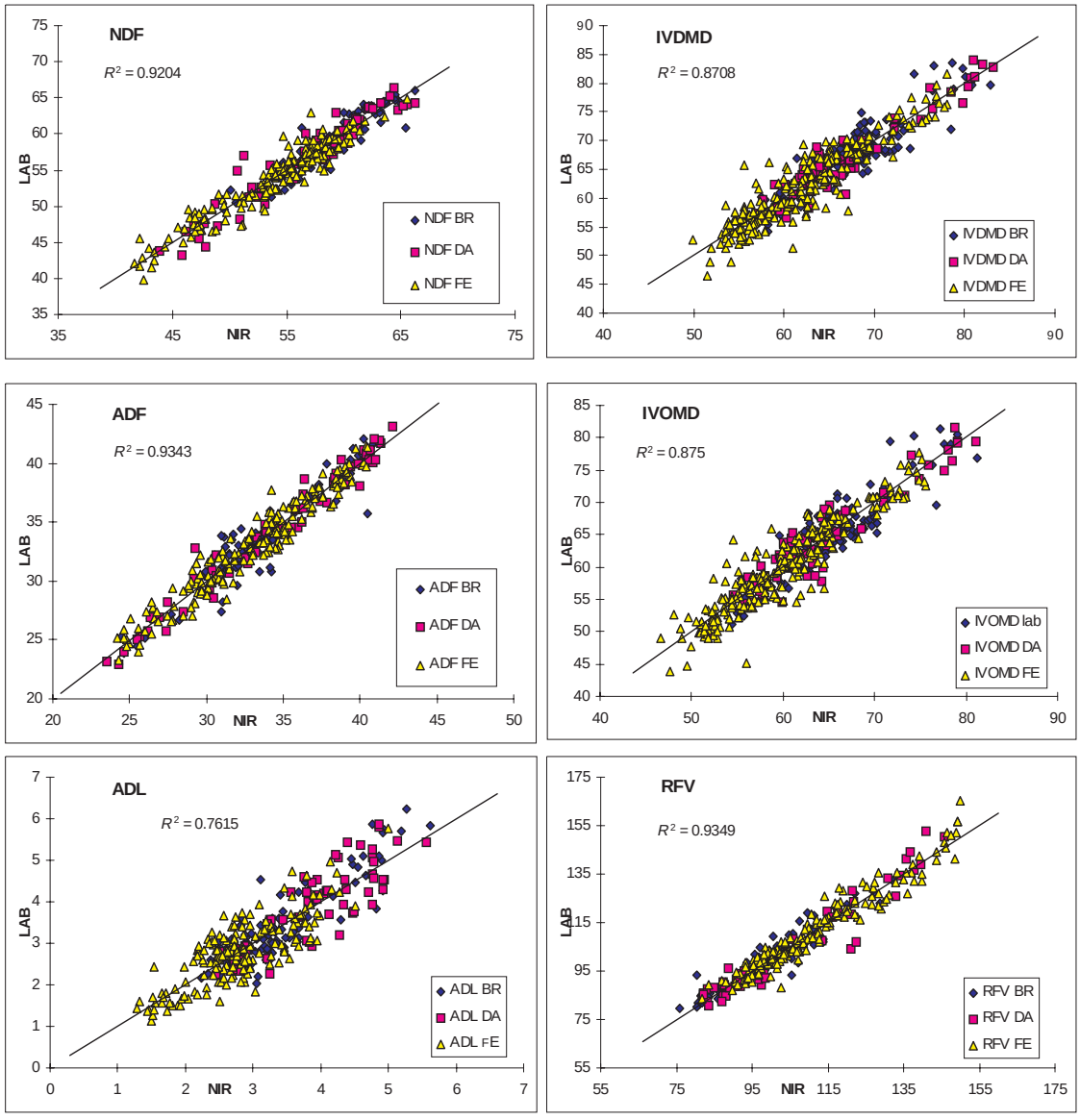

Figure 4. NIR reflectance spectroscopy predicted vs reference values for cell wall composition, digestibility and feeding value in global clone population for the three perennial grasses.

