Prediction of Chemical Composition from Semi-natural Grassland by NIR Spectroscopy

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Summary

The objective of this research was to examine three techniques for prediction of the chemical composition by NIR spectroscopy (1100 – 2500 nm) from semi-natural grassland: modified partial least squares (MPLS) regression; partial least square (PLS) regression and principal component regression (PCR). A spectral data for a total of 150 samples originated from seminatural grassland were used. Standard errors of calibration (SEC) for crude proteins (CP) were 6.52, 4.87 and 6.94 for MPLS, PLS and PCR, while standard errors of cross validation (SECV) were 8.16, 6.13 and 7.56 respectively. SEC for organic matter (OM) were 7.69, 7.61 and 7.37 for MPLS, PLS and PCR, while SECV were 8.08, 8.27 and 7.57 respectively. Higher SEC and SECV were reported for neutral detergent fibre (NDF) and acid detergent fibre (ADF) content than reported for CP and OM content.

Hyperspectral analysis by PLS resulted in the highest accuracy for the estimation of crude protein, organic matter and neutral detergent fibre and acid detergent fibre, while MPLS was the best in predicting acid detergent fibre. The greatest accuracy in this research was achieved for CP, then NDF, OM, and finally ADF content. Prediction for NDF, OM, and especially ADF content should be improved in the future by involving specific semi-natural grassland samples.

Key words

semi-natural grassland, chemical composition, NIR spectroscopy, PLS, MPLS, PCR

Introduction

Forage nutritive value changes mostly with plant maturity but is also affected by many other factors, including genotypic variation, plant species and variety specifics, plant morphology, chemical and physical composition, climate, environment, soils and management practices (Fales and Fritz, 2007).

Forage nutritive value is extremely important in animal production systems in determining nutritionally balanced feed rations, evaluating forage management practices (growing, harvesting and storage) and marketing (Dale et al., 2012). Ruminant productivity is affected by forage quality: the higher the quality of the forage, the greater the productivity and economic return (Fales and Fritz, 2007).

The main factors determining forage nutritive value are nutrient concentration and digestibility (Collins and Fritz, 2003). The nutrient concentration in feed and forage is related to chemical composition, which can be determined by NIR spectroscopy or wet chemistry methods. The NIR technique in comparison with classical chemical methods is non-destructive, nonpolluting, fast and relatively inexpensive per analysis.

For this reasons NIR spectroscopy has become widely used as an analytical method in many areas (Mark and Campbell, 2008), thus in agriculture (Roberts et al., 2003). For studying grassland and meadow hay, Vazquez de Aldana (1996) developed a calibration model to predict ash content. NIR spectroscopy has also been used to predict dry matter (DM), crude protein (CP), ash, in vitro organic matter digestibility (OMD), acid detergent fiber (ADF) and neutral detergent fiber (NDF) of forages in broad-based calibration models (Garcia and Cozzolino, 2006), to determine the chemical composition, in vitro OMD and in vitro gas production in feed and forage (Lavrenčić et al., 2001), to estimate CP concentration in fresh grass silage (Vranić et al., 2005), to predict chemical composition from semi-natural grassland (Vranić et al., 2010), or to estimate protein and net energy content in maize at various stages of development (Volkers et al., 2003). NIR spectroscopy has been successfully applied to predict the nutritive value of a variety of forages, feed stuffs, and young legumes and grasses with R^2 values of 0.90 or higher (Lenné et al., 2003; Stuth et al., 2003; Cozzolino and Morón, 2004; García and Cozzolino, 2006).

The objective of this research was to examine three techniques for prediction of CP, organic matter (OM), neutral detergent fibre (NDF), and asid detergent fibre (ADF) from semi-natural grassland: modified partial least squares (MPLS) regression; partial least square (PLS) regression, and principal component regression (PCR).

Materials and methods

Experimental design and plant sampling

The study was conducted at the Grassland Research Center owned by the University of Zagreb Faculty of Agriculture. The Center is located at 638 meters above sea level in the Nature Park Medvednica.

In the ten year period (2003-2014) farm yard manure (FYM) was applied on the seminatural grassland community *Arrhenatheretum medioeuropaeum* to determine the effect of quantity, time and frequency of FYM application on sward chemical composition. The experiment was conducted as a randomized block design with four replications and ten fertilizing treatments with incorporation of 30 and 50 kg ha⁻¹ FYM each fall, each autumn, every third fall, every third autumn, and a control classical fertilization treatments with mineral fertilizers (NPK 8:26:26) every fall in the amount of 500 kg ha⁻¹ and calcium ammonium nitrate (KAN) (27% N) in the quantity of 600 kg ha⁻¹. FYM was manually applied and evenly distributed on plots 6 x 2 m each (12 m²) with 0.5 m spacing between plots.

