THE PLS-CV MODEL FOR DETERMINATION OF TOTAL CRUDE PROTEIN CONTENT IN FORAGES FROM A PERMANENT MEADOW (GRĂDINARI; CARAS-SEVERIN) USING NIR SPECTROSCOPY (1100-2200 NM)

MODELUL PLS-CV PENTRU DETERMINAREA CONȚINUTULUI TOTAL DE PROTEINĂ BRUTĂ DIN FURAJELE PROVENITE DE PE O PAJIŞTE PERMANENTĂ (GRĂDINARI; CARAŞ-SEVERIN) UTILIZÂND SPECTROSCOPIA NIR (1100-2200 NM)

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Abstract: *In this paper are presented our researches regarding the determination of total crude protein content (%) of forages samples, harvested in March 31st, 2008, from a permanent meadow situated near Grădinari (Caraş Severin), using the obtained spectra between 1100-2200 nm of NIR Spectroscopy. The permanent meadow was organized in ten trials with different doses of organic and mineral fertilizations, and five replicates for each trial. The mathematical model (PLS-Cross Validation model) for total crude protein content (%) determination by NIRS method was created with the values obtained using wet chemical Kjeldahl method and those for reflectance from NIR spectra (1100-2200nm). For the statistical interpretation of obtained results was used UNSCRAMBLE software. The external calibration was made using samples harvested from the same permanent meadow, but in October, 2007.*

Rezumat: *În această lucrarea ştiinţifică sunt prezentate cercetările noastre legate de determinarea conţinutului total de proteină brută (%) din probele de furaje recoltate la data de 31 martie 2008 de pe o pajişte permanentă de lângă Grădinari (Caras Severin), utilizând spectrele obţinute între 1100-2200 nm prin spectroscopie NIR. Pajiştea permanentă a fost organizată în zece variante experimentale care au fost fertilizate cu diferite doze de îngrăşăminte organice şi minerale. Pentru fiecare variantă au fost înfiinţate cinci repetiţii. Modelul matematic (PLS-Cross Validation) pentru determinarea conţinutului total de proteină brută (%) prin metoda NIRS a fost creat folosind rezultatele obţinute prin metoda chimică Kjeldahl şi valorile reflectanţelor din spectrele NIR (1100-2200nm). Pentru interpretarea statistică a rezultatelor obţinute s-a utilizat soft-ul UNSCRAMBLE. Calibrarea externă a fost realizată cu probe de furaje recoltate de pe aceeaşi pajişte permanentă, dar în luna octombrie 2007.*

Key words: *NIR Spectroscopy, PLS-Cross Validation, total crude protein content, forages*
Cuvinte cheie: *Spectroscopie NIR, PLS-Cross Validation, conţinut total de proteină, furaje*

INTRODUCTION

Proteins represent essential elements for animal organism, being responsible for a lot of functions [JARRIGE & ALDERMAN, 1987]: are present in the structure of nucleic acids, determine the protein synthesis from milk, are fundamental components of the cells and tissues, and protect the animal's organism against the pathogens [GEORGESCU coord. et al, 2007]. Appreciatively 50% of proteins are found in muscles and 30% in hide and conjunctive tissues.

Proteins did not represent a fix structure, being in permanent renewing processes. In each moment a part of them are depolymerised in amino acids and replaced with other proteins

new synthesized [JARRIGE, 1988]. That's way is so important that the forages must contains proteins in sufficient quantities to cover the nutritive necessities of animals, function of physiological state [MOISUC&ĐUKIČ, 2002].

The Kjeldahl method is used for determination of total nitrogen content, and then, multiplying the results with a coefficient equal with 6.25, is possible to calculate the total protein content. But this is a chemical method which request a long time to perform the analyses, a high consume of regents and qualified human resources.

Near Infrared Spectroscopy (NIRS) can be used like alternative method for the determination of total protein content of forages. But first is necessary to obtain a good mathematical model for calibration. NIRS is a non-destructive method, is very fast, not ask the usage of reagents [WILSON, 1994].

It was established that when the sample has high protein content than in NIR spectrum the predominant picks will be those specifics for protein bonds absorption, at the following wavelengths: 1190, 1488, 1735, 1974, 2054 and 2162 nm [COZZOLINO & LABANDERA, 2002; WILLIAMS & NORRIS, 2001; ROBERTS et al, 2004]. That's way for performing the mathematical model presented in this paper we choose the spectral domain 1100 – 2200 nm.

