PREDICTION OF CHEMICAL COMPOSITION AND ENERGY VALUE OF GRASS SILAGE BY NEAR-INFRARED REFLECTANCE SPECTROSCOPY

NAPOVEDOVANJE KEMIČNE SESTAVE IN ENERGIJSKE VREDNOSTI TRAVNE SILAŽE Z BLIŽNJO INFRARDEČO REFLEKSIJSKO SPEKTROSKOPIJO

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ABSTRACT

One hundred and eighteen grass silage samples with known chemical composition and in vitro determined concentration of net energy for lactation (NEL) were scanned over the wavelength range from 1100 to 2500 nm at 8 nm intervals. Calibration equations for the prediction of crude protein (CP), crude fibre (CF), crude fat (F), crude ash (A), dry matter of air-dried samples (DM) and NEL were developed by the use of principal component analysis (PCA) and modified partial least squares regression technique (mPLS). NIRS demonstrated high predictive ability for CP (R^2 = 0.97), CF (R^2 = 0.96) and A (R^2 = 0.94). Moderate accuracy was characteristic for F and DM (R^2 = 0.81 and 0.79). Crude protein, F and DM deviations from reference methods were comparable to those which are expected by the use of the same reference methods in different laboratories. The determination coefficient for in vitro assessed NEL concentration was 0.76. Seventy-seven percent of samples lied within acceptable limits of ± 0.3 MJ NEL kg^{-1}DM. Suboptimal sample distribution, i.e. small number of samples in classes below 4.6 and above 6.0 MJ NEL kg^{-1}DM was observed. It seems that deviations of NIRS predicted values from the reference values were related to the concentration of NEL. It was concluded that NIRS shows the potential for reliable determination of chemical composition and energy value of grass silage.

Key words: grass silage/ net energy for lactation/ chemical composition/ NIRS

IZVLEČEK

Stoosemnajstim vzorcem travne silaže z znano kemično sestavo in in vitro določeno vsebnostjo neto energije za laktacijo (NEL) smo v valovnem območju med 1100 in 2500 nm na vsakih 8 nm izmerili spektre odbite bližnje infrardeče svetlobe. S pomočjo analize glavnih komponent (PCA) in regresijske metode modificiranih delnih najmanjših kvadratov (mPLS) smo razvili umeritvene enačbe za napovedovanje vsebnosti surovih beljakovin (SB), surove vlaknine (SVl), surovih maščob (M), surovega pepela (P), suhe snovi zračno suhih vzorcev (DM) in NEL. Metoda NIRS je bila zelo dobra pri napovedovanju SB (R^2 = 0,97), SVl (R^2 = 0,96) in A (R^2 = 0,94). Za M in DM je bila značilna zmerna točnost (R^2 = 0,81 in 0,79). Pri SB, M in DM so bila odstopanja od referenčnih metod primerljiva z odstopanji, ki jih lahko pričakujemo pri izvajanju istih referenčnih metod v različnih laboratorijih. Determinacijski koeficient za in vitro ocenjeno koncentracijo NEL je znašal 0,76. Sedeminsedemdeset odstotkov vzorcev je ležalo znotraj sprejemljivih meja ± 0,3 MJ NEL kg^{-1}SS. Za vzorce travnih silaž je bila značilna ne-optimalna porazdelitev vzorcev, t.j. majhno število vzorcev v razredih pod 4,6 in nad 6,0 MJ NEL kg^{-1}SS. Izgleda, da so odstopanja med NIRS ocenjenimi vrednostmi in referenčnimi vrednostmi povezana z vsebnostjo NEL. Sklenili smo, da je z NIRS metodo mogoče zanesljivo oceniti kemično sestavo in energijsko vrednost travne silaže.

