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PREDICTION OF CHEMICAL COMPOSITION AND ENERGY VALUE OF HAY BY NEAR-INFRARED REFLECTANCE SPECTROSCOPY (NIRS)

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ABSTRACT

One hundred and fifty-eight hay 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 dry matter (DM), crude protein (CP), crude fibre (CF), crude fat, ash and NEL were developed by the use of principal component analysis. NIRS demonstrated high predictive ability for CP (R^2 = 0.98), CF (R^2 = 0.95) and ash (R^2 = 0.94). Only moderate accuracy was characteristic for DM (0.87) and crude fat (R^2 = 0.75). With exception of ash deviations from reference methods are comparable to those which are expected by the use of the same reference methods in different laboratories. NIRS has a high ability to predict *in vitro* assessed NEL concentration (R^2 = 0.89). More than 95% of samples lied within acceptable limits of ± 0.3 MJ NEL kg^{-1} dry matter (DM). Despite suboptimal sample distribution, i.e. small number of samples in classes below 4.4 and above 5.6 MJ NEL kg^{-1} DM, the deviations of NIRS predicted values from reference values were not related to concentration of NEL. It was concluded that NIRS is rapid and reliable technique for determination of chemical composition and energy value of hay.

Key words: feed / hay/ net energy for lactation/ chemical composition/ NIRS

NAPOVEDOVANJE KEMIČNE SESTAVE IN ENERGIJSKE VREDNOSTI MRVE Z BLIŽNJO INFRARDEČO REFLEKSIJSKO SPEKTROSKOPIJO (NIRS)

IZVLEČEK

Stooseminpetdesetim vzorcem mrve z znano kemično sestavo in *in vitro* določeno vsebnostjo neto energije za laktacijo (NEL) smo na vsakih 8 nm izmerili spektre reflektirane bližnje infrardeče svetlobe v valovnem območju med 1100 in 2500 nm. S pomočjo analize glavnih component smo razvili umeritvene enačbe za napovedovanje vsebnosti suhe snovi (SS), surovih beljakovin (SB), surove vlaknine (SVl), surovih maščob, pepela in NEL. Metoda NIRS je bila zelo dobra pri napovedovanju SB (R^2 = 0,98), SVl (R^2 = 0,95) in pepela (R^2 = 0,94). Za surove maščobe in SS je bila značilna zmerna točnost (0,75 in 0,87). Z izjemo pepela so bila odstopanja od referenčnih metod primerljiva z odstopanji, ki jih lahko pričakujemo pri izvajanju istih referenčnih metod v različnih laboratorijih. Metoda NIRS je bila zelo dobra tudi pri napovedovanju *in vitro* ocenjene vsebnosti NEL (R^2 = 0,89). Več kot 95 % vzorcev je ležalo znotraj sprejemljivih meja ± 0,3 MJ NEL kg^{-1} sušine (SS). Kljub neoptimalni porazdelitvi vzorcev, t.j. majhnemu številu vzorcev v razredih pod 4,4 in nad 5,6 MJ NEL kg^{-1} SS, odstopanja z NIRS ocenjenih vrednosti od referenčih vrednosti niso bila povezana z vsebnostjo NEL. Sklenili smo, da je NIRS hitra in zanesljiva metoda za ocenjevanje kemične sestave in energijske vrednosti mrve.

Ključne besede: krma / mrva/ neto energija za laktacijo/ kemična sestava/ NIRS

INTRODUCTION

The nutritive value of forages can be estimated on the basis of *in vivo* or *in vitro* digestibility trials or on the basis of chemical composition and digestibility coefficients from tables. All this methods have several drawbacks. *In vivo* digestibility methods can not be used in every-day praxis because experimental animals and large quantities of forages are needed. Besides, they are laborious, time-consuming and costly. The time and quantity of forage required for determination of digestibility can be reduced by introduction of *in vitro* methods (Tilley and Terry, 1963; Menke *et al.*, 1979). However, the problem of experimental animals remains. For a long time, nutritive value has been estimated on the basis of chemical composition and digestibility coefficients from tables. The main deficiency of this method is inaccuracy. Both, systematic error due to inadequacy of tables as well as unexplained deviations from *in vivo* or *in vitro* assessed NEL concentration were observed in samples of fresh forages, hays and silages (Babnik and Verbič, 2000; Babnik *et al.*, 2001, 2002). There is a substantial need for fast, accurate and simple methods for the estimation of nutritive value of forages. In recent years, the use of near infrared reflectance spectroscopy (NIRS) has expanded dramatically. To relate NIRS optical measurements to the chemical composition or nutritive value of forages the process of calibration has to be carried out. The aim of the present study was to examine the reliability of NIRS to predict chemical composition and energy value of hay.

