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NIRS determination of non-structural carbohydrates, water soluble carbohydrates and other nutritive quality traits in whole plant maize with wide range variability

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Abstract

The aim of this work was to study the potential of near-infrared reflectance spectroscopy (NIRS) to predict non-structural carbohydrates (NSC), water soluble carbohydrates (WSC), *in vitro* organic dry matter digestibility (IVOMD), organic matter (OM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF) and starch in samples of whole plant maize with a wide range of variability. The samples were analyzed in reflectance mode by a spectrophotometer FOSS NIRSystems 6500. Four hundred and fifty samples of wide spectrum from different origin were selected out of 3,000 scanned for the calibration set, whereas 87 independent random samples were used in the external validation. The goodness of the calibration models was evaluated using the following statistics: coefficient of determination (R^2), standard error of cross-validation (SECV), standard error of prediction for external validation (SEP) and the RPD_{CV} and RPD_P indexes [ratios of standard deviation (SD) of reference analysis data to SECV and SEP, respectively]. The smaller the SECV and SEP and the greater the RPD_{CV} and RPD_P, the predictions are better. Trait measurement units were g/100 g of dry matter (DM), except for IVOMD (g/100 g OM). The SECV and RPD_{CV} statistics of the calibration set were 1.34 and 3.2 for WSC, 2.57 and 3 for NSC and 2.3 and 2.2 for IVOMD, respectively. The SEP and RPD_P statistics for external validation were 0.74 and 4.7 for WSC, 2.14 and 2.5 for NSC and 1.68 and 1.6 for IVOMD respectively. It can be concluded that the NIRS technique can be used to predict WSC and NSC with good accuracy, whereas prediction of IVOMD showed a lesser accuracy. NIRS predictions of OM, CP, NDF, ADF and starch also showed good accuracy.

Additional key words: coefficient of determination; modified partial least-squares; organic matter; partial least-squares.

Introduction

Forage maize (*Zea mays* L.) is an important source of fodder for dairy farms in northwest of Spain, where the silage dependence for cow feeding extends over five to seven months per year. The near infrared spectroscopy (NIRS) technique allows rapid determination of forage nutritional quality compared to routine analysis in laboratories of animal nutrition, without destroying or contaminating the samples (Williams, 2001).

This technique has been used to estimate nutritional quality traits of forage from the 1970's to the present (Norris *et al.*, 1976; Shenk *et al*., 1976; Garrido *et al*., 1993; Shenk & Westerhaus, 1995). The technique has also been recommended by different authors as an adequate method for evaluating quality traits such as crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), lignin (ADL), hemicellulose (HCEL), starch, pH, etc. in maize whole plant or maize silage (Valdes *et al.*, 1987; Reeves *et al.*, 1989; Amari & Abe, 1997; De Boever *et al.*, 1997; Cozzolino *et al.*,

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Abbreviations used: ADF (acid detergent fiber); AS (ashes); CP (crude protein); DM (dry matter); IVOMD (*in vitro* organic matter digestibility); MPLS (modified partial least-squares); NDF (neutral detergent fiber); NIRS (near infrared spectroscopy); NSC (non-structural carbohydrates); OM (organic matter); PLS (partial least-squares); RPD_{CV} and RPD_P (ratio of SD of reference analysis data to SECV and SEP, respectively); SECV (standard error of cross validation); SEP (standard error of prediction for external validation); WSC (water soluble carbohydrates).

2001, 2003, 2006; Flores, 2004; Campo *et al.*, 2007, 2010; Riboulet *et al.*, 2008). There have been few studies on the determination of non-structural (NSC) and water soluble carbohydrates (WSC). The usefulness of the NIRS technique to determine NSC and non-protein nitrogen in forage has been questioned by some authors (Vasquez *et al.*, 2004), since starch may interfere with the cellulose in some infrared wavelengths.

The NSC content is an important component in animal feed, comprised mainly of starch, sugars and fructans. The NSC form part of the energy reserve of the plant, which can be immediately used by animals and have positive influence on utilization of other nutrients (MacGregor *et al.*, 1983). The total NSC content in the forage is more important than the separation in different fractions concerning the energy balance of animal feed (Castro, 2000). The NSC concentration in the maize stover is inversely correlated with the starch content in the maize grain due to translocation of NSC from the stover to ear. This translocation supplies starch into the grain when the plant is approaching maturity. The NSC content increases the digestibility in the stover, whereas the starch content increases it in the grain. Thus the digestibility of the maize whole plant remains stable during the last weeks of plant ripening because it is minimally affected by the NSC translocation from the stover to the grain (Murray *et al.*, 2008).

