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STANOVENIE SKUTOČNE STRÁVITEĽNÝCH DUSÍKATÝCH LÁTOK V TENKOM ČREVE METÓDOU NIRS V KRMIVE PRE PREŽÚVAVCE

DETERMINATION OF PROTEIN DIGESTIBLE IN INTESTINE BY NIRS-METHOD IN FORAGES FOR RUMINANTS

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The aim of the work was to find the possibility for using the methods of near infrared reflectance spectroscopy (NIRS) to analyse bulky feedstuff from permanent (PG), oversown (OG) and temporary grassland (TG) in the treatments at different fertilization rates (0 NPK, P30K60, N90PK, N180PK) in the three-regim cuts. Near infrared reflectance spectroscopy (NIRS) method of analysis was used to determine protein digestible in intestine (PDI) when energy is limiting (PDIE) and when nitrogen is limiting (PDIN) in forage for ruminants. Herbage samples were taken from permanent (PG), oversown (OG) and temporary (TG) grasslands managed at different NPK fertiliser application and under three-cut utilisation system. The samples were analysed by reference methods and their spectra were measured by NIRS. A correlation between the reference method data and the collected spectra was used to develop an equation "FORAGE.EQA" for forage analysis. An identification of samples with NIRS was conducted by the analysis of main components. Parameters of quality FORAGE.EQA are determined by the standard error of calibration (SEC), SEC_{PDIE} = 1.93 g kg^{-1}, SEC_{PDIN} = 2.09 g kg^{-1}; the standard error of cross validation (SECV), $SECV_{PDIE}$ = 2.60 g kg^{-1}, $SECV_{PDIN}$ = 2.52 g kg^{-1}, and the coefficient of correlation (RSQ) RSQ_{PDIN} = 0.99 showed a very strong dependence. A strong dependence was found at RSQ_{PDIE} = 0.91. The quality of calibration model was tested in the validation file VFORAGE.CAL.

Key words: forage, grasslands, NIRS, PDIE, PDIN

Improvement in animal performance enhances the requirements for nutrients and their effective utilization by animals. Effective nutrients degradability in rumen is an important characteristic of forage quality for ruminants and indicates supplementation of animal and ruminal microorganisms with energy and nitrogen. Much research has been conducted on the effective nutrient utilization by ruminants (Čerešňáková et al., 2003; Pozdíšek and Bjelka, 2002) and rumen degradability of plants protein supplements (Šimko et al., 2006; Čerešňáková et al., 2002). Grasslands are an important source of forage in the diet of ruminants mainly in upland and mountain regions. Forage quality is affected by many factors, including the characteristics of the environments (soil, temperature, precipitation, light conditions), grassland management (fertilization, cutting, grazing) and preservation techniques (Juráček et al., 2001; Gallo et al., 2006; Bíro et al., 2002; Gálik, 2007). The profitable production and the improvement in animal performance from grassland – based systems depend on both the botanical composition and the plant maturity stage. Botanical diversity of meadows and pastures affects the crude protein content of forage and is directly related to growth potential of the animals (Pozdíšek and Bjelka, 2002). The crude protein content is an important limiting nutritional factor. Therefore, in order to adequately supply the nutritional needs of the ruminants, the evaluation of crude protein content of forage is needed. The evaluation of crude protein (CP) according to the protein digestible in intestine (PDI) system is based on utilisable nitrogen sources in the small intestine of ruminants. These sources consist of microbial protein synthesised in forestomachs and of non-degradable CP of forage and their proportions – according to van Straaten (Sommer, 1994) – depend on an extent of degradation and on

