

ORIGINAL RESEARCH ARTICLE

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Using near infrared reflectance spectroscopy for estimating nutritional quality of *Brachiaria humidicola* in breeding selections

Johanna Mazabel¹  | Margaret Worthington^{1,2}  | Valheria Castiblanco¹  |
Michael Peters¹  | Jacobo Arango¹ 

¹ International Center for Tropical Agriculture (CIAT), Km 17, Recta Cali-Palmira, Palmira, Valle del Cauca, Colombia

² Current address, Dep. of Horticulture, University of Arkansas, 306 Plant Sciences Bldg., Fayetteville, AR 72701, USA

Correspondence

Jacobo Arango, International Center for Tropical Agriculture (CIAT), Km 17, Recta Cali-Palmira, Palmira, Valle del Cauca, Colombia.
Email: j.arango@cgiar.org

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Abstract

Brachiaria humidicola (BH) (syn. *Urochloa humidicola*) is an important forage grass in the tropics due to its capacity to grow in nutrient-deficient soils, tolerate waterlogging, and inhibit soil nitrification. A major objective of BH breeding is to improve its nutritional quality. Therefore, a rapid and low-cost method is needed to assess main quality parameters such as neutral detergent fiber (NDF), acid detergent fiber (ADF), in vitro dry matter digestibility (IVDMD), and crude protein (CP). This study developed models using near infrared reflectance spectroscopy (NIRS) to predict concentrations of these parameters toward breeding. Samples were collected from BH trials located in different regions of Colombia, scanned for NIRS (400–2,500 nm), analyzed with wet chemistry as reference values, and used to build the chemometric models. Results from wet chemistry showed wide variability in terms of dry matter percentage for NDF (51.6–76.2%), ADF (26.1–46.1%), IVDMD (41.5–78.3%), and CP (2.8–12.8%). The NIRS models were validated using an independent set of samples and have coefficients of determination (R^2) and one minus the variance ratio ($1 - VR$) values in the range of .9 and .95, suggesting a good correlation between reference-lab and NIRS-predicted values. The standard errors of cross validation (SECV) for IVDMD, NDF, ADF, and CP were 1.59, 1.18, 0.74, and 0.53%, respectively. Prediction efficiency (ratio of performance to standard deviation, RPD) for all parameters was above 3.0, except for CP (2.6). Calibrations obtained present an adequate adjustment and predictive tendency, making them suitable for selection and BH breeding.

Abbreviations: $1 - VR$, coefficient of determination for cross-validation minus one variance ratio; ADF, acid detergent fiber; BH, *Brachiaria humidicola*; CIAT, International Center for Tropical Agriculture; CP, crude protein; CV, coefficient of variation; IVDMD, in vitro dry matter digestibility; NDF, neutral detergent fiber; NIRS, near infrared reflectance spectroscopy; PCA, principal component analysis; R^2 , coefficient of determination for calibration; RPD, ratio of performance to standard deviation; SD, standard deviation; SEC, standard error calibration; SECV, standard error of cross validation; SEL, standard error laboratory; SEP, standard error of prediction; SNV, standard normal variate; SNVD, standard normal variate and detrend.

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1 | INTRODUCTION

Brachiaria humidicola (Rendle) Schweick (BH) [syn. *Urochloa humidicola* (Rendle) Morrone & Zuloaga] is a perennial, stoloniferous grass native to eastern and south-eastern Africa, and was introduced to Colombia in 1973 by the International Center for Tropical Agriculture (CIAT). It is tolerant to waterlogging and adapted to acidic soils with high Al saturation and low fertility (Pérez & Lascano, 1992). Another valuable attribute of BH is its excellent weed suppressive ability, which can be attributed to its strongly stoloniferous growth and ability to maintain good ground cover under high animal stocking rates (Cook et al., 2020).

By 2050, the world population is expected to increase and reach almost 9.7 billion people. Agriculture will have to produce almost 50% more food, fodder, and biofuel than it produced in 2012 (High Level Panel of Experts [HLPE], 2016). Thus, there is pressure on the livestock industry to meet the challenge of providing environmentally friendly and nutritious forage. Livestock plays an important economic role in many food systems, providing income, wealth, and employment. In addition, being able to produce high-quality forage secures high live weight gains and health of cattle and therefore income for livestock farmers (OEDC/FAO, 2017). Accurate knowledge of forage quality parameters is crucial in safeguarding a sustainable livestock industry. Since a large portion of what an animal eats ends up as excrement (between 50 and 90% of feed C, N, and P), chemical constituents need to be appropriately determined in forages to reduce environmental pollution caused by animal production (HLPE, 2016).

