Zb. Bioteh. Fak. Univ. Ljubl., Kmet. Zooteh., 78(december 2001)2, 151–160.

http://www.bfro.uni-lj.si/zoo/publikacije/zbornik

Original scientific paper
Izvirni znanstveni prispevek

DETERMINATION OF CHEMICAL COMPOSITION, *IN VITRO* DRY MATTER DIGESTIBILITY AND *IN VITRO* GAS PRODUCTION OF FORAGE BY NEAR-INFRARED REFLECTANCE SPECTROSCOPY

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Received September 04, 2001, accepted October 08, 2001.
Delo je prispelo 04. septembra 2001, sprejeto 08. oktobra 2001.

ABSTRACT

One hundred dried samples of forage, harvested three or four times during 1997 and 1999 in different morphological stages were analysed for chemical composition, *in vitro* dry matter digestibility (IVDMD; Tilley and Terry, 1963) and *in vitro* gas production (Menke and Steingass, 1988) by laboratory methods and by near infrared reflectance spectroscopy (NIRS). NIRS predicted the main chemical components, such as crude protein, crude fibre, neutral detergent fibre, acid detergent fibre, with a very high degree of accuracy (i.e. the determination coefficient (R^2) varied from 93.5 % for ADF to 98,2 % for NDF), while the coefficients of determination of crude ash, ether extract and acid detergent lignin were 79.6, 85.1 and 82.3 %, respectively, with standard errors of cross validation (SECV) exceeding 9.0 %. The IVDMD was also determined with a high degree of accuracy (R^2 = 94.3 %; SECV = 4.3 %), such as parameters of gas production "B" (total gas production) and "A" (constant factor describing the decay in specific gas production rate), estimated with the Gompertz model, which have R^2 and SECV values of 93.0 and 3.8 % and 94.5 and 8.0 %, respectively. The parameter "C" (the specific fermentation rate) was predicted with a somewhat lower degree of accuracy (R^2 = 78.3 and SECV = 9.6 %).

Keywords: animal nutrition / forage / chemical composition / *in vitro* dry matter digestibility / *in vitro* gas production / analytical chemistry / near infrared reflectance spectroscopy (NIRS)

DOLOČANJE KEMIČNE SESTAVE, *IN VITRO* PREBAVLJIVOSTI SUHE SNOVI IN *IN VITRO* PRODUKCIJE PLINA Z BLIŽNJO INFRARDEČO REFLEKSIJSKO SPEKTROSKOPIJO

IZVLEČEK

Sto posušenim vzorcem voluminozne krme, košenih tri do štirikrat na leto v različnih morfoloških stadijih razvoja v letih 1997 in 1999, smo določili kemično sestavo, *in vitro* prebavljivost suhe snovi (IVPSS; Tilley in Terry, 1963) in *in vitro* produkcijo plina (Menke in Steingass, 1988) z laboratorjskimi metodami in z uporabo bližnje infrardeče refleksijske spektroskopije (NIRS). Rezultati te raziskave so pokazali, da lahko z NIRS predvidimo vsebnosti najpomembnejših kemičnih snovi, kot so surove beljakovine, surova vlaknina, v nevtralnem detergentu netopna vlakna (NDV) in v kislem detergentu netopna vlakna (KDV) z veliko stopnjo točnosti (koeficienti determinacije (R^2) so bili v razponu od 93,5 % za KDV do 98,2 % za NDV), medtem ko so bili determinacijski koeficienti za surovi pepel, surove maščobe in v kislem detergentu netopni lignin 79,6, 85,1 in 82,3 %, s standardno napako navzkrižnega preverjanja

(SECV) vedno večjo od 9,0 %. IVPSS je bila ocenjena z visoko stopnjo točnosti (R^2 = 94,3 %; SECV = 4.3 %), podobno pa tudi parametra plinskega preskusa "B" (skupna produkcija plina) in "A" (stalni dejavnik zmanjševanja specifične stopnje hitrosti produkcije plina), ki smo jih ocenili z Gompertzovim modelom, ki sta imela R^2 in SECV 93,0 % in 3,8 % ("B") ter 94,5 % in 8,0 % ("A"). Parameter "C" (specifična stopnja hitrosti fermentacije) smo ocenili z nekoliko manjšo točnostjo, saj je bil R^2 le 78,3 % in SECV 9,6 %.