MATERIAL AND METHODS

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

One hundred and fifty-eight hay samples were collected during the period of four years. All samples were dried in a ventilated oven at 60 °C and ground with the Brabender mill to pass 1 mm screen. Analyses of moisture, crude protein, crude fibre, crude ash and crude fat were done according to the methods described by Naumann and Bassler (1976). Samples were also tested with the *in vitro* Hohenheim gas test (Menke *et al.*, 1979) using the modified method as described by Blümmel and Ørskov (1993). About 200 mg of sample was exactly 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}), crude fat and crude fibre (CF) (both in g kg^{-1} DM) using the regression equation proposed by Aiple *et al.* (1995) (equation [1]).

$$NEL = 2.88 + 0.0754 \times GV_{24} + 0.000398 \times \text{crude fat} \times \text{crude fat} - 0.00340 \times CF \qquad [1]$$

Near-infrared reflectance spectroscopy (NIRS)

Samples were scanned in full rectangular cups with NIRSystems 6500 Monochromator (Foss NIRSystem, Silver Spring, MD). The system operated with software equipment (WinISI – version 1.50) in the region 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, which are considered to add no spectral variation to the population, were also eliminated. SELECT algorithm was used for this purpose.

RESULTS AND DISCUSSION

Chemical composition and concentration of net energy for lactation

Chemical composition and energy concentration in forage 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.

Table 1. Chemical composition (g kg^{-1} DM) and concentration of net energy for lactation (NEL, MJ kg^{-1} DM) in samples of hay (n = 158)
Preglednica 1. Kemična sestava (g kg^{-1} SS) in koncentracija neto energije za laktacijo (NEL, MJ kg^{-1} SS) v vzorcih mrve (n = 158)

	Mean ± SE Sredina ± SE	Minimum Najmanj	Maximum Največ
Dry matter Suha snov	966 ± 14	916	983
Crude protein Surove beljakovine	111 ± 28	56	200
Crude fibre Surova vlaknina	312 ± 37	216	390
Crude fat Surove maščobe	20.9 ± 6.4	6.1	36.5
Ash Pepel	78.0 ± 19.2	30.6	154.8
NEL	4.97 ± 0.41	3.87	5.98

SE – standard error/ standardna napaka; NEL – net energy for lactation/ neto energija za laktacijo

Accuracy of calibration equations

Determination coefficients (R^2), standard errors of calibration (SEC) and standard errors of cross validation (SECV) of calibration equations are presented in Table 2. The highest accuracy of calibration equations was achieved for CP, CF and ash which have the highest R^2 (0.94–0.98) and relatively low SEC and SECV values. Similar parameters of calibration equations for CP and CF were reported in the literature (Norris *et al.*, 1976; Herrero *et al.*, 1996; Bruno-Soares *et al.*, 1998; Park *et al.*, 1998; Lavrenčič *et al.*, 2001). In agreement with previous work (Berarado *et al.*, 1997; Bruno-Soares *et al.*, 1998; Park *et al.*, 1998) are also results for ash, although, somewhat lower R^2 were also reported (De Boever *et al.*, 1996; Lavrenčič *et al.*, 2001). The dry matter determination coefficient (R^2) was slightly lower (0.87). Considerably lower accuracy of NIRS prediction was recorded for crude fat where the R^2 value was only 0.75 (Table 2). Constituents that are present in small quantities are known to fall under the category with the lowest accuracy and crude fat in hay is undoubtedly one of them. The poor predictive power of NIRS for fat can be ascribed also to the heterogenous nature of crude fat in grassland forages (De

Boever *et al.*, 1996) which includes besides tryglicerides also waxes, pigments and other compounds (Kircgeßner, 1997). The reports on capacity of NIRS for prediction of crude fat are contradictory. Some studies (Berardo *et al.*, 1997, Lavrenčič *et al.*, 2001) reported significantly higher R^2 than in present study (0.97 and 0.85) while in others the R^2 was lower (0.56–0.57, De Boever *et al.*, 1996).

