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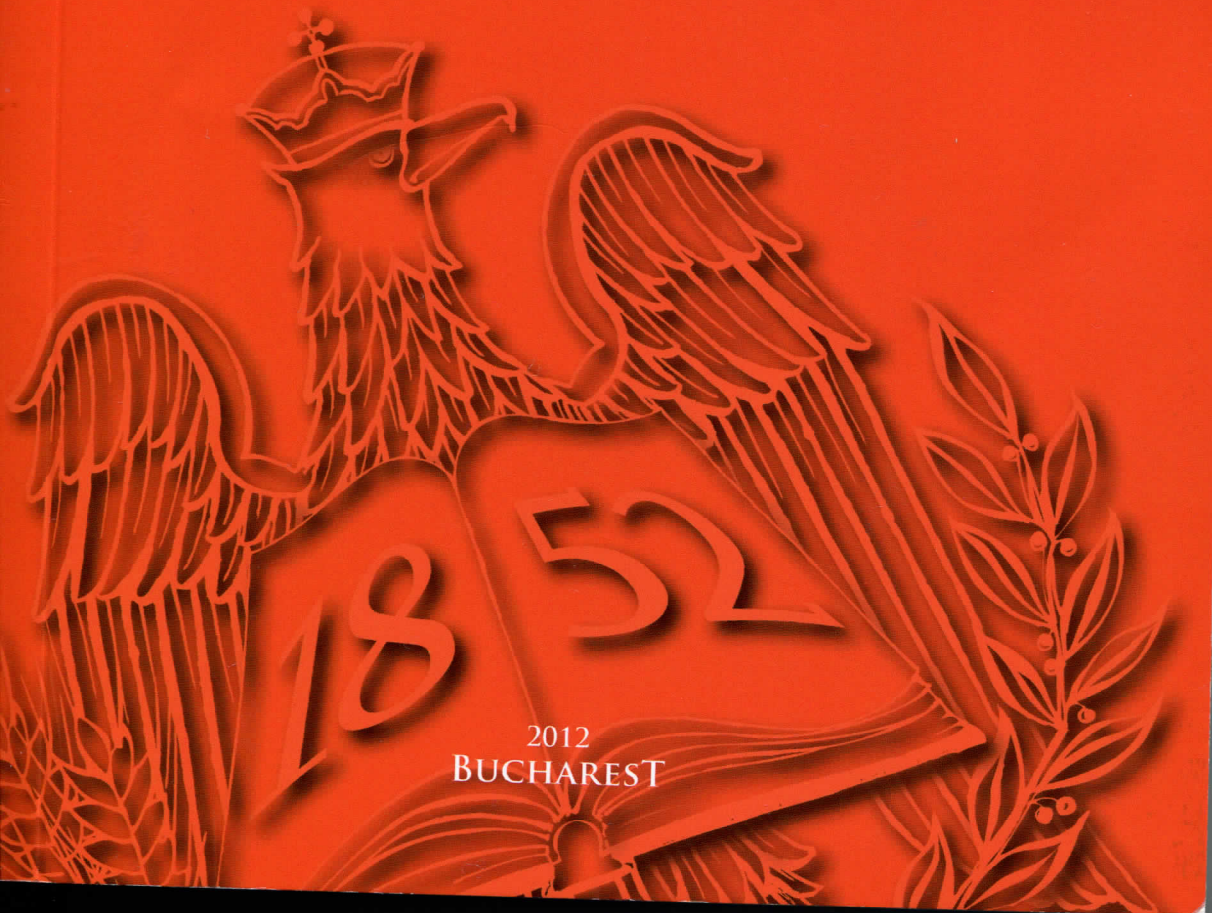

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DETERMINATION OF ROMANIAN ALFALFA CRUDE PROTEIN AND CRUDE FIBER CONTENTS AS WELL AS *IN VITRO* ORGANIC MATTER DIGESTIBILITY BY NIR SPECTROMETRY