Ključne besede: travna silaža/ neto energija za laktacijo/ kemična sestava/ NIRS

POVZETEK

Namen raziskave je bil preučiti zanesljivost metode merjenja odboja bližnje infrardeče svetlobe (NIRS) pri napovedovanju kemične sestave in energijske vrednosti travne silaže. Stoosemnajstim vzorcem travne silaže z znano kemično sestavo in in vitro ocenjeno vsebnostjo neto energije za laktacijo (NEL) smo izmerili spektre odbite bližnje infrardeče svetlobe. Odbito bližnjo infrardečo svetlobo vzorcev smo merili v 8 nm intervalih v razponu valovnih dolžin od 1100 do 2500 nm. S pomočjo analize glavnih komponent (PCA) smo število spektralnih spremenljivk zmanjšali na neodvisne vire variabilnosti in jih pretvorili v številsko obliko. S pomočjo regresijske metode modificiranih delnih najmanjših kvadratov (mPLS) smo razvili umeritvene enačbe za ocenjevanje kemične sestave in neto energije za laktacijo (NEL).

Vzorci travnih silaž so se v sestavi in vsebnosti NEL med seboj zelo razlikovali (preglednica 1). Ocenjujemo, da smo z vzorci pokrili razpon, ki je značilen za travno silažo s slovenskih kmetij. Največjo točnost umeritvenih enačb smo dosegli pri SB (R^2= 0,97), SVl (R^2= 0,96) in P (R^2= 0,94), nekoliko slabšo pa pri M in DM (R^2= 0,81 in 0,79) (preglednica 2). Koeficient determinacije umeritvene enačbe za in vitro ocenjeno vsebnost NEL je bil 0,76, kar je slabše od literaturnih podatkov za lastnosti, ki so povezane z vsebnostjo NEL (in vivo ter in vitro prebavljivost).

Zanesljivost umeritvenih enačb smo testirali na vzorcih, ki so bili na podlagi Mahalanobisove razdalje med sosednjimi vzorci (NH<0,6) izločeni iz postopka umerjanja NIRS analizatorja. Za kazalnike zanesljivosti smo uporabili meje ponovljivosti (RL_{RM}) in sprejemljive meje (AL) (Preglednica 3). Približno 50 % z NIRS ocenjenimi vrednosti za surovo vlaknino in surove maščobe ter 65,4 % za suho snov zračno suhih vzorcev je bilo znotraj RL_{RM}. Pri surovih beljakovinah in surovem pepelu je bilo takih vrednosti okrog 30 %. Glede na sprejemljive meje (AL) je bilo pri surovih beljakovinah, surovih maščobah in suhi snovi zračno suhih vzorcev znotraj AL več kot 95 % vrednosti. Pri surovi vlaknini je bilo znotraj sprejemljivih meja 88,5 % vzorcev, pri pepelu pa samo 50,0 %.

Ugotovili smo, da je znašalo povprečno individualno odstopanje med NIRS ocenjenimi vsebnostmi NEL in in vitro ocenjenimi vrednostmi 0,17 MJ kg^{-1} SS, največje odstopanje pa je bilo 0,61 MJ kg^{-1}SS. Več kot tri četrtine (76,9 %) vzorcev travnih silaž je odstopalo od in vitro ocenjenih vrednosti za manj kot 0,3 MJ NEL kg^{-1} SS.

Na podlagi Mahalanobisove razdalje je bilo za umerjanje NIRS analizatorja od 118 vzorcev izbranih le 92. Ugotovili smo, da porazdelitev vzorcev glede vsebnosti NEL ni bila enakomerna. V razredih pod 4,6 MJ in nad 6,0 MJ NEL kg^{-1} SS se je nahajalo premalo vzorcev (grafikon 1). Analiza odstopanj z NIRS ocenjenih vrednosti od referenčnih vrednosti po razredih je pokazala, da je majhno število vzorcev v teh razredih vplivalo na zanesljivost ocenjevanja vsebnosti NEL z NIRS metodo (grafikon 2).

Sklenili smo, da so rezultati sicer dobri, da pa bi lahko z načrtnim dodajanjem manjkajočih vzorcev travnih silaž te rezultate še izboljšali.

