THE INFLUENCE OF SAMPLE PARTICLES SIZE ON THE DETERMINATION OF TOTAL NITROGEN CONTENT FROM FORAGES BY NIR SPECTROSCOPY, USING MINIMUM REFLECTANCE VALUES

INFLUENTA MĂRIMII PARTICULELOR DE PROBĂ ASUPRA DETERMINĂRII CONȚINUTUL TOTAL DE AZOT DIN FURAJE PRIN SPECTROSCOPIE NIR, UTILIZÂND VALORILE MINIME ALE REFLECTANTELOR

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Abstract: *The main objective of this scientific paper was to study the influence of forages samples particles size on the determination of total nitrogen content (TNC) by NIR Spectroscopy. For this, correlations were realized between the values obtained for TNC using classical Kjeldahl method and the minimum reflectance values for four frequencies from NIR spectra. We used two particles sizes for forage samples: bulk (sieve with 1 mm diameter) and fine (sieve with 0.3 mm diameter). The correlation coefficient R^2 obtained for regression equation of bulk particles size was equal with 0.8212, smaller than that for fine particles size R^2=0.90542092. It is possible to observe that the usage of fine particles size improves significantly the correlation coefficient R^2 of regression equation.*

Rezumat: *Obiectivul principal al acestei lucrări ştiinţifice a fost studierea influenţei mărimii particulelor probelor de furaje în determinarea conţinutului total de azot (TNC) prin spectroscopie NIR. Pentru aceasta s-au realizat corelaţii între valorile obţinute pentru conţinutul total în azot prin metoda clasica Kjeldahl şi valorile minime ale reflectantelor pentru patru frecvente din spectrele NIR. S-au utilizat două dimensiuni pentru particulele de furaje: grosiere (sita cu diametrul de 1 mm) şi fine (sita cu diametrul de 0.3 mm). Coeficientul de corelaţie R^2 pentru ecuaţia de regresie în cazul particulelor grosiere a fost de 0.8212, mai mic decât cel obţinut în cazul particulelor fine R^2=0.90542092. Se poate observa că utilizarea particulelor de probă mai fine îmbunătăţesc semnificativ coeficientul de corelaţie R^2 al ecuaţiei de regresie.*

Key words**:** *total nitrogen content, NIR Spectroscopy, forages*
Cuvinte cheie**:** *conţinut total de azot, spectroscopie NIR, furaje*

INTRODUCTION

NIR Spectroscopy (Near Infrared Reflectance Spectroscopy) is a non-destructive qualitative and quantitative method of analysis [Wilson, 1994]. The main advantages of NIR Spectroscopy technique used to monitor the quality of fodder plants from pastures are the following:

- it is a non-destructive method, the same sample can be used for another determination [RUANO-RAMOS et al, 1999];
- it is a very quickly method, each spectrum can be measured in 30 seconds [LANDAU et al, 2006];
- it is not necessary a long time for the sample preparation [MORON & COZZOLINO, 2003];
- a single scan permits to determine in the same time many constituents of analysed sample;

- it does not require the usage of reagents; not a destructive method for the environment [FOLEY et al, 1998].

The necessity to study NIRS method applications in our country to characterize the nutritive quality of pastures forages refers both to calibrate this method for the plants species existing in different pedo-geographically zones from Romania and to organize a national database. However, to calibrate this method is necessary to know which is the influence of particles size on the NIR spectra quality and accuracy of NIRS method.

The main objective of this study was to determine the influence of forages particle size on the total nitrogen content (TNC) analyzes by Near Infrared Reflectance Spectroscopy. For this, two sizes of forages samples harvested from Gradinari permanent pasture (Caras-Severin County) were analysed for total nitrogen content using both NIR Spectroscopy and classic Kjeldahl methods. Then it was made correlations between the values obtained for TNC using NIR Spectroscopy with those obtained using classic Kjeldahl method, depending on forages particles size.

MATERIAL AND METHODS

Samples preparation:

Samples were harvested on October 2007, in triplicate, from a permanent pasture from Gradinari, a collinear village situated in Caras-Severin District. The main species founded on permanent pasture from Gradinari were: *Festuca rupicola* and *Calamagrostis epigeios*. Other species were *Antohoxanthum odoratum, Briza media, Poa pratensis, Trifolium arvense, Trifolium medium, Genista tinctoria, and Lotus corniculatus.* From the other botanical families was presented *Filipendula vulgaris.*

The permanent pasture was organized in ten experimental variants with different doses of fertilization. For each variant were made five repetitions. The fertilization doses for all the ten experimental variants are present in Table 1:

Table 1

The fertilization doses for all the ten variants of Gradinari permanent pasture

Experimental variants	Characterization of variants
GV1	unfertilized variant
GV2	20 t manure
GV3	40 t manure
GV4	60 t manure
GV5	20 t manure + 50 P
GV6	20 t manure + 50 P + 50 K
GV7	20 t manure + 50 N + 50 P + 50 K
GV8	100 N + 50 P + 50 K
GV9	150 N + 50 P + 50 K
GV10	100 + 100 N + 50 P + 50 K

G – Gradinari; V – experimental variant

Each sample harvested from the ten experimental variants was dried at room temperature (appreciatively 22^{o}C) for two weeks and then and divided in two sizes: bulk (sieve

with 1 mm diameter) and fine (sieve with 0.3 mm diameter). TNC values by Kjeldahl represent the mean of values obtained for harvested samples for each experimental variant.

