DETERMINATION OF ALFALFA CRUDE FIBER, NDF, ADF AND LIGNIN CONTENT BY NIR SPECTROMETRY

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Abstract

From all forage crops, which together with meadows have a major contribution in ensuring the forage base,, alfalfa crop (*Medicago sativa* L.) occupies a position of great importance. This plant is distinguished by its forage value, high cultivation area and high digestibility, and from the point of view of farmers and world's agricultural sciences is considered to be the "Queen of Fodder Herbs". In order to determine the quality of alfalfa, a series of classical analysis for cellulose, NDF, ADF and ADL were performed. This paper proposes a simple and nondestructive technique for rapid determination of these organic substances, method called „Near Infrared Spectrophotometry" (NIRS). In this purpose samples of alfalfa (*Medicago sativa* L., variety Eugenia) were obtained at the Experimental Teaching Station, Mănăştur and Cojocna Farm, in the years 2008-2009. Alfalfa crop was seeded by randomized block method with two experimental factors. The highest content of crude fiber, NDF and ADL was recorded on the phenophase of seeds formation (33.21%, 70.31% , respectively 15.15%). The lowest content recorded (31.14%) was the ADF content during the phenophase - seed formation. Good results (successful results) were obtained for the calibration of NIRS device (SEP = 1.058 [CF], 0964 [NDF], 1041 [ADF] and 1209 [ADL]). This system allows us to use NIRS technique for determining organic matter derived from alfalfa to feed and for other feed quality determination.

Key words: NIRS, alfalfa, crude fiber, NDF, ADF, ADL.

On the current agricultural vision, fodder production obtained from the permanent grassland, temporary grassland and forage crops, is an integral part of agricultural land management [Dale, 2011]. A fair assessment regarding the quality of forage grass originating from meadows requires an overall analysis on the data regarding the botanical composition of pastures, the nutrient and mineral content and digestibility of fodder produced [Rotar *et al*, 2005]. From all forage crops, which together with meadows have a major contribution in ensuring the forage base,, alfalfa crop (*Medicago sativa* L.) occupies a position of great importance. This plant is distinguished by its forage value, high cultivation area and high digestibility, and from the point of view of farmers and world's agricultural sciences is considered to be the "Queen of Fodder Herbs".

From agrobiological point of view, alfalfa gathers a number of particularities: resistance to drought and low temperatures, good revaluation of irrigation water, high capacity for regeneration after mowing, high rate of competitiveness [Rotar, 1993]. According to archaeological information or ancient philosophers writings, *Medicago sativa* L. crop has been taken in culture with 4000 years î.c. in regions of southwest Asia. Over the actual territory of our country, alfalfa was grown at first in Transylvania and on the late eighteenth century in Banat. Alfalfa has a great economic importance, illustrated by its high ecological plasticity, high production recorded, of more than 50 t/ha green mass/3 sewn, in natural conditions and over 80 t/ha green mass/4 sewn under irrigation, or between 10-15 t/ha of hay, and also by its high digestibility.

This paper proposes a simple, nondestructive and elegant technique for determination of quality parameters (crude fibre, fibre detergent neutru [NDF], fibre detergent acid [ADF], lignine [ADL]), method called „Near Infrared Spectrophotometry" (NIRS). Crude fibre is the most common polyglucide composed of glucose residue from the plant world, being the main component of cell walls. It presents a macromolecular, homogeneous polyglucide, consisting of several scrap β - D - glucopiranosis, united by ties β-1, 4 glycosidic bonds. Crude fiber is found together with a several number of

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substances encrusted: lignin, hemicellulose, pectins, resins, minerals, etc.. [Diaconescu et al, 2007]. NDF is the amount of protective substances obtained from residue after boiling a fodder sample in neutral detergent solution. NDF residue, actually contains very little pectic substances, but may contain negligible amounts of products like starch, nitrogenous substances and tannins [Jarrige et al, 1988]. The amount of protective substances residue obtained after boiling the sample feed with detergent solution is called ADF. ADF content is regularly higher than the crude fiber from forage, these features being closely related, since both are an estimate of the amount of cellulose + lignin. [Jarrige et al, 1988]. NIRS method allows a large number of repetitions, has accuracy and high precision [Dale et al., 2012]. Also, because it is based on the use of chemicals without the need for preliminary preparation of samples, is considered to be a "clean technology" – supporting sustainable agriculture dogmas. [Vidican *et al*., 2000].

MATHERIAL AND METHOD

Alfalfa samples were obtained from the Experimental Teaching Station Mănăştur - Farm Cojocna in 2008-2009, from experience with alfalfa which was established using randomization blocks method and two experimental factors (mineral fertilization and period of harvest). Alfalfa (variety Eugenia) was sown in spring 2007 and fertilized with chemical fertilizers in different compositions, depending on the doses proposed for each variant. The first variant of this experience, the control-variant was kept with the natural fertility of the soil. To assess the quality of alfalfa forage , laboratory samples were obtained by dividing the analytical (in the amount required by method) and chemical control was performed (crude fiber, NDF, ADF and ADL). Each quality parameter mentioned above was analyzed both by classical determinations: Weende (cellulose) method and Van Soest method (NDF, ADF and ADL) and modern determination (NIRS). This paper aims to highlight the determination of quality parameters using NIRS analysis and the results are based on data obtained by classical analysis. For NIRS technique samples were prepared after the standard model, that have been ground to smaller than 1 mm. These were scanned before being subjected to classical analysis. A part of the results obtained by classical analysis were used for model calibration and another part for model validation.