INTRODUCTION

Reliable assessment of the nutritive value of forages is a prerequisite for quantitative approach in ruminant nutrition. In Slovenia, the energy value of feeds for dairy cattle is usually expressed in terms of net energy for lactation (NEL). Two thirds of the total agricultural area in the country is covered by grassland [11] and therefore hay and grass silage are among the most important components of ruminant diets. Concentration of net energy for lactation in forages can be estimated on the basis of in vivo or in vitro digestibility trials. However, these methods are costly, time consuming and laborious. Experimental animals are also needed but they can not be used in every-day praxis. For a long period, NEL in feeds has been estimated on the basis of chemical composition and digestibility coefficients from tables. However, the accuracy of this method is questionable. For the mentioned method Babnik and Verbič [2] reported that the average individual deviation from in vitro estimated values of NEL was 0.53 MJ NEL kg^{-1} while the maximum individual deviation may be as high as 1.36 MJ NEL kg^{-1} DM.

In the last decades, near infrared reflectance spectroscopy (NIRS) has been successfully used for the estimation of chemical composition and nutritive value of grass silage [9, 10, 13]. The aim of the present study was to examine the reliability of NIRS to predict chemical composition and in vitro assessed NEL of grass silage samples in Slovenia. A wide range of samples, from intensively managed artificial meadows to late cut permanent meadows, was covered.

MATERIAL AND METHODS

Chemical composition and estimation of net energy for lactation (NEL)

One hundred and eighteen grass silage samples were collected during the period of four years. All samples were dried in a ventilated oven at 60 °C and ground with Brabender mill to pass 1 mm screen. Analyses of crude

protein (CP), crude fibre (CF), crude ash (A), dry matter of air-dried samples (DM) and crude fat (F) were done according to the methods described in the Methodenbuch [7]. Samples were also tested with the in vitro Hohenheim gas test [6] using a modified method [3]. Two hundred milligrams of sample was weighed in graduated 100 ml glass syringes. Thirty ml of inoculum (rumen liquor and artificial anaerobic saliva) was added to each syringe and incubated in water bath at 39°C for 24 hours. Gas volume for each sample was measured in triplicates. Deviations caused by rumen liquor activity were corrected according to the standard hay sample which was provided by the University of Hohenheim. Net energy for lactation (NEL, in MJ kg^{-1} DM) was calculated on the basis of corrected gas volume produced in 24 hours (GV_{24}), F and CF (both in g kg^{-1} DM) using a regression equation from literature [1].

$$NEL = 2.88 + 0.0754 \times GV_{24} + 0.000398 \times F \times F - 0.00340 \times CF$$

Near-infrared reflectance spectroscopy (NIRS)

Samples were scanned in quarter rectangular cups with NIRSystems 6500 Monochromator (Foss NIRSystem, Silver Spring, MD). The system operated with software equipment (WinISI – version 1.50) in the range from 1100 to 2500 nm at 8 nm intervals to give 173 data points for each sample spectrum. Data points were recorded as absorbance values (log (1/R); where R means reflectance). Principal component analysis (PCA) method was used to reduce spectral data to independent sources of variation and converted to scores to replace the spectra. At the same time samples with atypical spectral characteristics were eliminated using the CENTER algorithm which eliminates samples with the Mahalanobis distance (GH) bigger than 3.0 from the mean of the file. Samples with Mahalanobis distance (NH) smaller than 0.6 between neighbours were considered to add no spectral variation to the population. Therefore, they were also eliminated. SELECT algorithm was used for this purpose. Calibration equations were developed by the use of modified partial least squares (mPLS) regression technique.

