THE PLS-CV MODEL FOR THE DETERMINATION OF TOTAL CRUDE PROTEIN CONTENT OF FORAGES HARVESTED IN MAY FROM A PERMANENT MEADOW (GRĂDINARI; CARAŞ-SEVERIN), USING NIR SPECTROSCOPY

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ABSTRACT - This paper presents our studies regarding the determination of total crude protein content (%) from forages, using NIR Spectroscopy. The forage samples were harvested in the middle of May 2008 from a permanent meadow situated in Caraş-Severin County, near Grădinari. The permanent meadow was organized in ten trials with different doses of organic and mineral fertilizations and five replicates for each trial. The fertilization of permanent meadow was made using: GP1 - unfertilized variant, GP2 - 20 t sheep manure, GP3 - 40 t sheep manure, GP4 - 60 t sheep manure, GP5 - 20 t sheep manure + 50 P_2O_5, GP6 - 20 t sheep manure + 50 P_2O_5 + 50 K_2O, GP7 - 20 t sheep manure + 50 N + 50 P_2O_5 + 50 K_2O, GP8 - 100 N + 50 P_2O_5 + 50 K_2O, GP9 - 150 N + 50 P_2O_5 + 50 K_2O, GP10 – (100 + 100) N + 50 P_2O_5 + 50 K_2O. The main species founded on the permanent meadow from Grădinari were: *Festuca rupicola* and *Calamagrostis epigeios*. Other species were *Antohoxanthum odoratum, Briza media,* *Poa pratensis, Trifolium arvense, Trifolium medium, Lotus corniculatus* and *Filipendula vulgaris*. The mathematical model (PLS-Cross Validation model) for the determination of total crude protein content (%) by NIR method was performed using the values of reflectance from NIR spectra and those obtained with chemical Kjeldahl method. For the statistical interpretation we used UNSCRAMBLE software. The external calibration was made using forage samples harvested from the same permanent meadow, but in March 2008.

Key words: NIRS, PLS-CV, forage, crude protein content

REZUMAT – Modelul PLS-CV pentru determinarea conţinutului de proteină brută din furajele recoltate în luna mai, de pe o pajişte permanentă (Grădinari, jud. Caraş-Severin), folosind spectroscopia NIR. Lucrarea prezintă studiile privind determinarea conţinutului total de proteină brută (%) din furaje, folosind spectroscopia NIR. Mostre de

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furaje au fost recoltate la mijlocul lunii mai, anul 2008, de pe o pajişte permanentă, situată lângă Grădinari, jud. Caraş-Severin. Pajiştea permanentă a fost organizată în 10 experienţe, la care s-au aplicat diferite doze de îngrăşământ organic şi mineral, în cinci repetiţii pentru fiecare experienţă. Fertilizarea pajiştii permanente a fost realizată astfel: GP1 – varianta nefertilizată, GP2 - 20 t îngrăşământ natural de oi, GP3 - 40 t îngrăşământ natural de oi, GP4 - 60 t îngrăşământ natural de oi, GP5 - 20 t îngrăşământ natural de oi + 50 P_2O_5, GP6 - 20 t îngrăşământ natural de oi + 50 P_2O_5 + 50 K_2O, GP7 - 20 t îngrăşământ natural de oi + 50 N + 50 P_2O_5 + 50 K_2O, GP8 - 100 N + 50 P_2O_5 + 50 K_2O, GP9 - 150 N + 50 P_2O_5 + 50 K_2O, GP10 – (100 + 100) N + 50 P_2O_5 + 50 K_2O. Principalele specii care au fost găsite pe pajiştea permanentă din Grădinari au fost: *Festuca rupicola* şi *Calamagrostis epigeios*. Alte specii au fost *Antohoxanthum odoratum, Briza media, Poa pratensis, Trifolium arvense, Trifolium medium, Lotus corniculatus* şi *Filipendula vulgaris*. Modelul matematic (Modelul PLS) pentru determinarea conţinutului de proteină brută (%) prin metoda NIR a fost realizat folosind valorile reflectanţei din spectrul NIR şi cele obţinute prin metoda Kjeldahl. Calibrarea externă a fost realizată folosind mostre de furaje recoltate de pe aceeaşi pajişte permanentă, dar în luna martie a anului 2008.

