RESEARCH PAPER

Prediction of the nutritive value of whole plants and morphological fractions of forage sunflower by near-infrared reflectance spectroscopy and empirical equations

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Abstract

S. Pereira-Crespo, A. Botana, M. Veiga, L. González, C. Resch, R. Lorenzana, M. P. Martínez-Diz, D. A. Plata-Reyes, and G. Flores-Calvete. 2023. Prediction of the nutritive value of whole plants and morphological fractions of forage sunflower by near-infrared reflectance spectroscopy and empirical equations. Int. J. Agric. Nat. Resour. 46-57. This technical note sought to examine the ability of near-infrared reflectance spectroscopy (NIRS) to predict the chemical content and organic matter digestibility (OMD) of whole plants and the morphological components of forage sunflower. Empirical models for the prediction of OMD values from chemical components were developed, and their predictive ability vs. NIRS models was assessed. The total set of samples (n=147) was composed of whole plants (n=14) and morphological components (n=133) from different experiments performed at Galicia (Spain) and were scanned using a Foss NIR System 6500 instrument. The reference values of OMD corresponded to *in vitro* determinations (n=112 samples) from laboratory incubation tests using rumen fluid. The predictive capacity of the NIRS models was assessed by the coefficient of determination value in external validation (r^2), showing good to excellent quality prediction of OMD and chemical components with values of $r^2 \geq 0.88$. However, the estimation of lignin did not show predictive utility ($r^2=0.40$). Using the NIRS models to predict the OMD of whole plants and morphological components of forage sunflower led to a decrease in the standard error in external validation, in contrast to the best empirical equation through the chemical components of samples (from ±8.25 to ±3.23%). This technical note showed that NIRS is a suitable technology, providing a rapid assessment of forage sunflower. However, these results should be considered preliminary, as they are based on a limited number of samples, and it is desirable to improve the performance of NIRS equations by increasing the dataset in future works.

Keywords: Chemical composition, digestibility, empirical models, NIRS.

Introduction

Maize (*Zea mays* L.) silage is the main conserved forage consumed in Galician dairy farms, and it is calculated that approximately 75% of dairy milk production in Galicia comes from farms that feed cows with total mixed rations based on maize silage (Flores-Calvete et al., 2017). The predominant climate in the milk production area in Galicia is humid-Atlantic, although relatively frequent episodes of seasonal droughts during the summer can compromise the forage maize yield, particularly in areas of sandy or shallow soils. Sunflower (*Helianthus annuus* L.) is a crop characterized by drought tolerance (Tan & Tümer, 1996), and it is increasingly considered an alternative to maize in such areas.

The efficient use of home-grown forage in dairy farms requires an advanced feed evaluation system that provides a fast, inexpensive and accurate assessment of nutritive value. *In vivo* evaluation provides the reference energy (i.e., digestibility) values of ruminant feeds but is not applicable in routine analysis because of its high labor intensity and price (Gosselink et al., 2004). Different methods can be substituted for *in vivo* evaluation, assessing the digestibility of forages, such as regression equations from chemical components, *in vitro* methods, and near-infrared reflectance spectroscopy (NIRS). An empirical model is a methodology based on mathematical relationships between digestibility and chemical parameters of samples determined by wet chemical analysis.

As an alternative to *in vivo* measurement, the *in vitro* method described by Tilley and Terry (1963) has been widely recognized as one of the most useful methods for estimating OMD, which requires incubation first with buffered rumen liquor and then with acid pepsin solution. NIRS has been applied broadly, and numerous works have recognized it as a fast, accurate and cost-effective tool to estimate forage quality (Lobos et al., 2019; Pereira-Crespo et al., 2022a, 2022b). This technical note sought to test the suitability

of NIRS for estimating the chemical composition and OMD of whole plants and morphological components of forage sunflower, comparing the predictive capacity of NIRS for OMD with empirical models developed from the same set of samples.

Material and Methods

Forage sample set

Samples of sunflower forage (n=147) were collected from different experiments performed at A Coruña and Lugo in Galicia (Spain) over five years. The total samples included whole plants (n=14) and morphological components (n=133), representing a wide range of varieties, harvest data, phenological stages and agronomic management. The different morphological components were vegetative fraction (leaves and stalks), capitulum (including receptacle, bracts and inflorescence or seeds), stalks, leaves, receptacle and seeds. The samples were oven-dried (80 °C; 16 h) and ground through a 1 mm sieve.

Laboratory determinations of the chemical components

The chemical components analyzed were organic matter (OM), neutral detergent fiber (NDF), acid detergent fiber (ADF), cellulose (CEL), lignin (LAD), crude protein (CP), water soluble carbohydrates (WSC), nonstructural carbohydrates (NSC) and ether extract (EE). The parameters were determined according to the procedures described by Pereira-Crespo et al. (2022 a, b).

