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The prediction of forage maize digestibility by near infrared reflection spectroscopy

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Abstract

The possibilities of Near Infrared Reflection Spectroscopy (NIRS) to predict the organic matter digestibility (OMD) of fresh forage maize were examined. Cellulase digestibility, corrected to in vivo level, served as reference method. Calibration was based on 261 samples, varying in OMD from 68.0 to 80.3% and validation occurred on 58 samples (71.7-75.9% OMD). With a scanning IA-500 monochromator the best equation, based on the second derivative of the reflected energy at wavelengths 1620 and 1664 nm, had a standard error of prediction (SEP) of 0.65%. The repeatability of the prediction amounted to 0.49% and was smaller than that of the reference analysis. The best equation, developed for a simulation of an IA-450 filter-apparatus, had a SEP of 0.74%. Cross-validation on the calibration set showed the validity of the calibrations for a wide range of digestibility. NIRS-predicted OMD was highly correlated with the reference OMD, whereas calculated OMD, based on constant digestion coefficients for the ears and the stalk+leaves, did not show any relationship.

Keywords: forage maize, digestibility, NIRS

Introduction

The main evaluation criteria for testing new maize varieties are earliness, resistance against lodging and the yield of digestible organic matter. Up to now, the latter characteristic is based on the assumption for all varieties that the digestibility of the ears (cob + grains + husks) is 80% and of the stalk + leaves 60%. This value, calculated from constant coefficients, does not account for varietal differences in digestibility. Such an evaluation system further implies that the ears and the rest of the plant have to be separately harvested, which is labour-intensive and not representative for practice.

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The digestibility of feeds for ruminants is conventionally determined by experiments with sheep. However, such in vivo trials are not suited for screening, because they take three to four weeks, ask large amounts of feed, are labour-intensive and expensive. These disadvantages are for a great part avoided by using simulation methods, based on the in vitro incubation of a feed sample with rumen fluid or commercial enzymes. For breeding purposes, however, these techniques remain too time-consuming and cumbrous. The development of the Near Infrared Reflectance Spectroscopy (NIRS) technology during the last two decades opened new perspectives for a fast and accurate quantitative and qualitative evaluation of diverse organic materials. Apart from its rapidity, this physical technique is non-destructive, asks no chemical reagents and produces no pollutants. The principle of the technique is the selective light-absorption by chemical bounds in the wavelength range of 1,100 to 2,500 nm, of which the intensity is proportional to their concentration. However, the relationship between the concentration of a separate constituent and the absorbed energy has to be indirectly derived, because the spectrum of a sample is the sum of the spectral bands of the separate constituents and varies with the measuring conditions. This implies the measurement of many samples, representative for the normal variation, of which the parameter(s) of interest is (are) determined following a classical method. The second phase of the calibration consists of the optimization of the mathematical model between the reference and the spectral data. Finally, the calibration equation has to be validated against an independent sample set.

In this paper the possibilities of NIRS to predict the in vivo digestibility of fresh forage maize from variety trials will be examined.

Materials and methods

The maize samples for the calibration set comprised 100, 54 and 107 samples from the National Institute for Plant Breeding, harvested in 1990, 1991 and 1992, respectively. The samples of 1990 originated from 10 standard varieties, grown at four centres and from 30 experimental varieties of two centres. The samples of 1991 were from 9 standard varieties of six centres. Those of 1992 came from two series of 9 standard varieties (from which 13 were different), both grown at three locations, further extended with 53 samples with low and high digestibility. The samples of the validation set concerned fresh maize from the own cultures of the National Institute for Animal Nutrition, consecutively harvested from 1987 to 1992. From differences in variety and harvest date 10 samples were selected from 1987, 9 from 1988, 6 from 1989, 7 from 1990, 7 from 1991 and 19 from 1992. All samples originated from chopped whole plants with exception of the calibration samples of 1990, which were composed afterwards in proportion to the dry matter yields of the ears and the rest of the plant.