The WSC concentration composed mainly of sugars and fructans is a determining factor in the ensilage of maize under anaerobic conditions, because WSC is the substrate for the growth of lactobacilli needed for acid lactic fermentation (Smith, 1973). Legumes and grasses from temperate climate accumulate starch in the vegetative parts and grain, while the grasses accumulate also fructans in the vegetative parts. Starch degradation is slower than WSC in rumen, what promotes greater use of nitrogen by the animal.

Therefore, it seems important the need for accurate, simple and rapid analysis of the different components of the forage maize at harvest, such as NSC, WSC, *in vitro* organic matter digestibility (IVOMD), CP, ADF, NDF and starch to know the nutritive quality value of the forage maize harvested and take decisions on animal feeding.

The aims of this work were: (i) to determine specific NIRS calibration equations for WSC, NSC, IVOMD and other nutritive quality traits such as CP, ADF, NDF, starch and organic matter (OM) in whole plant forage maize with wide range variability; (ii) to estimate the accuracy of the NIRS technique for these traits by using an external validation set; and (iii) to study the relationships among the nutritive quality traits evaluated in whole plant forage maize.

Material and methods

Sample selection for analysis

Between 2002 and 2010, a large amount of samples from maize whole plant was gathered from trials conducted in different locations of Galicia (Spain) and cultivated under diverse environmental conditions. In order to create wide range calibration equations, the selected samples came from wide genetic diversity sources (inbred lines, experimental hybrids and commercial hybrids), different origin (Germany, France and Spain), different tillage systems (conventional and organic) and different maturity stages. The final plant density was about 9 plants m^{-2} in most of the trials where samples were collected. More than 3,000 samples were analyzed by NIRS, of which 450 were selected for calibration and 87 for external validation. Sample selection was based on expanding the large variability of spectra by adding appropriate outlier samples to previous calibrated equations after analysis of their nutritive components by reference methods. In addition, an external validation set of 87 random independent samples from the original 3,000 samples was formed and analyzed by using the reference methods for nutritional traits except for IVOMD, WSC and starch where only 50, 69 and 69 samples were used, respectively.

The PROC CORR of SAS statistical package (SAS, 2008) was used to analyze the simple correlation between carbohydrates and the rest of the nutritional traits.

Reference analysis

Fresh samples of whole plant forage maize were taken to determine the dry matter (DM) content. The DM content was analysed by drying 300 g of each representative sample in an oven at 80°C with a flux of 85% renewed air for 16 hours (Castro, 1996). Afterward, samples were ground through a 1 mm screen in a Christy & Norris 20.32-cm mill.

The analysis of residual moisture and ashes (AS) was performed in a thermogravimetric analyser TGA-601 (LECO Co., MI, USA). The OM content was ex-

pressed as 100 minus AS corrected by residual moisture. Nitrogen content was analyzed by Kjeldhal and a colorimetric determination in an autoanalyzer Bran + Luebbe. Factor of conversion to CP was 6.25 (method no. G-188-97 Rev 2, Bran + Luebbe, Analyser Division, Norderstedt, Germany). The ADF and NDF contents were analysed according to Goering & Van Soest (1970) method in a Fiberted digester (Foss Tecator AB, Sweden). The WSC and NSC contents were determined by a colorimetric method in an autoanalyzed Bran + Luebbe according to Castro (2000). Every sample was duplicated in each analysis, and reanalyzed if the difference between duplicates was larger than 5%.

The IVOMD was determined according to Tilley & Terry (1963). Samples were analyzed in series of 80-120 units. In order to adjust for variation of IVOMD estimates among series, 30-50 problem samples were duplicated in each series; in addition one rumen fluid blank sample was duplicated and four standard reference samples, which remained the same over all series, were quadruplicated. Duplicated problem samples were reanalyzed if their difference was larger than 5%. Any quadruplicated standard was eliminated if the difference between the highest and the lowest value was larger than 5%. Possible differences in digestibility among series were adjusted by the following equation (Flores *et al.,* 2003):

$$Dx_{ADx} = \frac{Dref}{Dref_i} Dx_i$$

where Dx_i, and Dx_{ADx}, are the *in vitro* digestibility means of sample *x* in series *i* and the adjusted *x* sample, respectively; $Dref_i$ and *Dref* are the *in vitro* digestibility means of the reference samples estimated in series *i* and the known true means, respectively.