Ključne besede: prehrana živali / voluminozna krma / kemična sestava / *in vitro* prebavljivost suhe snovi / *in vitro* produkcija plina / analitska kemija / bližnja infrardeča refleksijska spektroskopija (NIRS)

INTRODUCTION

In many North-European countries fresh forage represents the main component of diets for ruminants, especially during the spring and summer seasons. A knowledge of the nutritive value of forage is indispensable in planning forage utilisation through the year and to develop an optimal feeding regime for ruminants. Furthermore, the optimisation of feeding strategy is essential to control forage wastage and minimise enviromental effects. The chemical composition and digestibility of fresh forage can be estimated on the basis of herbage species, stage of maturity at harvest, climatic conditions and fertilizer application (Park *et al.*, 1998). Wet chemical and other laboratory methods, which are used to provide information about the chemical composition and fermentability and/or digestibility of nutrients, have been used traditionally to characterize the forage and to predict their nutritive value. However, these methods have several drawbacks; they are time-consuming, costly and in some instances hazardous chemicals are involved.

The use of *in vitro* techniques based on rumen fluid or commercial enzymes, has been extensively utilised to replace laborious, time-consuming and expensive digestibility trials with animals. However, *in vitro* analyses are not real-time, and can still be too long to follow the day-by-day modification of nutritive values of forage. Accurate information on a range of issues, such as chemical composition, digestibility and fermentability of nutrients, are needed in a relatively short time. To provide controlled feeding regimes for ruminants faster, simpler and more accurate predictions of the nutritive value and chemical composition of forage are necessary.

All the drawbacks of *in vivo* and *in vitro* analyses can be eliminated by near infrared reflectance spectroscopic (NIRS) analysis, which is now recognized as a valuable tool in the accurate determination of the chemical composition, digestibility parameters and gas production parameters of a wide range of forages (Shenk and Westerhaus, 1985; Redshaw *et al.*, 1986; Givens *et al.*, 1997; Herrero *et al.*, 1997; Park *et al.*, 1998; Adesogan *et al.*, 1998). The main advantages of NIRS are rapidity, multiplicity of analyses, reduced sample size, minimal or no sample preparation, non-destructiveness and reduced cost of analysis. Moreover, no reagents are required and no wastes are produced. NIRS is a physical method of analysis and is based on the absorption of radiation at different wavelengths in the near infrared region of the electromagnetic spectrum, as measured by the proportion of incident radiation which is diffusely reflected (Deaville and Baker, 1993). The ability of NIRS to determine various quality parameters in highly variable sample types is due to the rotational and vibrational energies associated with hydrogen bonding. In the near infrared region, the covalent bonds that involve hydrogen are particularly dominant and in the contexts of food, those involving C-H, O-H, N-H and possibly S-H and C=O are responsible for the majority of the observed absorption (Murray, 1986; Deaville and Baker, 1993).

The objective of this study was to examine the potential of NIRS to produce accurate, robust calibrations, developed on the spectra dried forage spectra, to predict chemical composition, *in vitro* digestibility and gas production parameters.

MATERIAL AND METHODS

Six grass species, Italian ryegrass (*Lolium multiflorum*), perennial ryegrass (*Lolium perenne*), timothy (*Phleum patense*), orchardgrass (*Dactylis glomerata*), red fescue (*Festuca rubra*), meadow fescue (*Festuca pratensis*) and red clover (*Trifolium pratense*) grown in pure stands on Mengeško polje, Slovenia, were harvested in 1997. In 1999 the collection of grass and clover samples was repeated, except that fescues and orchardgrass were not sampled. Each species was harvested four times and within each of the harvests crops were sampled at different morphological stages. The exceptions were the third consecutive harvest where orchardgrass, red and meadow fescue, perennial ryegrass and timothy were sampled only once in the vegetative stage of growth, while Italian ryegrass and red clover were sampled in the vegetative, boot and bud stages and in the flowering stage of growth. Another exception was the fourth harvest (autumn harvest) in which all samples were cut at vegetative stage of growth. A total number of 100 samples were available, 60 samples obtained in 1997 and 40 samples in 1999.

Immediately after sampling, forages were stored for a week at –21°C, thawed for one day and dried at temperatures below 50 °C. Air dry samples were then ground to pass a 1 mm screen (Alpine, Ausburg, Germany) and stored at room temperature in a dark room until analysed.

Samples of dried forage were analysed for crude protein (CP), ash, ether extract (EE), crude fibre (CF) according to Neumann and Bassler (1986) and for neutral detergent fibre, acid detergent fibre (ADF) and acid detergent lignin (ADL), according to Goering and Van Soest (1970).

A mature nonlactating Friesian cow, weighing approximately 700 kg and fitted with a permanent rumen cannula was used as the donor of rumen liquid for *in vitro* dry matter digestibility (IVDMD) and for *in vitro* gas production. The cow was fed with average quality hay (103 g CP and 290 g CF in kg DM) *ad libitum* and 2 kg of commercial compound feed (180 g/kg crude protein) in 2 equal meals at 7.30 and 16.30 h, with an additional mineral supplement (50 g) once a day. The diet was calculated to cover the energy and protein requirements of maintenance (DLG, 1997). The animal was given the diet 14 days before the beginning of the *in vitro* trials.