RESULTS AND DISCUSSIONS

Total crude fiber content, and the protein varies between very wide limits, and it mostly depends on the phase of development which makes harvesting alfalfa. Cellulosic substances are organic residue obtained after two consecutive hydrolysis (one in an acidic environment, the other

in alkaline medium) [Dale, 2011]. Content in cell walls (NDF), crude fiber and hemicellulose that is intensified with increasing vegetation, ie with increasing age [Jarrige et al, 1988]. Holland et al, 2008, believe that to obtain a high quality harvest, alfalfa should be harvested in development stage in which the leaves are present in greatest amount before to develop strains, cell walls deposits. The analysis regarding ADF content was originally intended as a preparatory step to determine lignin and cellulose determination [Van Soest, 1994]. Weak acid reagent dissolve hemicellulose, leaving most of the cellulose. Determination of NDF and ADF content is often used in order to estimate the hemicellulose. Holland et al, (2008) states that leaves suffer small changes during the growing process of alfalfa and that cellulose and lignin content, ie content of ADF, and the stem is increased significantly. Several methods have been used to isolate or to oxidize lignin in ADF, prompting acid detergent lignin (ADL). Digestibility of NDF content and ADF content are highly variable mostly due to the composition of lignin [De Boever et al, 1999]. Pecetti et al, (2001) states that there is a close connection between cellulose content and lignin content.

Table 1 presents the calibration picture, the calibration curve for crude fiber, NDF, ADF and ADL made for alfalfa, the number of samples used in calibration (N), average value of raw pulp, minimum and maximum amount which has been taken for making the model of calibration calculation, standard error (SD), standard error of calibration (SEC), coefficient of multiple determination (R^2), standard error of cross validation (SECV), standardized error of prediction (SEP) and coefficient of determination (1-VR). The calibration curve for cellulose has a standard error for cross validation of 1.35, a low coefficient of multiple determinations for calibration (0.92) and a report SECV/SD of 0.28. The calibration results are similar to those of other authors for the same type of biological material: thus cross-validation standard error obtained Iantcheva et al, (1999) is 3.12, and the regression coefficient obtained is similar to that of Brogna, et al, (2009) ie 0.87. NDF calibration curve has a large standard error for cross validation (1.70), a low coefficient of multiple determination for calibration (0.93) and a report SECV/SD of 0.23.

The calibration results are similar to those of other authors for the same type of biological material: thus cross-validation standard error obtained Iantcheva et al, (1999) is 2.88, Sheaffer et al, (2000), obtaining 8.10 , Brogna et al, (2009) 2.45, and the regression coefficient obtained is similar to that of Sheaffer et al, (2000) 0.98,

Brogna et al, (2009) .93. ADF calibration curve results shows a cross-validation standard error greater (1.46), a moderate multiple coefficient of

determination for calibration (0.94) and a report SECV/SD of 0.25.

Table 1

Calibration models for Alfalfa				
Content	CF	NDF	ADF	ADL
N	771	357	318	205
Mean	27.67	43.92	31.89	7.36
SEC	1.32	1.57	1.35	0.6
R^2	0.94	0.95	0.94	0.93
SECV	1.35	1.7	1.46	0.68
SEP	1.058	0.964	1.041	1.209
SD	5.37	7.31	5.72	2.24
SD/SEC	3.97	7.69	6.15	2.46
SECV/SD	0.25	0.23	0.25	0.3

The calibration results are similar to those of other authors for the same type of biological material: thus cross-validation standard error obtained Iantcheva et al, (1999) is 2.97 Sheaffer et al, (2000), obtaining 9.80, Brogna et al, (2009) 1.56; and the regression coefficient obtained is similar to that of Sheaffer et al, (2000) 0.98, Brogna et al, (2009). NDF calibration curve has a large standard error for cross validation (1.70), a low coefficient of multiple determination for calibration (0.93) and a report SECV/SD of 0.23.

