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NIR Spectroscopy: A Non-destructive Method for PUFAs Determination of Forage from Permanent Grassland

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Abstract. The objective of this study was to develop a non-destructive and rapid method for polyunsaturated fatty acids (PUFAs) content of forage from grassland using near infrared spectroscopy (NIRS). To accomplish this desiderate it was harvested forages in June from ten experimental trials of a permanent grassland situated in the south – west part of Romania, in Banat County, fertilized organic (fermented sheep manure), mineral and organic – mineral. On the permanent grassland the mineral fertilizers were applied yearly since 2003 and the organic fertilizer at each two years. For multivariate analysis to perform the NIR model was used a supervised technique, partial least squares (PLS) regression. The NIRS model was characterized by the following parameters: R^2 = 0.9888, RMSEC = 222.5, RMSEP = 215.7. The differences between the chemical results and those predicted recommend like promising the NIRS method for PUFAs contents prediction of forages from permanent grassland in studied conditions.

Keywords: near infrared spectroscopy, polyunsaturated fatty acids, grassland, feed, quality.

INTRODUCTION

Many of spontaneous plants from permanent grasslands (haylands and pastures) are veritable threshold for farmers in animals' nutrition when have desirable chemical compounds. In Romania grasslands represents the most sustainable sources of forages for ruminants because they are the cheapest feedstuffs. To obtain a high quality of forages from these surfaces must be the main objective of farmers. But also it is important to keep the biodiversity of species from grassland, both in intensive or ecological systems (Plantureux *et al.,* 2005). The good practices of grassland management are recommended to obtain a production with high quality, in enough quantity to assure security of animal's feed, with a good production / price ratio and to assure the health of environment.

Fatty acids profile of forage from grassland becomes an important parameter of quality in our days, since many epidemiological studies have shown their benefits on human health (Aydin *et al.,* 2001; Howe *et al.,* 2006; Larque *et al.,* 2002; Lemaitre *et al.,* 2003; Simopoulos, 1989, 2004). For consumers the main source for fatty acids is the food. But the fatty acids' profile of dairy products and meat from cattle and sheep depends by the animals diets (Clapham *et al.,* 2005). That's way it is important to determine the PUFAs contents of plants species which compose the floristic matrix of forages.

The chemical method to monitor PUFAs component, gas–chromatography coupled with mass spectrometry (GC MS), requires time to develop the external or internal calibration, and it is expensive regarding the used reagents and solvents. An alternative for the wet method can be NIRS, a rapid, non-destructive, low-cost and environmental-friendly method, because it does not need reagents (Pereira *et al.,* 2008; Valdes *et al.,* 2006). The goal of this research was to develop a NIR calibration model for PUFAs content determination using reflectance values of 39 spectra and the chemical results for this parameter by GC MS. The

spectra were scanned for the 800 – 2500 nm spectral range. PLS regression was choosing for multivariate analysis to perform the NIRS calibration model.

MATERIALS AND METHODS

The forage samples were harvested in June 2009 and were transported to laboratory and conditioned in conformity with Harmanescu (2012). The forages were collected from a permanent grassland on Calcic Luvisol situated in the south – west part of Romania (45.151^0 N / 21.538^0 E), at maximum 200 m above sea level. In the period 2007 - 2009 the multiannual average of precipitations was 842 mm. The experimental field was fertilized first in 2003, organic with maximum 60 t/ha fermented sheep manure, organic – mineral and exclusively mineral with maximum 50 kg/ha P_2O_5, 50 kg/ha K_2O and 200 kg/ha N. The mineral fertilizers were applied yearly, while the organic at two years. It was organized in 10 trials in multiple randomized stage blocks, with five replications.

The floristic matrix was established gravimetrically. It was present among *Poaceae* mainly *Festuca rupicola* (maximum 52%), *Calamagrostis epigejos* (maximum 13%) and *Poa pratensis* (maximum 5%). From *Fabaceae* were identified dominant *Trifolium repens* (maximum 38%) and in maximum 6% was *Lathyrus pratensis.* From other botanical families were especially *Galium verum*, *Inula britanica*, *Rosa canina*, *Filipendula vulgaris* (Harmanescu, 2009).

For polyunsaturated fatty acids (linoleic acid + linolenic acid) chemical determination was used gas – chromatography coupled with mass spectrometry. The equipment GC MS QP 2010 (Shimadzu) was used. The external calibration and the parameters for GS MS technique were those presented by Harmanescu (2012). The extraction method was a direct transmethylation in one-step technique (BF_3/ methanol) described by Weston et al. (2008) and adapted by Harmanescu (2012).