IVDMD was determined according to Tilley and Terry (1963), while *in vitro* gas production was determined according to the procedure described by Menke and Steingass (1988). Gas production was measured after 0, 2, 4, 6, 8, 10, 12, 24, 48, 72 and 96 hours. The *in vitro* gas production data were fitted with the Gompertz model (Beuvink and Kogut, 1993):

$$Y_t = B \cdot e^{-C \cdot e^{-At}} \qquad [1]$$

where: Y_t is the gas produced at time "t", "B" is the total gas produced (ml 100 mg^{-1} DM); "C" is the specific fermentation rate which is affected by a constant factor "A" describing the decay in specific gas production rate (Beuvink and Kogut, 1993); and "t" is time in hours. Parameter values and curve fitting were estimated by the Marquardt compromise of a non-linear regression method, using Statistical Analysis System software (PROC NLIN) (SAS Institute Inc., 1989).

Near-infrared spectra of forage samples were obtained with a Perkin Elmer Spectrum One NTS FT-NIR Spectrometer (Perkin Elmer, Monza, Italy) equipped with Perkin Elmer NIRA Sample Spinner. About 10–20 g of forage contained in a 7.8 mm i.d. Petri disk were placed over the NIRS source; the sample and Petri disk rotated during readings. Thirty spectra for each sample were obtained in about 60 seconds.

Optical values were taken at 2 nm intervals over the wavelenght range 1000 to 2500 nm. Calibration equations were calculated from all measured samples with Spectrum Quant+ v4.51 software using a Principal Component regression (PCR+) algorithm (Perkin Elmer, Monza,

Italy). For all forage samples the prediction of chemical component contents, IVDMD and parameters of gas production was obtained.

Statistical Analysis System software (SAS Institute Inc., 1989) was used to calculate simple linear regression equations among chemical composition, IVDMD and *in vitro* gas production parameters determined by laboratory analyses (LAB) and between chemical composition, IVDMD and *in vitro* gas production parameters determined by NIRS.

RESULTS AND DISCUSSION

As expected, we obtained a large variation of chemical components, and of IVDMD and *in vitro* gas production parameters (Table 1). This large variation was a result of different harvesting and sampling in various morphological stages of growth. Additionally, using two plant families also enlarged the variation. The differences between the minimum and maximum values were the greatest for ADL and CP contents; they varied by more than 6 and 5 times, respectively. However, other chemical constituents, IVDMD and *in vitro* gas production parameters also had wide ranges of values, often exceeding 100 %.

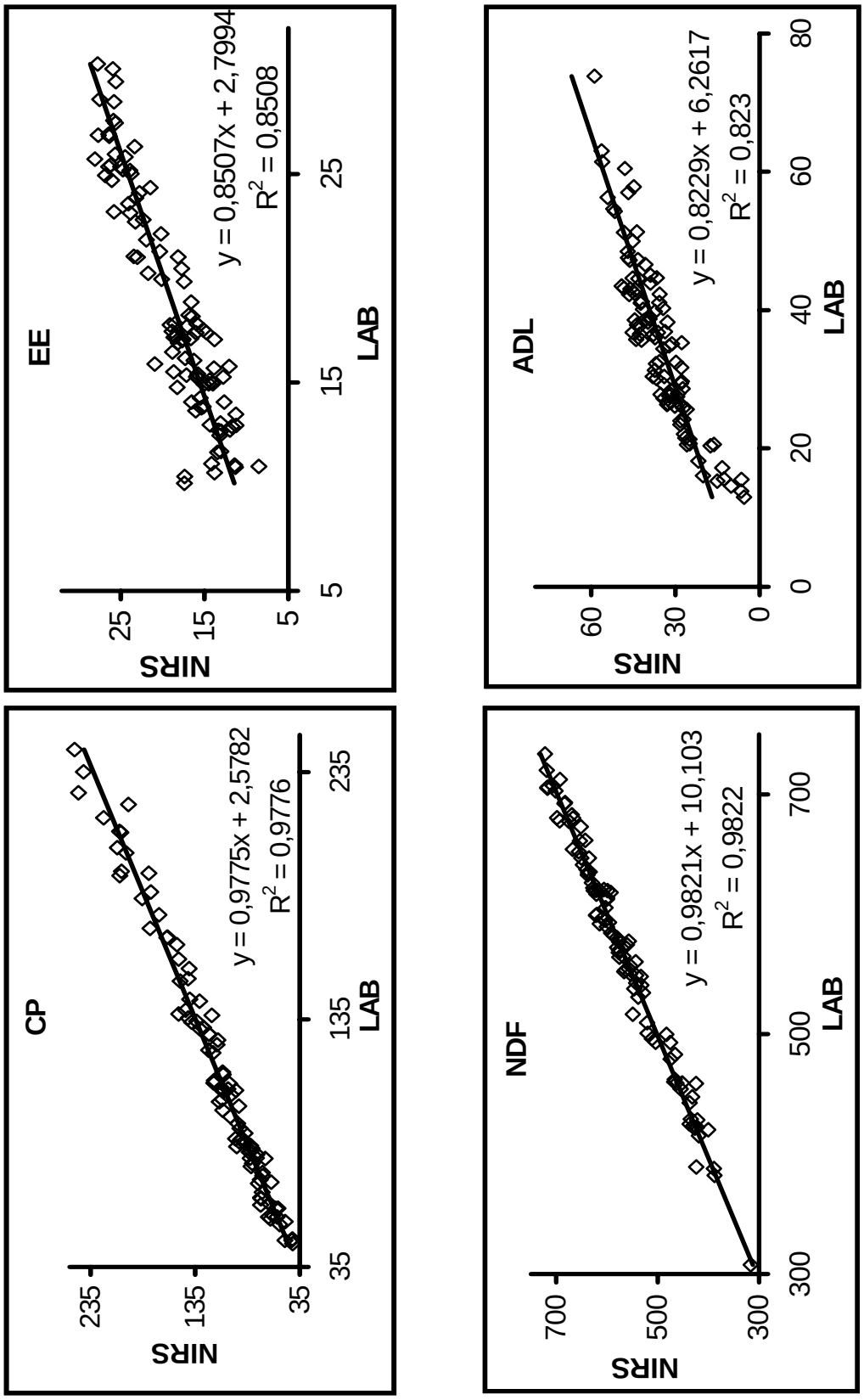
Table 1. Composition (g kg^{-1} dry matter) and a range of values for samples used in calibration and cross validation of NIRS equations (n = 100)