The experimental plots were mowed with a self-propelled mower at the tasseling phase of the dominant grass to a height of 5 cm. To avoid marginal impact, the strip of 1.2 m width was harvested at the middle of each plot. Chemical composition of first spring cut forage samples from 2006, 2007 and 2014 (total of 120 samples) was analysed. Spectral data from 30 additional samples from 1st cut in 2015, not analysed by wet chemistry, were used in the experiment.

Chemical analysis

Plant mass was weighed and subsamples (about 500 g) dried at a temperature of 60°C in a fan-assisted oven (ELE International, UK) to a constant weight to determine dry matter (DM) concentration and finally DM yield ha⁻¹. Dried forage samples were grounded with hummer mill (Overhung Disintegrator, Christy Hunt, UK) to pass a 1 mm screen.

The ash contents were measured by igniting the samples in a microwave oven (Milestone PIYRO, Italy) at 550°C for 3 h. The total N concentrations were determined by the Kjeldahl method (AOAC 1990, ID 954.01) using a Gerhardt nitrogen analyser. In addition, N concentration was expressed as crude protein (CP) (total N x 6.25) g kg⁻¹ DM.

Neutral detergent fibre (NDF) and acid detergent fibre (ADF) contents were analysed using the procedure described by Van Soest et al. (1991) utilising Ancom Filter Bag Technology with an Ancom Fibre nalyser (Ancom Technology, USA).

Spectral data collection

A spectral data for a total of 150 samples originated from seminatural grassland were used for calibration development. Out of them, four samples were detected as ouliers and excluded from further calculation. A calibration set of 94 samples and a validation set of 22 samples was chosen randomly for this investigation. A repeatability file set consisted of 30 spectral samples not analysed by wet chemistry.

Before scanning, the samples were re-dried at 105°C for at least three hours. Three independent scans were recorded from each of 150 samples, using a NIRSystem Model 6500 spectrometer (Foss-NIRsystem, Sweden) fitted with a sample transport module and a product sample cup (5 x 6.5 cm). Samples were scanned (32 scans co-added) using the ISI SCAN Version 1.0 (Infrasoft International, Port Matilda, PA, USA) from 1100 to 2498 nm in reflectance mode (R mode: PbS detector). Data were collected every 2 nm (700 data points per spectrum). The mean spectral value of each sample was calculated using the WIN ISI III Version 1.5. (Infrasoft International, Port Matilda, PA, USA)

Analysis of Spectral Data

Calibration models were developed with the software WinISI II from Foss, NIRSystems (Silver Spring, MD), testing different

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mathematical treatments of the spectra (differentiation order, subtraction gap, smoothing interval), with or without applying Standard Normal

Variate (SNV) and Detrend for scatter correction of the spectra. SNV scales each spectrum to have a standard deviation of 1.0 to help reduce particle size effects, and Detrend removes the linear and quadratic curvature of each spectrum (ISI, 1999). In performing measurements with the NIRSystem Model 6500 spectrometer, hundreds of data are generated for each sample and a principal component analysis (PCA) data reduction method was needed to facilitate data interpretation. Two different mathematical treatments were applied during the calibration development 1, 4, 4, 1, and 1, 8, 8, 1, where the first number indicates the order of the derivative (0 represents no derivative, 1 the first derivative and 2 the second derivative of log 1/R), the second is the gap in the data points over which the derivative is calculated, the third and fourth numbers refer to the number of the data points used in the first and second smoothing, respectively.