the forage retention time in the forestomachs (Chrenková et al., 2000). The assessment of CP in the nutritive value of feedstuff is characterised by two values of PDI, namely PDIE (protein digestible in intestine when energy is limiting) and PDIN (protein digestible in intestine when nitrogen is limiting). The lower value is the real value of feed if fed without supplements. The higher value is the potential value that can be reached if forage is fed together with a suitable supplement. At calculating PDI of feeding rations, the PDIN and PDIE values of the ration components must be calculated separately. The real PDI is then the lower value either of total PDIN or of total PDIE. Recent research on nutritive value of grassland forage showed the great variability in nutrient content and plant quality among plant species as well as among the cultivars of grasses and legumes (Pozdíšek et al. 2002). Plant species diversity in interaction with environmental variables affects the dynamics and variability of nutritive value and requires a reliable method to determine the grassland forage quality. Several studies confirmed good predictive ability of NIRS for trace minerals in legumes (Cozzolino, D. and Moron, A., 2004) and protein digestibility (De Boever et. al., 2002) as well. The use of NIRS in evaluation of quality of agricultural production was reported by Dardenne et al. (2006) and Kalinin et al. (2008). The aim of this study was to evaluate the use of NIRS for predicting the protein digestible in intestine in forage from grasslands for ruminant nutrition.

Materials and methods

In our three-year research, a content of protein digestible in intestine (PDI) when energy is limiting (PDIE) and when nitrogen is limiting (PDIN) was studied in herbage from permanent (PG), oversown (OG) and temporary (TG) grasslands managed at three-cut utilization system and different NPK fertiliser treatments: 1) $N_0P_0K_0$; 2) $P_{30}K_{60}$, 3) $N_{90}P_{30}K_{60}$ and 4) $N_{180}P_{30}K_{60}$. The trial site was at 460 m altitude, 5–30° slope inclination, NNE exposure in a moderately warm region (mean annual daily temperature 7.7 °C), mean annual rainfall 853 mm, soil type rendzina, and soil texture loamy clay. The trial was divided into three blocks: permanent (PG), oversown (OG) and temporary (TG) grassland. Temporary grassland (TG) has been developed at the trial site for 40 years and has been used by cutting and grazing. TG was established by grassland renovation and OG was installed by direct drilling of grass/clover mixture into native permanent grassland. For TG and OG establishment, the following grass/clover mixture and seed rates were used (table 1).

Herbage samples were taken from all the grassland types three times during the growing season at harvest dates. The first harvest date was at the start of heading stage of dominating grass species in the sward, the second harvest date followed 4–5 weeks after the first one and the third harvest date was 6–8 weeks after the second harvest date. Samples were oven-dried at 60 °C, ground to particle size of 1 mm and analysed by the reference laboratory methods. At the site, arable land was turned to grassland and used by cutting and grazing. Botanical composition of PG was dominated by *Trisetum flavescens, Dactylis glomerata, Poa pratensis, Trifolium repens* and *Taraxacum officinale* at 73.9–88.8 % ground cover and also by *Trifolium pratense, Lolium perenne, Arrhenatherum elatius, Achillea millefolium* and *Falcaria vulgaris.* In OG, *Dactylis glomerata, Lolium perenne, Trifolium pratense* and *Trifolium repens* were the overdrilled species, *Trisetum flavescens, Taraxacum officinale* as well as *Poa pratensis* (in dry years) remained from the original sward and mean ground cover was 84.7–89.1 %. The temporary grassland was cut once to control weeds and then the meadow species typical for the sward before ploughing returned. The botanical composition of TG differed from PG and OG throughout the trial by presence of *Festuca arundiancea* and *Trifolium pratense* and also by higher proportions of *Dactylis glomerata* and *Lolium perenne* at all the treatments. The proportions of *Trisetum flavescens, Arrhenatherum elatius* and *Bromus mollis* were markedly low.

Herbage samples were taken from all the grassland types, oven-dried at 60 °C, ground to particle size of 1 mm and analysed by the reference laboratory methods. The content of CP was determined by the Kjeldahl method (Directive1993/28/EHS). After the analysis, the samples that did not comply with the analytical tolerance for laboratory results of nutrient content were selected (as defined by "Analytical tolerance" – Decree No.39/1/2002-100 issued by Ministry of Agriculture of the Slovak Republic). The data obtained by the analysis and the tables specifying the nutritive value of forage were used to calculate PDIE and PDIN. The samples were measured by "NIRS 6500" apparatus and absorption spectra were collected. The samples were identified using the method of main components. The samples were centred and those with the spectrum distance (Δ) in disagreement with the other population were excluded. The collected spectra file was extended by including the PDIE and PDIN parameters. The modified partial least squares regression (MPLS) was chosen from the NIRS 6500 software database of linear regression methods. A correlation between the results of reference laboratory methods and the NIRS spectra of FORAGE.CAL file by MPLS was used to develop the