Preglednica 1. Sestava (g kg^{-1} suhe snovi) in razpon vrednosti vzorcev, uporabljenih pri umerjanju in navzkrižnem preverjanju NIRS-ovih enačb (n = 100)

Component Sestavina	Minimum Najmanj	Maximum Največ	Mean ± SE‡ Sredina ± SE
Crude protein Surove beljakovine	44	244	115 ± 5.03
Crude ash Surovi pepel	36	137	79 ± 1.95
Ether extract Surove maščobe	10	30	19 ± 0.54
Crude fibre Surova vlaknina	163	387	282 ± 5.24
NDF NDV	308	734	565 ± 9.35
ADF KDV	209	444	326 ± 5.30
ADL KDL	13	74	35 ± 1.23
IVDMD § IVPSS	467	818	664 ± 11.32
B	19.8	33.2	26.06 ± 0.30
C	1.57	3.07	2.271 ± 0.035
A	0.069	0.187	0.1175 ± 0.003

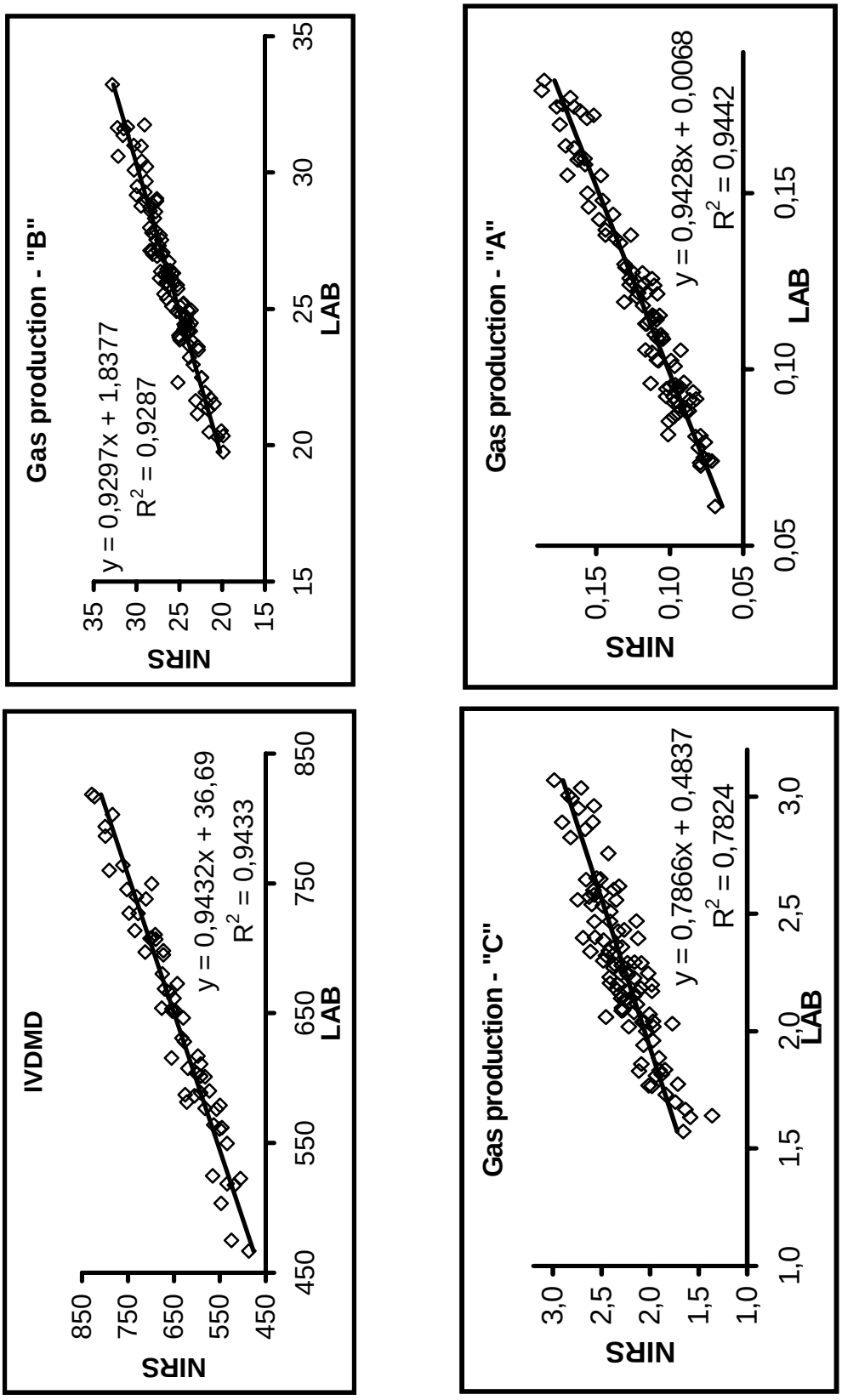
§ n = 60, ‡ SE = standard error/ standardna napaka