Cuvinte cheie: NIR, PLS-CV, furaje, conţinutul de proteină brută

INTRODUCTION

The nutritive value of forage represents the measure in which this feed can satisfy the animal nutrition necessities (Moisuc&Đukič, 2002). It is important that forage properties correspond to the metabolic and digestive particularities and influence positively the animal production, breeding, health and reproduction function (Crăiniceanu *et al.,* 2006). Protein is one of the most important nutritive parameters, a complex organic substance, which contains nitrogen, considered the fundamental chemical factor of vital processes (Şumălan, 2006).

Found in vegetal and animal cells, protein represents the support of genetic material, protects the animal organism against the pathogens and regulates the hydro-metabolism, participates at digestive, hormonal and reproductive activities (Georgescu *et al.,* 2007).

The chemical Kjeldahl method used for the determination of total crude protein requests reagents and electrical energy consumption qualified human resources and long time for performance. NIRS (Near Infrared Spectroscopy) is considered at present an alternative non-destructive method for the determination of this parameter, being a fast method without reagents consumption (Wilson, 1994). The aim of this study was to calibrate a NIR method using PLS-CV regression model and the results for forages harvested in the middle of May 2008, from a permanent meadow found in Grădinari (Caraş Severin County), for the determination of total crude protein content (%).

MATERIALS AND METHODS

The mathematical model for NIRS calibration was performed using PLS - CV (Partial Last Square – Cross Validation) regression model, reflectance

values from NIR spectra and the results for total crude protein for forages harvested in the middle of May 2008, from a permanent pasture situated near Grădinari (Caraş-Severin County). The altitude of permanent pasture is around 190 m; soil is brown clay-illuvial; annual average temperature is around $10.4^{\circ}C$. The experimental filed was fertilized with mineral, organic-mineral and organic fertilizers during 2003-2007. The mineral fertilizers were 15:15:15 NPK complex, ammonium nitrate, superphosphate, potassium salt, applied annually. We have used fermented sheep manure as organic fertilizer, applied every two years.

The permanent pasture was organized in ten trials with different doses of fertilizers, using the method of randomized plots, in multiple blocks with five replicates for each trial: V1 – unfertilized trial, V2 – 20 t/ha sheep manure, V3 – 40 t/ha sheep manure, V4 – 60 t/ha sheep manure, V5 – 20 t/ha sheep manure + 50 P_2O_5 (Kg/ha), V6 – 20 t/ha sheep manure + 50 P_2O_5 (Kg/ha) + 50 K_2O (Kg/ha), V7 - 20 t/ha sheep manure + 50 P_2O_5 (Kg/ha) + 50 K_2O (Kg/ha) + 50 N (Kg/ha), V8 = 100 N (Kg/ha) + 50 P_2O_5 (Kg/ha) + 50 K_2O (Kg/ha), V9 = 150 N (Kg/ha) + 50 P_2O_5 (Kg/ha) + 50 K_2O (Kg/ha), V10 = (100 + 100) N (Kg/ha) + 50 P_2O_5 (Kg/ha) + 50 K_2O (Kg/ha).

The main species found on the permanent pasture of Gradinari were *Festuca rupicola* and *Calamagrostis epigeios*. Other plant species were *Antohoxanthum odoratum*, *Briza media*, *Poa pratensis*, *Trifolium arvense*, *Trifolium medium*, *Genista tinctoria*, *Lotus corniculatus* and *Filipendula vulgaris*.

Total nitrogen content was determined chemically using the Kjeldahl method in conformity with AOAC (method 978.04). Digestion of samples

(appreciatively 1g forages) was made with concentrated H_2SO_4 and cupric catalizator in DK6 Heating Digester Unit from Velp Scientific. Distillation of samples was made with the UDK 127 Distillation Equipment from Velp Scientific. The 95% sulphuric acid, 0.1N sulphuric acid solution used for titration and 4% boric acid solution for ammonium capture were of pure grade (Merck, Germany). All solutions were prepared using deionized water. For all the samples, the determination was made in triplicate and the results were reported to dry matter. The total crude protein content was calculated by multiplying total nitrogen content with 6.25.

NIRS spectra were scanned with V 670 Spectrophotometer instrument by Abble-Jasco in the range 800-2500 nm. For all the samples the scan was made in triplicate.

The obtained mathematical model using Partial Last Square – Cross Validation regression model was performed with UNSCRAMBLE software.