All reagents were analytical and chromatographic purity grade. They were purchased from Merck (Hohenbrunn, Germany), Sigma–Aldrich (St. Louis, MO, USA), Supelco Inc. (Bellefonte, PA, USA) and Grace (USA).

The NIR spectra were collected with V670 Spectrophotometer instrument by Able-Jasco. The total number of calibration spectra was 39, for the forages from permanent grassland dried and grounded, scanned from 800 to 2500 nm. The NIR model was performed using partial least square regression (PLS) for PUFAs implemented in Panorama software (Variant 3, LabCognition, 2009).

RESULTS AND DISCUSSION

The PUFAs contents of forages from grasslands, reported like linoleic and linolenic acids, determined by GC MS, were in range 1815 – 11099 mg/kg. These chemical results, the reflectance values from NIR spectra and the PLS regression were used to create the calibration model of a rapid and non-destructive method. The PLS regression represents a supervised multivariate analyses technique.

For PUFAs determination each forage sample was chemically analyzed and NIR scanned like mixed forage and the three components *Poaceae*, *Fabaceae* and other botanical families, reported to dry matter (DM). The predictions of NIR model with ten factors for PUFAs content of 39 forages from permanent grassland is shown in Fig. 1.

Fig. 1. The NIRS predicted values versus chemical results of PUFAs (mg/kg) on 800 – 2500nm spectral range

The statistic parameters of NIRS calibration model were: R^2 (coefficient of determination) = 0.9888 and RMSEC (root mean square error of calibration) = 222.5. To verify that this mathematical model can be used to predict PUFAs it was validated. This desiderate was accomplish choosing a samples set which represents 50% of total forages number used for NIR calibration model. These samples set were harvested from the same permanent grassland with the calibration samples and were scanned with the same equipment in NIR domain, and then on based of these spectra were predicted with NIR model the PUFAs contents of forages.

The statistical parameters for validation procedure were: R^2 = 0.9787 and RMSEP (root mean square error of prediction) = 215.7. The differences between the PUFAs contents obtained by chemical method (GC MS) and those predicted by NIR model for validation set forage samples are shown in Tab. 1.

Tab. 1.

The differences between PUFAs results (mg/kg) obtained by chemical method (GC MS) and those predicted by NIR model for forage samples (validation set)

Samples name	GC MS results (mg/kg) (1)	NIR predicted results (mg/kg) (2)	Residual (mg/kg) (3) = (1) – (2)	Residual (%) (3) x 100 / (1)
101b	6754	6750	4	0.06
101c	3065	2941	124	4.05
102a	4357	4232	125	2.87
102d	6254	6409	-155	-2.48
103b	8006	8082	-76	-0.95
104c	7555	7894	-339	-4.49
104d	11099	10663	436	3.93
105d	5816	6147	-331	-5.69
105b	7873	7705	168	2.13
106a	3710	4177	-467	-12.59
106b	6402	6592	-190	-2.97
107d	4144	4303	-159	-3.84
107a	3042	2802	240	7.89
107b	6906	6824	82	1.19
108a	3918	3943	-25	-0.64
109a	2817	2755	62	2.20
109c	4417	4443	-26	-0.59
110b	9833	9827	6	0.06
110c	4337	4159	178	4.10
110d	2852	2954	-102	-3.58
a - *Poaceae*, b - *Fabaceae*, c - other botanical families, d – mixed forages				

The results from Tab. 1 are promising. The differences between the values of chemical method and those predicted by NIR model were under 1% in the 25% of studied cases, 40% were between 1% - 3%, 30% between 3% - 5% and 15% higher than 5%. The predictability of NIR method is smaller than the chemical method, it is true, but with higher number of forage samples from grassland, more diversified, which characterized better the overtones specific for different profiles of PUFAs from plants, its accuracy can be improved.

A high number of forage samples used for NIR calibration is justified by the complex chemical matrix of plants species, present in the green grass mass, dominant being perennial, which assure the biodiversity of grassland. The advantages like the low – cost of analysis because it doesn't need expensive reagents and solvents with ultra high purity, the short time of analysis, a good accuracy when a sufficient number of samples are used for calibration, human resources which can learn quickly to work with these kinds of equipments, the possibilities of portable equipment acquisition, recommend this method in farmers' practices.

CONCLUSIONS

In this research was tried the possibilities to use NIRS in polyunsaturated fatty acids prediction of forages from a permanent grassland situated in the south – west part of Romania. The obtained values representing the differences between the chemical results and those predicted by NIR model show that this rapid, non-destructive and low-cost spectroscopic method can be use with accuracy for PUFAs contents prediction of forages from studied permanent grassland in studied conditions.

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