NDF / NDV = neutral detergent fibre / v nevtralnem detergentu netopna vlakna; ADF / KDV = acid detergent fibre / v kislem detergentu netopna vlakna; ADL / KDL = acid detergent lignin / v kislem detergentu netopni lignin; IVDMD / IVPSS = *in vitro* dry matter digestibility / *in vitro* prebavljivost suhe snovi; B = total gas production (ml 100 mg^{-1} DM) / skupna produkcija plina (ml 100 mg^{-1} suhe snovi); C = specific fermentation rate / specifična stopnja hitrosti fermentacije; A = constant factor of the decay in specific gas production rate / stalni dejavnik zmanjševanja specifične stopnje hitrosti fermentacije



Graph 1. Relationship between laboratory determined (LAB) and NIRS predicted contents of CP, EE, NDF and ADL of forage samples.

Grafikon 1. Povezava med vsebnostjo surovih beljakovin (CP), surove maščobe (EE), NDV (NDF) in KDL (ADL) v vzorcih voluminozne krme, določenih v laboratoriju (LAB) in napovedanih s pomočjo NIRS.



Graph 2. Relationship between laboratory determined (LAB) and NIRS predicted values of IVDMD and gas production parameters ("B", "C" and "A") of forage samples.
Grafikon 2. Povezava med vrednostmi IVPSS in parametri produkcije plina ("B", "C" in "A") iz vzorcev voluminozne krme določenih v laboratoriju (LAB) in napovedanih s pomočjo NIRS.

The results of calibration and validation are presented in Table 2. The rightness of a selected calibration equation was estimated with three statistical parameters: the standard error of calibration (SEC), the coefficent of determination (R^2) and the standard error of cross validation (SECV). The first two parameters estimate the variance of chemical analyses and *in vitro* trials explained by the NIRS spectrum (R^2) and the accuracy of the measurement (SEC).
Chemical components, such as CP, CF, NDF, ADF had high values of R^2, exceeding 90 %, while lower R^2 were obtained for crude ash, ether extract and ADL (79.6, 85.1 and 82.3 % respectively).

Table 2. Accuracy of the NIRS calibration and cross validation (n = 100)
Preglednica 2. Točnost umeritve in navzkrižne preveritve z NIRS-om (n = 100)

Component Sestavina	R^2, % [c]	SEC [c]	SECV [c]
Crude protein Surove beljakovine	97.8	8.1 (7.0 %)	10.4 (9.0 %)
Crude ash Surovi pepel	79.6	9.1 (11.5 %)	9.4 (11.9 %)
Ether extract Surove maščobe	85.1	2.2 (11.7 %)	1.7 (9.0 %)
Crude fibre Surova vlaknina	95.3	12.3 (4.4 %)	14.8 (5.2 %)
NDF [a] NDV	98.2	13.6 (2.4 %)	16.8 (3.0 %)
ADF [a] KDV	93.5	14.1 (4.3 %)	15.8 (4.8 %)
ADL [a] KDL	82.3	5.5 (15.7 %)	6.2 (17.5 %)
IVDMD [ab] IVPSS	94.3	23.2 (3.5 %)	27.7 (4.3 %)
B [a]	93.0	0.86 (3.3 %)	1.00 (3.8 %)
C [a]	78.3	0.175 (7.7 %)	0.218 (9.6 %)
A [a]	94.5	0.0078 (6.7 %)	0.0094 (8.0 %)