Determination of Total Nitrogen Content (TNC)

Total Nitrogen content was determined chemically using Kjeldahl method [GERGEN, 2004]. Digestion of samples (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). All solutions were prepared using deionized water. For all the samples the determination were made in triplicate.

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

Statistical interpretation of obtained data using multivariate analyses was performed with Statistica-6 software.

RESULTS AND DISCUSIONS

The results obtained for TNC for the bulk size of samples, using Kjeldahl method, and the minimum reflectance values for main four frequencies from NIR spectra are present in Table 2. The main frequencies with minimum reflectance values were: L1 - 1467 nm, L2 - 1730 nm, L3 - 1930 nm and L4 - 2107 nm.

Table 2

TNC for the bulk size of samples, determined using Kjeldahl method, and minimum reflectance values for four frequencies (L1 – L4) obtained from NIR spectra

Experimental variants	TNC Kjeldahl %	L1 1467 nm	L2 1730 nm	L3 1930 nm	L4 2107 nm
GV1b	1.09	56.2073	59.7144	44.2542	46.8326
GV2b	1.36	58.1737	61.2698	47.0139	48.8586
GV3b	1.47	59.8141	62.9778	48.0224	51.2464
GV4b	1.74	59.3857	62.6463	46.8284	49.9022
GV5b	1.22	57.6536	60.9776	45.8836	48.2380
GV6b	1.25	58.2396	61.3060	47.1005	48.7289
GV7b	1.19	55.9085	59.5121	44.5554	46.9812
GV8b	1.05	57.6484	61.3440	46.8536	48.6453
GV9b	1.39	59.4977	62.7440	47.8669	49.9799
GV10b	1.10	57.6659	61.6967	45.8959	48.1168

G – Gradinari; V – experimental variant, b - bulk size of the samples

The results obtained for bulk size of samples were interpreted with Multiple Regression Analysis, using Statistica-6 software. On the basis of statistical parameter, the regression equation for N% calculations with minimum reflectance values of the four frequencies is:

$$\mathbf{TNC\% = -3.50 + 0.47L1 - 0.26L2 - 0.20L3 + 0.06L4}\ (R^2=0.821200,\ p<0.04127) \quad [1]$$

The graphical presentation of correlation between N% by Kjeldahl method and predicted values by regression equation on the basis of frequencies with minimum reflectance is presented in Figure 1:

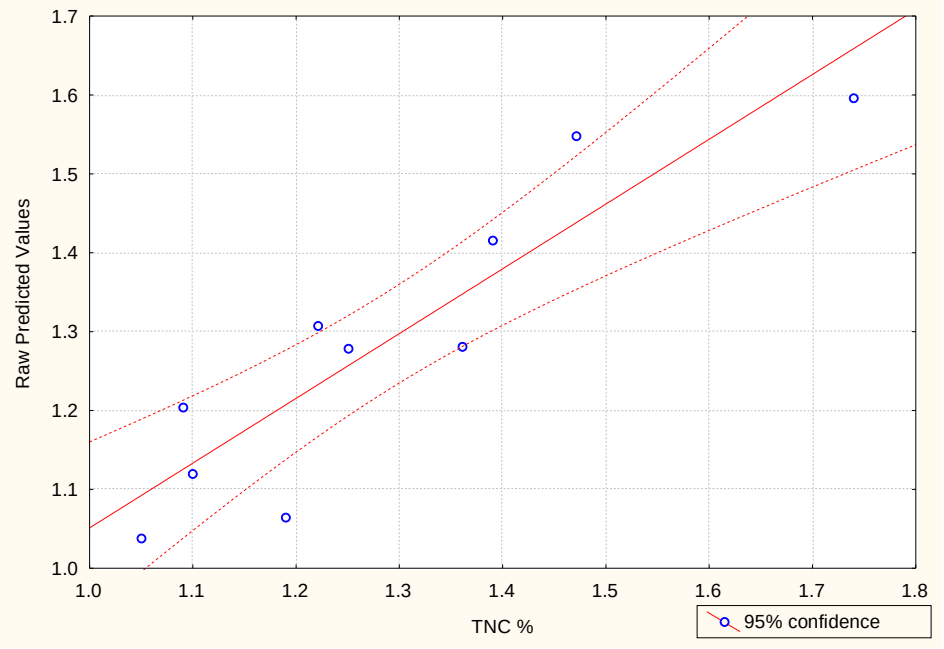

Figure 1. Correlation between N% by Kjeldahl method for bulk size of samples and predicted values by regression equation on the basis of frequencies with minimum reflectance