At CIAT, two separate *Brachiaria* (Trin.) Griseb. (syn. *Urochloa* P. Beauv.)-breeding programs are maintained, one for the species within the agamic complex {palisade grass [*B. brizantha* (Hochst. ex A. Rich.) Stapf], signal grass [*B. decumbens* (Stapf) R.D. Webster], and ruzigrass [*B. ruziziensis* (R. Germ. and C.M. Evrard)]} and the other for the more distantly related BH. Breeding in both programs is based on a recurrent scheme that pursues two basic objectives: (a) to accumulate genetic gain in nutritional quality and other traits through cycles of selection, and (b) to intentionally and deliberately exploit heterosis in improved hybrid apomictic genotypes (Miles, 2007). While the improvement of nutritional quality parameters is an important breeding objective, assessment of these parameters in the laboratory using traditional methods of chemical analysis is time consuming and expensive. Thus, the number of genotypes that can be evaluated each breeding cycle is limited.

It is necessary to quickly and precisely measure the nutritional quality parameters of many genotypes so that decisions about parents and culls can be made early during each breeding cycle. Near infrared spectroscopy provides

Core Ideas

- Chemometric models for nutritional quality in *Brachiaria humidicola* are robust and reliable.
- New NIRS models enable breeders to evaluate more genotypes for nutritional quality.
- Models must be regularly monitored by comparing predictions with reference methods.

a cost-effective solution to perform evaluations of quality parameters on a large number of selected genotypes each cycle. Forage feed value related to animal production potential can be characterized by the amount of digestible energy intake and how effectively this energy can be used. Traditionally, wet chemical analyses and in vivo experiments have been used to determine forage feed value (usually expressed in units of energy and protein). These traditional methods are laborious, time consuming, expensive, and generally impractical on a large scale. In the 1970s, near infrared reflectance spectroscopy (NIRS) was shown to be effective and useful to estimate forage quality constituents (Norris, Barnes, Moore, & Shenk, 1976). With further improvements in the 1980s and 1990s, including new instrumentation and calibration techniques, forage quality parameters could be predicted faster, and without the use of chemical reagents and production of chemical waste (Givens, De Boever, & Deaville, 1997). Also referred to as a rapid decision tool, non-destructive, and valid alternative technique, which represents a radical shift from conventional chemical methods (Li et al., 2016; Lobos, Gou, Hube, Saldaña, & Alfaro, 2013). The NIRS has enabled livestock producers and forage researchers to quickly and cheaply estimate forage nutrient composition (Johnson et al., 2017; Paz, Silva, & Rêgo, 2019).

Widely used as a quantitative and qualitative analysis method, NIRS technology requires the development of prediction models that involve multivariate analysis and analytical chemistry to extract the most relevant information (Lucio-Gutiérrez, Coello, & Maspocho, 2012). The NIRS technology is based on measurements of the near infrared light spectrum between 780 and 2,500 nm and provides information about the main structural elements and/or functional groups associated with living organisms: CH, OH, NH, SH, and C = O. These functional groups all respond to radiation in the near infrared frequency range (Rivera, Medina, & Cerón, 2018; Valenciaga & Saliba, 2006).

Several recent studies focused on the development and validation of NIRS equations to predict the chemical composition of tropical forages. Sandoval, Bueso, and Velez

(2008) developed calibrations for tropical forage species from Honduras, Nicaragua, El Salvador, and Guatemala, including guineagrass (*Panicum maximum* Jacq. [syn. *Megathyrsus maximus*] 'Tobiata' and 'Tanzania' and the *Brachiaria* hybrid cultivar Mulato. Similarly, Monrroy, Gutiérrez, Miranda, Hernández, and García (2017) developed models for *Brachiaria* to estimate neutral detergent fiber (NDF), acid detergent fiber (ADF), and crude protein (CP) contents. Molano, Cortes, Ávila, Martens, and Muñoz (2016) developed models for IVDMD of tropical forages with *Brachiaria* interspecific hybrids and BH at times of regrowth following defoliation. All these recent studies have shown the potential of NIRS chemometric models to efficiently evaluate nutritional parameters of tropical forages. The main objective of this study was to develop chemometric models based on measurements taken in the near infrared that can predict the contents of NDF, ADF, in vitro dry matter digestibility (IVDMD), and CP for breeding populations in the CIAT BH improvement program.