[a] = Abbreviations are defined in Table 1 / Okrajšave so razložene v preglednici 1; [b] = n = 60; [c] = R^2 = determination coefficient / koeficient determinacije; SEC = standard error of calibration / standardna napaka umeritve; SECV = standard error of cross validation / standardna napaka navzkrižne preveritve (in brackets the error percentage / v oklepajih odstotek napake)

The highest R^2 was calculated for NDF (98.2 %) followed by those of CP (97.8 %). Similar R^2 for NDF and CP were also obtained by other reserchers (Coleman *et al.*, 1993; Garcia-Ciudad *et al.*, 1993; Park *et al.*, 1998; Ruano-Ramos *et al.*, 1999).

The SEC expressed in terms of percentage ranged from 2.4 % for NDF to 15.7 % for ADL, with values being generally lower than 10 %. Some SEC values exceeded the 10 % level, such as those for crude ash, ether extract and ADL. Generally all SEC percentages were above those reported by Berardo (2000) for the same chemical components measured in alpine forages.

The percentage values of SECV reflected those of SEC, the former being always higher than the corresponding SEC values. The lowest and the highest SECV percentages were observed for the same chemical components of SEC, i.e. NDF (3.0 %) and ADL (17.5 %).

Low variance (R^2) and high percentage values of SEC and SECV for crude ash (79.6 %, 11.5 % and 11.9 %, respectively) agree with the results reported by De Boever *et al.* (1993) for

compound feeds and raw materials. However, Ruano-Ramos *et al.* (1999) determined the ash content by NIRS with an R^2 of 90.3 % for grass samples from semi-arid grasslands. The reason for the low variance in ash determination in our study could be that the constituents of ash were in ionic form or are in the form of salts which did not reflect near infrared energy. Only covalent bonds involving hydrogen are responsible for the majority of the observed absorption (Murray, 1986; Deaville and Baker, 1993). However, if mineral constituents of ash are associated with organic complexes or are chelated, than NIRS could be successfully applied to determine ash content (Clark *et al.,* 1987; Shenk *et al.,* 1992).

According to Berardo (2000) higher SECV values for crude fat and ADL content could be attributed to their complex structure and composition. Crude fat is defined by several components such as pure fats, fatty acids, chlorophyll, waxes etc., which are all soluble in petroleum ether and thus have different chemical properties. Neutral detergent fibre, which had very high R^2 values, is also characterised by a very complex structure, although it is constituted by components with very similar chemical properties, which give similar reflectances of near infrared light. Acid detergent lignin, which in this study had the lowest R^2 and higher SECV, is also a complex mixture, but differently composed from NDF. In other words, the determination of ADL contents needs more analytical measurements and all of them increase the analytical error. In any case, the SECV of all analysed components has the same grade of variability as their SEC, which is also presented in Graph 1, where the relationships between results obtained by laboratory analyses and those obtained by NIRS are examined.

The prediction of IVDMD by NIRS was very accurate (R^2 = 94.3 %; Graph 2), confirming the results obtained in other studies (De Boever *et al.*, 1996; Marten, 1989; Coleman, 1993; Rabotnikof *et al.*, 1995). The prediction of total potential gas production (B) and a constant factor describing the decay of microbial activity (A) was very accurate, R^2 being 93.0 and 94.5 %, respectively. The SEC and SECV percentages for "B" were 0.86 and 1.00 % and for "A" 6.7 and 8.0 %. Only the specific rate of gas production was predicted less accurately, with somewhat greater SEC and SECV values. Herrero *et al.* (1996) reported that NIRS did not accurately predict gas production parameters obtained with the Ørskov and McDonald model (McDonald, 1981); values of R^2 in the calibration of the parameters "a", "b" and "c" were 50 %, 40 % and 50 %, respectively. The correlations between these parameters obtained *in vitro* and by NIRS were very low, ranging from 0.51 for parameter "a" and 0.77 for parameter "a+b", considerably lower than the values obtained in the present research for the "B" of the Gompertz model (Graph 2), which corresponds to the parameter "a+b" of the exponential model. Herrero *et al.* (1996) noted that the reasons for the failure of NIRS to accurately predict gas production parameters could be the choice of model used to estimate the parameters, along with the non-linearity kinetics and the random nature of the population used for calibration. The latter two reasons could also account for the lower accuracy when estimating parameter "C" of the Gompertz model.

CONCLUSIONS

The results of this study have shown that FT-NIRS analysis provides accurate prediction of chemical components, especially for CP, CF, NDF and ADF, but the prediction of EE, ash and ADL content was only moderate. This technique can also be used to provide accurate predictions of the digestibility and the total gas production, which together with chemical composition are used to calculate the content of the metabolizable or net energy in forage.