Determination coefficient of calibration equation for NEL was 0.89. The value is similar to literature data for other biological parameters of forages, like *in vivo* or *in vitro* digestibility (0.87 to 0.94; Norris *et al.*, 1976, Park *et al.*, 1997; Bruno-Soares *et al.*, 1998 and Lavrenčič *et al.*, 2001). Determination coefficients between *in vitro* assessed concentrations of NEL and concentrations, which were estimated on the basis of digestibilities from tables or published equations, were considerably lower (from 0.54 to 0.60, Babnik *et al.*, 2001).

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

	R^2	SEC	SECV
Dry matter Suha snov	0.87	4.6	6.1
Crude protein Surove beljakovine	0.98	3.6	4.7
Crude fibre Surova vlaknina	0.95	9.0	12.6
Crude fat Surove maščobe	0.75	3.1	3.6
Ash Pepel	0.94	4.7	6.4
NEL	0.89	0.15	0.17

* 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 and bigger than 0.6 between neighbours were included. The number of selected samples was 110.
* 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 in večjo od 0,6 med sosednjimi vzorci. Število izbranih vzorcev je bilo 110.
ABREVIATIONS: R^2 – coefficient of determination; SEC – standard error of calibration; SECV – standard error of cross validation
OKRAJŠAVE: R^2 – koeficient determinacije; SEC – standardna napaka umeritve; SECV – standardna napaka navzkrižnega preverjanja

Reliability of NIRS method was tested on samples which were on the basis of Mahalanobis distance excluded from the calibration set (Table 3). Data are presented as deviations of NIRS predictions from the reference values which were obtained using wet chemistry or *in vitro* method. With exception of crude fat, more than 50% of predictions lied within repeatability limits (RL %) of reference methods. We should have in our mind, that repeatability limits are referred to results obtained using the same reference method in the same laboratory by the same operator using the same equipment. They are usually used to test the duplicates and therefore the criteria are relatively strong. Acceptable limits (AL), which are in case of chemical constituents defined by reproducibility limits, are much higher. They are referred to differences obtained using the same reference method in different laboratories by different operators using different

equipment. The criteria is met if 95% of results lies within these limits (AL %) and it is obvious, that NIRS method meets the criteria for dry matter, crude protein and crude fat, while the value for crude fibre is close to the target value (Table 3). Having in mind that reproducibility limits are referred to the results obtained by the same method and that two different methods (wet chemistry and NIRS) were compared in present experiment, also the result for crude fibre can be considered as satisfactory. The criteria of acceptable limits for ash content were not fulfilled and this shows that the use of NIRS for ash determination is limited (De Boever in sod., 1996; Lavrenčič in sod., 2001) and more attention is needed.

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 hay [a)]

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

	AID	MID	RL	RL %	AL	AL %
Dry matter Suha snov	6.5	22.5	7.6	60.4	29.0	100
Crude protein Surove beljakovine	3.7	–21.7	3.9	64.6	16.8	97.9
Crude fibre Surova vlaknina	8.5	30.2	9.3	58.3	19.5	93.7
Crude fat Surove maščobe	3.1	–9.6	2.0	45.8	7.9	95.8
Ash Pepel	5.1	21.4	5.0	56.2	10.1	87.5
NEL	0.12	0.39	/	/	0.30	95.8

[a)]Indicators of reliability are based on samples which were on the basis of Mahalanobis distance excluded from calibration procedure (n= 48)

[a)]Kazalci zanesljivosti temeljijo na vzorcih, ki so bili na podlagi Mahalanobisove razdalje izločeni iz postopka kalibracije (n= 48)