The maize of the calibration set was dried at 78 ± 2°C for 72 hours. The samples of 1990 were ground with a Fritsch mill and those of 1991 and 1992 with a Gondar mill, both provided with a 1 mm sieve. The maize of the test set was dried at 60-70°C and ground with a Brabender mill through a 1 mm sieve.

Residual moisture was determined by drying at 103 ± 2°C for 3 hours and ash content was obtained after ignition in a furnace at 600°C.

The organic matter digestibility (OMD) was determined in duplicate following an enzymatic procedure (De Boever et al., 1986). It consists of three steps:

1. pepsin in 0.1 N hydrochloric acid for 24h at 40°C,
2. pepsin in 0.1 N hydrochloric acid for 45' at 80°C,
3. cellulase T. viride (BDH) for 24h at 40°C.

Because many versions of enzymatic methods are used, it is recommended to publish digestibility results in a uniform way. Therefore, in each run 3 standard samples of maize silage with in vivo OMD's of 68.1, 73.6 and 77.5 %, respectively, were analyzed in duplicate to correct the enzymatic digestibility of the unknown samples to the in vivo digestibility level. This correction increased the mean digestibility level with about 2%-units and halved the standard deviation between the samples.

NIRS analysis was carried out by means of an Infraalyzer 500 monochromator (Bran & Luebbe, Norderstedt, Germany) in the wavelength range from 1,100 to 2,500 nm with steps of 4 nm. From each sample two cups were filled and measured on different days. To obtain a more evenly balanced distribution of the calibration set, the 50% spectrally most interesting samples were selected by a Picks programme (Bran & Luebbe, 1989). Calibration was done by multiple linear regression analysis using either raw log 1/R data, first or second derivatives or principal components as independent variables. Segment and gap amounted to 8 and 14 nm for first and to 24 and 30 nm for second derivatives. A calibration was also developed for the more routinely used Infraalyzer 450 provided with 19 filters. Calibrations were evaluated by testing them on the validation set. The best performing equations, based on the whole spectrum and the filter set, were further cross-validated on the calibration set. In this test, alternately two-thirds of the calibration samples were used for calibration and one third for validation, so that each sample was tested independently; the standard errors of the 3 partial validations were then averaged.

Finally, the prediction accuracy of NIRS was compared with the use of constant digestibility coefficients of 80% and 60% for the ears and the stalk+leaves, respectively, for 36 samples of the validation set.

Results

In Table 1 the residual moisture content and the enzymatic OMD, corrected to in vivo level, are given for the calibration and the validation samples. The residual moisture content tended to be higher for samples of earlier harvest years. Averaged per year, digestibility was rather constant (72.2-75.1%). Within years, the variation in digestibility was greater for the calibration than for the validation samples. The mean ± standard deviation and range of the calibration set after picks and the validation set amounted to 74.3 ± 2.3, 68.0-80.3% and 73.6 ± 1.1, 71.7-75.9%, respectively. The repeatability of the corrected cellulase digestibility, calculated as the standard deviation of the differences between the first two determinations, amounted to 0.6%.

Table 1. Residual moisture content and in vivo corrected organic matter digestibility (OMD) of maize samples.

		Residual moisture (%)		In vivo corr. OMD (%)	
		$\bar{x}\pm s_x$	range	$\bar{x}\pm s_x$	range
Calibration					
1990	100	6.8±0.7	4.7-8.1	75.1±1.5	71.2-77.5
1991	54	4.5±0.4	3.4-5.1	72.2±1.6	68.5-76.0
1992	107	5.2±0.8	3.2-7.2	74.5±2.5	68.0-80.3
Validation					
1987	10	8.4±0.3	8.0-8.9	73.2±1.0	71.9-75.4
1988	9	8.1±0.6	7.1-9.2	72.9±0.8	71.7-74.1
1989	6	8.4±0.3	8.1-9.0	74.0±1.0	72.7-75.3
1990	7	7.6±0.9	6.3-9.1	73.7±1.1	72.0-75.4
1991	7	6.5±0.9	4.6-7.2	74.1±0.7	73.2-75.5
1992	19	4.0±1.0	2.3-5.6	73.9±1.2	72.2-75.9