Organic matter digestibility determinations

The values of OMD were only obtainable for 112 samples of the total set. The OMD was determined using the *in vitro* digestion technique developed by Tilley and Terry (1963) and modified by Alex-

ander and McGowan (1966), with rumen liquor from fistulated cattle. The incubations *in vitro* of each sample were carried out per duplicate; if the difference between duplicates passed 5% of the mean of the determination, the incubation was repeated. In each *in vitro* series, 4 reference samples, which have known *in vivo* OMD, were included with the objective of checking variability among different series. The *in vitro* OMD values were corrected by these four reference samples. Consequently, the *in vitro* OMD values of 112 samples were expressed as *in vivo* OMD values by a regression equation. Measurement of the *in vivo* values of 4 reference samples was carried out with five castrated male sheep, which were kept individually in metabolic cages. The experimental procedures of this technical note, which involved animals (cow and sheep), were approved by the local ethics committee, according to Spanish legislation.

Development of regression equations for estimating digestibility

The association between digestibility values and chemical components was tested using linear regression (simple and multiple) and correlation analysis, accompanied by a stepwise regression process. The univariate and multivariate models that had a high percentage of explained variation in the dependent variable were selected through the procedures CORR, STEPWISE, GLM, and REG of the statistical software SAS version 9.4. Leave-one-out cross-validation (LOO-CV) was applied to create the predictive models, and then, these models were subjected to external validation as described in Pereira-Crespo et al. (2022b).

Spectral acquisition and data analysis

The spectral measurement was obtained from samples dried and ground through a 1-mm screen. For each sample, two subsamples were scanned using a Foss NIR System 6500 monochromator in

the wavelength range of 1100-2500 nm. The final spectrum of each sample was the average of two subsample measurements. Spectral information was obtained as reflectance (R), and the absorbance data were recorded as log (1/R). WinISI II software was used to carry out data analysis and the development of chemometric models.

Prior to carrying out NIR calibrations, the structure and variability in the population was studied using the CENTER algorithm included in the WinISI II software package (Shenk & Westerhaus, 1991). This algorithm performed a principal component analysis (PCA) and calculated the global Mahalanobis distance (GH) of each sample to the center of the population in an n-dimensional space. The samples with GH>3 were identified as spectral outliers and removed. A validation set of samples (for external validation), completely independent of the calibration set, was used to validate the accuracy of each calibration model. The set of samples was divided randomly into two sets: a calibration set containing approximately 75% (112 samples) and an external validation set containing the remaining 25% (35 samples).

The sample collection was divided into a calibration set (75% of the samples; n=112) and validation set (25% of the samples; n=35) using the CENTER and SELECT algorithms included in WinISI II software (Shenk & Westerhaus, 1991), which represent the spectral variation in both datasets.

Forty-eight different spectral pretreatments based on 4 scatter correction procedures combined with twelve mathematical treatments were carried out as described in Pereira-Crespo et al. (2022b). Modified partial least squares regression (MPLS) was used to develop the NIRS models for all parameters. The cross-validation procedure was used to choose the optimal number of terms for each model, and then 4 groups of cross-validations were used to avoid overfitting.

The statistics used to select the best model were standard error of cross validation (SECV), standard

error of external validation (SEP), coefficient of determination calculated in cross-validation (1-VR) and external validation (r^2). Furthermore, the residual prediction deviation (RPD, ratio between the standard deviation of the reference data to SEP and the range error ratio index (RER, ratio between the range of the reference data toSEP) (Williams, 2001; Williams, 2014) were applied to evaluate the prediction ability of the models. Additionally, the best models of calibration were externally validated using a set previously created and evaluated by the procedure proposed by Shenk et al. (2001).

To determine the influence of the error of the reference method on the prediction performances of the NIRS models obtained, the standard error of the laboratory (SEL) of all reference methods was calculated. The standard errors of the reference method considered in this technical note were the same as those in Pereira-Crespo et al. (2022b).

Results

Empirical equations to predict digestibility

The set of samples (n=112) of whole sunflower plants with available OMD data and chemical composition (Table 1) was composed of 20% of samples of whole plants, and the remaining percentages were distributed, in approximately equal proportions, between the vegetative (plants without capitulum, leaves, and stems) and inflorescence (capitulum with different proportions of fruit) components. The mean compositions (and range) expressed as %DM were as follows: OM, 84.4 (76.8 to 97.3); CP, 8.0 (2.3 to 19.7); NDF, 43.3 (22.2 to 74.1); ADF, 33.7 (17.9 to 61.9); CEL, 27.7 (10.5 to 51.5); ADL, 6.7 (2.5 to 14.0); EE, 11.7 (0.0 to 52.4); NSC, 13.3 (0.7 to 38.4); and WSC, 12.7 (0.8 to 36.5). The mean value for the digestibility of organic matter was 52.6% (range, 31.8 to 73.9%).