2 | MATERIALS AND METHODS

2.1 | Description of samples

Three sets of samples were used in this study. A first group corresponded to 54 BH hybrids grown at CIAT Headquarters in Palmira, Valle del Cauca, Colombia (3°30'16.9" N, 76°21'26.5" W) in single-plant experimental units of 1 m² in a randomized complete block design (RCBD) with three replications. The soil type in this site was a Vertisol and during the period of the experiment, the average temperature was 25 °C and average annual precipitation was 1,250 mm. All experimental units were harvested on three different sampling dates in January, March, and May of 2016. The second group was a population of 18 BH hybrids planted at La Libertad experimental station of AGROSAVIA in Meta (Villavicencio city), Colombia (4°03'38.8" N, 73°27'16.9" W) in a RCBD with six replicates; soil type Oxisol, average temperature 25 °C and 3,800 mm of average rainfall per year. For the second group, all experimental units were harvested in November 2016. The third group of 16 samples were BH cultivars collected from grazed pastures in six farms in the Department of Casanare, Colombia, during July and September 2015. The pastures were managed following the usual practices of the producers in each farm. The Casanare site had a typical Oxisol soil type, an annual mean rainfall of 2,250 mm, and a temperature range of 26–30 °C. The samples were collected using a 50 by 50 cm quadrant in 10 random points within farmers pasture field 8 wk after the last grazing event by cattle. Additional information about these 16 samples can be found in Arango et al. (2016).

Plants in the Palmira and Villavicencio sites were harvested following 8 wk of regrowth after a uniform cut to 8-cm height. Plots were unfertilized and irrigated only when necessary. Each sample consisted of 200–300 g of harvested fresh matter. The sampled tissue was dried in a conventional oven at 60 °C for 72 h, ground using a Retsch SM 100 cutting laboratory mill (Retsch GmbH) with 1-mm sieve size, packed in plastic bags and labelled for later use in the chemical and spectral analyses. The samples from the six Casanare pastures were collected using a 50- by 50-cm quadrant at 10 randomly selected points in each field and a 250 g subsample from each quadrant was taken to CIAT's forage quality lab for analysis. The samples collected in Casanare were at a similar stage of regrowth as the samples from the breeding plots in Palmira and Villavicencio. Additional information about the 16 samples from pastures in Casanare can be found in Arango et al. (2016).

2.2 | Chemical composition

The chemical analyses were performed at the CIAT forages and animal nutrition quality laboratory. Concentrations of NDF and ADF were measured sequentially, according to operating instructions, using an ANKOM 2000 fiber analyzer (Ankom Technology, 2011) and according to the methods of Van Soest and Robertson (1985). Crude protein was determined using a FOSS Kjeltac 8100 (Foss Company) according to the guidelines of the Association of Official Analytical Chemists AOAC, Method 2001.11 (AOAC International, 2002). An estimation of IVDMD was done by the method described by Tilley and Terry (1963). All parameters were analyzed in duplicate and mean values were used to construct the calibration models. The entire set of 614 samples were evaluated for NDF, ADF, and IVDMD. Due to limitations on access to equipment, only a subset of 73 random samples were evaluated for CP.

2.3 | Spectral analysis

The samples were scanned using a FOSS model 6500 spectrophotometer (FOSS NIRSystems Inc.) with spectral range 400–2,500 nm. Absorbance was recorded as log 1/R at 2-nm intervals. Each sample was packed into two separate quartz cells and spectral readings were taken from each using ISIScan software (version 2.71 FOSS and Infrasoft International). The two spectra of each sample were averaged.

2.4 | Calibration and validation

Near infrared spectra models were developed using WinISI IV Software version 4.9 (FOSS Analytical software, 2012).

The population of spectral samples was divided into two random groups: the first group was used as the calibration set (419 samples for NDF, ADF, and IVDMD, and 53 samples for CP) and a second group was used as an external validation set (180 samples for NDF, ADF, and IVDMD, and 20 samples for CP). The recorded NIRS values for all samples fell within the expected concentration range variability of BH hybrids in both calibration and validation sets.

The calibration procedure used regression methods with modified partial least squares (MPLS) and data transformations such as standard normal variate and detrend (SNVD) and derivative mathematics to reduce the correlation among spectral data points of a full spectrum. Spectral transformations, including SNVD, were used to correct for variations of physical nature (such as particle texture and size). Standard normal variate scales each spectrum to have a standard deviation of 1.0 to help reduce particle size effects. Detrend removes the linear and quadratic curvature of each spectrum. In addition, to extract relevant chemical information, different mathematical treatments were applied to the models on the first and second derivative. Furthermore, a full range of 400–2,500 nm was recorded but only the spectral range between 1,100–2,500 nm was used for spectral pre-processing.