Table 2 gives the calibration errors (SEC) for equations based on log 1/R data, first and second derivatives, involving 2, 3, 4 or 5 wavelengths, together with the corresponding validation errors (SEP). The number of wavelengths, at which IA-500 equations performed best, was 5 for log 1/R data, 5 for first derivatives and 2 for second derivatives. A regression, using the first 7 principal components, had a SEC of 0.99 and a SEP of 1.01%. Overall best performance (SEC = 0.83% ; SEP = 0.65%) was obtained with the second derivative equation based on the reflections at the wavelengths 1620 and 1664 nm. The best equation, developed for the IA-450 apparatus, was based on the reflections at 6 filters (1680, 1722, 1940, 2100, 2208 and 2270 nm) and resulted in a SEC of 0.82% and a SEP of 0.74%.

The SEP and bias of the whole spectrum - and the filter calibration for the separate years are given in Table 3. The SEP-values showed little variation among years, despite the first three years were not represented in the calibration set. The monochromator calibration gave some systematic underestimation of maize from '89 and '92, whereas a positive bias was detected in '90. The filter calibration overestimated the maize from '87.

Table 2. Calibration (SEC) and prediction errors (SEP) of the NIRS-equations.

	Number of wavelenghts							
	2		3		4		5	
	SEC	SEP	SEC	SEP	SEC	SEP	SEC	SEP
log 1/R	1.23	1.10	0.95	1.51	0.76	0.90	0.74	0.80
1st derivative	0.94	1.35	0.89	1.78	0.87	1.83	0.72	0.87
2nd derivative	0.83	0.65	0.79	0.68	0.73	0.71	0.70	0.80

Table 3. SEP and bias of NIRS calibrations for the maize validation samples of different years.

Years	IA-500		IA-450	
	SEP	Bias	SEP	Bias
1987	0.58	0.29	0.96	0.65
1988	0.29	–0.05	0.61	–0.06
1989	0.71	–0.34	0.76	–0.06
1990	0.78	0.42	0.47	0.30
1991	0.50	0.07	0.70	0.26
1992	0.77	–0.41	0.76	–0.24
All	0.65	–0.07	0.74	0.09

The calibrations were further examined by cross-validation on the calibration set to get an idea of their performance on samples with a wider range in digestibility than the validation samples. This test resulted in SEP's of 0.79 and 0.84% for the calibration based on the whole spectrum and the filterset, respectively.

The repeatability, calculated as the standard deviation of the differences between two scans on different days, was with 0.49% smaller than that of the reference analysis.

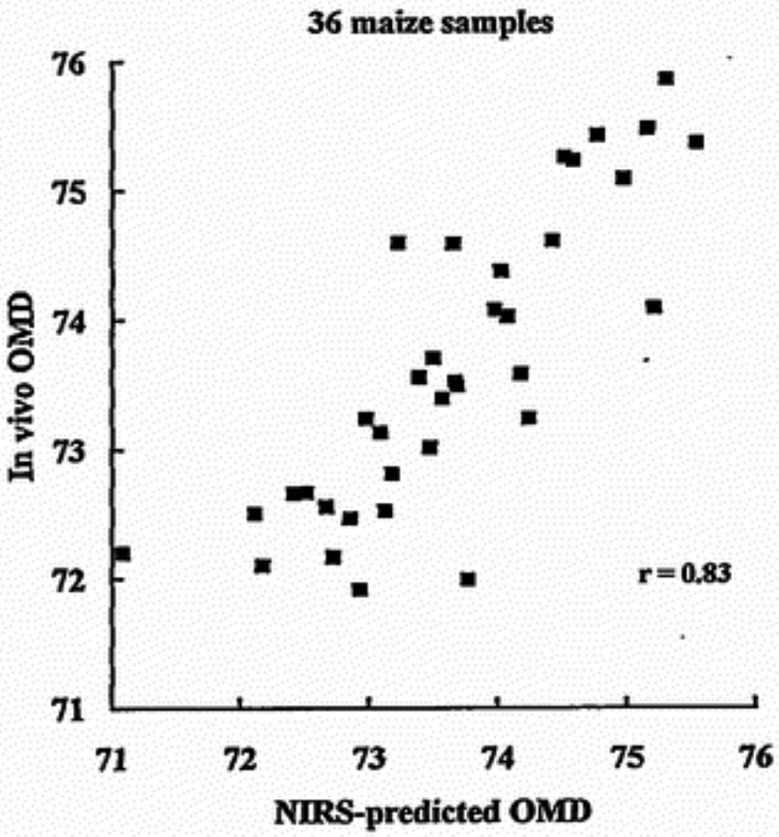
Figure 1 shows the relationships between the reference OMD and the NIRS-predicted as well as the calculated OMD. This comparison was done for the 36 samples of the validation set, from which the portions of the ears and the stalk+leaves in the dry matter were known. Mean and standard deviation of in vivo, NIRS and calculated OMD amounted to 73.6 ± 1.2, 73.6 ± 1.0 and 70.0 ± 1.2, respectively. NIRS-predicted OMD was highly correlated with the reference OMD ($r = 0.83$), whereas the calculated OMD did not show any relationship.