Finally, starch content was estimated as the difference between NSC and WSC content (Flores, 2004). The DM was expressed as g/100 g of fresh matter, IVOMD as g/100 g OM and the rest of the evaluated traits were expressed as g/100 g DM.

NIRS analyses

Two samples of ~2.5 g were taken from ~100 g of dried and ground whole maize plants. The duplicate samples were analysed in a monochromator spectrophotometer Foss NIRSystems 6500 using the spinning module with a 5-cm diameter quartz-window cup completely filled. The WinISI II, v1.5 (ISI, 2000) statistical software was used to record the spectral data, calibration, validation and results analysis.

Calibration

Calibration equations were performed by regression between spectral data and reference analysis data. The statistical procedures were PLS (partial least-squares) and MPLS (modified partial least-squares). The spectral range 1,100-2,500 nm was used with a scatter correction SNV (standard normal variate) and d-trend (Barnes *et al.,* 1989). The automatically elimination procedure of WinISI II was applied to eliminate outliers by using one pass for outlier elimination. The samples with $T > 2.5$ and $H > 3$ (Mahalanobis distance) were considered outliers.

The fitted calibration equations were selected on the basis of the highest values of the determination coefficient (R^2) of calibration and the RPD_{CV} index [ratio of the standard deviation (SD) of the reference analysis to the standard error of cross validation (SECV)] (Williams, 2007). RPD_{CV} estimates below 1.5 indicate that the equation cannot be used for prediction; between 1.5 and 2 the equation can distinguish between high and low values; between 2 and 2.5 indicate that acceptable quantitative predictions can be made; between 2.5 and 3 predictions are good; and values above 3 indicate that the predictions are excellent (Saeys *et al.*, 2005). SECV values were also taken into account to establish levels of predictions.

External validation

In the external validation the R^2 and the standard error of prediction (SEP) were estimated. The utility of prediction models was evaluated by RPD_P (ratio between SD of the validation set to the SEP) (Williams & Sobering, 1996; Saeys *et al.*, 2005). No outliers were eliminated in the external validation set.

Results

Table 1 shows the range between maximum and minimum values, mean and SD for the nutritional value traits of the calibration and the external validation sets analyzed by reference methods. Chemical variation found in the reference analyses of nutritional traits could be considered acceptable and wide enough for

Table 1. Mean, standard deviation (SD) and range (maximum and minimum) of nutritive traits analyzed by reference methods in whole plant forage maize from the calibration set, which included 450 samples, and the validation set with 87 samples

Trait[1] (g/100 g DM)	Calibration				External validation			
	Mean	SD	Max	Min	Mean	SD	Max	Min
OM	96.36	0.87	97.77	91.55	96.56	0.69	97.64	92.59
CP	6.03	1.20	9.80	2.88	6.20	0.84	8.50	3.27
NDF	47.51	5.44	63.42	30.77	44.97	4.54	56.33	34.47
ADF	24.45	3.43	36.49	16.64	22.67	3.15	31.36	16.17
IVOMD	68.83	4.95	80.12	50.52	71.65	2.62	77.76	65.10
WSC	9.14	4.29	24.16	1.10	10.16	3.49	22.77	4.04
NSC	39.26	7.79	75.92	16.43	41.49	5.32	51.34	27.40
Starch	30.37	9.03	69.15	3.20	31.51	5.82	43.88	14.32

[1] DM: dry matter. OM: organic matter. CP: crude protein. NDF: neutral detergent fiber. ADF: acid detergent fiber. IVOMD: *in vitro* organic matter digestibility (g/100 g OM). WSC: water soluble carbohydrates. NSC: non-structural carbohydrates.

the development of the aimed calibration equations. The ranges of variation on the validation set were within the range of variation of the calibration set for all evaluated parameters.

Table 2 shows the correlation coefficients between nutritional traits analyzed by chemical methods. Positive high correlations were obtained between NDF and ADF (0.96), NSC and both OM and starch (0.69 and 0.88, respectively), OM and starch (0.64) and between IVOMD and WSC (0.62). Negative high correlations were found between both NDF and ADF and the following traits IVOMD (–0.38, –0.48), NCS (–0.93, –0.88) and starch (–0.76, –0.70, respectively).