For transforming the measured spectral data into the sample properties producing model that describes the relationship between spectral data and chemical composition, i.e. crude protein (CP), organic matter (OM), neutral detergent fibre (NDF) and acid detergent fibre (ADF), three methods were applied: modified partial least squares (MPLS), principal component regression (PCR) and partial least squares (PLS). When developing the model equations, cross-validation is recommended in order to select the optimal number of factors and to avoid overfitting (Davies and Williams, 1996). Validation errors were combined into a standard error of cross-validation (SECV) (Shenk and Westerhaus, 1995). The statistics used to select the best equations were correlation coefficient (R^2) and the standard error of cross-validation (SECV). The predictve capacity of the model obtained was evaluated with the standard prediction error encountered in the NIR model (SEP). Furthermore, the risk of mistake with the equations under practical conditions was very low or almost nil when using the standardised H statistic (Mahalanobis distance) during routine analysis of unknown samples (Martens and Naes, 2001). After the number of principal components had been calculated, detection of anomalous spectra was accomplished using the Mahalanobis distance (H statistic), establishing H>3 as the limit value for spectral reasons.
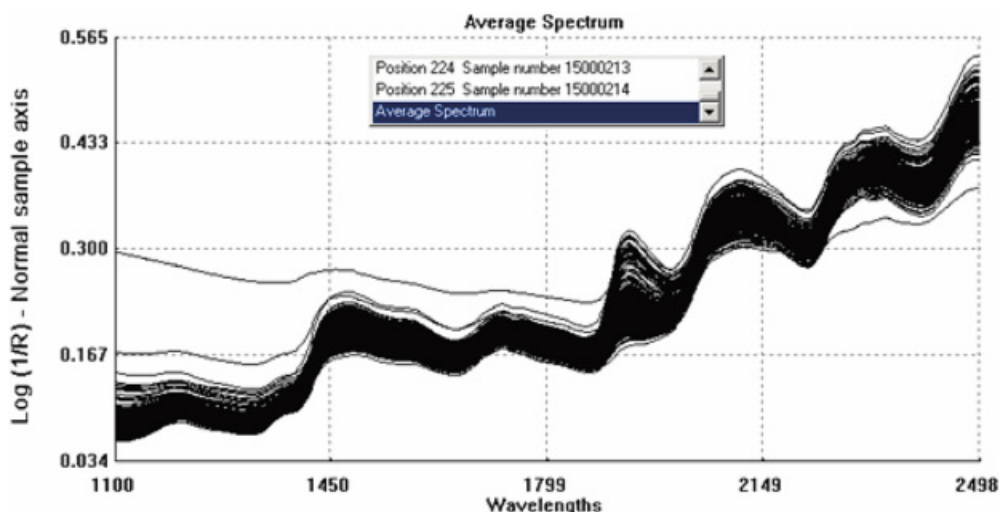
Table 1. Average chemical composition of samples used in the experiment (n=120)

Parameter	Minimum	Maximum	Mean	SD
CP (g kg^{-1} DM)	106.00	210.64	152.44	33.46
OM (g kg^{-1} DM)	882.41	940.33	909.53	12.70
NDF (g kg^{-1} DM)	381.63	658.34	498.73	71.92
ADF (g kg^{-1} DM)	283.57	458.33	342.47	28.24

DM, dry matter; CP, crude protein; OM, organic matter; NDF, neutral detergent fibre; ADF, acid detergent fibre; SD, standard deviation.

Results and discussion

The usefulness of a NIR prediction depends, on the one hand, on the accuracy of the results with respect to reference data, and on the other, on the level of error we are prepared to accept and how fast we can have the results available to make important management decisions. In the case of forage management, the change in CP and fibre content could be important features in deciding when to harvest for forage conservation, or the removal of animal stock from a given paddock.

Chemical composition of samples used in the experiment is presented in Table 1. The compositional data for the samples (Table 1) showed a wide variability in composition of analytical data. It can be seen that calibration set consisted of samples from leafy to more mature forage, with CP and NDF content ranging from 106 to 210.6 g kg^{-1} DM and 381.63 to 658.34 g kg^{-1} DM, respectively.

A broad distribution of values is desirable when a set of samples is selected for the development of NIR calibrations, as a way to have a better representation of the universe to be predicted subsequent in routine analysis. The requirements for the calibration samples are that the samples are similar enough to each other to represent a single population but diverse enough to allow independent resolution of each constituent of interest (Williams, 2001).

The spectra from samples scanned by reflectance (average of three readings) are presented in Figure 1. It can be seen from the figure, four of scanned samples showed a different spectra

Figure 1. The raw spectra from samples scanned by reflectance (average of three readings)

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Table 2. Calibration statistics of the prediction of semi-natural grassland for chemical composition by three chemometric methods