MATERIAL AND METHODS

The permanent meadow chooses like experimental field is situated near Grădinari (Caraş-Severin District), in a hill region of Banat County. It was located on a brown argiloiluvial soil and was organized in ten trials using the method of randomized plots, in multiple stage blocks with five replications. The fertilizers were applied over a five year period 2003-2007. The fertilization doses were: 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 average height of permanent meadow is around 190 m and the slope of relief is around 30 grades. The multi-annual average of temperature is 10.4^oC.

The floristically composition of permanent meadow from was made using geo-botanical method (Braun Blanque). The main species 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 forages from the permanent meadow were harvested at March 31st, 2008. The sample harvested from the ten trials were dried in shadow, appreciatively at 24-28^oC, for two weeks and then were grounded and sieved under 0.3 mm particle diameter.

The mathematical model for the determination of total crude protein content using NIR Spectroscopy (Near Infrared Spectroscopy) was obtained using the results for this parameter by chemical Kjeldahl method and the reflectance values from NIR spectra between 1100-2200 nm. Partial Last Square – Cross Validation model implemented in UNSCRAMBLE software was choose to perform the model.

Total nitrogen content determination using Kjeldahl method was made in conformity with AOAC [method 978.04]. Digestion of forages (appreciatively 1g) was made with concentrated H_2SO_4 and cupric catalizator in DK6 Heating Digester Unit from Velp Scientific. Distillation of samples was made with 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). For all the analysed samples the determination were made in triplicate. The results were reported to dry matter. The total crude protein content was calculated multiplying total nitrogen content with 6.25.

NIRS spectra were scanned with V 670 Spectrophotometer instrument by Abble-Jasco in the range 1100-2200 nm. For all the samples the scan was performed in triplicate.

RESULTS AND DISCUSSIONS

The mathematical model PLS – Cross Validation obtained for the correlation between total crude protein (%) results determined by Kjeldahl method and the reflectance values from NIR spectra, scanned only for characteristic protein bonds 1100-2200 nm, is shown in Figure 1. The correlation coefficient R^2 for PLS – CV model (1100-2200 nm) is equal with 0,587. The main principal component (PC) is five and the distribution of analyzed samples depending on PC1 and PC2 is one grouped, in all the four quadrates.

For the external validation of the mathematical model were used the all the NIR spectra scanned for forages harvested from the same permanent meadow, but in autumn of 2007. Then, for these samples was determined the total crude protein content by chemical Kjeldahl method, used like reference method. The results for total crude protein obtained by chemical method and those predicted with mathematical model PLS – CV (1100-2200 nm) is presented in Table 1:

Table 1

The results for total crude protein obtained chemically with Kjeldahl method and those predicted with mathematical model PLS – CV (1100-2200 nm) for the forages harvested from permanent meadow in autumn of 2007

Samples harvested in 2007	Real TCP Content by Kjeldahl (%)	TCP Content predicted with PLS – CV (1100-2200nm) (%)	PLS–CV (1100-2200nm) deviation for TCP prediction	The differences between predicted and real TCP (%)
gp1m	6.810	9.159	4.465	2.349
gp2m	8.500	14.333	4.068	5.833
gp3m	9.190	10.521	4.922	1.331
gp4m	10.870	13.886	3.801	3.016
gp5m	7.620	10.673	3.647	3.053
gp6m	7.810	12.831	3.953	5.021
gp7m	7.440	10.139	4.995	2.699
gp8m	6.560	11.600	4.642	5.040
gp9m	8.690	14.312	3.567	5.622
gp10m	6.870	9.082	4.198	2.212

TCP – Total Crude Potein

From Table 1 it is possible to observe that the differences between predicted and real TCP (%) are included in the range 1.331 – 5.833 %. These differences are generally smaller than mathematical model deviation of PLS – CV (1100-2200 nm), but are still high reported to the TCP values. That means the PLS – CV (1100-2200 nm) must be performed with a high number of forages samples harvested from the experimental field, to cover better the seasonally variation of TCP depending on floristically composition or the nutritive elements content of soil.