NIRS calibration

Table 3 summarizes the best statistics of calibration and cross-validation for the evaluated traits. The best predictions were achieved with the statistical method PLS, the second derivative and eight cross-validation sets for OM, CP, ADF and IVOMD. Statistical method MPLS, the second derivative and four cross-validation sets provided the best predictions for NSC and starch, whereas MPLS, the first derivative and four cross-validation sets were the most adequate for NDF and WSC.

The R^2 of calibration showed satisfactory values for most of the tested parameters: 0.93 0.92, 0.91, 091, 0.90, and 0.90 for CP, ADF, OM, NDF, WSC and starch, respectively. The IVOMD and NSC only reached R^2 values 0.77 and 0.87, respectively. The SECV were similar to those of calibration (SEC), with values 0.26, 0.32, 1.68, 1.03, 2.53, 1.38, 2.69 and 2.99 for OM, CP, NDF, ADF, IVOMD, WSC, NSC and starch respectively.

The RPD_{CV} values were higher than 3.0 for most of the nutritive traits evaluated 3.8, 4.1, 3.4, 3.5, 3.2, 3.0 and 3.4 for OM, CP, NDF, ADF, WSC, NSC and starch, respectively. Most authors indicate that NIRS predictions can be used if RPD_{CV} values are above 2.5 (Edney *et al*., 1994; Mouazen *et al*., 2005; Saeys *et al*., 2005). Only IVOMD was below this threshold with an RPD_{CV} value 2.2.

Table 2. Coefficients of correlation between cell wall components of 450 samples of whole plant forage maize analyzed by reference methods

Components[1]	CP	NDF	ADF	IVOMD	WSC	NSC	Starch
OM	–0.49***[2]	–0.52***	–0.49***	–0.03[ns]	–0.19***	0.69***	0.64***
CP		–0.09*	–0.20***	0.35***	0.12**	–0.09*	–0.18***
NDF			0.96***	–0.38***	0.09*	–0.93***	–0.76***
ADF				–0.48***	0.05[ns]	–0.88***	–0.70***
IVOMD					0.62***	0.18***	–0.16***
WSC						–0.22***	–0.63***
NSC							0.88***

[1] See Table 1. [2] Statistical significant levels * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. ns: non-significant $p > 0.05$.

Table 3. Calibration and cross-validation statistics for nutritional value traits in whole plant forage maize obtained by regression

Trait[1] (g/100 g DM)	Adjustment model		Calibration[4]		Cross-validation[5]		
	SP[2]	MT[3] (a,b,c,d)	SEC	R^2	SECV	R^2_{CV}	RPD_{CV}
OM	PLS	2,8,4,1	0.23	0.91	0.26	0.88	3.8
CP	PLS	2,8,4,1	0.29	0.93	0.32	0.92	4.1
NDF	MPLS	1,4,4,1	1.61	0.91	1.68	0.91	3.4
ADF	PLS	2,8,4,1	0.98	0.92	1.03	0.91	3.5
IVOMD[2]	PLS	2,8,4,1	2.30	0.77	2.53	0.72	2.2
WSC	MPLS	1,4,4,1	1.34	0.90	1.38	0.89	3.2
NSC	MPLS	2,4,4,1	2.57	0.87	2.69	0.85	3.0
Starch	MPLS	2,4,4,1	2.67	0.90	2.99	0.87	3.4

[1] See Table 1. [2] SP: statistical procedure (PLS: partial least-squares, MPLS: modified partial least-squares). [3] MT: mathematical treatment (a: order of derivative of log10(1/R) where R is the ratio of the intensity of light reflected from the sample to that reflected from a reference surface; b: the gap, *i.e.,* number of wavelength points used to calculate the derivative; c: number of points used for the first smoothing; d: the number 1 means that the second smoothing was not applied). [4] SEC: standard error of calibration. R^2: coefficient of determination of calibration. [5] SECV: standard error of cross-validation. R^2_{CV}: coefficient of determination of cross-validation. RPD_{CV}: ratio of standard deviation of reference analysis to SECV.