Assessment of the precision of the NIRS method

Reliability of NIRS method was tested on samples which were excluded from the calibration set on the basis of Mahalanobis distance between neighbours (NH< 0.6). Results were compared to the reference values which were obtained using wet chemistry and in vitro method. Average individual deviation (AID) of predicted value from reference value was calculated as ($AID=\sum|d_i|/n$) where d_i is individual deviation of predicted value from reference value. Deviations of NIRS results from reference values were also compared to repeatability limits of reference methods (RL_{RM}). Adequate standards require that the absolute difference between two independent results obtained using the same reference method on identical test material in the same laboratory by the same operator using the same equipment within a short time should in not more than 5 % of cases exceed RL_{RM}. For CP, RL_{RM} is 2.28 g kg^{-1} + 0.0147 CP (ISO 5983:1997(E)), for CF it is 3 % of individual reference value (73/46/EEC), for crude fat it is 2 g kg^{-1} (for samples containing less than 50 g of crude fat kg^{-1}; 98/64/EC) and for ash it is 5 g kg^{-1} (for samples containing from 50 to 200 g of crude ash kg^{-1}; ISO 5984-1978 (E)). Acceptable limits (AL) were defined as reproducibility limits which refer to independent results obtained using the same reference method on identical test material in different laboratories by different operators using different equipment. Standards require that the absolute difference between two single test results should in not more than 5 % of cases exceed reproducibility limit. Reproducibility limit for CP is 12.8 g kg^{-1} + 0.0361 CP (ISO 5983:1997(E)). As reproducibility limits for other constituents of forages are not available they were estimated on the basis of ratio between reproducibility and repeatability limits for available feedstuffs (ISO 6865: 2000(E), ISO 6492: 1999(E), ISO 5984: 2002(E)) and repeatability defined in EU directives for crude fibre and fat (73/46/EEC, 98/64/EC) or standard for ash (ISO 5984-1978 (E)). AL for NEL concentration (± 0.3 MJ) is our own estimate.

RESULTS AND DISCUSSION

Chemical composition and the concentration of net energy for lactation

Chemical composition and NEL concentration of grass silage samples is presented in Table 1. A wide range in chemical composition and NEL concentration was covered and it can be considered that samples covered the range typical for samples from Slovenian farms [12].

Accuracy of calibration equations

Determination coefficients (R^2), standard errors of calibration (SEC) and standard errors of cross validation (SECV) for calibration equations are presented in Table 2. The highest R^2 values (0.94-0.97) were achieved with calibration equations for CP, CF and A. The values are similar to data from literature [4, 10]. Lower accuracy of NIRS prediction was obtained for F (R^2= 0.81). The reports on the prediction of F concentration in grass silage show variable results. Park and co-workers [10] reported that R^2 for the prediction of F concentration in undried grass silage samples was 0.88. In the American study [5] R^2 for legume-grass silages was less than 0.75. Considerably lower R^2 (0.56 and 0.57) for dried

Table 1: Chemical composition (g kg^{-1}DM) and concentration of net energy for lactation (NEL, MJ kg^{-1}DM) in samples of grass silage
(n = 118)
Preglednica 1:Kemična sestava (g kg^{-1}SS) in koncentracija neto energije za laktacijo (NEL, MJ kg^{-1}SS) v vzorcih travnih silaž (n = 118)

	Mean ± SE Sredina ± SE	Minimum Najmanj	Maximum Največ
DM / SS (g/kg)	961 ± 11	912	980
CP / SB	150 ± 30	93	222
CF / SVl	281 ± 40	183	386
F / M	32.8 ± 7.7	16.5	52.3
A / P	111.2 ± 41.5	48	290
NEL	5.4 ± 0.55	4.06	6.87

ABREVIATIONS: DM – dry matter of air-dried samples; CP – crude protein; CF – crude fibre; F – crude fat; A – crude ash; NEL – net energy for lactation; SE – standard error
OKRAJŠAVE: SS – suha snov zračno suhih vzorcev; SB – surove beljakovine; SVl – surova vlaknina; M –surove maščobe; P – surovi pepel; NEL – neto energija za laktacijo; SE – standardna napaka

Table 2: Accuracy of the NIRS calibration equations *
Preglednica 2: Točnost umeritvenih enačb za NIRS *

	R^2	SEC	SECV
DM / SS (g/kg)	0.79	5.3	7.5
CP / SB	0.97	5.6	9.0
CF / SVl	0.96	7.9	10.8
F / M	0.81	3.6	4.7
A / P	0.94	10.4	14.2
NEL	0.76	0.27	0.33