4) In all cases the application of the first and the second derivatives give better results than no derivative. The three best treatments 1.10.5, 2.5.5 or 2.10.5 for all parameters are determined. The use of the raw spectral data without mathematical treatment is never selected for global perennial grass clone calibrations. This is the same for the specific calibrations for *Festuca arundinacea* Schreb. The use of the raw data is selected in some cases: *Dactylis glomerata* L. database for ADF, ADL, DDM with 0.0.1 and for RFV with 0.0.5; *Bromus inermis* Leyss. database for Cellulose, DMI and RFV with 0.0.1.

5) The maximum number of terms in global calibrations is 14 for both IVDMD, IVOMD and the minimum is six for ADL. In specific calibrations, the maximum and minimum numbers of terms are as fol-

Table 5. Comparison of the SECV values obtained with the best specific calibrations for the three perennial grasses and the best regressions for global clone calibration samples.

			Dactylis glomerata L.			*Festuca arundinacea Schreb*			*Bromus inermis Leyss.*		
Criteria	*N Glo*	*T Glo*	*T* Spe	*SECV Glo*	*SECV Spe*	T Spe	*SECV Glo*	*SEP Spe*	T Spe	*SECV Glo*	*SEP Spe*
NDF (%DM)	327	10	6	2.02	1.98	8	1.62	1.62	4	1.65	1.58
ADF (%DM)	327	9	6	1.22	1.16	5	1.21	1.20	4	1.68	1.54
ADL (%DM)	327	6	2	0.68	0.57	7	0.49	0.50	5	0.51	0.53
HEMI (%DM)	327	13	8	1.44	1.63	9	1.60	1.56	4	1.20	1.37
CELLU%DM)	327	8	9	0.91	0.95	5	1.15	1.15	8	1.42	1.36
LIGNIF(rel%)	327	10	2	1.03	0.94	4	0.89	0.87	5	0.80	0.86
IVDMD (%)	418	14	4	2.60	2.43	6	2.96	2.98	7	3.00	2.96
IVOMD (%)	418	14	6	3.02	2.62	6	2.86	2.90	6	2.81	2.88
DDM (%)	327	9	8	0.91	0.90	5	0.94	0.93	4	1.30	1.20
DMI (%bw)	327	11	8	0.09	0.09	8	0.07	0.07	7	0.06	0.06
RFV (rel%)	327	11	7	5.64	5.75	8	4.39	4.43	7	4.73	4.50

Glo = Global; Spe = specific

low: for *Dactylis glomerata* L. nine for cellulose and only two for ADL; for *Festuca arundinacea* Schreb. nine for hemicellulose and four for lignification; for *Bromus inermis* Leyss. eight for cellulose and four for NDF, ADF, hemicellulose and DDM. Due to the reduced numbers of samples, the cross validation selected less numbers of terms in specific calibrations (average = 6 terms) than in global calibration (average = 10.5 terms).

It may be proposed that the accuracy of prediction for quality parameters will be better by specific calibrations for each plant culture than by the global broad-based calibrations including more plant cultures in point of view of better homogeneity of the specific populations. But we observed higher coefficients of determination and smaller *SEC* for cell wall components, digestibility and nutritive value, excluding ADL and lignification only for *Dactilis glometrata* L. For the two other cultures *Festuca arundinacea* Schreb. and *Bromus inermis* Leyss. the *SEC* are higher and coefficients of determination are smaller than in global calibrations.

In order to test the versatility of the global NIR reflectance spectroscopy equations, predictions of all quality parameters were compared to predictions made by specific calibrations (Table 5). The standard errors of prediction of specific single species calibrations with smaller number of terms were lower in 60% of the cases than those obtained from the best global calibrations with smaller numbers of terms.