Discussion

The tendency of an increasing moisture content for samples of earlier harvest years was due to the longer storage time, because determination occurred soon after or before NIRS-analysis, which for all samples was carried out in the period 1992-93.

The range in digestibility between different varieties was larger than in a study of some 10 years ago (De Boever et al., 1983); particularly in 1992 some experimental varieties exceeded the standard varieties. In view of an adequate ranking of varieties, the level of repeatability of the reference results can be considered satisfactory. Anyway, the cellulase method is better repeatable than an in vivo experiment. For 50 maize silages, of which digestibility (mean: 74%) was obtained with 5 non-lactating cows or 5 adult wethers, an average variation coefficient of 1.55% was calculated (De Boever et al., 1988). Moreover, the repeatability of the reference method is very important for the indirect NIRS technique, since the calibration error cannot become smaller.

The best performing calibration based on second derivatives contained fewer terms and was more accurate than the best equation based on log 1/R data or first de-



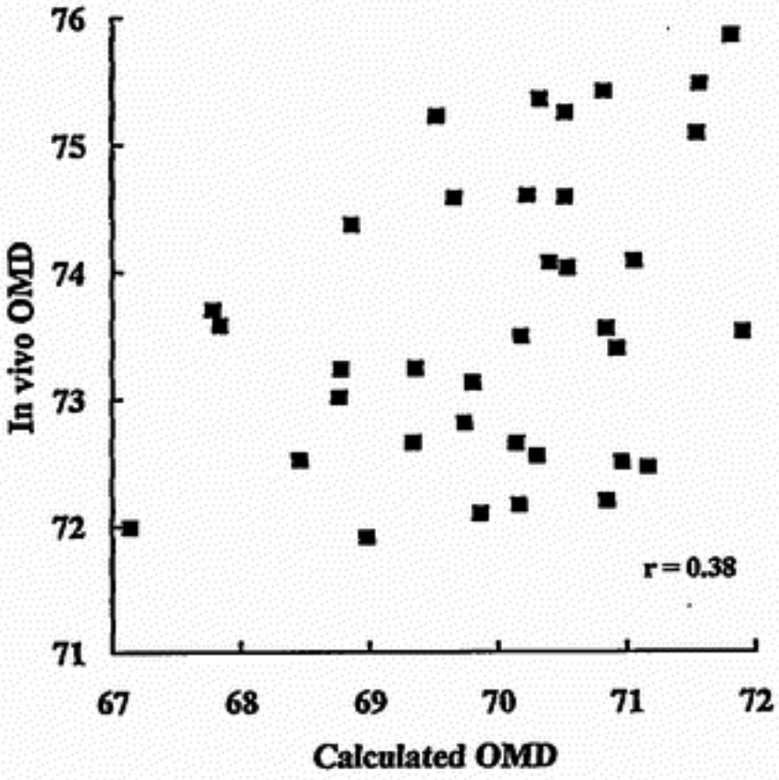


Figure 1. a. Correlation between NIRS predicted and in vivo OMD; b. correlation between calculated and in vivo OMD.