* Calibration equations were developed on the basis of representative samples which were selected on the basis of their spectral characteristics. Only the samples with the Mahalanobis distance smaller than 3.0 from the mean (GH) and bigger than 0.6 between neighbours (NH) were included. The number of selected samples was 92.
* Umeritvene enačbe so bile razvite na podlagi reprezentativnih vzorcev, ki so bili izbrani na podlagi značilnosti njihovih spektrov. Vključeni so bili le vzorci z Mahalanobisovo razdaljo manjšo od 3,0 od povprečja (GH) in večjo od 0,6 med sosednjimi vzorci (NH). Število izbranih vzorcev je bilo 92.
ABREVIATIONS: R^2 – coefficient of determination; SEC – standard error of calibration; SECV – standard error of cross validation; other abbreviations are explained in Table 1.
OKRAJŠAVE: R^2 – koeficient determinacije; SEC – standardna napaka umeritve; SECV – standardna napaka navzkrižnega preverjanja; ostale okrajšave so razložene v preglednici 1.

Table 3: Indicators of reliability of NIRS method for prediction of chemical composition (in g kg^{-1} DM) and energy value (in MJ kg^{-1} DM) of grass silage [a)]

Preglednica 3: Kazalci zanesljivosti metode NIRS za napovedovanje kemične sestave (v g kg^{-1}SS) in energijske vrednosti (v MJ kg^{-1}SS) travnih silaž[a)]

	AID	MID	RL_{RM}	RL_{RM} %	AL	AL %
DM / SS	5.6	-14.4	7.5	65.4	28.9	100.0
CP / SB	5.9	-19.0	4.4	30.8	18.0	96.1
CF / SVl	9.3	23.4	8.8	50.0	18.4	88.5
F / M	2.2	8.3	2.0	53.8	7.9	96.1
A / P	12.1	-31.9	5.0	30.8	10.1	50.0
NEL	0.17	-0.61	/	/	0.30	76.9

[a)]Indicators of reliability are based on samples which were on the basis of small Mahalanobis distance between neighbours excluded from calibration procedure (n= 26)

[a)]Kazalci zanesljivosti temeljijo na vzorcih, ki so bili na podlagi majhne Mahalanobisove razdalje med sosednjimi vzorci izločeni iz postopka kalibracije (n= 26)

ABBREVIATIONS:

AID – average individual deviation of predicted value from reference value; **MID** – maximal individual deviation of predicted value from reference value; **RL_{RM}** - repeatability limits of reference methods; **RL_{RM} %** - percentage of samples within repeatability limits of reference method; **AL** – acceptable limits; **AL %** - percentage of samples within acceptable limits; other abbreviations are explained in Table 1.

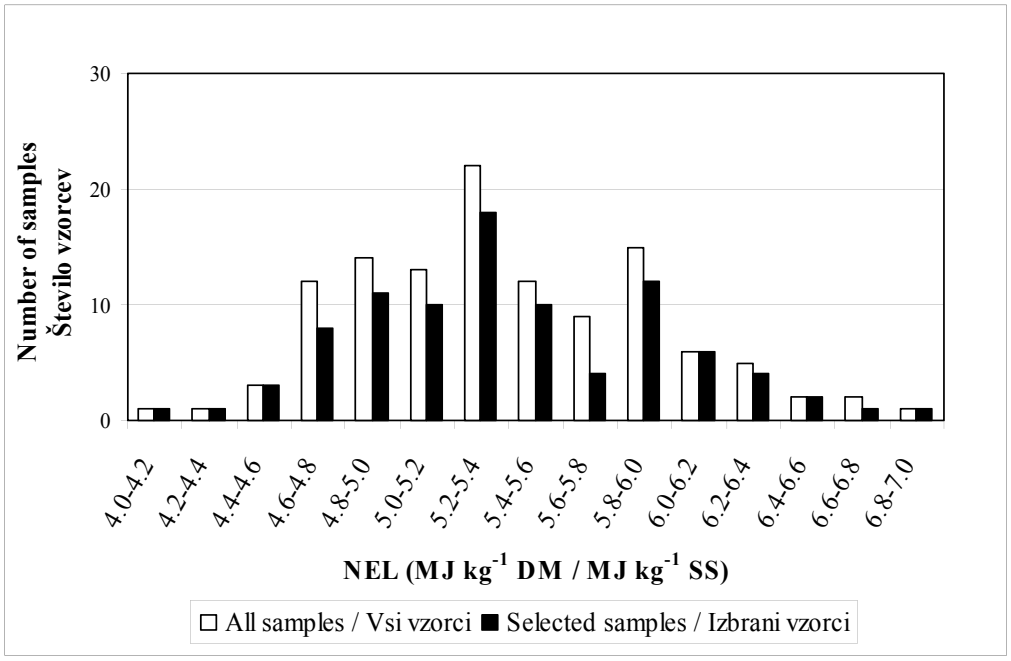
OKRAJŠAVE:

