FIRST DERIVATIVE IN NIR SPECTROSCOPY CALIBRATION FOR PROTEIN CONTENT OF FORAGES FROM HILL PERMANENT GRASSLAND

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Abstract

The aim of this study was to perform a NIR calibration model for crude protein content determination of forages using first derivative of 294 spectra and chemical Kjeldahl values. The forages samples were harvested in June from hill permanent grassland (Grădinari, Caraş-Severin District), fertilized with sheep manure and/or mineral fertilizers. The annual average temperature in this region was around 10.4°C and the soil Calcic Luvisol. The fermented sheep manure was applied at each two years, while the mineral fertilizers yearly, since 2003. The matrix of floristic composition was established gravimetrically. PLS (Partial Least Squares) regression, implemented in Panorama program (version 3, LabCognition, 2009), was used to obtained the "NIR-CP" model. The statistical parameters (R^2=0.9303; RMSEC=0.88; SD=3.11) and the differences between predicted and references values make promising this calibration model for practice evaluation of crude protein content for forages from grassland harvested in this period of year.

Key words: feed, infrared, quality, PLS regression, plants

In the context of consumer's safety, health and security, the crude protein analyse is one of the main parameters request in feed quality characterisation. This procedure is also available for forages from grasslands, the cheapest source for animal's nutrition in our days (Moisuc et al., 2001). The forages quality in crude protein content depends by soil properties, climatic conditions, flow of substances in agro-ecosystem, harvesting, preserving techniques and storage. That's way for the farmers are so important to be able to determine this parameter quickly, without reagent consumption and very cheap, during the entire forage chain.

The chemical methods are recognized for high reagents consumption, long time requesting and depend by human resources qualification. Classical chemical are considered like reference methods, only these being accepted in disputed issue cases. But in practice can be also developed other methods, in infrared for example, more rapid, easy to use and low-coast, based on calibration with spectral information and reference chemical result (McClure, 1994; Bernson, 2003).

NIRS (Near Infrared Spectroscopy) can be considered like alternative in practice for chemical methods in crude protein content determination of forages from permanent grasslands. The NIR range (800 – 2500 nm) is recognized as the overtones region, where absorb different hydrogenic bounds vibrations: -OH, -CH, -NH (Burns and Ciurczak, 2001; McClure, 1994). Generally the sample's compounds in minimum 0.1% concentration can be predicted quantitatively by NIR technique (Cen and He, 2007).

For calibration a NIR model is necessary to follow some steps: the selection of the optimum number of samples to train the device; the choosing of regression equation depending by the number of analysed parameters; the testing and monitoring of the performance and accuracy of obtained model with another samples set than that used for calibration (McClure, 1994; Rodriguez-Nogales, 2006; Hoyer, 1997).

In common practice when the used NIR spectra does not give satisfactory results, it can be apply the partial derivatives, usually first or second (Osbourne et al., 1993; Valdes et al., 2006). Often is used for the differentiation the algorithm Savitzky-Golay (Kramer and Ebel, 2000; Cen and He, 2007). The aim of our study was to perform a "NIR-CP" calibration model using the results for crude protein by chemical Kjeldahl method for all 50 forages samples and the first derivative (Savitzky-Golay algorithm) of whole spectral range (800 – 2500 nm) of obtained 294 spectra, to optimize the extraction of useful information.

The multivariate analyses for NIR calibration was realized by PLS (Partial Least Squares) regression, which is similar to principal component regression, but use both chemical results and spectral data to obtained the factors characteristics for calibration model (Gonzales-Martin et al., 2007).

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MATERIAL AND METHOD

Experimental field

The permanent grassland from Grădinari (Caraș-Severin District) were organized in ten randomized plots, in multiple stage blocks: V1- unfertilized trial; V2, V3, V4 – fermented sheep manure (20 to 60 t/ha), V5, V6, V7 – organic and mineral fertilizers (20 t/ha sheep manure and combination of 50 kg/ha of P_2O_5, K_2O, N); V8, V9, V10 – only mineral fertilizers (50 kg/ha P_2O_5, 50Kg/ha K_2O and different N doses: 100, 150, 200 kg/ha). The mineral fertilizers were: 15:15:15 NPK complex, ammonium nitrate, superphosphate, potassium salt. The mineral fertilizers were applied yearly, while the fermented sheep manure at each two years. For each trial was made five replications.