rivatives. The effect of using second derivatives consists in the resolution of overlapping peaks and the removal of baseline shifts (Hruschka, 1990). In our study baseline variations could be expected from differences in sample moisture content, in particle size and in harvest years. In view of future application, building in of many variables is certainly an advantage. The robustness appeared from the excellent performance of the second derivative equation with two terms on the validation samples, ground with another mill than the calibration samples and of which half originated from other harvest years. This equation was also better than the one based on principal components, a data compression technique claimed to eliminate collinearity among wavelengths. The slightly higher prediction error by cross-validating the calibration set showed the validity of the calibration for a wider range of digestibility than that of the validation set. The filter calibration was almost as accurate as the monochromator calibration. The higher number of terms in the former equation obviously compensated for the absence of a filter in the relevant 1620 - 1664 nm region and for the baseline variations. Interpretation of the wavelengths selected is not always clear-cut, but in this case 1620 and 1664 nm could almost certainly be traced back to indigestible cell wall material or lignin (Norris et al., 1976 ; Deaville and Baker, 1993).

Our calibration was more accurate than those published in the literature. In Table 4 an overview is given of NIRS calibrations to predict the digestibility of forage maize. Pure comparison is difficult because of differences in the nature and range of

Table 4. Literature overview of calibrations to predict digestibility of forage maize.

Reference	Nature of samples	n	Digestibility[2]	Range	Apparatus	SEC	SEP
Moe and Carr, 1985	silage	142	IVDOM	38.5-74.7	FQA51[3]	5.4	4.0
Valdes et al., 1987	fresh	90	IVDMD	60.2-83.8	IA-400[3]	1.6	1.6
Van Es et al., 1987	silage, fresh, cobs	154	IVDOMD*	50.0-78.0	IA-500[4]	1.7	1.5
			CDOMD*	46.1-79.0	IA-500	1.5	1.3
Biston & Dardenne, 1988	silage, fresh	90	COMD	64.0-81.6	PSCO6250[4]	0.8	1.1
			vivo OMD	63.1-77.7	PSCO6250	1.5	1.6
			vivo OMD	63.1-77.7	IA-450[3]	2.0	2.1
Reeves III et al., 1989	silage[1]	59	IVDDM	72.7-89.8	PSCO6350[4]	2.2	1.4
Mainka, 1990	fresh	147	IVOMD	64.1-82.3	PSCO6250	1.9	1.6
This study	fresh	134	COMD*	68.0-80.3	IA-500	0.8	0.7
					IA-450	0.8	0.7

[1] silages were scanned in undried form
[2] IV = in vitro Tilley and Terry; C = cellulase
* corrected to in vivo digestibility level
[3] filter instruments
[4] monochromators

the samples, in the nature and expression form of digestibility and in the apparatus. General tendencies are that the accuracy is better for fresh than for ensiled maize and for cellulase than for in vivo or rumen fluid digestibility. The worse results with maize silage as compared with fresh maize may originate from the difficulty in obtaining a representative sample from the former because of the presence of volatile fermentation products. As discussed earlier, the reproducibility of the reference digestibility, which is lower in vivo or with rumen fluid than with commercial enzymes, highly determines the calibration error. Filter instruments, at least those provided with 19 filters, can predict maize digestibility almost as accurate as scanning monochromators.

Current ranking of maize varieties using constant digestion coefficients for the ears and the rest of the plant is not only labour-intensive but also not correct. Prediction of OMD by NIRS, on the other hand, is very fast and more accurate. For 918 in vivo experiments with maize silage, involving 175 genotypes, Barrière et al. (1992) also did not find a relationship between the grain content and the OMD, whereas cellulose digestibility explained two-thirds of the variability.

Conclusion

Considering its rapidity and accuracy to predict digestibility, NIRS is the appropiate technique to screen maize varieties. Because a NIRS-equation is only valid within the variation of the calibration set, its performance should regularly be checked. After each new harvest, a certain percentage of the samples should be analyzed in a classical way to adjust for a possible bias and the calibration should eventually be recalculated for extreme samples or outliers.

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