We evaluated five mathematical treatments without scatter correction (original data) and with scatter correction SNVD (0, 0, 1, 1; 1, 1, 1, 1; 2, 1, 1, 1; 1, 4, 4, 1; 2, 4, 4, 1) where the first number is the derivative (0 for raw spectra, 1 for first derivative, and 2 for second derivative). The second number is the gap over which the derivative is calculated, the third digit is the number of data points in a running average or first smoothing, and the fourth digit (by default is 1) is the number of data points in the second smoothing. A principal component analysis (PCA) was conducted before the development of equations to define the spectral thresholds of the populations of study and to identify spectral outliers. As a result, 15 samples with spectral differences were classified as atypical. Three outlier samples were identified for the CP model and 12 outliers were identified for all other models. These outlier samples were removed from the calibration set (PCA analysis was performed using NIRS values only).

The validation process used a different validation test set (180 samples for NDF, ADF, and IVDMD and 20 samples for CP), not included in the calibration set according the method suggested by Liebmann, Filzmoser, & Varmuza (2010) to obtain the parameters R^2 , standard deviation (SD), standard error calibration (SEC), standard error of prediction (SEP), and ratio of standard deviation to standard error of prediction (RPD), a measure of bias and predictive capacity coefficient. The criteria used for model selection were R^2 and 1-VR coefficients closest to 1.0, the lowest standard error of cross validation (SECV) and RPD

values greater than 3.0 following Williams, Dardenne, & Flinn, 2017.

3 | RESULTS AND DISCUSSION

3.1 | Laboratory analysis of NDF, ADF, IVDMD, and CP

The entire set of samples, calibration set, and validation set had similar distributions for each parameter with similar mean values, SD and coefficients of variation (CV) (Table 1). The observed concentrations for four parameters of NDF, ADF, IVDMD, and CP ranged between 51–76, 26–45, 41–78, and 2–12%, respectively. A large sample population ($n = 614$) was used for most variables, with the exception of CP ($n = 73$). However, according to Pasquini (2003) and others, the number samples utilized for a calibration set should be in the range of 50–100 samples, depending on the complexity and variability parameter of interest assessed. In this case (i.e., CP), the variability of the BH hybrids used for CP model and other parameters were represented in both calibration and validation sets suggesting that the CP population size is adequate for initial model development. The highest CV was observed for CP, of approximately 28% in all three sets. The standard error of laboratory (SEL) values calculated between duplicates using the reference method for NDF, ADF, IVDMD, and CP were 0.72, 0.32, 1.7, and 0.11%, respectively. These low SEL values demonstrated that laboratory procedures used to measure each attribute were robust. The randomly selected external validation set covered the range of composition for NDF, ADF, and IVDMD, while the CP validation set ranged from 4.24 to 12.12%. In the case of CP, the validation set lacked samples representative of the very lowest portion of the total range of values in the entire set (2.8–4.24%).

3.2 | Spectral analysis and calibration

Table 2 shows the selected treatments (1,4,4,1 for ADF and IVDMD; 2,4,4,1 for NDF and CP). The calibration models were developed using pre-processing spectra data with PCA, SNVD, and MPLS regressions. The spectra were collected in the visible NIRS range. This spectra type have mainly overtones and combination bands of hydrogen groups, and the absorption peaks have weak intensity. When the full spectrum is involved in a model, it becomes more complex (Lin et al., 2017). The selection of an optimal spectral range is important, as it contributes to the model's overall performance. Models with optimized spectral ranges have lower prediction error and

TABLE 1 Descriptive statistics of chemical composition for the entire set, calibration set, and validation set for chemical parameters, in percent (%) of dry weight

Parameter	Entire set						Calibration set					Validation set				
	Min.	Max.	Mean	SD	CV	SEL	Min.	Max.	Mean	SD	CV	Min.	Max.	Mean	SD	CV
%NDF ^a	51.63	76.25	64.83	4.83	7.45	0.72	51.63	76.25	64.73	4.73	7.31	51.97	75.77	64.59	5.07	7.85
%ADF ^a	26.06	45.99	34.32	3.35	9.75	0.32	26.06	42.57	34.33	3.38	9.85	27.62	45.99	34.29	3.66	10.67
%IVDMD ^a	41.46	78.33	66.31	5.43	8.19	1.7	41.36	78.33	66.35	5.13	7.73	41.46	77.59	66.22	6.08	9.18
%CP ^b	2.81	12.77	8.14	2.29	28.13	0.11	2.81	12.77	8.13	2.31	28.41	4.24	12.12	8.20	2.32	28.29

Note. SD, standard deviation; CV, coefficient of variation; SEL, standard error laboratory; Min., minimum; Max., maximum; NDF, neutral detergent fiber; ADF, acid detergent fiber; IVDMD, in vitro dry matter digestibility; CP, crude protein.