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Abstract

Alfalfa (Medicago sativa L.) is a high quality forage which has been used worldwide. The superiority of alfalfa lies in its high yield, high protein content and high digestibility. The aim of this study was to develop a simple, fast and non-destructive method, named Near Infrared Spectroscopy (NIRS) to determinate alfalfa quality. To realize this study, alfalfa samples were obtained from Mănăştur Experimental Station – Farm Cojocna in 2008–2009, in one experiment carried out using randomization blocks design with two experimental factors (mineral fertilization and period of harvest). Alfalfa quality was first determinated on 48 samples by classical analyses: crude protein CP (AOAC, 1990), crude fiber CF (Fiber Cap, FOSS, DK) and in vitro organic matter digestibility OMDrt (DeBoever, 1986). Then the samples were scanned by NIRS. Calibration models were performed by PerkinElmer Spectrum Quant + 4.21 program (USA) on the 48 samples determinated by classical analysis. The results showed fully confirmed by acceptable coefficients of determination and standard error of cross-validation (R2=0.96 for CP, 0.94 for CF, 0.98 for OMDrt and SECV=0.77 for CP, 1.35 for CF, 1.13 for OMDrt). Successful results for prediction of other 176 alfalfa samples were then obtained using these calibration models: SEP=0.869 for CP, 1.058 for CF, 1.058 for OMDrt). The highest CP and OMDrt values of alfalfa were obtained in bud stage (22.0% and 66.0% respectively). While for CF, the highest content was registered in the seed formation stage (46.0%). The NIRS technique offers us the possibility to determinate rapidly and easily Romanian alfalfa important parameters, but the system could also be used for the determination of other constituents.

Keywords: *alfalfa, NIRS, crude protein, crude fiber, in vitro organic matter digestibility*

INTRODUCTION

In current agriculture, forage production, obtained from permanent grasslands, temporary grasslands and forage crops is an integral part of agricultural land management [1]. A correct appreciation of feed quality requires a set of general analyses such as: botanical composition, organic and mineral contents, palatability and organic matter digestibility, as well as dry matter intake [2].

Alfalfa (*Medicago sativa* L.) is a high quality forage used worldwide. The superiority of alfalfa lies in its high yield, high protein content and high digestibility. It is considered by researchers as the 'queen of forages' [3]. Archaeological informations and antic scientific papers pointed out that alfalfa was cultivated first in South-West of Asia already on 4000 years before Christ [4]. In Romania, alfalfa was first cultivated in the last part of XVIIIth century in Transylvania and Banat

Country [4]. On the agrobiologic point of view alfalfa presents some particularities such as: water resistance, low temperature resistance, good utilization of irrigation water, high capacity of regeneration after harvest period and high competitive supply [3]. Alfalfa has also a high economic importance, manifested by its high ecological plasticity and high productivity. In natural conditions, in Romania alfalfa can offers more than 50 t/ha of green mass on 3 harvests/year and in irrigation conditions more than 80 t/ha of green mass on 4 harvests/year [3]. Jarrige, [5] pointed out that alfalfa has lower proportion of leaves in the first stage of vegetation in comparison with gramineae. But advancing in vegetation stages, alfalfa crude protein content and digestibility value decrease while cell wall content increases. The main components of cell walls are fibers. The organic matter digestibility of the feed depends mainly on the fiber content and its digestibility. Digestibility decreases with the proportion of cell walls and their level of lignification in the plant. Organic matter digestibility of the feed during the first cycle of vegetation depends almost exclusively on the stage of development. Organic matter digestibility of the feed (~60%) at accrued harvest stages has the same significant high value, no matter the place, year and the level of nitrogen fertilization [5]. Routine analyses of forages are necessary to evaluate their nutritive values and calculate optimal and well balanced diets for ruminants. NIRS is a non-destructive method of analysis based on diffuse reflectance of ground samples. NIRS was applied first in the agricultural sector by Norris in the 1960's to determine moisture content in soybeans [6]. More recently, Dale et al. [7] reviewed a lot of studies regarding the use of NIRS and NIR-HSI. This technology was used particularly for fast determination of the nutrients' concentration and feeding value of dried and fresh crop materials [8-11]. NIRS technique has become a 'clean technology' very used in sustainable agriculture [10] because it is using a reduced amount of chemical substances (4, 12-13). The aim of this study was to develop a simple, fast and non-destructive method to determine alfalfa quality (crude protein CP, crude fiber CF and *in vitro* organic matter digestibility OMDrt), based on Near Infrared Spectroscopy (NIRS system).