ABBREVIATIONS: AID – average individual deviation of predicted value from reference value ($AID=\sum|d_i|/n$), where d_i is individual deviation of predicted value from reference value; MID – maximal individual deviation of predicted value from reference value; RL – Repeatability limits of reference methods. 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. For CP RL is 2.28 g kg^{-1} + 0.0147 CP (ISO 5983:1997(E)), for CF is 3% of individual reference value (73/46/EEC), for crude fat 2 g kg^{-1} (for samples containing less than 50 g of crude fat kg^{-1}; 98/64/EC) and for ash 5 g kg^{-1} (for samples containing from 50 to 200 g of crude ash kg^{-1}; ISO 5984–1978 (E)); RL % – percentage of samples within repeatability limits of reference method; AL – Acceptable limits. AL were defined as reproducibility limits. The absolute difference between two independent results obtained using the same reference method on identical test material in different laboratories by different operators using different equipment 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 repeatabilities 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 is our own estimate; AL % – percentage of samples within acceptable limits

OKRAJŠAVE: AID – povprečno individualno odstopanje napovedane vrednosti od referenčne vrednosti ($AID=\sum|d_i|/n$), kjer je d_i individualno odstopanje napovedane vrednosti od referenčne vrednosti; MID – največje individualno odstopanje napovedane vrednosti od referenčne vrednosti; RL – meje ponovljivosti referenčnih metod.

Absolutna razlika dveh neodvisnih rezultatov dobljenih z isto referenčno metodo na enakem preizkusnem materialu v istem laboratoriju z istim osebjem in opremo v kratkem času ne bi smela več kot v 5 % preseči RL. Za SB so RL 2,28 g kg^{-1} + 0,0147 SB (ISO 5983:1997(E)), za SVl so 3 % od posamezne referenčne vrednosti (73/46/EEC), za surove maščobe 2 g kg^{-1} (za vzorce ki vsebujejo manj kot 50 g of maščob kg^{-1}; 98/64/EC) in za pepel 5 g kg^{-1} (za vzorce, ki vsebujejo od 50 do 200 g pepela kg^{-1}, ISO 5984–1978 (E)); RL % – odstotek vzorcev znotraj mej ponovljivosti referenčne metode; AL – sprejemljive meje. AL so bile definirane kot meje obnovljivosti. Absolutna razlika dveh neodvisnih rezultatov dobljenih z isto referenčno metodo na enakem preizkusnem materialu v različnih laboratorijih z različnim osebjem in opremo ne bi smela več kot v 5 % preseči mej obnovljivosti. Meje obnovljivosti za SB so 12,8 g kg^{-1} + 0,0361 SB (ISO 5983:1997(E)). Ker meje obnovljivosti za druge sestavine voluminozne krme niso dostopne smo jih ocenili na podlagi razmerja med dostopnimi mejami obnovljivosti in ponovljivosti za druga krmila (ISO 6865: 2000(E), ISO 6492: 1999(E), ISO 5984: 2002(E)) in ponovljivosti, ki jih določajo smernice EU za surovo vlaknino in surove maščobe (73/46/EEC, 98/64/EC) oz. standard za pepel (ISO 5984–1978 (E)). AL za vsebnost NEL je naša ocena; AL % – odstotek vzorcev znotraj sprejemljivih meja

Predicted NEL values deviate from *in vitro* determined values on average for 0.12 MJ kg^{-1} DM (Table 3). The maximal deviation was 0.39 MJ kg^{-1} DM. Both values were considerably lower than corresponding values for predictions based on digestibility coefficients from DLG tables (0.29 and 1.12 MJ NEL kg^{-1} DM) or GfE equations (0.27 and 1.59 MJ NEL kg^{-1} DM) (Babnik *et al.*, 2001). More than 95% of samples lied within acceptable limits (AL) of ± 0.3 MJ NEL kg^{-1} DM (Table 3). The results suggest a comparative advantage of the NIRS method over the estimates based on chemical composition and equations from literature or digestibility factors from tables.