The correlation matrix between chemical parameters and the OMD of whole sunflower plants and their morphological components (Table 2) showed a significant ($p<0.001$) positive correlation between OMD and NSC and WSC values (r=0.67 in both cases) as well as the CP value (r=0.53). In contrast, a negative correlation, which had a lower intensity but was significant ($p<0.001$), was found between OMD and NDF and ADF (r=-0.52 and -0.51, respectively). For the whole plant group, the OMD correlation coefficient values were -0.66 and -0.55 for NDF and ADF and 0.58, 0.59, and 0.39 for NSC, WSC,

Table 1. Chemical composition and digestibility of fresh samples of whole sunflower plants and their morphological components (n=112).

Parameter	Mean	Standard deviation	Coefficient of variation	Maximum	Minimum
Organic matter (%DM)	88.4	4.7	5.3	97.3	76.8
Crude protein (%DM)	8.0	4.2	52.2	19.7	2.3
Neutral detergent fiber (%DM)	43.3	13.0	30.0	74.1	22.2
Acid detergent fiber (%DM)	33.7	9.2	27.2	61.9	17.9
Cellulose (%DM)	27.7	9.3	33.5	51.5	10.5
Acid detergent lignin (%DM)	6.7	2.3	35.0	14.0	2.5
Ether extract (%DM)	11.7	13.9	119.0	52.4	0.0
Nonstructural carbohydrates(%DM)	13.3	9.4	70.9	38.4	0.7
Water soluble carbohydrates (%DM)	12.7	9.2	72.2	36.5	0.8
Organic matter digestibility (%)	52.6	12.6	23.9	73.9	31.8

and CP, respectively. Such values approximately reproduce the relationships obtained for the whole collection and demonstrate the effect of maturity on the reduction of the OMD of the whole sunflower plant. This finding aligns with a progressive increase in fiber fractions and a decrease in protein and nonstructural carbohydrates, which are partially transformed into oil that accumulates in the seeds of the plant.

The multivariate model with the highest variance explained in the external validation process (Table 3), which includes NSC, EE, and CP as predictors, had a coefficient of determination value (1-VR) of 0.76 and an SECV prediction of ±6.09% in the cross-validation when estimating OMD. The removal of EE from the equation slightly worsened the prediction, reducing 1-VR to 0.71 and increasing SECV to ±6.68%. The quadratic equation with NSC as the only predictor showed a significant loss of accuracy, reducing the coef-

ficient of determination to 0.45 and significantly increasing the prediction error to ±9.23%.

The corresponding values of r^2 and SEP for the three OMD prediction models based on the external validation were 0.66 and ±8.25%, 0.62 and ±8.84%, and 0.38 and ±10.1%, respectively. Such findings indicate an overall poor predictive ability with a high digestibility estimation error. Shenk et al. (2001) indicated that the values of the coefficient of determination in the cross validation (1-VR) between 0.70 and 0.89 provide a good fit for the prediction model. However, according to Williams (2001), RPD values between 1.5 and 2.0 allow for a basic qualitative prediction. Considering these criteria, the first two equations would be useful for an approximate qualitative prediction (high, medium, and low values) of digestibility. However, coefficient of determination values slightly higher than 0.60 in the external validation, RPD_{ev} values ≤1.5, and high predic-

Table 2. Correlation matrix between chemical composition parameters and the digestibility of the organic matter of fresh samples of whole sunflower plants and their morphological components.

		CP	NDF	ADF	CEL	ADL	EE	NSC	WSC	OMD
OM	*r*	-0.48	0.16	0.14	0.16	-0.27	-0.12	0.51	0.48	0.07
	p	***	NS	NS	NS	**	NS	***	***	NS
CP	*r*	-	-0.71	-0.68	-0.76	0.29	0.46	-0.01	0.00	0.53
	p		***	***	***	**	***	NS	NS	***
NDF	*r*	-	-	0.89	0.87	-0.03	-0.59	-0.31	-0.31	-0.52
	p			***	***	NS	***	***	***	***
ADF	*r*	-	-	-	0.94	0.14	-0.56	-0.27	-0.28	-0.51
	p				***	NS	***	**	**	***
CEL	*r*	-	-	-	-	-0.10	-0.14	-0.15	0.24	-0.39
	p					NS	NS	NS	**	***
ADL	*r*	-	-	-	-	-	-0.10	-0.14	-0.15	0.24
	p						NS	NS	NS	**
EE	*r*	-	-	-	-	-	-	-0.31	-0.31	-0.14
	p							***	***	NS
NSC	*r*	-	-	-	-	-	-	-	0.99	0.67
	p								***	***
WSC	*r*	-	-	-	-	-	-	-	-	0.67
	p									***