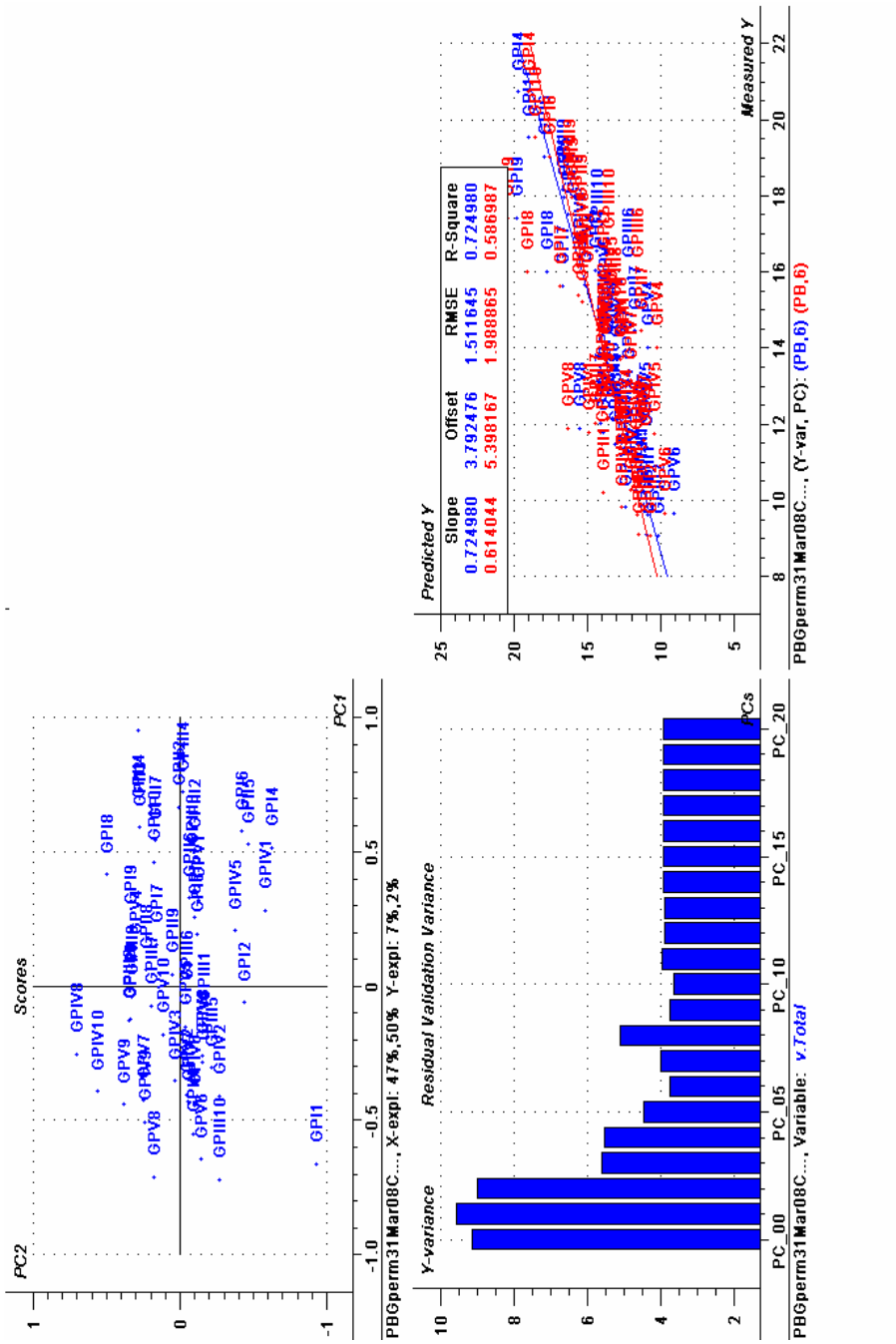

Figure 1. The mathematical PLS – Cross Validation model for the correlation between total crude protein results determinated by Kjeldahl method with the reflectance values from NIR spectra (1100-2200nm

CONCLUSIONS

The PLS – CV (1100-2200 nm) must be perfected using a high number of forages samples harvested from the experimental field, to characterize better the seasonally variation of TCP depending on floristically composition or the nutritive elements content of the soil.

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