AID – povprečno individualno odstopanje napovedane vrednosti od referenčne vrednosti; **MID** – največje individualno odstopanje napovedane vrednosti od referenčne vrednosti; **RL_{RM}** - meje ponovljivosti referenčnih metod; **RL_{RM} %** - odstotek vzorcev znotraj mej ponovljivosti referenčne metode; **AL** – sprejemljive meje; **AL %** - odstotek vzorcev znotraj sprejemljivih mej; ostale okrajšave so razložene v preglednici 1.

samples of grass silage were reported by De Boever et al. [4]. Authors mentioned that relatively low prediction accuracy for F in this study [4] could be associated with the change in extraction method and heterogeneous nature of F in grassland forages.

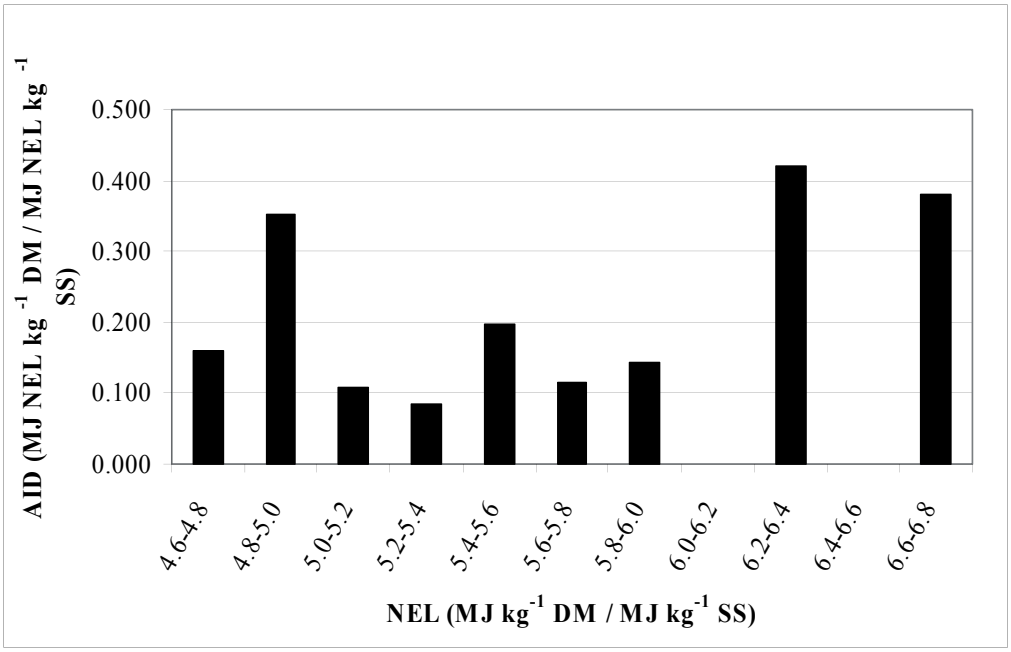
The determination coefficient of the equation for the prediction of NEL concentration was 0.76. The value is lower than the values reported for the prediction of in vivo digestibility of grass silage (0.87 and 0.88) [8, 9]. Lower determination coefficient could be caused by soil contamination or relatively small number of grass silage samples included in the procedure of calibration equation development. Nevertheless, determination coefficients obtained in this study were significantly higher than R^2 between in vitro assessed concentrations of NEL and concentrations, which were estimated on the basis of digestibility coefficients from tables or published equations (from 0.29 to 0.49) [2].