DM: dry matter; OM: organic matter (%DM); CP: crude protein (%DM); NDF: neutral detergent fiber (%DM); ADF: acid detergent fiber (%DM); CEL: cellulose (%DM); ADL: acid detergent lignin (%DM); EE: ether extract (%DM); NSC: nonstructural carbohydrates (%DM); WSC: water soluble carbohydrates (%DM); OMD: OM digestibility (%); *r* : Pearson's correlation coefficient; *p*: significance (***$p<0.001$; **$p<0.01$; *$p<0.05$; NS $p>0.05$: *not significant*)

Table 3. Prediction equations of organic matter digestibility based on the chemical parameters of fresh samples of whole sunflower plants and their morphological components.

Equation				Calibration		Cross-validation		External validation				
				R²	SEC	1-VR	SECV	r²	SEP	SEPc	Bias	Slope
OMD =	29.10	+0.79NSC	+1.94CP	-0.23EE	0.77	5.97	0.76	6.09	0.66	8.25	8.00	-1.956	0.775
s.e.	±1.44	±0.061	±0.148	±0.047								
p	***	***	***	***	***		***		***			
OMD =	27.81	+0.89NSC	+1.59CP		0.73	6.55	0.71	6.68	0.62	8.84	8.56	-2.168	0.747
s.e.	±1.55	±0.063	±0.143									
p	***	***	***		***		***		***			
OMD =	37.23	+1.53NSC	-0.0190NSC²		0.46	9.24	0.45	9.23	0.38	10.15	10.14	-0.360	0.736
s.e.	±2.24	±0.327	±0.0093									
p	***	***	*		***		***		**			

OMD: organic matter digestibility (%); DM: dry matter; NSC: nonstructural carbohydrates (%DM); CP: crude protein (%DM); EE: ether extract (%DM); *p*: significance (***p<0.001; **p<0.01; *p<0.05; NS not significant p>0.05); R²: coefficient of determination of the calibration; SEC: standard error of calibration; 1-VR: coefficient of determination in cross-validation; SECV: standard error of cross-validation; r²: coefficient of determination in external validation; SEP: standard error of prediction; SEPc: standard error of prediction corrected for bias

tion error (higher than 8 OMD units) indicate limited usefulness of the empirical models for predicting the digestibility of sunflower plants and their morphological components.

NIRS equations for the prediction of chemical components and digestibility

The mean values (and range of variation) of the chemical constituents in the calibration and external validation collections (Table 4), expressed as %DM, were as follows: OM, 88.9 (76.8 to 97.3) and 88.3 (76.7 to 97.5); CP, 7.3 (1.0 to 19.7) and 8.33 (2.4 to 18.7); NDF, 43.1 (21.8 to 80.1) and 42.30 (24.7 to 74.1); ADF, 34.6 (16.7 to 63.7) and 33.8 (19.0 to 62.0); CEL, 28.9 (12.4 to 57.5) and 27.0 (10.5 to 51.4); ADL, 6.2 (2.5 to 14.0) and 7.3 (2.5 to 13.7); EE, 12.3 (0.3 to 57.2) and 7.8 (0.5 to 53.9); NSC, 13.0 (0.7 to 45.0) and 14.9 (1.3 to 38.4); and WSC, 12.2 (0.8 to 43.9) and 14.6 (1.1 to 36.5). The corresponding mean OMD was 51.0% in the calibration group (range, 33.6 to 73.9%) and 55.2% in the validation group (range, 31.8 to 73.9%). The wide range found for all parameters is maintained in both groups and reflects the high diversity of samples that compose both collections.

The prediction models were obtained with SNV+D as spectral pretreatment; second derivative for OM, CP, NDF, ADF, CEL, and OMD; and first derivative for ADL, EE, NSC, and WSC. The number of anomalous samples removed from the calibration set with respect to the initial number of samples ranged from 0.8 to 4.4% for the NIRS calibrations for estimating the chemical parameters, with 4.5% for OMD. The values were all clearly below the maximum value of 20% recommended by Shenk and Westerhaus (1995) for the development of NIRS calibration equations.