The soil of permanent grassland was Calcic Luvisol. The annual average temperature was around 10.4°C. The fertilisation of permanent grassland begins in 2003.

Samples

The analysed forages were harvested in June from al 50 trials, from representative surface. The matrix of floristic composition was determined gravimetrically in laboratory.

From Poaceae familly dominant was *Festuca rupicola*, followed by *Calamagrostis epigejos*. *Trifolium repens* and *Lathyrus pratensis* were presented from Fabaceae family. From other botanical family were present: *Rosa canina*, *Filipendula vulgaris*, *Galium verum* and *Inula britanica*. After harvesting the samples were oven dried at 70°C and grounded.

NIR calibration model

The NIR mathematical model for crude protein content determination ("NIR-CP") was performed by PLS regression. For multivariate analyses was choose Panorama software (Variant 3, LabCognition, 2009). All the 50 dried and grounded samples were scanned with V670 Spectrophotometer instrument by Abble-Jasco in the range 800 - 2500 nm.

For „NIR-CP" calibration model like input data were used the reflectance values from 294 NIR spectra and the results obtained using the chemical data by Kjeldahl method in conformity with JAOAC 954.01/1990.

For external validation was used other samples set harvested from the same permanent grassland, in the same month and year.

RESULTS AND DISCUSSIONS

The „NIR-CP" model with first derivative of whole spectral domain presents a decreasing variation of PRESS parameter, 7 factors, prediction of crude protein presented in fig. 1, and the statistical parameters from tab. 1.

Table 1

Statistical parameters of "NIR-CP" model with first derivative on whole spectral domain

R^2	0.9303
RMSEC	0.88
SD	3.11

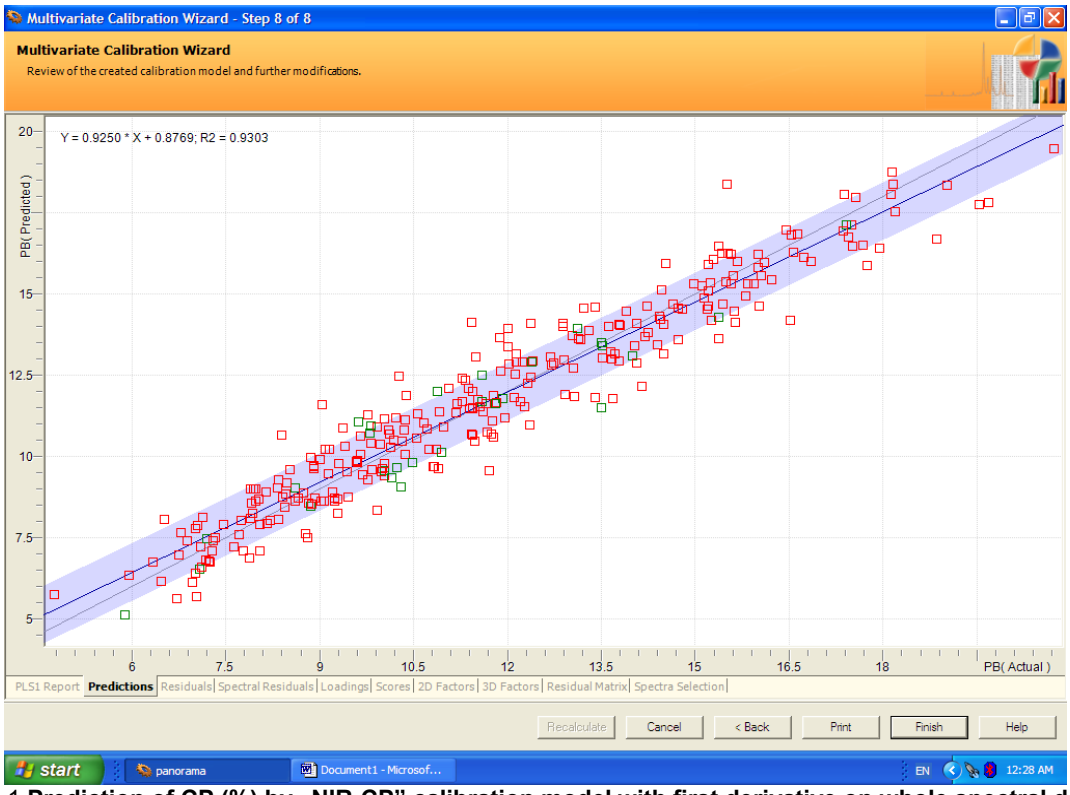

Figure 1 **Prediction of CP (%) by „NIR-CP" calibration model with first derivative on whole spectral domain**