MATERIAL AND METHOD

Alfalfa samples were obtained from Mănăştur Experimental Station – Farm Cojocna in 2008–2009, in one experiment carried out using block randomization design with two experimental factors (mineral fertilization and period of harvest). The experiment started in spring 2007, with the seeding of alfalfa. The mineral fertilization was applied each year in early spring in different levels of graduation: unfertilized or fertilized with N_{50}, $N_{50}P_{50}$, $N_{50}P_{50}K_{50}$, N_{100}, $N_{100}P_{75}$ or $N_{100}P_{75}K_{75}$. The samples were collected directly in the field in the harvest period (plant height 30 cm, bud, flowering and seed formation), then were dried at 60°C in oven 2 days. In the next step, the samples were milled using a 5 mm screen (Grindomix GM 200, Retsch, Haan, Germany) and then milled through a 1 mm sieve (Cyclotec™ 1093, Tecator, Sweden).

Alfalfa quality was determinated on 48 samples by classical analyses: crude protein (CP) (Kjeldhal method [14]), crude fiber (CF) (Weende Scheme - Fiber Cap, FOSS, DK) and *in vitro* organic matter digestibility (OMDrt) (DeBoever method [15]). The same 48 samples were scanned for NIRS analyses to perform calibration model (36 samples) and validation model (12 samples); other 176 samples from the same experiment were then scanned for prediction. Spectra were collected over the wavelengths range 10000 to 4000 cm^{-1} (1000-2500 nm) at 8 cm^{-1} resolution with 2 cm^{-1} step, in two repetitions. Each spectrum is a mean of 32 scans of the same sample.

For the mathematical models, the program PerkinElmer Spectrum Quant + 4.21 was used. The algorithm used for calibration models was Partial Least Squares (PLS). To perform a robust calibration model, spectra were treated by following mathematical treatment: 2nd Derivative (order: 3, window: 15 pt) and a cross validation was also used: leave one out, using 18 PLS factors.

RESULTS AND DISCUSSIONS

The CP, CF and OMDrt contents of alfalfa determinated by classical methods are presented in table 1. The results were similar to results showed by Varga [12], Vintilă [16] and Vîntu et al., [13]. And it can be pointed out those higher levels of crude protein content in the first and second harvested stages where obtained because of a great proportion of leaves than stems in these two stages compared to flowering and seed formation [17].

Table 1. Crude protein (CP), crude fiber (CF) and in vitro organic matter digestibility (OMDrt) of alfalfa harvested at different stages determinated by classical methods

Harvest stage	CP	CF	OMDrt
Plant height 30 cm	14.23% - 19.79%	27.92% - 34.37%	57.30% - 63.66%
Bud	14.38% - 17.64%	30.33% - 33.75%	63.39% - 65.83%
Flowering	12.87% - 17.44%	32.03% - 39.82%	52.95% - 59.86%
Seed formation	12.53% - 15.19%	38.05% - 44.03%	38.05% - 44.03%

The PLS algorithm for calibration model was performed on the spectra with the help of data obtained by classical analyses. The characteristics of alfalfa calibration models for CP, CF and OMDrt are presented in table 2. The report SD/SECV allows a comparison of the equations developed, which are independent of units and have been used by other authors. The ratio SD/SECV of 2.5-3.0 has been considered appropriate for measuring samples quality, but to ensure the models robustness, values of at least 3.0-5.0 are necessary [18]. The SD/SECV ratio was 4.18, 3.97 6.70 for CP, CF and OMDrt respectively. The ratios for CP and CF were in the limits presented by Williams and Sobering, [18]. But the ratio for OMDrt was higher than the limits; this means that it will be necessary to perform more classical analyses for the OMDrt model. The results obtained for calibration and validation models were similar to those of other authors for the same type of biological material (table 3).

Table 2. Characteristics of alfalfa calibration models

Content	CP	CF	OMDrt
N	48	48	48
Mean	15.95	27.67	64.7
SEC	0.72	1.32	0.99
R^2	0.96	0.94	0.98
SECV	0.77	1.35	1.13
SEP	0.869	1.058	1.058
SD	3.22	5.37	7.58
SD/SECV	4.18	3.97	6.70

Legend: N: samples taken in calibration model, Mean: the mean value, SEC: the standard error of calibration, R2: the coefficient of determinations, SECV: the standard error for cross validation, SEP: the standard error of prediction, SD: the standard deviation.

Table 3. The coefficient of multiple determinations (R2) and the standard error for cross validation (SECV) of alfalfa calibration models

Calibration models for	R^2	SECV	Reference
CP	0.96	0.96	Sheaffer, et al, [19];
	0.95	0.94	Velasco, et al, [20];
	0.92	0.91	Iantcheva et al., [21];
	0.95	0.94	Brogna et al., [22].
CF	0.87	-	Brogna et al., [22];
	-	3.12.	Iantcheva et al., [21].
OMDrt	-	2.97	Iantcheva et al.,[21];
	0.87	3.70	Brogna et al., [22].