External validation NIRS

The standard errors of predictions for the external validation (SEP) were generally lower than the SEC estimates. The values of R^2 were 0.88, 0.83, 0.89, 0.88, 0.62, 0.95, 0.84 and 0.85 for OM, CP, NDF, ADF, IVOMD, WSC, NSC and starch, respectively (Table 4). As happened in the calibration procedure, the external validation accuracy was also low for IVOMD, with low R^2 value 0.62 and moderate SEP value 1.68. The SEP values for the rest of traits were good, because SEP values less than one third of the SD of the reference data confirm a very good calibration equation (Kennedy *et al*., 1996). The RPD_P values in the external validation (Table 4) were 3.5, 2.6, 3.0, 2.9, 1.6, 4.7, 2.5 and 2.6 for OM, CP, NDF, ADF, IVOMD, WSC, NSC and starch, repectively. Except for IVOMD the other analyzed traits exceed the value of 2.5. This confirms that the developed model can make good predictions for those nutritional trais.

Table 4. External validation statistics obtained by regression for the estimation of nutritional traits in whole plant forage maize

Trait[1] (g/100 g DM)	SEP[2]	R^2	RPD_P
OM[2]	0.20	0.88	3.5
CP	0.33	0.83	2.6
NDF	1.52	0.89	3.0
ADF	1.10	0.88	2.9
IVOMD[3]	1.68	0.62	1.6
WSC	0.74	0.95	4.7
NSC	2.14	0.84	2.5
Starch	2.26	0.85	2.6

[1] See Table 1. [2] SEP: standard error of prediction; R^2: coefficient of determination for external validation; RPD_P: ratio of standard deviation of reference analysis in external validation to SEP. [3] IVOMD: *in vitro* organic matter digestibility (g/100 g OM).

Fig. 1, with eight parts (a), (b), (c), (d), (e), (f), (g) and (h), shows the relationship between the reference analysis of the external validation sample set and NIRS predicted values for OM, CP, NDF, ADF, IVOMD, WSC, NSC, and starch, respectively. The R^2 of prediction and the RPD_P were also shown in each part.

Discussion

Positive correlation coefficients between NSC and nutritive traits such as OM (0.53) and starch (0.93) were found by Flores (2004) in whole plant maize, as we found in this work. The same author found negative correlation coefficients between WSC and starch (–0.67), NSC and ADF (–0.78) and IVOMD and ADF (–0.74), which were similar to those presented in this work (Table 2), except for the last one, which was higher in magnitude than ours (Table 2). Mechin *et al*. (2001) found negative correlations between IVOMD and NDF (–0.69) and starch and NDF (–0.84) in maize silage; these values were higher in magnitude than those presented in this paper (–0.38 and –0.76, respectively). Likewise, Cozzolino *et al*. (2001) reported