The results obtained for TNC for the fine size of analyzed samples, using Kjeldahl method, and the minimum reflectance values for main four frequencies from NIR spectra are present in Table 3:

Table 3

TNC for the fine size of samples, determinated using Kjeldahl method, and minimum reflectance values for four frequencies (L1 – L4) obtained from NIR spectra

Experimental variants	TNC Kjeldahl %	L1 1467 nm	L2 1730 nm	L3 1930 nm	L4 2107 nm
GV1f	1.21	56.2329	59.2145	45.9727	48.8619
GV2f	1.81	60.8255	62.5471	54.0750	56.4170
GV3f	1.55	59.1496	62.5243	46.3918	49.8791
GV4f	1.97	61.4183	64.3856	49.2799	52.7644
GV5f	1.35	58.5283	61.4301	47.3079	50.3489
GV6f	1.30	59.4252	62.3996	48.9282	51.1742
GV7f	1.33	57.8975	60.8537	47.9981	51.2536
GV8f	1.17	58.5509	62.1816	48.6481	51.1669
GV9f	1.50	61.4069	64.3417	51.2120	53.7976
GV10f	1.17	60.3846	64.1690	50.1550	52.9356

G – Gradinari; V – experimental variant, f - fine size of the samples

The results obtained for fine size of samples were interpreted also with Multiple Regression Analysis. Using the statistical parameter of this case was established the regression equation for N% calculations with minimum reflectance values of the four frequencies:

TNC% = - 4.10 + 0.47L1 - 0.35L2 - 0.28L3 + 0.25L4 (R^2=0.90542092, p<0.00898) [2]

The graphical presentation of correlation between N% by Kjeldahl method for fine size of samples and predicted values by regression equation on the basis of frequencies with minimum reflectance is presented in Figure 2:

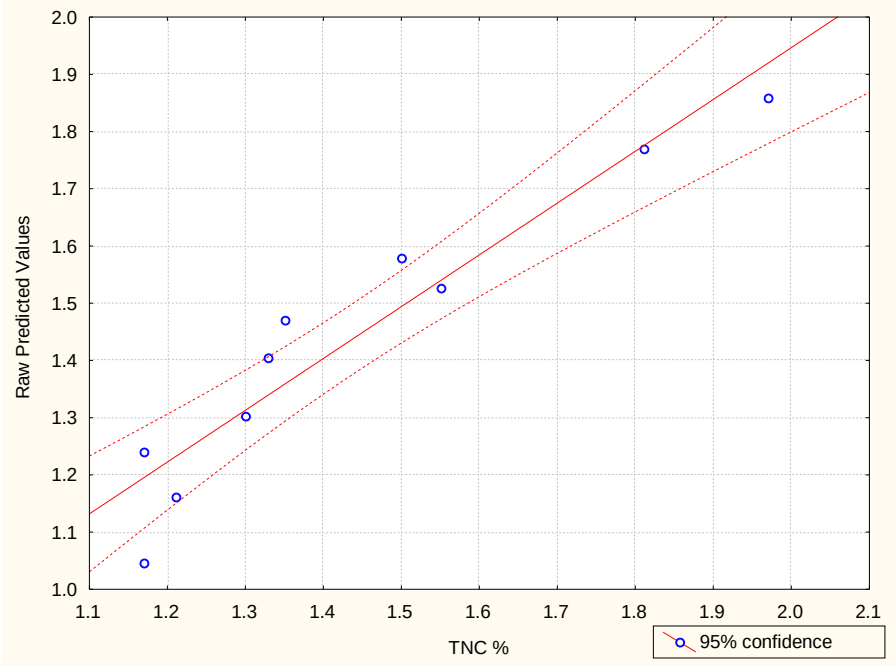

Figure 2. Correlation between N% by Kjeldahl method for fine size of samples and predicted values by regression equation on the basis of frequencies with minimum reflectance

On the basis of regression coefficients we can observe that equation [2] obtained from data of fine size samples is better than equation [1] in prediction of TNC values for analyzed forages. Both equations are constructed on the basis of four independent variables, L1, L2, L3 and L4, which represent the minimum reflectance values from NIRS spectra. The obtained equations for NTC calculation from NIRS data spectra are true only for the NTC values in the 1-2%. For higher values it is necessary to calculate a new equation with adequate samples.

CONCLUSIONS

For the analyzed forage samples harvested from Gradinari permanent pasture the best correlation coefficient was obtained for fine size of particles, R^2 = 0.90542092. That means that for practical application in TNC determination by NIRS is very important to obtain a fine powder of samples with diameter of particles under 0.3 mm. Only in this way the results obtained for NTC by NIRS is appropriate to real data. Also is important that forages samples sizes used to calibrate the NIR spectrometer being prepared in the same conditions with those for analyzed sample. In the next work we intend to study the influence of sample particles size on the determination of TNC from forages by NIR Spectroscopy, using maximum values of reflectance.

LITERATURE

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