RESULTS AND DISCUSSION

The mathematical model of NIRS method calibration for determination of total crude protein, using PLS-CV regression model, the results determined by chemical Kjeldahl method and the reflectance values from NIR spectra, obtained with Spectra Manager are shown in *Figure 1*.

The results for total crude protein of forages harvested in the middle of May, determined by Kjeldahl method are presented in *Table 1*.
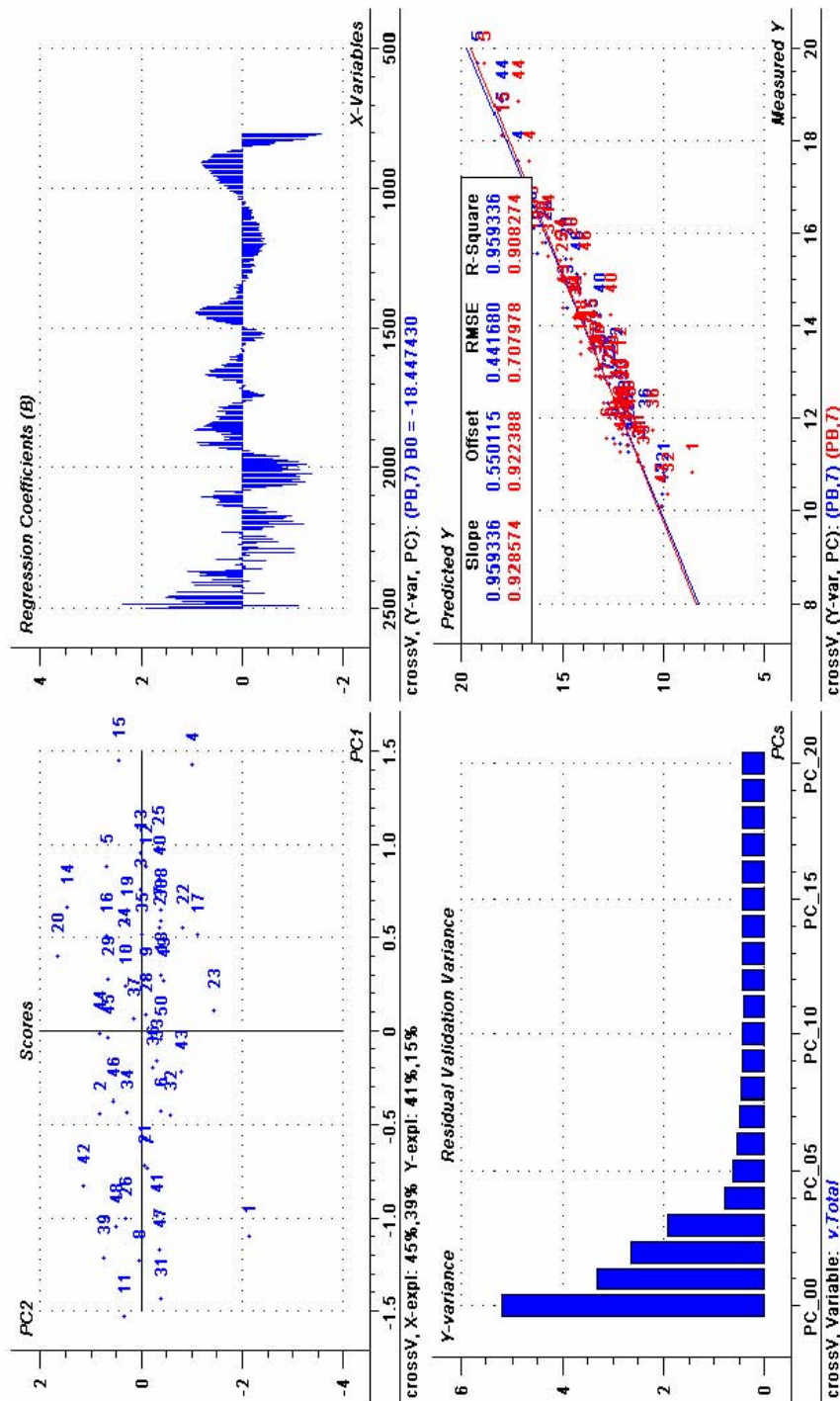
Figure 1 - PLS–Cross Validation regression model of NIRS method calibration for total crude protein determination, performed using forage samples harvested in the middle of May 2008