Successful results for prediction of other 176 alfalfa samples were also obtained by NIRS analysis (SEP=0.869 for CP, 1.058 for CF, 1.058 for OMDrt – table 1). This system offers us the possibility of using NIRS technique for alfalfa organic substances determination. The accuracy and reliability of the prediction of e.g. the CP, CF or OMDrt of a sample by this NIRS technique has been totally dependent on the

accuracy and reliability of the classical determination of CP, CF or OMDrt. It is the reason why it is absolutely necessary to perform accurate analyses by classical techniques.

Table 4. Crude protein (CP), crude fiber (CF) and *in vitro* organic matter digestibility (OMDrt) of alfalfa harvested at different harvest stage determinated by NIR spectrometery

Harvest stage	CP	CF	OMDrt
Plant height 30 cm	12.83% - 20.61%	15.96% - 33.61%	50.07% - 63.57%
Bud	14.32% - 22.04%,	19.53% - 35.06%	46.46% - 65.99%
Flowering	12.06% - 20.14%	17.56% - 38.47%	45.07% - 61.58%
Seed formation	11.47% - 16.83%	18.33%- 46.06%	33.16% - 45.14%.

The *in vitro* OMDrt values of alfalfa in harvest stage were indirectly related to the CF content and directly proportional to the CP content. During the years of the experience, 2008 and 2009, CP levels and OMDrt were the highest at bud stage. A lower OMDrt and a highest CF content were obtained in seed formation stage, showing the role of CF in the OMDrt; the higher the crude fiber content is, the lower the digestibility is. It can be seen in table 5 that our results obtained by NIRS method were similar to different results obtained by classical or NIRS analysis. The results are presented in function of the harvest stage (bud, flowering and seed formation).

Table 5. Crude protein (CP), crude fiber (CF) and *in vitro* organic matter digestibility (OMDrt) of alfalfa harvested at different stages

Harvest stage	CP	CF	OMDrt
Bud	20.07% [12]; 24.05% [13]; 19.80% [16]; 22.60% [19]; 19.90% [23]; 16.00% [24]; 23.03% [25].	28.70% [12]; 21.40% [13]; 33.40% [24]; 25.49% [25].	50.09% [23]; 63.00% [24].
Flowering	18.47% [12]; 21.17% [13]; 18.10% [16]; 19.20% [19]; 19.40% [23]; 13.20% [24]; 33.09% [25].	31.03% [12]; 28.50% [13]; 35.70% [24]; 29.66% [25].	51.20% [23]; 62.00% [24].
Seed formation	15.92% [12]; 16.45% [13]; 17.50% [16]; 16.40% [19]; 17.40% [23]; 12.30% [24]; 16.37% [25].	34,46% [12]; 32.00% [13]; 33.60% [24]; 40.18% [25].	45.40% [23]; 60.00% [24].

In the first year of experiment a lower content of crude protein was obtained compared to second year for the different harvest stages, because in the first year of the experiment the alfalfa is installing in the yield and the nitrogen is used for this [3]. The same effect was pointed out by Decruyenaere et al., [11] and Stanacev et al., [26]. According to the harvest stage it was observed a reduction of CP and an increase in CF in the latest harvest which could be explained by to the evolution of stems and leaves [17] leaves containing more CP and less CF than stems. Moreover Heinriches [27] and Babinec et al. [28] pointed out that losses of leaves are important because the protein concentration was higher in leaves than in stems. The crude protein content and the crude fiber contents vary between very wide limits, depending largely on the development stage of alfalfa [1].

The relative contribution of qualitative parameters mentioned above helps to lift organic matter digestibility up to 60%, contributing equally to protein and fiber content. From this it can be concluded that the digestibility and crude protein content decrease quickly, while crude fiber increase slightly and then remain constant. Demarquilly and Andrieu [17] noted that the whole plant digestibility of alfalfa has been correlated to the proportion of leaves and stems, because leaves are more digestible than stems.

CONCLUSIONS

Based on the samples supplied, it has been shown that NIRS and PLS can be used to determine CP, CF and *in vitro* OMDrt of alfalfa.

This preliminary study proves that NIRS is an extremely reliable, non-destructive and rapid technique for the prediction of quantitative chemical and physical properties of alfalfa from Romania.

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