The coefficients of determination (1-VR) and the prediction errors (SECV) in the cross-validation process were 0.89 and ±1.75% for OM, 0.98 and ±0.48% for CP, 0.98 and ±1.85% for NDF, 0.87 and ±4.13% for ADF, 0.86 and ±4.24% for CEL, 0.55 and ±1, 38% for ADL, 0.99 and ±1.10% for EE, 0.98 and ±1.25% for NSC, 0.98 and ±1.30% for WSC, and 0.95 and ±2.57% for OMD, respectively. According to the criteria defined by Shenk et al. (2001) to assess the quality of the fit of the equations as a function of the value of the coefficient of determination in the cross-validation, the obtained value was excellent (1-VR≥0.90) for the prediction of CP, NDF, EE, NSC, WSC,

Table 4. Calibration and cross-validation statistics of NIRS equations to estimate the chemical composition and organic matter digestibility of fresh samples of whole sunflower plants and their morphological components.

Parameter	MT	Calibration										Cross-validation		
		n	Outliers	T	Mean	SD	Minimum	Maximum	R^2	SEC	1-VR	SECV	RPDcv	RERcv
OM	2,5,5,1	112	3	9	88.9	4.9	76.8	97.3	0.93	1.39	0.89	1.75	2.8	11.7
CP	2,10,5,1	112	3	8	7.3	4.0	1.0	19.7	0.99	0.38	0.98	0.48	8.3	39.3
NDF	2,6,4,1	112	2	8	43.1	15.0	21.8	80.1	0.99	1.55	0.98	1.85	8.1	31.5
ADF	2,10,5,1	112	1	8	34.6	10.4	16.7	63.7	0.89	3.40	0.87	4.13	2.5	11.4
CEL	2,6,4,1	112	3	9	28.9	10.3	12.4	57.5	0.87	3.74	0.86	4.24	2.4	10.6
ADL	1,4,4,1	112	3	5	6.2	2.3	2.5	14.0	0.63	1.23	0.55	1.38	1.6	8.3
EE	1,10,10,1	110	5	9	12.3	14.5	0.3	57.2	1.00	0.96	0.99	1.10	13.2	51.7
NSC	1,4,4,1	112	5	9	13.0	10.0	0.7	45.0	0.99	1.10	0.98	1.25	8.0	35.6
WSC	1,4,4,1	112	5	8	12.2	9.8	0.8	43.9	0.99	1.10	0.98	1.30	7.5	33.0
OMD	2,10,5,1	87	4	8	51.0	12.4	33.6	73.9	0.97	2.19	0.95	2.57	4.8	15.6

| Parameter	External validation										
	n	Mean	SD	Minimum	Maximum	r^2	SEP	SEPc	Bias	Slope	RPDev	RERev
OM	35	88.3	5.89	76.7	97.5	0.88	2.12	2.12	-0.318	0.934	2.78	9.78
CP	35	8.33	4.79	2.42	18.7	0.95	1.04	1.05	0.122	1.039	4.62	15.7
NDF	35	42.3	13.4	24.7	74.1	0.97	2.35	2.38	0.081	0.959	5.74	21.0
ADF	35	33.7	10.3	18.9	61.9	0.88	3.69	3.74	0.098	0.933	2.81	11.6
CEL	35	26.9	11.1	10.5	51.4	0.90	3.53	3.54	0.505	0.965	3.15	11.6
ADL	35	7.27	2.37	2.52	13.6	0.40	1.89	1.85	0.503	0.893	1.25	5.89
EE	35	7.83	14.0	0.51	53.9	0.99	1.06	1.05	0.241	1.017	13.2	50.4
NSC	35	14.8	10.0	1.32	38.3	0.99	1.30	1.25	-0.410	1.022	7.74	28.4
WSC	35	14.6	9.57	1.07	36.4	0.97	1.77	1.79	-0.143	0.982	5.41	20.0
OMD	25	55.2	13.2	31.7	73.9	0.94	3.23	3.25	-0.560	1.002	4.12	13.0

MT: Mathematical treatment; SNV+D: standard normal variate+detrend; T: number of regression factors; SD: standard deviation; SEC: standard error of calibration; R^2: coefficient of determination of calibration model; SEC: standard error of calibration; 1-VR: coefficient of determination in cross-validation; SECV: standard error of cross-validation; RPDcv: SD/SECV; RERcv: ratio between range of values and SECV; r^2: coefficient of determination in external validation; SEP: standard error of prediction; SEPc: standard error of prediction corrected for bias; Bias: mean difference between the observed values and those predicted by the equation; RPDev: SD/SEP; RERev: ratio between range of values and SEP; DM: dry matter; OM: organic matter (%DM); CP: crude protein (%DM); NDF: neutral detergent fiber (%DM); ADF: acid detergent fiber (%DM); CEL: cellulose (%DM); ADL: acid detergent lignin (%DM); EE: ether extract (%DM); NSC: nonstructural carbohydrates (%DM); WSC: water soluble carbohydrates (%DM); OMD: organic matter digestibility (%)