Table 1 Botanical composition and seed rates

Botanical species (1)	Cultivar (2)	Seed rates (3)	
		seed in kg.ha^{-1} (4)	seed in % (5)
Dactylis glomerata L.	Rela	4	14
Festulolium	Felina	12	41
Lolium perenne L.	Metropol	8	28
Trifolium pratense L.	Sigord	3	10
Trifolium repens L.	Huia	2	7
Total seed rate (6)	–	29	100

Tabuľka 1 Botanické zloženie miešanky
(1) botanické druhy, (2) odroda, (3) výsevok ďatelinotrávnej miešanky, (4) výsevok v kg. ha^{-1}, (5) výsevok v %, (6) celkový výsevok

Table 2 Quality parameters for the developed calibration equation FORAGE.EQA

Variable (1)	Samples number (2)	Mean in g.kg^{-1} (3)	SEC in g.kg^{-1} (4)	SECV in g.kg^{-1} (5)	RSQ (6)	1-VR (7)
PDIE (8)	103	99.05	1.93	2.60	0.91	0.84
PDIN (9)	106	115.46	2.09	2.52	0.99	0.99

Tabuľka 2 Kvalitatívne parametere kalibračnej rovnice KRMIVO.EQA
(1) premenná, (2) počet vzoriek, (3) stredná hodnota (priemer), (4) štandardná chyba kalibrácie, (5) štandardná chyba krížového overovania, (6) koeficient determinácie, je kvadrátom korelačného koeficientu, (7) vyjadrenie proporcie referenčnej metódy krížového overovania predpokladanej hodnoty, (8, 9) nedegradované N-látky krmiva skutočne stráviteľné v tenkom čreve

Table 3 Quality parameters for the validation file VFORAGE.CAL

Variable (1)	Samples number (2)	Mean (LAB) in g.kg^{-1} (3)	Mean (NIRS) in g.kg^{-1} (4)	SEC in g.kg^{-1} (5)	BIAS (6)	SLOPE (7)	RSQ (8)
PDIE (9)	20	97.81	97.70	1.96	0.11	1.01	0.88
PDIN (10)	20	114.98	115.41	2.35	-0.43	1.01	0.99

Tabuľka 3 Kvalitatívne parametere rovnice z validačného súboru VRMIVO. CAL
(1) premenná, (2) počet vzoriek, (3) stredná hodnota (priemer) vypočítaná z laboratórnych výsledkov metódy podľa Kjeldahla, (4) stredná hodnota (priemer) vypočítaná z výsledkov z NIRS analyzátora, (5) štandardná chyba kalibrácie, (6) systematická chyba, (7) sklon smernice priamky, (8) koeficient determinácie, (9, 10) nedegradované N-látky krmiva skutočne stráviteľné v tenkom čreve

calibration equation FORAGE.EQA required to determine forage quality from herbage samples. The quality of this calibration equation was investigated by application of the methods of linear regression at these parameters: standard error of calibration (SEC), correlation coefficient – proportion of explained variance (RSQ), standard error of cross validation (SECV) and proportion of reference method variation explained by cross validation predicted values (1-RV).

Results and discussion

The calibration equation for determination of forage quality was developed by correlation between the results of reference laboratory methods and the NIRS spectra using MPLS. The quality parameters for the equation (mean, SEC, SECV, 1-VR, number of samples) are given in Table 2.

When 5 distant samples were excluded, mean PDIE was 99.05 g kg^{-1}, RSQ increased to 0.91, SEC was 1.93 g kg^{-1}, SECV was 2.60 g kg^{-1} and 1-VR was 0.84. After excluding 2 distant samples, mean PDIN was115.46 g kg^{-1}, SEC was 2.09 g kg^{-1}, RSQ was 0.99, SECV was 2.52 g kg^{-1} and 1-VR was 0.99. A comparison between the NIRS analytical method and the LAB method by ANOVA showed that the differences in PDIE and PDIN were not significant at the levels $P < 0.05$ and $P < 0.01$, respectively. The relationship between the methods was linear ($P_v = 0.917$). A test validation file VFORAGE.CAL was created with 20 samples randomly taken from the laboratory database of samples (taken also from other experimental sites) that had been analysed by NIRS as well as by reference methods and then determined by the FORAGE.EQA equation. A difference was found between the data obtained by the reference methods and those measured by NIRS. The quality parameters (means, SEP, BIAS, SLOPE, RSQ, number of samples) are given in Table 3.