Chemical parameter	Method applied	SEC	SECV	SEP	SEP(C)	SLOPE	BIAS	RSQc	RSQcv
				g kg⁻¹ DM					
CP	MPLS	6.52	8.16	6.38	5.18	0.98	4.0	0.96	0.93
	PLS	4.87	6.13	5.97	5.93	0.99	0.90	0.98	0.96
	PCR	6.94	7.56	6.22	6.30	1.00	0.00	0.95	0.94
OM	MPLS	7.69	8.08	8.38	8.46	1.82	-0.61	0.51	0.52
	PLS	7.61	8.27	8.12	8.20	1.02	-0.60	0.55	0.47
	PCR	7.37	7.57	8.48	8.51	1.05	-1.11	0.54	0.53
NDF	MPLS	24.96	28.17	26.45	26.77	0.98	1.93	0.86	0.83
	PLS	21.17	24.36	25.42	25.73	0.36	0.47	0.90	0.87
	PCR	30.81	32.24	28.06	28.41	1.00	-0.00	0.78	0.77
ADF	MPLS	14.31	19.47	20.78	24.08	1.07	2.50	0.69	0.45
	PLS	17.77	18.80	24.37	24.38	1.11	3.76	0.50	0.45
	PCR	18.05	18.75	24.59	24.60	1.11	3.78	0.48	0.46

CP, crude proteins; OM, organic matter; NDF, neutral detergent fibre; ADF, acid detergent fibre; n, number of samples; MPLS, modified partial least squares regression; PLS, partial least squeares regression; PCR, principal component regression; SEC, standard error of calibration; RSQc, the correlation coefficient; SECV, standard error of cross validation; SEP, standard error of prediction; RSQcv, coefficient of determination in the cross validation.

characteristics, thus detected as ouliers and were excluded from further calculation.

Calibration equations

After testing two mathematical treatments, the best calibrations were selected for each of three chemometric methods applied according to their cross validation parameters. PCA was applied to reduce the number of data and was guided by a variance-covariance matrix (Sharma, 1996) of the original NIR data. Table 2 shows the statistics of the calibrations for the different regression techniques analysed.

The equations obtained by chemometric methods for CP showed the highest statistics for certainty with all three chemometric methods applied, with R² of 0.95 or higher and a SECV of 8.16 or lower, i.e. lower than a third of the standard deviation (SD) of reference data. This relation between SECV and SD has been proposed as useful for evaluating an equation, which can be considered as reliable for prediction work when SD is more than three times higher than the SECV (Kennedy et al., 1996). The best results were provided by PLS despite the fact that MPLS is often more stable and accurate than the standard PLS algorithm (Geladi and Kowalski, 1986).

The advantage of PLS is the inclusion of the whole hyperspectral data range into the analysis, which results in lower losses of spectral information (Haaland and Thomas, 1988; Nguyen et al., 2006).

In MPLS, the NIR residuals at each wavelength, obtained after each factor has been calculated, are standardized (dividing by the standard deviations of the residuals at each wavelength) before calculating the next factor. It is also known that PLS regression is similar to PCR but uses both reference data (chemical, physical, etc.) and spectral information to form the factors useful for fitting purposes (Martens and Naes, 2001). Other chemical parameters involved in the investigations have not met the requirement for R² (of 0.95 or higher) while for SECV

that should be of 8.16 or lower met only OM with MPLS applied (Kennedy et al., 1996).

Another criterion that can be applied in calibration development is the ratio between SECV and the average of reference data for a given fraction, and in general the best equations also tended to show values below 0.1 for this relation. This was the case for all analytical parameters investigated by all three methods in the reflectance mode.

The chemical parameters involved in the research obtained coefficients of determination in the cross validation (1-VR) ranging from 0.45 to 0.96. The greatest 1-VR was achieved for CP (values of 0.93≤1-VR≤0.96) which was lower than 0.98 obtained for CP by Garcia and Cozzolino (2006) while predicting chemical composition of forages in broad-based calibration models or similar to those in predicting CP in temperate forages by NIR spectroscopy (Abrams et al., 1987; Roberts et al., 2004).

Hoffman et al. (1999) reported the same accurancy level for CP (R² = 0.96) in grass silage samples as achieved in this research by MPLS method. NIR prediction ability for ADF (0.48 – 0.69) and NDF (0.78 – 0.90) was the same or markedly higher than prieviously reported (R² = 0.77 and R² = 0.72, respectively) by Hoffman et al. (1999).

The most reliable chemometric method for CP, OM, and NDF prediction by NIR spectroscopy was PLS, while for ADF prediction was MPLS (Table 2).

Figure 2, 3, 4 and 5 show the relationship between chemical (LAB) data and data calculated from near infrared (NIR) spectral data for CP, OM, NDF and ADF content (g kg⁻¹ DM) investigated by the chemometric method of the highest predictive abiliy.