and OMD; good (1-VR between 0.70 and 0.89) for the prediction of OM, ADF, and CEL; and mediocre (1-VR between 0.50 and 0.69) for the prediction of ADL. Based on the criteria of Williams (2001) for assessing the predictive quality of the equations based on RPD_{cv} and RER_{cv} in the cross-validation, the quality was good (RPD_{cv}>3) for CP, NDF, EE, NSC, WSC, and OMD, allowing a quantitative prediction; acceptable (RPD_{cv} between 2.5 and 3.0) for OM and ADF, allowing qualitative prediction in ranges; mediocre for CEL (RPD_{cv} between 2 and 2.5), allowing only a basic classification of values (high, medium, and low); and poor for ADL (RPD_{cv}<2).

Figure 1 shows the scatter plots of reference data versus predicted values by NIRS for the external validation (independent) sets of chemical parameters and OMD.

For external validation, the values of the coefficient of determination (r^2) and the prediction error (SEP) were 0.88 and ±2.12% for OM, 0.95 and ±1.04% for CP, 0.97 and ±2.35% for NDF, 0.88 and ±3.69% for ADF, 0.90 and ±3.53% for CEL, 0.4 and ±1.89% for ADL, 0.99 and ±1.06% for EE, 0.99 and ±1.30% for NSC, 0.97 and ±1.77% for WSC, and 0.94 and ±3.23% for OMD, respectively. According to the criteria defined by Williams (2014), the predictive quality of the calibration models in the external validation was good for the prediction equations of CP, NDF, CEL, EE, NSC, WSC, and OMD, enabling quantitative predictions with high reliability on independent samples (RPD_{ev}>3); acceptable
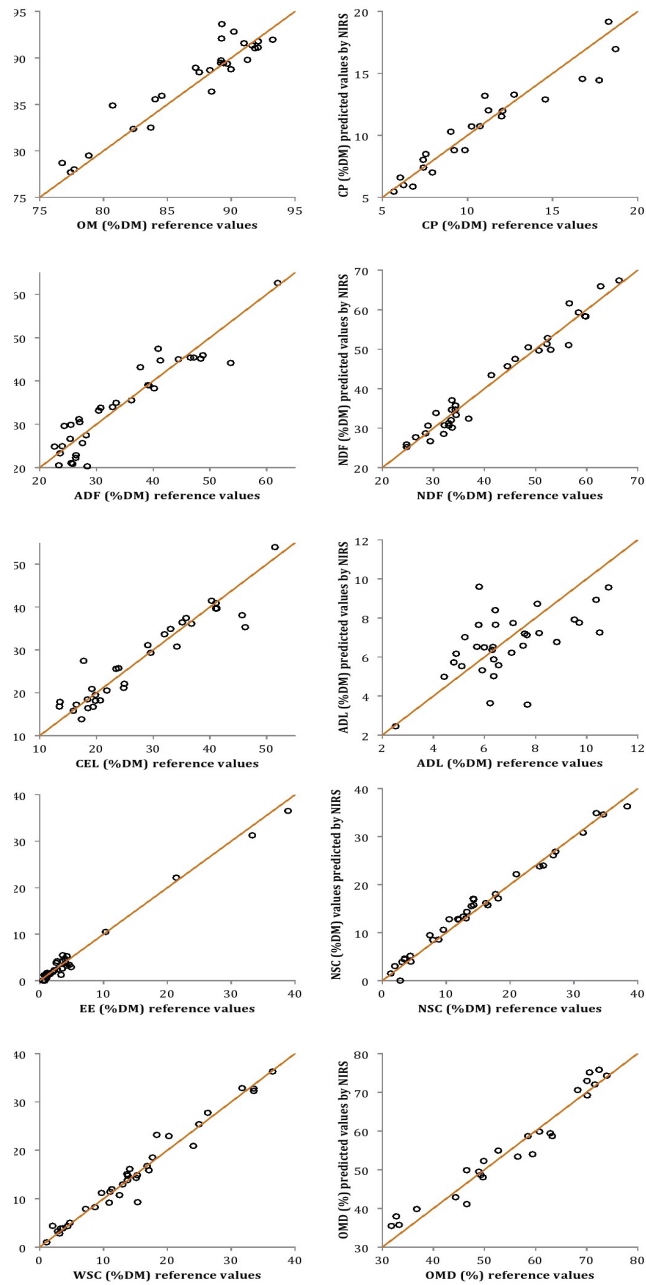
Figure 1. Plot of the chemical composition and organic matter digestibility values measured by reference methods and values predicted by the NIRS equations for fresh samples of whole sunflower plants and their morphological components.
DM: dry matter

for OM and ADF (RPD$_{ev}$ between 2.5 and 3.0), enabling predictions of sample quality ranges; and no utility for ADL (RPD$_{ev}$<1.5).

