Near infrared spectroscopy for measuring dry matter degradability of forages

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Introduction

Estimation of nutritive value of forages is of primary importance for the prediction of animal performance. Until recently, feed evaluation of forages was based on cutting date and chemical composition, because *in vivo* digestibility experiments with animals were very time-consuming and expensive. Several *in vitro* techniques have been developed to predict digestibility and nutritive value of feeds for ruminants. Among them *in vitro* gas production procedure,[1–3] which indirectly records digestion kinetics using gas produced from feeds incubated with rumen fluid, is gaining popularity because it is a low-cost and highly reproducible procedure. This method has provided better predictions of the *in vivo* digestibility and energetic values of forages than other *in vitro* techniques.[2,4,5]

Near infrared (NIR) spectroscopy has proved to be a successful tool for estimating the chemical composition and nutritive value of various forages.[6] NIR has also been applied to the prediction of dry matter and crude protein rumen degradability.[7–9]

The objective of this study was to investigate the potential of NIR for prediction of degradability characteristics of alfalfa hay, meadow hay, alfalfa silage and maize silage, estimated by the gas production method.

Material and methods

Forage samples

The forages investigated included 43 alfalfa hay samples, 30 maize silage samples, 43 alfalfa silage samples and 37 meadow hay samples. The samples were collected from four research institutes in Bulgaria, mainly from the Agricultural Faculty, Thracian University, Stara Zagora. Prior to ensiling, alfalfa was wilted to approximately 30–40% dry matter. Alfalfa hays were field-dried, from early to full bloom of alfalfa. Meadow hays were field-dried and predominant forage species were *Phleum pratense, Agrostis alba, Dactylis glomerata, Bromus intermis*, *Festuca rubra* and a smaller percentage of legumes such as *Lotus corniculatus*, *Trifolium pratense* and *Medicago sativa*.

In vitro gas production procedure

The gas production test was done at the Institute for Animal Nutrition, Hohenheim, Germany, basically by using the procedures of Menke *et al.*[1] and Menke and Steingass.[2]

Rumen liquor was obtained from two lactating dairy cows, fitted with rumen fistula. The diet of the cows consisted of grass hay and concentrate. Collections were made by dipping into the ventral portion of the rumen before morning feeding. The rumen fluid was mixed with a buffer medium in ratio 1 : 2.

All incubations were done in 100 ml calibrated glass syringes. Six syringes from each sample were incubated in two runs. Gas production was recorded at 2, 4, 6, 8, 12, 24, 32, 48 and 72 h. The corrections with blank (without forage) were done.

The exponential model $y = a + b\,(1 - e^{-ct})$ described by Orskov and McDonald[10] and McDonald[11] was used to calculate the extent and rate of gas production, where y—*in vitro* gas production (ml) at time, $(a + b)$—potential gas production and c—rate of gas production per hour. For calculating effective gas production at different rumen outflow rates (k) the following model by McDonald[11] was used:

$$y = (a + b)c/(c + k)e^{-(c + k)t}$$

Near infrared analysis

NIR reflectance spectra of the samples were obtained on dried samples using an NIRSystem 4250 spectrophotometer (NIRSystem, Silver Spring, MD, USA), using a rotating sample cup. The spectral

Table 1. The range and standard deviation of the tested parameters.

Forages	V_{24}, ml	$a + b$, %	$c \times 100$	EGP	
				$k = 0.03$	$k = 0.06$
Alfalfa hay ($n = 43$)					
Mean	36.77	43.24	8.71	31.78	25.14
Minimum	22.38	36.02	4.31	20.70	14.30
Maximum	42.65	48.83	11.17	37.00	30.30
SD[a]	5.38	3.92	1.73	4.70	4.64
Corn silage ($n = 30$)					
Mean	48.56	60.08	6.78	42.78	33.64
Minimum	40.51	54.33	4.56	37.50	29.30
Maximum	54.49	66.01	9.45	47.00	38.40
SD	3.72	3.08	1.08	2.71	2.51
Alfalfa silage ($n = 43$)					
Mean	26.32	32.8	6.96	23.10	17.84
Minimum	13.25	21.25	1.13	12.20	8.60
Maximum	36.34	53.07	11.59	32.10	26.10
SD	6.17	6.40	2.06	5.49	4.94
Grass hay ($n = 37$)					
Mean	33.69	48.92	4.89	30.17	21.98
Minimum	27.05	40.22	3.47	24.60	16.70
Maximum	43.52	62.36	6.50	37.70	26.60
SD	3.40	4.75	0.81	2.58	2.08