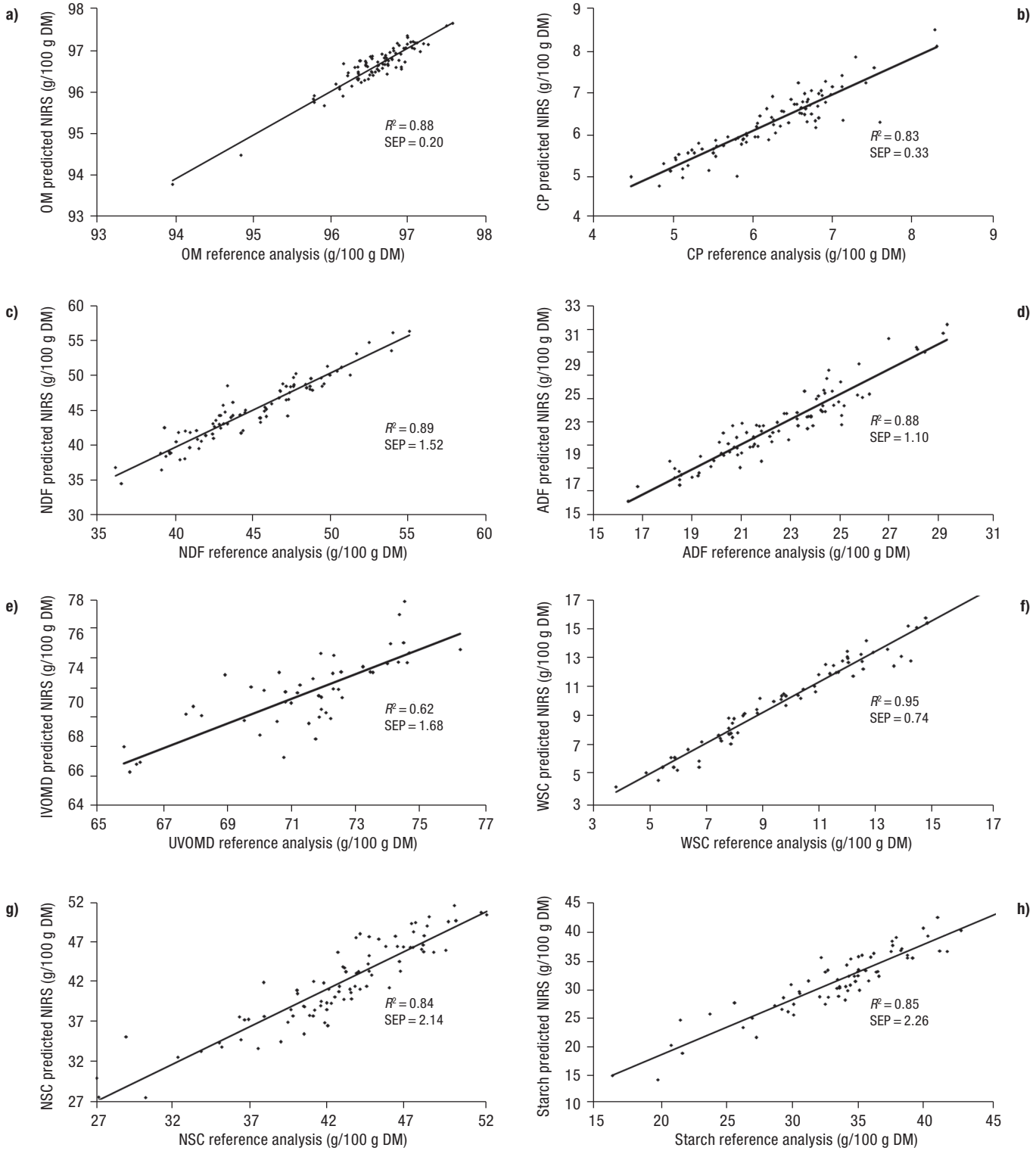

Figure 1. Relationships between reference chemical analysis values from external validation and NIRS predicted values in whole plant forage maize for organic matter (OM), crude protein (CP), neutral detergent fiber(NDF), acid detergent fiber (ADF), *in vitro* organic matter digestibility (IVOMD), water soluble carbohydrates (WSC), non-structural carbohydrates (NSC) and starch in parts (a), (b), (c), (d), (e), (f), (g) and (h), respectively. Two statistics, the coefficient of determination of prediction (R^2) and the standard error of prediction (SEP) are shown in each part.

correlation coefficients between IVOMD and NDF (–0.49) and between ADF and NDF (0.79) with the same sign but higher and smaller in magnitude than those presented in Table 2, respectively.

Values of R^2 from the calibration procedure were higher than 0.87 for all nutritional traits except for IVOMD 0.77. Therefore, according to the Williams (2001, 2007) criteria, the predictions were excellent for OM, CP, NDF and ADF, good for WSC, NSC and starch, whereas only acceptable quantitative predictions for IVOMD were obtained. Cozzolino *et al*. (2001) showed similar R^2 values for CP 0.96, NDF 0.98 and ADF 0.96, and higher R^2 values for IVOMD 0.98 than those found in this work.

The moderate accuracy found for IVOMD predictions in this work might be due to two main factors: (i) the wide range of samples used in the calibration procedure in comparison with other studies, which makes that the prediction equation is useful for a large universe of samples but with loss of precision for some of the samples; and (ii) especially the lesser accuracy in the reference method, which uses biological rumen fluid samples, in comparison with more precise chemical methods.

In the revision of scientific literature on calibration equations, different authors have found similar, better or worst accuracy parameters than those found in this study for the different nutritive traits. Similar values of R^2 for CP (0.90), and smaller values for IVOMD, ADF and NDF (0.40, 0.81 and 0.84, respectively) were shown by Cozzolino *et al.* (2006) in comparison with results of this work (Table 3). Reeves *et al*. (1989) achieved similar R^2 values for CP (0.83), ADF (0.86) and NDF (0.85) and smaller values for IVOMD (0.53) than in this work (Table 3). Park *et al*. (2005) also reported values of calibration parameters R^2 and SECV in silage maize (0.96 and 1.05 for ADF, 0.93 and 2.14 for NDF, and 0.88 and 0.29 for CP, respectively), which were similar to those obtained in this study (Table 3), whereas their estimates 0.92 and 1.73 for IVOMD were better than those found in our study. The estimation of SECV for IVOMD presented in this work 2.53 was similar to those found in several studies of silage maize 2.1 in De Boever *et al*. (1997), 2.79 in Castro *et al*. (2001) and 2.45 in Castro *et al*. (2004).