The validity of the equations to assess the robustness and reliability of external samples in routine analysis indicates an irregular performance of the equations. In addition to the confirmed uselessness of the ADL prediction equation, the most robust models were obtained for estimating ADF, CEL, EE, NSC, and WSC, which met the criteria of r²≥0.60, bias=±0.60SEC, SEPc≤1.30 SEC, and

a slope of 0.9 to 1.1, as indicated by Shenk et al. (2001) and Shenk and Westerhaus (1991). In contrast, SEPc exceeded the aforementioned threshold value for the rest of the equations, indicating the existence of variability in the external sample set that is not ideally reflected in the calibration set. Such findings suggest the need to expand the collection of calibration samples.

Discussion

Characteristics of samples of whole sunflower plants and their morphological components

The INRA Tables (2010) indicate that the mean values and range of variation of the chemical composition between the end of the vegetative stage and the brown capitulum were (in %DM) 86.9 (84.2 to 89.1), 13.3 (10.5 to 16.5), 40.4 (38.0 to 42.4), and 27.5 (24.6 to 30.7) for OM, CP, NDF, and ADF, respectively. Furthermore, the mean for OMD was 67.7% (range, 62.0 to 76.0%). A recent study carried out at Galicia by Sainz-Ramírez et al. (2020) sought to evaluate the agronomic and nutritional performance of a sunflower forage variety harvested between the flowering and dough seed stages. Based on their findings, CP (from 9.4 to 8.6% DM), WSC (from 16.9 to 10.6% DM), NDF (from 41.8 to 36.8% DM), and OMD (67.0 to 58.4%) decreased with plant maturity. The ranges of chemical composition values for samples of whole sunflower plants used in this technical note (in %DM, OM: 80.7 to 92.7; CP: 5.9 to 16.6; NDF: 32.0 to 52.2; ADF: 23.9 to 40.2; ADL: 4.7 to 11.9; EE: 1.4 to 44.1; NSC: 4.0 to 23.3), as well as those of OMD (48.9 to 69.1%), are comparable to those reported, showing the variability of the collection used for development of the prediction models.

Empirical equations to predict digestibility

There are no studies in the literature that refer to the chemical equations for OMD predic-

tion of sunflower plants, fresh and as silage. The OMD prediction equations based on the chemical composition of the samples collected in this technical note showed an overall poor predictive behavior. The great sample variability in the calibration set, which integrated morphological components with whole plants, caused the relatively high value of the coefficient of determination in the calibration (R^2 above 0.70 for the two best models), which does not avoid a high prediction error, close to ±6% in the cross-validation and increasing up to ±8% in the external validation. Such findings indicate that the variability in the population of independent samples in the calibration set was not sufficiently represented.

NIRS equations for predicting chemical components and digestibility

The NIRS models developed in this technical note for estimating the nutritional value of sunflower forage showed good quality for predicting the CP, NDF, CEL, EE, NSC, and WSC contents of the samples, as well as the OMD values. As a result, quantitative predictions with high reliability on independent samples were obtained, despite the lower quality found for predicting OM and ADF and the lack of utility for predicting ADL. In general, prior studies have reported the good quality of NIRS predictions to estimate CP, EE, and WSC contents, with more variable results obtained for the prediction of the remaining parameters. Fassio et al. (2007) assessed the potential of predicting the chemical composition and digestibility of sunflower plants, fresh and as silage, by NIRS using 73 fresh forage and 50 silage samples obtained at an experimental station in Uruguay. For fresh and silage samples, the most accurate estimates were obtained for CP (R^2>0.85; SECV=±0.99 and ±1.01%, RPD=2.9 and 2.3) and ash (R^2>0.85; SECV=±1.1 and ±0.82%; RPD 2.2 and 2.9). The prediction of EE (only for silage samples) was very accurate, with R^2=0.94, SECV=1.5, and RPD=4.4. In contrast,

the prediction of ADF and NDF was mediocre, with $R^2<0.70$ and RPD<2.0. The authors reported the difficulty associated with obtaining robust calibrations (RPD>3) to predict the sunflower chemical composition.

Recently, Saha et al. (2017) studied the usefulness of NIRS calibrations to predict a wide range of chemical composition parameters of soybean and sunflower plants and their leaf and inflorescence fractions using samples of plants, leaves, and reproductive parts at different maturity stages of sunflower (n=72) and soybean (n=48). The reliability of the prediction models, applied to the analysis of independent samples, was excellent ($r^2>0.90$ and RPD>3.0) for estimating ash, CP, EE, ADL, and WSC contents and approximate (r^2 between 0.80 and 0.66 and RPD between 2.5 and 2.0) for estimating ADF, NDF, and CEL. These findings indicate that the same models can be applied to adequately quantify the nutritional composition of both forages. Furthermore, these models can be used reliably in routine analysis of external samples.