[a]SD-standard deviation

data were collected as log (1/*R*) values in the wavelength range from 1618 to 2320 nm. Using ISI NIR 3 software (Infrasoft International, Port Matilda, PA, USA), calibration equations were developed by modified PLS as the regression method. Examined parameters were potential gas production ($a + b$), rate of gas production c, gas production volume at 24 h incubation and effective gas production (EGP) at a rumen outflow rate of 0.03 h^{-1} and 0.06 h^{-1}. Gas production at 24 h was selected for calibration, because this volume had been used in the regression equation for the prediction of metabolisable energy (Menke and Steingass, 1988). Several methods for scatter correction, such as detrend (DT), standard normal variate (*SNV*), combination of DT + SNV and different mathematical treatment (first and second derivative), different segment length over which the derivative was taken and different length of the smoothing segments, were used. Cross-validation was used to test the calibration equations. Selection of the best equations was made on the base of lowest standard error of calibration (*SEC*) and standard error of cross-validation (*SECV*) and highest calibration coefficient of determination R^2 and cross-variation coefficient of determination CVr^2, respectively.

Results and discussion

The range and standard deviation of degradation characteristics obtained by fitting the data for gas production to the exponential equation of McDonald[11] and gas volume after 24 h incubation are summarised in Table 1. The potential gas produced ($a + b$) is highest in the case of maize silage and lowest for alfalfa silage which agrees with their *in vivo* digestibility of organic matter. Effective gas production decreased with increasing of rumen outflow passage rate. At the same passage rates, effective gas production is highest from corn silage and lowest from alfalfa silage.

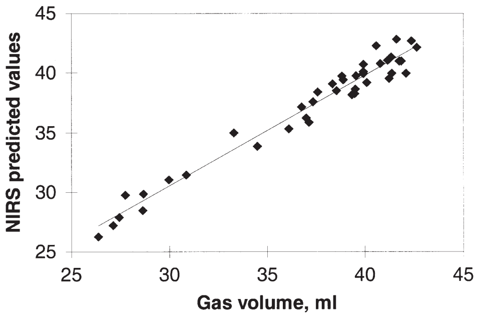

Figure 1. Relationship between actual and NIR predicted values of gas volume after 24 h incubation for alfalfa hay.

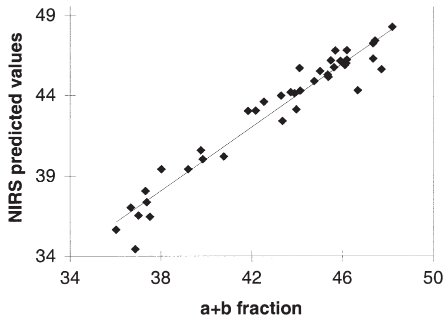

Figure 2. Relationship between actual and NIR predicted values of potential gas production (*a* + *b*) for alfalfa hay.

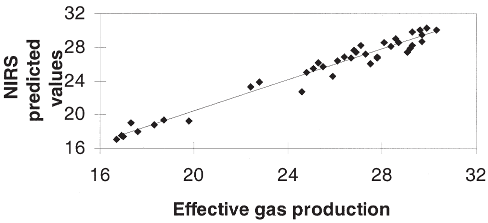

Figure 3. Relationship between actual and NIR predicted values of effective gas production at rumen outflow rate of 0.06 h^{-1} for alfalfa hay.

Statistical results of best NIR calibration equations for predicted the tested parameters are

Table 2. The statistical results of NIR calibrations for prediction of *in-vitro* gas production degradability of tested forages.