The statistical analysis for evaluating the significant differences between clones from each of three perennial grasses for all the parameters was performed using ANOVA General linear model procedure of the SAS software (Table 6).[17] The reference laboratory values have been used when they were available. The missing data have been replaced by the NIR predicted values coming from the best models. Only *Festuca arundinacea* Schreb. clones show

Table 6. The ANOVA General linear model (GLM - SAS). Analysis of variance for the clones of the three perennial grasses based on the predicted and actual values.

Dactylis glomerata L. Clones N = 15 59 samples, 3 years, 3 cuts				*Festuca arundinacea* Schreb. Clones N = 40 211 samples, 3 years, 3 cuts				*Bromus inermis* Leyss. Clones N = 34 186 samples, 3 years, 3 cuts			
Variable	Mean		*SD*	Variable	Mean		*SD*	Variable	Mean		*SD*
ADF	30.89	*	0.95	NDF	52.13	***	1.02	ADF	32.17	**	0.90
CELLU	26.29	**	0.82	ADF	30.81	***	0.78	ADL	3.51	***	0.17
				ADL	3.05	*	0.19	LIGNIF	6.26	***	0.34
				HEMI	21.86	***	0.64				
				CELLU	27.97	***	0.72				
				LIGNIF	5.85	*	0.34				
				IVDMD	62.88	*	1.43				
				IVOMD	59.64	*	0.68				
				DDM	64.74	***	0.65				
				DMI	2.31	***	0.04				
				RFV	116.05	***	3.11				

Significance: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

statistically significant differences between them for all parameters investigated. The others two forage perennial grasses show statistically significant differences just for some parameters, which may be due to a smaller number of clones investigated and probably because the variations between cuts and years are very large. The differences between clones of *Festuca arundinacea* Schreb. for the parameters

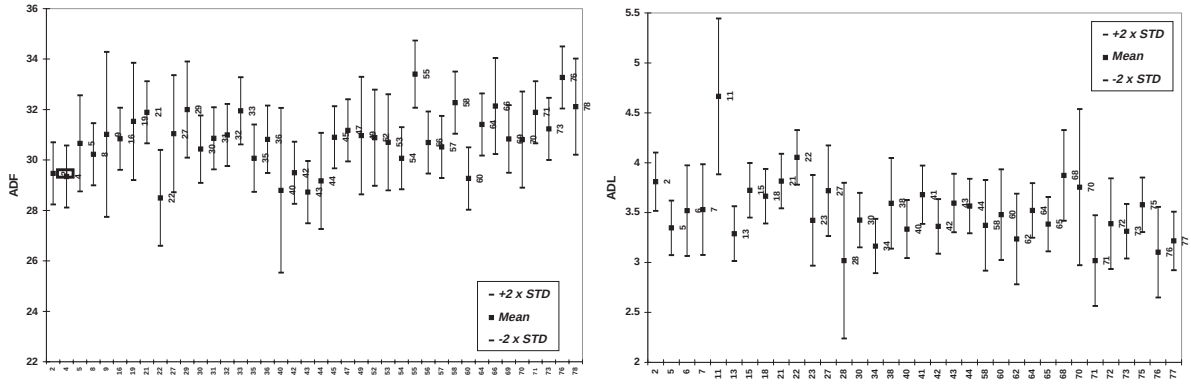

Figure 5. Mean values and standard deviations for ADF and ADL of *Festuca arundinacea* Schreb. clones

ADF and ADL are displayed by the mean values and the means plus and minus two standard deviations (Figure 5).

In conclusion the standard errors of prediction of specific single species calibrations with smaller numbers of terms were lower in 60% of the cases than those obtained from the best global calibrations with higher numbers of terms. On average, *SECV*s from specific calibrations are better than those from global calibrations, but the differences are quite small, and for the prediction of totally new samples (new crops, another years), the global calibrations will detect less outlier samples and will probably be more robust and more efficient. Even with very high variability between cuts and years, NIR reflectance spectroscopy is able via ANOVA GL Models to sort clones on their feeding value and to provide relevant information for the breeding programmes.

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