The predictive capacity of OMD in sunflower samples using the NIRS models in this technical note showed a categorical superiority compared to empirical models. In fact, the NIRS technique, in the external validation stage, showed a higher coefficient of determination (0.66 vs. 0.94) and a reduction in the prediction error by 60% with respect to the best empirical equation model, decreasing from ±8.25 to ±3.23%. The difficulty of obtaining consistent correlations between OMD and chemical constituents is highlighted in the study by Andrieu et al. (1981), where only mean reference values could be provided for the OMD of sunflower plants when nutritional values of French forages were available, with values of 76% at the end of the vegetative state and 64% at the consistent seed stage.

In the study by Fassio et al. (2007), the prediction error in the cross-validation (SECV) of the NIRS equation for estimating the OMD of fresh forage samples of sunflower was ±2.1%, showing acceptable accuracy. In contrast, the SECV was higher for the sunflower silage samples (±3.5%). The values of the coefficient of determination in the calibration (R^2) and the RPD_{cv} in the cross-validation ranged from 0.61 to 2.8 for the OMD prediction equations using fresh forage samples and 0.83 to 2.1 using silage samples. The SECV in this technical note (±2.5%) is slightly higher than that reported for fresh samples by previous authors. Nonetheless, both the coefficient of determination in the calibration (R^2=0.97) and the RPD_{cv} in the cross-validation (4.8) showed a better predictive capacity of the NIRS equations based on the collection of samples of whole sunflower plants and their morphological components.

The results of this technical note should be considered preliminary, as they are based on a limited number of samples. The calibrations performed by NIRS need to be thought about with caution during their utilization as routine analysis. Therefore, adding new samples to the database and expanding NIRS calibrations to ensure reliable results are essential.

Conclusion

The NIRS models for predicting the OMD of whole plants and morphological components of forage sunflower showed superior predictive capacity to empirical equations based on chemical parameters. This technical note reveals the potential to predict the chemical characteristics and digestibility of samples of whole plants and morphological components of forage sunflower by NIRS and, therefore, an alternative for determining these parameters in relation to conventional analytical methods. However, it should be noted that NIRS equations should be interpreted with caution because additional research is required to improve the performance by including more samples in the database.

Resumen

S. Pereira-Crespo, A. Botana, M. Veiga, L. González, C. Resch, R. Lorenzana, M. P. Martínez-Diz, D.A. Plata-Reyes, y G. Flores-Calvete. 2023. Predicción del valor nutricional de la planta entera y las fracciones morfológicas del girasol forrajero mediante espectroscopia de reflectancia en el infrarrojo cercano y ecuaciones empíricas. Int. J. Agric. Nat. Resour. 46-57. En esta nota técnica se evalúa la capacidad de la técnica NIRS para estimar la composición química y la digestibilidad de la materia orgánica de la planta entera de girasol (n=14) y sus componentes morfológicos (n=133) aprovechado para forraje, y se desarrollan ecuaciones empíricas basadas en parámetros químicos para la estimación de la digestibilidad de la materia orgánica (DMO), comparando su capacidad predictiva con la obtenida mediante NIRS. La información espectral de las muestras secas y molidas se realizó en un instrumento Foss NIRSystem 6500. Los valores de referencia de DMO (n=112) corresponden a determinaciones mediante incubaciones *in vitro* con líquido ruminal. Las calibraciones NIRS obtenidas para la predicción de todos los parámetros mostraron valores del coeficiente de determinación en validación externa (r^2) de calidad buena a excelente para la predicción de DMO y composición química, con valores de r^2 iguales o superiores a 0.88. Sin embargo, la ecuación NIRS de estimación del contenido en lignina no mostró utilidad predictiva (r^2=0.40). La tecnología NIRS mostró una superioridad predictiva de la DMO, comparada con la mejor ecuación empírica, permitiendo reducir el error de predicción de validación externa, desde ±8.25% a ±3.23%. Se concluye que las ecuaciones NIRS desarrolladas son una herramienta útil para la evaluación nutricional rápida y precisa de la planta entera de girasol y sus componentes morfológicos. Sin embargo, estos resultados deben considerarse como preliminares, ya que se basan en un número limitado de muestras y es deseable mejorar la robustez de las ecuaciones NIRS mediante el incremento de la colección de muestras.

Palabras clave: Composición química, digestibilidad, modelos empíricos, NIRS.

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