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makes harvesting alfalfa. The NIRS analysis of the crude fiber content of alfalfa obtained higher values on phenophase - seed formation between 18.33% and 46.06%, phenophase - flowering between 17.56% and 38.47%, harvest 9.53% and 35.06%, but lower the phenophase - average size 30 cm plants which are between 15.96% and 33.61%. NDF content has, in samples of alfalfa, small values on phenophase - average size 30 cm plant between 41.84% and 50.29%, the phenophase - germ values between 44.33% and 54.08% , the phenophase - flowering between 45.20% and 56.97%, and the phenophase - seed formation between 52.25% and 70.31%. ADF content consists of cellulose and lignin content, so alfalfa has higher values in phenophase - seed formation between 39.25% and 51.65%, the phenophase - flowering between 32.13% and 42.05%, the phenophase - germ, between 32.96 % and 39.33%, and the phenophase - medium size plant 30 cm between 31.14% and 37.54%. ADL, or lignin content in alfalfa is higher in phenophase - seed formation between 9.15% and 15.15%, the phenophase - flowering between 7.28% and 10.49%, the phenophase – germ between 7.53% and 10.33%. The phenophase - size average plant 30 cm between 7.02% and 10.04%. Good results (successful results) were reached in order to calibrate NIRS device (SEP = 1.058 [CF], 0.964 [NDF], 1.041 [ADF] and respectivelly 1.209 [ADL]). This system offer us the posibibily to use NIRS technique in determination of organic substances not only from alfalfa forage but also in other determination regarding forage quality. Results regarding alfalfa's quality in different vegetation phenophasses were reached by NIRS technique and there are distinguished on the scheme and the new system used by the classical method. As it can be observed the results we obtain

are similar to those reached by different researchers from our country and abroad. (*tabel 2*).

Table 2

Results reached for Alfalfa's quality in different phenophases of vegetation (phenophase of early bloom, phenophase of flowering and seed formation phenophase)

Harvest stage	Weende Scheme	Van Soest System		
	Crude fiber	NDF [%]	ADF [%]	ADL [%]
Bud	☞28.70 (Varga, 1973) ☞21.40 (Vintilă, 1989) ☞25.49 (Homolka et al., 2008) ☞33.40 (INRA, 2006)	☞37.50 (Sheffield, et al. 2000) ☞48.80 (Yu et al, 2003) ☞35.20 (Marković, et al., 2007) ☞36.00 (Holland, et al., 2008) ☞35.10 (Homolka, et al.,2008)	☞26.85 (Homolka et al., 2000) ☞31.60 (Sheaffer et al., 2000) ☞32.10 (Yu et al, 2003) ☞34.10 (INRA, 2006) ☞16.☞58 –42.62 (Marković et al., 2007) ☞26.00 (Holland et al, 2008)	☞20.40 (Yu et al, 2003) ☞4.20 (Marković et al, 2007) ☞9.27 (Homolka et al, 2008)
Flowering	☞31.03 (Varga, 1964) ☞28.50 (Vintilă, 1989) ☞29.66 (Homolka et al., 2008) ☞35.70 (INRA, 2006)	☞42.70 (Sheaffer, et al., 2000) ☞46.90 (Yu et al, 2003) ☞55.20 (INRA, 2006) ☞43.59 (Marković et al, 2007) ☞37.00 (Holland et al, 2008) ☞41.12 (HOMOLKA et al, 2008)	☞31.81 (Homolka et al, 2000) ☞35.90(Sheaffer et al, 2000) ☞29.40 (Yu et al, 2003) ☞ 35.60 (INRA, 2006) ☞17.65 – 47.19 (Marković et al, 2007) ☞28.00 (Holland et al, 2008)	☞19.90 (Yu et al, 2003), ☞4.64 (Marković et al, 2007) ☞8.98 (Homolka et al, 2008)
Seed formation	☞34,46 (Varga, 1964) ☞32.00 (Vintilă, 1989) ☞40.18 (Homolka et al., 2008) ☞33.60 (INRA, 2006)	☞49.20 (Sheaffer et al, 2000) ☞49.6 (Yu et al, 2003) ☞53.70 (INRA, 2006) ☞47.67 (Marković et al, 2007) ☞42.00 (Holland et al, 2008) ☞43.03 (Homolka et al, 2008) ☞47.50 (Vintu et al., 2012)	☞28.89 (Homolka et al, 2000) ☞41.40 (Sheaffer et al, 2000) ☞31.00 (Yu et al, 2003), ☞34.20 (INRA, 2006) ☞18.74 – 49.94 (Marković et al, 2007), ☞32.00 (Holland, et al., 2008) ☞42.30 (Vintu et al., 2012)	☞21.30 (Yu et al, 2003) ☞4.90 (Marković et al, 2007) ☞11.31 (Homolka et al, 2008)

CONCLUSIONS

The analyze system using NIRS technique can be successfully applied for determination of forage quality, meaning: crude protein, crude ash, crude fat, crude fiber, content of NDF, ADF content, lignin content, organic matter digestibility corn for silage, alfalfa and feed on natural pastures. The work detailed in this paper illustrates that it is possible, using FT-NIR spectroscopy, to determine a certain number of crude fiber, NDF, ADF and ADL content in alfalfa samples with accuracy similar to the reference method.

Based on the samples supplied, it has been shown that NIR and PLS can be used to determine crude fiber, NDF, ADF and ADL content of alfalfa with good correlation coefficients and low errors. This preliminary study proves that NIR spectroscopy is an extremely reliable, non-destructive and rapid technique for the prediction of quantitative chemical and physical properties.

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