The FT-NIRS technique offers considerable opportunities to support animal production rationing systems. With the use of this technique the results could be obtained within 30 hours after harvest (sampling), including transport of the samples to the laboratory and preprocessing

(drying and grounding), which are the most time consuming steps. However, as the analyses could also be performed on fresh material with great accuracy (Park *et al.*, 1998), then the results could be obtained even sooner, just after the samples are transported to the laboratory.

POVZETEK

Namen raziskave je bil preučiti, kako točno lahko z bližnjo infrardečo refleksijsko spektroskopijo (NIRS) določimo kemično sestavo, *in vitro* prebavljivost suhe snovi (IVPSS) in parametre *in vitro* produkcije plina vzorcev voluminozne krme, košenih večkrat na leto v različnih morfoloških stadijih razvoja. Reflektirano bližnjo infrardečo svetlobo z vzorcev smo merili v 2 nm intervalih v razponu valovnih dolžin med 1000 in 2500 nm. Dobljene spektralne podatke smo z algoritmom regresije najpomembnejših elementov (principal component regression – PCR) prilagodili parametrom kemične sestave, prebavljivosti in fermentabilnosti. Med procesom umerjanja smo izvedli tudi popolno navzkrižno preverjanje, kar nam je omogočilo določitev umeritvene enačbe, ki je imela največji koeficient determinacije (R^2) in najmanjše standardne napake umeritve (standard error of calibration – SEC) in navzkrižnega preverjanja (standard error of cross validation – SECV). Rezultati te raziskave so pokazali, da lahko z NIRS predvidimo vsebnosti najpomembnejših kemičnih snovi, kot so surove beljakovine, surova vlaknina, v nevtralnem detergentu netopna vlakna (NDV), v kislem detergentu netopna vlakna (KDV) z veliko stopnjo točnosti (koeficienti determinacije so bili v razponu od 93,5 % za KDV do 98,2 % za NDV), medtem ko so bili determinacijski koeficienti za surovi pepel, surove maščobe in v kislem detergentu netopni lignin (KDL) nekoliko manjši (79,6–85,1 % za surovi pepel in KDL), ter SECV, ki je bila vedno večja od 9,0 %. Tudi IVPSS smo ocenili z visoko stopnjo točnosti (R^2 = 94,3 %; SECV = 4,3 %), kar velja tudi za parametra plinskega testa "B" (skupna produkcija plina) in "A" (stalni dejavnik zmanjševanja specifične stopnje hitrosti produkcije plina), ki smo jih ocenili z Gompertzovim modelom, ki sta imela R^2 in SECV 93,0 % in 3,8 % ("B") ter 94,5 % and 8,0 % ("A"). Parameter "C" (specifična stopnja hitrosti fermentacije) smo ocenili z nekoliko manjšo točnostjo kot parametra "B" in "A" (R^2 = 78,3 % in SECV = 9,6 %). Z NIRS relativno točno napovemo vsebnost zelo raznolikih kemičnih sestavin in tudi parametrov prebavljivosti ter fermentabilnosti, ki nam omogočajo hitro oceno hranilne vrednosti krme, kar je zelo pomembno pri pripravi obrokov, ki vključujejo pašo oziroma prilast, saj se njihova hranilna vrednost izredno hitro spreminja.

REFERENCES

Adesogan, A.T./ Owen, E./ Givens, D.I. Prediction of the *in vivo* digestibility of whole crop wheat from *in vitro* digestibility, chemical composition, in situ rumen degradability, *in vitro* gas production and near infrared reflectance spectroscopy. Animal Feed Science and Technology, 74(1998), 259–272.

Berardo, N. Applicazioni della tecnica NIRS per la valutazione di foraggi dell'ambiente alpino italiano. Rivista Agronomica, 34(2000), 95–99 (Supplement 1).

Beuvink, J.M.W./ Kogut J. Modeling gas production kinetics of grass silages incubated with buffered ruminal fluid. Journal of Animal Science, 71(1993), 1041–1046.

Clark, D.H./ Mayland, H.F./ Lamb, R.C. Mineral analysis of forages with near infrared reflectance spectroscopy. Agronomy Journal, 79(1978), 485–490.

Coleman, S.W. / Murray, I. The use of near-infrared reflectance spectroscopy to define nutrient digestion of hay by cattle. Animal Feed Science and Technology, 44(1993), 237–249.

De Boever, J.L./ Cottyn, B.G./ De Brabander, D.L./ Vanacker, J.M./ Boucque, C.V. Predicting the feeding value of grass silages by chemical parameters, *in vitro* digestibility and near-infrared reflectance spectroscopy. Animal Feed Science and Technology, 60(1996), 103–115.