Different non-destructive methods were investigated to predict plant properties and nutrient status by measuring reflectance of the incident light on the leaf (Zhao et al., 2005), canopy (Hansen and Schjoerring, 2003), or landscape level (Serrano et al., 2002). However, similar studies on the estimation of forage
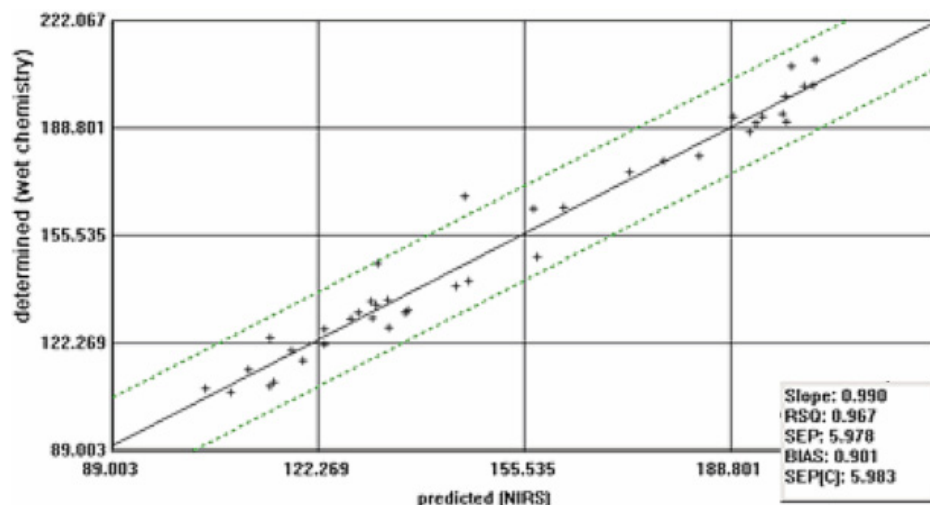
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Figure 2. The relationship between partial least square (PLS) data calculated from near infrared (NIR) spectral data and the measured chemical (LAB) data for crude protein (g kg^{-1} DM) investigated by PLS method
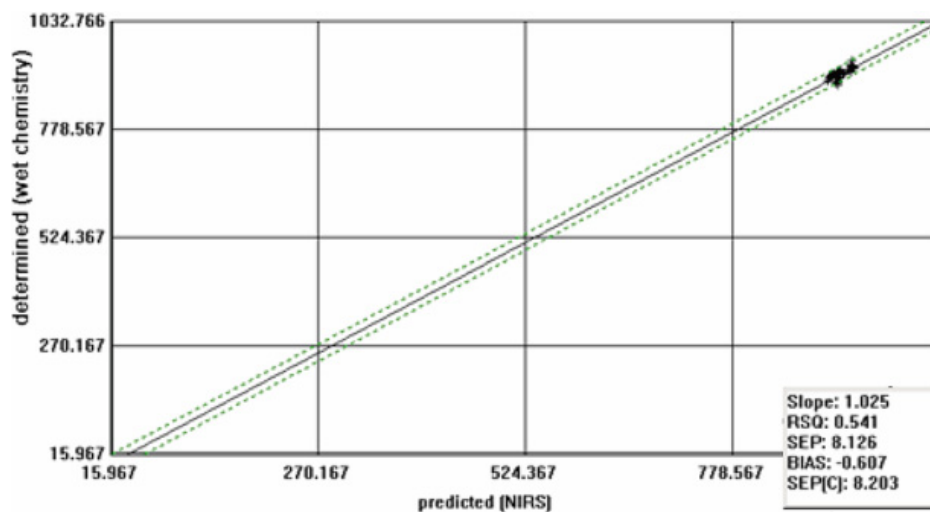