The prediction determines optimum parameter values. At PDIE content prediction, the standard error was ± 1.96 g kg^{-1}. The line slope was 1.01 and RSQ was 0.88 at means of LAB file 97.81 g kg^{-1} and NIRS file 97.70 g kg^{-1}, respectively. The differences in PDIE ranged between -3.77 and 3.05 g kg^{-1}. At PDIN content prediction, the standard error was 2.35 g kg^{-1}, mean was 114.98 g kg^{-1} in LAB file and 115.41 g kg^{-1} in NIRS file. The standard error of difference was 1.25 g kg^{-1}. The line slope was 1.01 and RSQ was 0.99. The differences in PDIN content between the LAB and NIRS sample files ranged from – 6.99 to 2.59 g kg^{-1}.

Conclusions

By comparison between the grassland types, the highest content of the investigated parameters was found in PG but the standard error was much higher than the one recorded at OG and TG. The assessment of PDI values at the range of fertiliser treatments showed the lowest standard error at $P_{30}K_{60}$. Higher standard errors were found at all the N-fertiliser application treatments and the highest value was recorded at N180PK treatment.

From this study it is concluded that NIRS provides rapid analyses of forage quality at a large number of samples and can be used for prediction of protein digestible in intestine in practical forage evaluation. However, this would not be possible without the current development of chemometric methods and the existing efficient computers allowing for statistical assessments of collected spectra in correlation with analytical, chemical and physical properties of the samples analysed.

Súhrn

Koreláciou výsledkov z laboratórnych referenčných metód a NIR spektier vzoriek metódou lineárnej regresie MPLS sme vytvorili vyhodnocovací model, tzv. kalibračnú rovnicu FORAGE.EQA pre analýzu ukazovateľov PDIE a PDIN objemových krmív z trávnych porastov. Parametre kvality kalibrácie sú dané nízkymi odchýlkami predikcie SEP, SED, vysokým RSQ (blízky 1,0) a smernice priamky SLOPE (blízkej 1,0). Korelačný koeficient PDIN mali veľmi silnú závislosť (0,99) a silnú závislosť pre ukazovateľ PDIE (0,91). Hodnoty SECV ukazovateľov PDIE a PDIN sú do 10%, takže kalibráciu možno označiť ako veľmi spoľahlivú. Chemometrickými metódami zo softvéru NIRS sme overili kvalitu kalibračnej rovnice na validačnom súbore vzoriek. Na základe výsledkov môžeme konštatovať, že ukazovatele PDIE, PDIN spĺňali kritériá parametrov lineárnej regresie. V práci sme zistili, že v porastoch TTP boli najvyššie

hodnoty aritmetického priemeru PDIE a PDIN, ale s najvyššími hodnotami smerodajnej odchýlky, oproti porastom PTP a DTP, ktoré sa vyznačovali nižšími hodnotami smerodajnej odchýlky. Teda typ TTP hoci poskytuje vysoké hodnoty aritmetického priemeru obsahu PDIE a PDIN, neposkytuje ich stabilné hodnoty. Dočasné a prisievané trávne porasty sa oproti poloprírodným trávnym porastom vyznačujú vyrovnanejším obsahom PDIE a PDIN, teda sú perspektívnejšie vo využití v krmivovej základni. Pri hodnotení ukazovateľov PDIE a PDIN vo variantoch s diferencovaným hnojením boli najnižšie hodnoty smerodajnej odchýlky prevažne v porastoch hnojených $P_{30}K_{60}$ a naopak najvyššie hodnoty boli vo variantoch s dusíkatým hnojením najmä však vo variante N180PK. Dusíkaté hnojenie N180PK výrazne vplýva na zvýšenie obsahu PDIN a PDIE.

Kľúčové slová: krmivo, NIRS, PDIE, PDIN, trávne porasty

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