Table 1 - Results for total crude protein of forages harvested in the middle of May, determined by Kjeldahl method

Samples	1st Replicate	2nd Replicate	3rd Replicate	4th Replicate	5th Replicate
GP1	10.827	12.412	12.737	11.071	11.200
GP2	15.517	16.100	12.379	11.776	14.396
GP3	17.557	15.934	15.422	14.196	18.861
GP4	19.680	18.130	15.111	13.110	13.656
GP5	11.559	15.997	12.889	11.744	15.136
GP6	13.507	12.322	13.074	13.395	10.102
GP7	12.141	13.646	11.445	14.147	11.772
GP8	11.960	15.571	15.802	10.939	11.431
GP9	13.404	15.440	12.923	14.235	12.348
GP10	13.057	13.039	11.661	10.352	11.273

G-Grădinari, P-permanent meadow, 1-10 – the trial number

The optimum number of main components for PLS – CV model is seven, the mathematical model being relatively simple. The distribution of forages harvested in the middle of May 2008, function of main two principal components PC1 and PC2 is one grouped, in all quadrates of xy-axis.

We have used two forage samples sets for validation of NIRS calibration; both proceed from the same place, the permanent meadow situated near Grădinari (Caraş-Severin). One set was formed by the samples harvested at the middle of May 2008 (*Table 2*) and was used for internal validation. The second set was represented by the forages harvested in March 2008 (Table 3) and was necessary for external validation. For these two sample sets, the total crude protein was determined using Kjeldahl method, like reference method, and after that was scanned with the NIR spectra for determination of this parameter with PLS-CV model.

The differences between the values for real CP and predicted CP of the forage samples used to perform internal validation were in the range 0.001 – 0.959. It means that the PLS-CV regression model can be used with success to determine total crude protein of forages harvested in this period of year from the permanent meadow situated near Grădinari (*Table 2*).

For external calibration, the differences between the values obtained for real crude protein by Kjeldahl method and those performed with PLS – CV model were in the range 0.137 – 6.589 (*Table 3*). Between these differences, there are still high values and this demonstrates that it is necessary to improve the mathematical PLS – CV model, introducing in calibration the NIRS method and the results obtained for CP of samples harvested at the end of March 2008.

Table 2 - Internal validation: results for total crude protein, determined with Kjeldahl method and predicted with PLS – CV model, for forages harvested in the middle of May 2008 from permanent meadow (Grădinari, Caraş-Severin)

Samples	Real CP content (%)	Predicted CP content (%)	Real CP - Predicted CP
P1	13.650	14.098	- 0.448
P2	13.070	13.238	- 0.168
P3	10.830	9.991	0.839
P4	13.400	13.399	0.001
P5	17.560	17.199	0.361
P6	12.740	12.724	0.016
P7	13.400	14.086	- 0.686
P8	15.420	15.139	0.281
P9	11.560	12.519	- 0.959
P10	15.570	16.253	- 0.683
P11	10.940	11.029	- 0.089

Table 3 - External validation: results for total crude protein, determined with Kjeldahl method and predicted with PLS – CV model, for forages harvested at the end of March 2008 from permanent meadow (Grădinari, Caraş-Severin)

Samples	Real CP content (%)	Predicted CP content (%)	Real CP - Predicted CP
P12	13.781	9.395	4.386
P13	20.731	14.142	6.589
P14	17.955	12.014	5.941
P15	19.029	14.044	4.985
P16	14.522	14.757	-0.235
P17	15.378	15.781	-0.403
P18	11.494	14.633	-3.139
P19	14.457	12.535	1.922
P20	12.012	12.813	-0.801
P21	14.058	12.472	1.586
P22	9.819	13.207	-3.388
P23	15.675	14.683	0.992
P24	14.786	12.120	2.666
P25	15.202	14.043	1.159
P26	11.388	11.525	-0.13

CONCLUSIONS

The PLS-CV regression model obtained with the results for CP of forages harvested in the middle of May 2008, by Kjeldahl method and the reflectance values from NIR spectra can be used successfully to determine this parameter for the forages harvested in this period of year.

The obtained mathematical PLS – CV model cannot be used to determine the CP of samples harvested at the end of March 2008 from the same experimental field, because the differences between real and predicted values are still high.

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