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

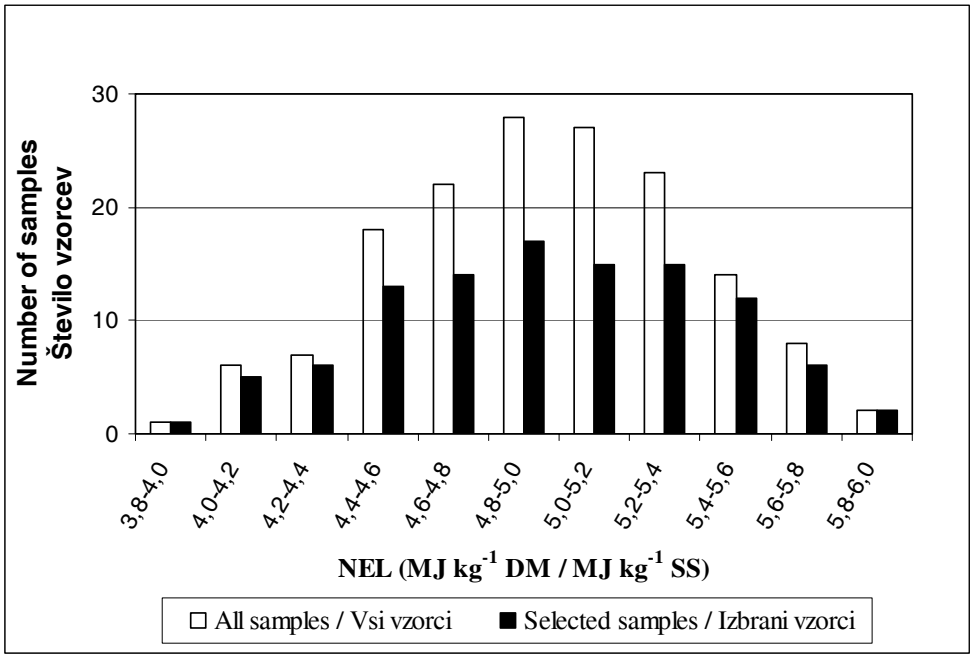

Figure 1. Distribution of total number of samples and number of samples which were selected for NIRS calibration with respect to NEL concentration.

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

Based on Mahalanobis distance only 110 from total 158 hay samples were selected for development of calibration equations. The number of selected samples is close to findings of Windham *et al.* (1989) that 50 to 100 samples are needed for narrow-based NIRS calibrations. However, although the number of selected samples seems adequate, it is obvious that the sample distribution is not optimal (Figure 1). While the number of samples in the range from 4.4 to 5.6 MJ NEL kg^{-1} DM was large, only few samples lied in ranges below 4.4 MJ and above 5.6 MJ NEL kg^{-1} DM. It seems that suboptimal distribution pattern does not affect the reliability of NEL predictions within individual classes considerably (Figure 2). Compared to classes with large number of samples, only slightly higher average individual deviation of predicted values from reference values was observed in class from 5.6 to 5.8 MJ NEL kg^{-1} DM. On the other hand, in class from 4.0 to 4.2 MJ NEL kg^{-1} DM, which was also characterised by small number of samples, the prediction of NEL was even better than on average. Some care is needed in interpretation of these data. We have to be aware that both extreme classes were tested only on the basis of few samples.

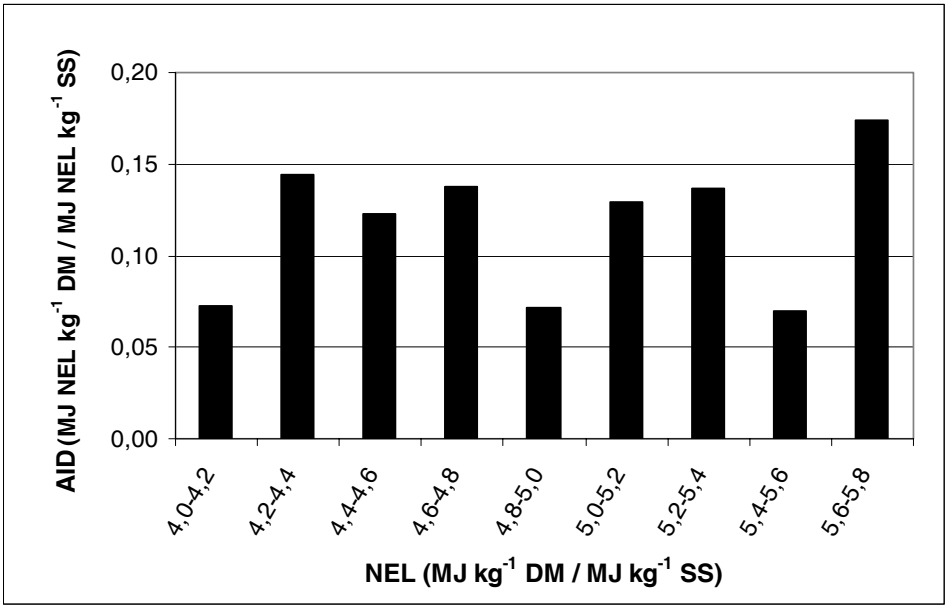

Figure 2. Average individual deviations of predicted NEL concentrations from reference values with respect to NEL concentration.