In relation to the RPD_{CV}, Cozzolino *et al*. (2006) found the same estimation for CP (4.1) than that presented in Table 3 and poorer values for the traits ADF (2.0), NDF (1.6) and IVOMD (1.0). The results of our study showed an RPD_{CV} value of 2.2 for IVOMD below the threshold value of 2.5. Therefore, according to Saeys *et al*. (2005), the IVOMD estimates could not be used to make accurate quantitative predictions for this trait, but are useful for approximate quantitative predictions. The RPD_{CV} index calculated according to the equation $1/\sqrt{1-R^2_{CV}}$ by Park *et al*. (2005) for IVOMD in silage maize was 2.1, which was similar to the value 2.2 showed in Table 3 of this work.

The calibration equations developed in this work yielded values of SECV and RPD_{CV} 1.38 and 3.2 for WSC, and 2.69 and 3 for NSC, respectively. These parameters indicate same or better accurate predictions than those showed by Castro *et al*. (2001) in silage maize, with values of SECV and RPD_{CV} 1.53 and 1.36 for WSC, and 3.47 and 3.1 for NSC, respectively.

The R^2 values in external validation were similar to those of Cozzolino *et al*. (2001) in the maize whole plant, 0.86, 0.83 and 0.98 for CP, NDF and ADF, respectively. These researchers concluded that NIRS can be used for estimating ADF, NDF and IVOMD with good accuracy; however they did not present external validation data for digestibility. Reeves *et al*. (1989) found R^2 and RPD_P estimates of 0.83 and 2.41 for CP, 0.87 and 2.72 for ADF, 0.82 and 2.61 for NDF and 0.82 and 1.46 for IVOMD, respectively, what indicates NIRS prediction parameters poorer than those obtained in this work.

According to these results, it can be concluded that the NIRS method can be used to predict WSC and NSC with very good accuracy, and IVOMD with a lesser precision but acceptable for establishing approximate quantitative differences. The other nutritional traits, OM, CP, NDF, ADF and starch, can be also predicted with good accuracy by NIRS.

The IVOMD needs further discussion because its determination by the reference method is associated with a greater error due to the sum of laboratory errors and the error associated with the animal variability concerning the rumen fluid. Thus the NIRS predictions for digestibility are generally less accurate than predictions for other chemical constituents analyzed by more precise wet reference methods. The SECV value for IVOMD in this work, 2.53, was lower than that achieved in other studies (Castro *et al.,* 2001) and slightly higher than the estimate obtained by De Boever *et al*. (1997) with a SECV of 2.1. Although in both cases NIRS estimates were based on reference to *in vivo* organic matter digestibility in silage maize. A previous

study attained very good IVOMD prediction parameters for the maize whole plant with estimates of R^2 and SECV 0.98 and 1.78, respectively, when 290 samples were analyzed and the MPLS statistical procedure was used (Cozzolino *et al*., 2001). In other study (Lovett *et al.*, 2004) the R^2 and RPD_{CV} estimations were 0.74 and 1.8, respectively, which were close to those presented in this work.

Cozzolino *et al*. (2006) proposed that the NIRS technology was an appropriate method to determine DM, CP and ADF in silage maize. De Boever *et al*. (1997) had also endorsed this technology as very good method to predict nutritional traits in silage maize (starch, ADF, NDF and CP); however its accuracy would be moderate for prediction of crude fiber and ADL and low for ash prediction. Lovett *et al*. (2004) also showed moderate prediction ability for IVOMD by NIRS.

In order to improve the estimate of IVOMD an increase in the reference method accuracy is necessary. Performing specific equations for different genotypes at different stages of maturity could improve the accuracy of the predictions because the genotype variability spanning the equations for this trait is large enough. However the accuracy of the equation developed for IVOMD was acceptable to distinguish between high and low values and to make approximate quantitative predictions.

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