Figure 3. The relationship between partial least square data calculated from near infrared (NIR) spectral data and the measured chemical (LAB) data for organic matter (g kg^{-1} DM) investigated by PLS method
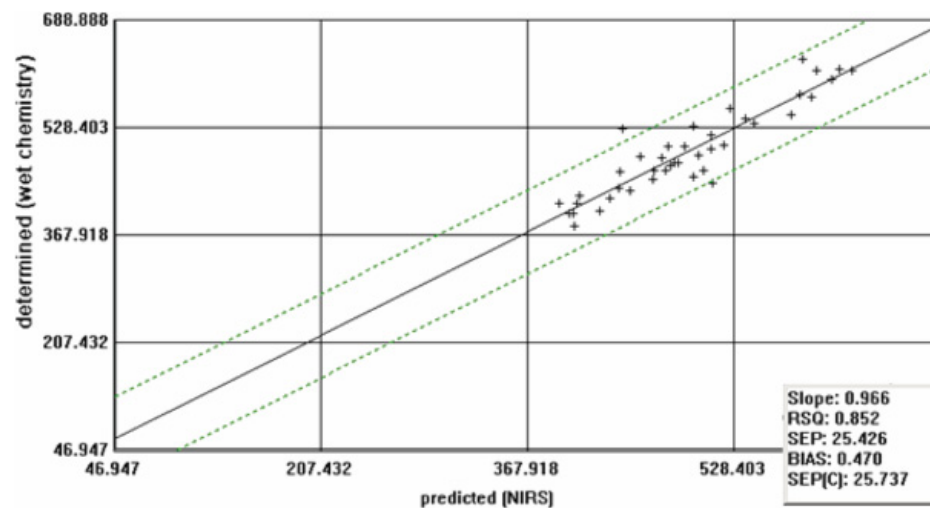

Figure 4. The relationship between partial least square (PLS) data calculated from near infrared (NIR) spectral data and the measured chemical (LAB) data for neutral detergent fibre (g kg^{-1} DM) investigated by PLS method

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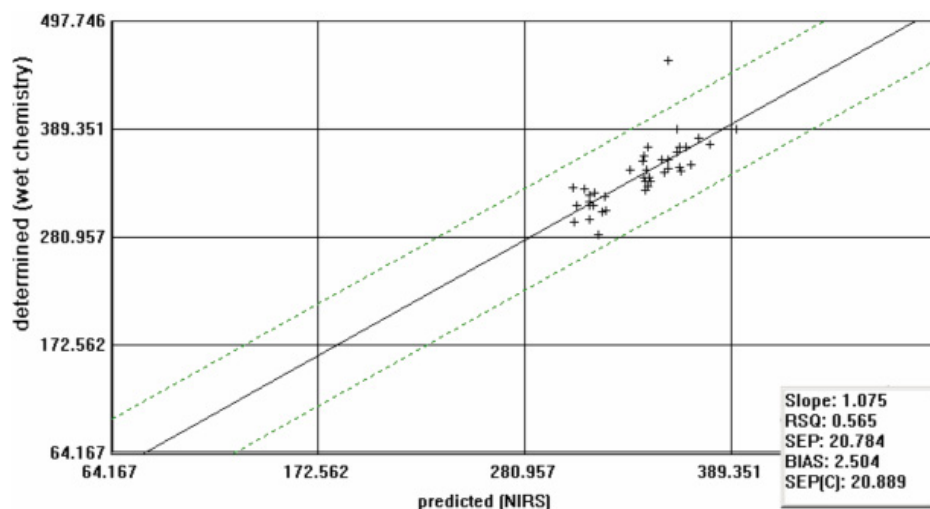Agric. conspec. sci. Vol. 81 (2016) No. 1

Figure 4. The relationship between partial least square (PLS) data calculated from near infrared (NIR) spectral data and the measured chemical (LAB) data for neutral detergent fibre (g kg^{-1} DM) investigated by PLS method

quality variables are limited and basically focused on nitrogen concentration (Gianelle and Guastella, 2007; Vranić et al., 2005, 2010). The accuracy of the NIR method for assessing the forage quality can be determined by the differences between NIR and reference methods that have a lower sampling error (Weiss and Pell, 2007).

Accurate information on nutritive values of swards is extremely useful in livestock and forage management. However, nutritive values of semi-natural swards can vary considerably within a field and during the growing period, due to disturbances such as lack of nutrients, frost, drought damage, or defoliation. Hence, a site specific determination of the nutritive values such as CP, OM, NDF and ADF in the field would help in detecting and quantifying forage heterogeneity and optimize field and forage management. The direct assessment of essential chamical parameters of semi natural forage swards in the field would be a major advance in the efficient and environmentally favorable management of grasslsand farming systems.

Conclusions

It was concluded that NIR spectroscopy shows the potential for reliable determination of CP of semi-natural grassland samples. Hyperspectral analysis by PLS resulted in the highest accuracy for the estimation of CP, OM, NDF and ADF while MPLS was the best in predicting ADF. The greatest accuracy in this research was achieved for CP, then NDF, OM, and finally ADF content. Prediction for NDF, OM, and especially ADF content should be improved in the future by involving specific semi-natural grassland samples.

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