Results of NIRS tested on samples which were not included in calibration procedure are presented in Table 3. In case of F and DM more than 50 % of samples lied within repeatability limits of reference methods (RL_{RM}) (53.8 and 65.4 %). Similar results were achieved for the prediction of CF where exactly 50 % of samples fulfilled this criterion. For the prediction of CP and A lower percentage (30.8 and 30.8 %) of samples met this requirement. Repeatability limits are referred to the results obtained using the same reference method in the same laboratory by the same operator using the same equipment. In our case where we compare NIRS results with the results of wet chemistry, the method, equipment and personnel were not the same and therefore these criteria are quite strong. Acceptable limits (AL) are a much better indicator of the quality of the results. They are higher and referred to the results obtained using the same reference method in different laboratories by different operators using different equipment. It is required that the difference between two results should in not more than 5 % of cases exceed AL. Although in our case the method was not the same, these requirements were fully met for the prediction of DM, CP and F (100, 96.1 and 96.1 %). For the prediction of CF content 88.5 % of samples met the criteria. The minimal percent of samples that lied within acceptable limits (50 %) was obtained for the prediction of A content. This shows that



Graph 1: Distribution of all samples and samples which were selected for NIRS calibration with respect to NEL concentration

Grafikon 1: Porazdelitev vseh vzorcev in vzorcev, ki so bili izbrani za umerjanje NIRS glede na vsebnost NEL



Graph 2: Average individual deviations of predicted NEL concentrations from reference values with respect to NEL concentration

Grafikon 2:Povprečna individualna odstopanja napovedanih vsebnosti NEL od referenčnih vrednosti glede na vsebnost NEL

the prediction of A content in grass silage samples could be problematic.

Predicted NEL values deviate from the in vitro determined values for 0.17 MJ kg^{-1} DM on the average (Table 3). The maximal deviation was 0.61 MJ kg^{-1} DM. Both values were much lower than corresponding values for predictions based on digestibility coefficients from DLG tables (0.53 and 1.36 MJ NEL kg^{-1} DM) or GfE equations (0.27 and 1.59 MJ NEL kg^{-1} DM) [2]. Almost seventy-seven (76.9) percent of samples lied within acceptable limits of ± 0.3 MJ NEL kg^{-1} DM (Table 3). The results suggest a comparative advantage of NIRS method over the estimates based on chemical composition and equations from literature or digestibility coefficients from tables.

Distribution of samples which were included in calibration equation and accuracy of prediction with regard to NEL concentration

Based on Mahalanobis distance 92 from the total of 118 grass silage samples were selected to develop calibration equations. In comparison with hay samples [14] the variability of grass silage samples was bigger. The distribution of grass silage samples was far from being optimal. A great deal of samples is missing, especially in the ranges below 4.6 MJ and above 6.0 MJ NEL kg^{-1} DM. It seems that suboptimal distribution pattern considerably affects the reliability of NEL predictions within individual classes (Graph 2). Average individual deviations (AID) of predicted values from reference values for individual classes with small number of samples (6.2-6.4; 6.6-6.8 MJ NEL kg^{-1} DM) ranged between 0.35 and 0.40 MJ NEL kg^{-1} DM while in classes with higher number of samples (5.0-6.0 MJ NEL kg^{-1} DM) they were considerably lower. Some care is needed in the interpretation of these data because the indicators of reliability are based only on 26 samples which were not included in the calibration set.

CONCLUSIONS

It may be concluded that NIRS is a rapid and reliable technique for determination of crude protein and crude fat content in grass silage samples. In both cases the deviations from the reference method were considerably lower than those required for the same reference method in different laboratories. Also, the results for crude fibre are close to that requirement. In the case of crude ash deviations between NIRS and reference method were considerably higher. This indicates that the prediction of ash concentration in grass silage by means of NIRS could be problematic. NIRS shows high ability to predict the in vitro assessed NEL concentration ($R^2 = 0.76$). Seventy-seven percent of samples lied within acceptable limits of ± 0.3 MJ NEL kg^{-1} DM. For better results more equally distributed samples of grass silage should be included in the calibration.

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