Slika 2. Povprečna individualna odstopanja napovedanih vsebnosti NEL od referenčnih vrednosti glede na na vsebnost NEL.

CONCLUSIONS

It is concluded that NIRS is rapid and reliable technique for the determination of chemical composition of hay samples. With exception of ash deviations from reference methods are comparable to those which are expected by the use of the same reference methods in different laboratories. NIRS has a high ability to predict *in vitro* assessed NEL concentration ($R^2 = 0.89$). More than 95% of samples lied within acceptable limits of ± 0.3 MJ NEL kg^{-1} DM. With respect to prediction of nutritive value NIRS has an important comparative advantage over the methods based on chemical composition and digestibility factors from tables.

POVZETEK

Namen raziskave je bil preučiti zanesljivost bližnje infrardeče refleksijske spektroskopije (NIRS) pri napovedovanju kemične sestave in vsebnosti NEL. Stooseminpetdesetim vzorcem mrve z znano kemično sestavo in *in vitro* ocenjeno vsebnostjo neto energije za laktacijo (NEL) smo izmerili spektre reflektirane bljižnje infrardeče svetlobe. Reflektirano 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 mrve so se v sestavi in vsebnosti NEL med seboj zelo razlikovali (preglednica 1). Ocenjujemo, da smo z vzorci pokrili razpon, ki je značilen za mrvo s slovenskih kmetij. Največjo točnost umeritvenih enačb smo dosegli pri surovih beljakovinah (R^2= 0,98), surovi vlaknini (R^2= 0,95) in pepelu (R^2= 0,94), nekoliko slabšo pa pri suhi snovi (R^2=0.87) in surovih maščobah (R^2= 0,75) (preglednica 2). Koeficient determinacije umeritvene enačbe za *in vitro* ocenjeno vsebnost NEL je bil razmeroma velik (0,89) in primerljiv z literaturnimi podatki 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 izločeni iz postopka umerjanja NIRS. Za kazalnike zanesljivosti smo uporabili meje ponovljivosti (RL) in sprejemljive meje (AL) (pregl. 3; okrajšave). Približno 50 % z NIRS ocenjenih vrednosti je bilo znotraj meja ponovljivosti (preglednica 3). Če upoštevamo sprejemljive meje, je bilo pri suhi snovi, surovih beljakovinah, surovih maščobah in NEL znotraj sprejemljivih meja več kot 95 % vrednosti. Pri surovi vlaknini je bilo takih 93,7 % vzorcev, pri pepelu pa 87,5 %. Glede na to, da so sprejemljive meje določene za isto metodo, mi pa smo primerjali dve različni metodi, smo lahko z rezultati zadovoljni.

Ugotovili smo, da je povprečno individualno odstopanje z NIRS ocenjene vsebnosti NEL od *in vitro* ocenjene vrednosti le 0,12 MJ kg^{-1} SS, kar je skoraj trikrat manj, kot če vsebnost NEL ocenimo na podlagi kemične sestave in prebavljivostnih koeficientov iz tabel. Podobno velja tudi za največja odstopanja, ki v primeru NIRS metode niso presegla 0,40 MJ kg^{-1} SS.

Na podlagi Mahalanobisove razdalje je bilo za umerjanje NIRS od 158 vzorcev izbranih le 110. Ugotovili smo, da porazdelitev vzorcev glede vsebnosti NEL ni bila enakomerna. V razredih pod 4,4 MJ in nad 5,6 MJ NEL kg^{-1} SS se je nahajalo le malo vzorcev (slika 1). Analiza odstopanj z NIRS ocenjenih vrednosti od referenčnih vrednosti po razredih je pokazala, da majhno število vzorcev v teh razredih ni vplivalo na zanesljivost ocenjevanja vsebnosti NEL z NIRS metodo (slika 2).

Sklenili smo, da je NIRS hitra in zanesljiva metoda za določanje kemične sestave in energijske vrednosti mrve.

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