De Boever, J.L./ Cottyn, B.G./ Vanacker, J.M./ Boucque, C.V. Recent developments in the use of NIRS for evaluating compound feeds and raw materials for ruminants. V: »NIR spectroscopy. Developments in agriculture and food.« Proceedings of the international conference on 12 October 1993, ADAS, Drayton, UK, 1993; 10 p.

Deaville, E.R./ Baker, C.W. Spectral interpretation in relation to feed characterisation. V: »NIR spectroscopy. Developments in agriculture and food.« Proceedings of the international conference on 12 October 1993, ADAS, Dryton, UK, 1993, 20 p.

DLG – Futterwerttabellen für Wiederkäuer, DLG Verlag, Frankfurt, 1997, 212 p.

Garcia-Ciudad, A./ Garcia-Criado, B./ Perez-Corona, M.E./ Vasquez de Aldana, B.R./ Ruano-Ramos, A.M. Application of near infrared reflectance spectroscopy to chemical analysis of heterogenous and botanically complex grassland samples. Journal of the Science of Food and Agriculture, 63(1993), 419–426.

Givens, D.I./ De Boever, J.L./ Deaville, E.R. The principles, practices and some future applications of near infrared spctroscopy for predicting the nutritive value of foods for animals and humans. Nutrition Research Review, 10(1997), 83–114.

Goering, H.K./ Van Soest, P.J. Forage fiber analyses (apparatus, reagents, procedures and some applications). Agricultural Handbook no. 379, ARS, USDA, Washington, D.C. 1970. 20 p.

Herrero, M./ Jessop, N.S./ Fawcett, R.H./ Murray, I./ Dent, J.B. Prediction of the *in vitro* gas production dynamics of kikuyu grass by near infrared reflectance spectroscopy using spectrally-structured sample populations. Animal Feed Science and Technology, 69(1997), 281–287.

Herrero, M./ Murray, I./ Fawcett, R.H./ Dent, J.B. Prediction of the *in vitro* gas production and chemical composition of kikuyu grass by near infrared reflectance spectroscopy. Animal Feed Science and Technology, 60(1996), 51–61.

Marten, G.C. Near infrared reflectance spectroscopy as an universal forage evaluation technique. Proceeding of the XVI International Grassland Congress, Nice, France, 1989, 885–886.

McDonald, I. A revised model for the estimation of protein degradability in the rumen. Journal of Agricultural Science, Cambridge, 96(1981), 251–252.

Menke K.H./ Steingass H. Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. Animal Research and Development, 28(1988), 7–55.

Murray, I. Near infrared reflectance analysis of forages. In: »Recent advances in animal nutrition – 1986« (eds.: Haresign, W./ Cole, D.J.A.), Butterworths, London, United Kingdom, 1986, 141–156.

Neumann, K./ Bassler, R. Methodenbuch, Band III. J. Neumann-Neudamm, 1976, 265 p.

Park, R.S./ Agnew, R.E./ Gordon, F.J./ Steen, R.W.J. The use of near infrared reflectance spectroscopy (NIRS) on undried samples of grass silage to predict chemical composition and digestibility parameters. Animal Feed Science and Technology, 72(1998), 155–167.

Rabotnikof, C.M./ Planas, G.M./ Silva-Colomer, J./ Stritzler, N.P. Near infrared reflectance spectroscopy (NIRS) for predicting forage quality of perennial warm-season grasses in La Pampa, Argentina. Annales de Zootechnie, 44(1995), 97–100.

Redshaw, E.S./ Mathison, G.W./ Milligan, L.P./ Weisenburger, R.D. Near infrared reflectance spectroscopy for predicting forage composition and voluntary consumption and digestibility in cattle and sheep. Canadian Journal of Animal Science, 66(1986), 103–115.

Ruano-Ramos, A.M./ Garcia-Ciudad, A./ Garcia-Criado, B. Determination of nitrogen and ash contents in total herbage and botanical components of grassland systems with near infrared-spectroscopy. Journal of the Science of Food and Agriculture, 79(1999), 137–143.

SAS Institute Inc. SAS/STAT. User's Guide, Version 6, 4th Edition, Vol. 2. Cary, NC, SAS Institute Inc., 1989, 846 p.

Shenk, J.S./ Westerhaus, M.O. Accuracy of NIRS instruments to analyse forage and grain. Crop Science, 25(1985), 1120–1122.

Shenk, J.S./ Workman, J.J./ Westerhaus, M.O. Application of NIR spectroscopy to agricultural products. In: Handbook of Near-Infrared Analysis« (eds. Burns, D.A./ Ciurczak, E.W.), Marcel Dekker, New York, 1992, 383–431.

Tilley, J.M.A./ Terry, R.A. A two stage technique for the *in vitro* digestion of forage crops. Journal of the British Grassland Society. 18(1963), 104–111.