Comparatively with the case of NIR calibration model for crude protein prediction without application of first derivative on spectra, when the statistical parameters were R^2 = 0.8630, RMSEC = 1.28, SD = 2.96, and the differences between chemical and predicted for 82% of samples used as validation set were under 1% (Harmanescu et al., 2010), the utilization of first derivative has improved R^2 (0.9303) and RMSEC (root mean standard error of calibration - 0.88) parameters, while SD (standard deviation) was 3.11.

The results of CP (%) prediction for the forages used like validation set using the „NIR-CP" calibration model with first derivative on whole spectral domain, are shown in tab.2.

Table 2

The results of CP (%) prediction for the forages, used like validation set, by „NIR-CP" calibration model with first derivative on whole spectral domain

Samples	Crude protein (%)		
	Chemical results (%)	Predicted values (%)	Differences between chemical and predicted (%)
101a	5.95	6.34	-0.39
101b	13.68	11.77	1.91
101c	8.40	10.67	-2.27
101d	7.90	8.10	-0.20
102a	6.78	7.67	-0.89
102b	1.29	16.06	-0.77
102c	9.04	11.58	-2.54
102d	8.52	9.60	-1.08
103a	8.61	9.045	-0.44
103b	14.44	14.21	0.23
103c	10.88	12.00	-1.12
103d	12.88	14.10	-1.22
104a	8.90	9.72	-0.82
104b	16.45	16.96	-0.51
104c	9.80	10.73	-0.93
104d	14.24	13.82	0.42
105a	6.47	6.16	0.31
105b	13.79	14.02	-0.23
105c	10.04	9.42	0.62
105d	9.60	9.82	-0.22
106a	7.23	6.79	0.44
106b	15.38	13.63	1.76
106c	9.76	11.28	-1.52
106d	10.00	9.62	0.38
107a	6.33	6.76	-0.43
107b	13.50	13.49	0.01
107c	8.88	8.52	0.36
107d	7.77	7.09	0.68
108a	7.10	7.20	-0.10
108c	9.14	9.48	-0.34
108d	7.91	8.58	-0.67
109a	7.05	7.89	-0.84
109b	12.00	13.94	-1.94
109c	9.2400	8.64	0.60
109d	7.4700	7.92	-0.45
110a	5.8800	5.13	0.75
110b	11.9200	11.79	0.13
110c	8.8200	8.56	0.26
110d	6.5200	8.05	-1.53

It can observe that the differences between chemical and predicted values for crude protein were in range [- 2.54 and 1.91]. A percent of 74.4 from them were under 1%, 20.5% higher than 1% and under 2%, and 5.1% higher than 2% and under 2.6%. Reported to SR EN ISO 5983-1/2006 and SR EN ISO 5983-1/AC/2010, the tolerance was established equal with 0.1% (mass fraction). The obtained differences "chemical versus predicted" indicate that the NIR calibration model cannot be used yet with the same precision with chemical method in crude protein prediction of forages from grassland, but can be introduce in practice for rapid determination of this parameter. This model will

can be improved that the number of samples used to perform the calibration will be higher, to characterize better the entire floristic matrix of harvested forages.

CONCLUSIONS

In this study was proposed a NIR methodology for crude protein determination of forages harvested in June from hill permanent grassland. For this partial least squares regression was used, with input data represented by results of chemical Kjeldahl method for this parameter and the reflectance values from whole 294 NIR spectra processed by first derivative. The obtained R^2 and RMSEC coefficients for NIR model were better than without the application of first derivative, but SD was a little higher. Good differences were calculated between the chemical and predicted values. That's means it is promising to use NIR technology with success to determine rapidly and cheap crude protein for the forages harvested in this period of year, with a favourable impact on the farmer's problems regarding the correct nutrition of animals.

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