Forage type	Parameter	Math. transf.	Scatter Correction	PLS factors	*SEC*	R^2	*SECV*	CVr^2	CV_{cv}, %
Alfalfa hay	$a + b$	2.10.5	DT	6	0.76	0.959	1.00	0.929	2.33
	c	1.5.5	DT	3	0.88	0.693	0.89	0.681	10.06
	V_{24}	2.5.5	SNV	5	1.07	0.953	1.22	0.928	3.28
	EGP_3	2.10.5	DT	6	1.10	0.936	1.27	0.914	3.97
	EGP_6	2.10.5	DT	6	0.84	0.962	1.12	0.932	4.39
Meadow hay	$a+b$	2.5.5	SNV+DT	6	1.75	0.870	2.51	0.728	5.16
	c	1.10.5	SNV	6	0.60	0.329	0.61	0.324	12.78
	V_{24}	2.10.5	SNV+DT	3	1.42	0.825	1.81	0.737	5.41
	EGP_3	2.5.5	SNV	3	1.02	0.799	1.20	0.724	4.00
	EGP_6	2.5.5	SNV	3	0.94	0.756	1.07	0.686	4.81
Maize silage	$a+b$	2.10.5	DT	2	1.43	0.802	1.74	0.701	2.90
	c	1.10.5	DT	4	0.42	0.804	0.61	0.584	9.17
	V_{24}	1.10.5	SNV	2	1.86	0.658	2.18	0.555	4.43
	EGP_3	2.10.5	DT	2	1.01	0.804	1.08	0.776	2.48
	EGP_6	1.10.5	DT	2	1.12	0.758	1.27	0.713	3.75
Alfalfa silage	$a + b$	1.5.5	DT	6	2.55	0.796	3.37	0.656	10.46
	c	2.10.5	SNV+DT	4	0.85	0.761	1.09	0.619	15.81
	V_{24}	1.5.5	SNV	5	2.55	0.830	3.55	0.686	13.45
	EGP_3	1.10.5	DT	4	2.75	0.752	3.25	0.670	14.26
	EGP_6	1.5.5	DT	6	2.10	0.826	2.48	0.766	14.07

presented in Table 2. Figures 1–3 graphically illustrates the relationship between laboratory determined and NIR predicted values of gas volume after 24 h incubation, potential gas production $(a + b)$ and effective gas production for alfalfa hay.

To obtain an idea of the predicted abilities of NIR, the coefficient of variation for the cross-validation procedure ($CV_{cv} = SECV$/mean value of the sample set) was calculated.

Accuracy of NIR calibration equations for effective gas production, which was a measure of degradability, degradability characteristics and gas volume after 24 h incubation, was either high or promising. The accuracy of determination of the rate of gas production was relatively low. The results for gas volume determination were in accordance with investigations by Herrero *et al.*,[12] who obtained *SEC* = 1.36 ml, *SECV* = 1.55 ml, 1–*RV* = 0.78 for gas volume after 24 h incubation for kikuyu grass. The alfalfa hay samples gave the most accurate results for all parameters tested.

Principal wavelengths were highlighted using simple linear regression. The first five wavelengths for each group of samples and parameters, ordered according to coefficient of determination obtained, are given in Table 3. The wavelength segments, which had high correlation with degradability of tested forages were 1660–1700 nm, 1760–1800 nm, 2040–2070 nm, 2100–2115 nm, 2260–2209 nm and 2310–2315 nm. Some of these regions were close to the regions linked to *in vivo* digestibility and *in sacco* dry matter degradability in fresh grass, grass silage and maize silage.[13,14] Deaville and Givens[15] reported that spectral regions of 1640 to 1690 nm and 2170 to 2290 nm are associated with low *in sacco* rumen degradability in all forages. These results indirectly confirmed a relationship between gas production measurements with *in vivo* digestibility and degradation characteristics of forages.

Table 3. The first five wavelengths with the highest single correlation between tested parameters and spectral data.

Forage type	Parameter	Wavelength, nm
alfalfa hay	$a + b$	2315, 2070, 1798, 1705, 2113
	c	2315, 2111, 2280, 2237, 2134
	V_{24}	2315, 2113, 2070, 1800, 1693
	EGP_3	2315, 2070, 1800, 2113, 1693
	EGP_6	2315, 2070, 2113, 1800, 1705
meadow hay	$a + b$	1800, 2098, 1794, 2115, 1761
	c	2211, 2270, 3202, 2319, 1781
	V_{24}	1800, 1656, 2260, 1786, 1761
	EGP_3	1800, 1761, 1656, 2098, 2115
	EGP_6	1800, 1761,1656, 2295, 2098
maize silage	$a + b$	1745, 1781, 2312, 1693, 2028
	c	2315, 2003, 2302, 2295, 1736
	V_{24}	2060, 2003, 1800, 2312, 1946
	EGP_3	2060, 2003, 1800, 2312, 1946
	EGP_6	2060, 2003, 1800, 2182, 2237
alfalfa silage	$a + b$	2040, 2134, 2111, 2312, 1961
	c	2134, 2197, 2211, 2040, 2111
	V_{24}	2040, 1975, 2312, 2110, 2134
	EGP_3	2040, 2134, 2312, 2110, 2211
	EGP_6	2040, 2134, 2111, 2312, 1961

In conclusion, near infrared spectroscopy gives possibilities for estimating the degradation characteristics of a large number of forages without losing accuracy.

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