^aFor NDF, ADF, and IVDMD the entire set was composed of 599 samples, the calibration set was composed of a subset of 419 samples and the validation set was composed of a separate subset of 180 samples. ^b For CP the entire set was composed of 70 samples, the calibration set was composed of a subset of 50 samples and the validation set was composed of a separate subset of 20 samples.

TABLE 2 Summary statistics of selected chemometrics models for estimation of neutral detergent fiber (NDF), acid detergent fiber (ADF), in vitro dry matter digestibility (IVDMD), and crude protein (CP) contents in *Brachiaria humidicola* (BH) hybrids

Parameter	Mathematical treatment	Scatter	Spectral region	Factors	Calibration				Cross validation		
					n	Mean	SD	SEC	R ²	SECV	1 – VR
			nm								
%NDF	2,4,4,1	SNVD	1,100–2,500	10	403	64.73	4.73	1.00	.95	1.18	0.93
%ADF	1,4,4,1	SNVD	1,100–2,500	11	402	34.33	3.38	0.69	.96	0.74	0.95
%IVDMD	1,4,4,1	SNVD	400–2,500	11	399	66.35	5.13	1.41	.92	1.59	0.90
%CP	2,4,4,1	SNVD	400–2,500	7	50	8.13	2.31	0.23	.99	0.53	0.95

Note. SEC, standard error calibration; R², coefficient of determination for calibration; SECV, standard error of cross validation; 1 – VR, one minus variance ratio or the coefficient of determination for cross validation; SNVD, standard normal variate and detrend.

better correlation with wet chemistry values (Pasquini, 2018). Our results identified the best prediction performance for IVDMD and CP models using the full spectral range (400–2,500 nm). In contrast, a reduced spectral region between 1,100–2,500 nm was selected for NDF and ADF models (Table 2). The difference between the SECV and SEC was small for all selected models. The optimal number of factors, which produced the minimum SECV, for ADF and IVDMD models were 11. In the case of NDF and CP models, 10 and 7 factors were selected as optimal, respectively.

The R² of models for NDF, ADF, IVDMD, and CP were 0.95, 0.96, 0.92, and 0.99, respectively. The values of SECV in the four models were 1.18, 0.74, 1.59, and 0.53%, respectively. Freitas, Santos, Tomich, and Franco (2016) reported slightly lower R² values of 0.89, 0.81 and 0.87 for NDF, ADF, and CP models in BH samples. Lopes (2011) obtained similar values for CP and NDF prediction curves developed from samples of palisade grass, *Brachiaria* hybrid cultivar Mulato, bermudagrass [*Cynodon dactylon* (L.) Pers.], African bermudagrass (*Cynodon nlemfuensis* Vanderyst.), guinea grass (*P. maximum* Jacq.) and elephant grass [*Penisetum purpureum* (Schumach.) Morrone (syn. *Cenchrus purpureus*)]. Overall, the models produced in this study compare favorably with models previously developed for tropical forages.

3.3 | External validation

In Table 3, the validation using external models are presented with samples different to the calibration set. Results from each parameter evaluated with wet chemistry (reference data) from all samples were compared with the NIRS prediction equations. The R² obtained showed good correlation for NDF (R² = .92), ADF (R² = .95), and IVDMD (R² = .93) (Table 3). The R² obtained for the CP model was lower (R² = .87) compared to the other models but

TABLE 3 External validation statistics of selected chemometric models obtained from regression equations of laboratory values of neutral detergent fiber (NDF), acid detergent fiber (ADF), in vitro dry matter digestibility (IVDMD), and crude protein (CP) in *Brachiaria humidicola* (BH) hybrids and near infrared reflectance spectroscopy (NIRS) predicted values for the validation set

Parameter	n	Mean	SD	SEP	R ²	Bias	RPD
%NDF	180	64.59	5.07	1.41	.92	0.04	3.62
%ADF	180	34.29	3.66	0.79	.95	–0.001	4.40
%IVDMD	180	66.22	6.08	1.55	.93	0.20	3.63
%CP	20	8.20	2.32	0.91	.87	–0.25	2.56

Note. SD, standard deviation; SEP, standard error of prediction; R², coefficient of multiple determination; RPD, ratio of performance to standard deviation (SD/SEP).

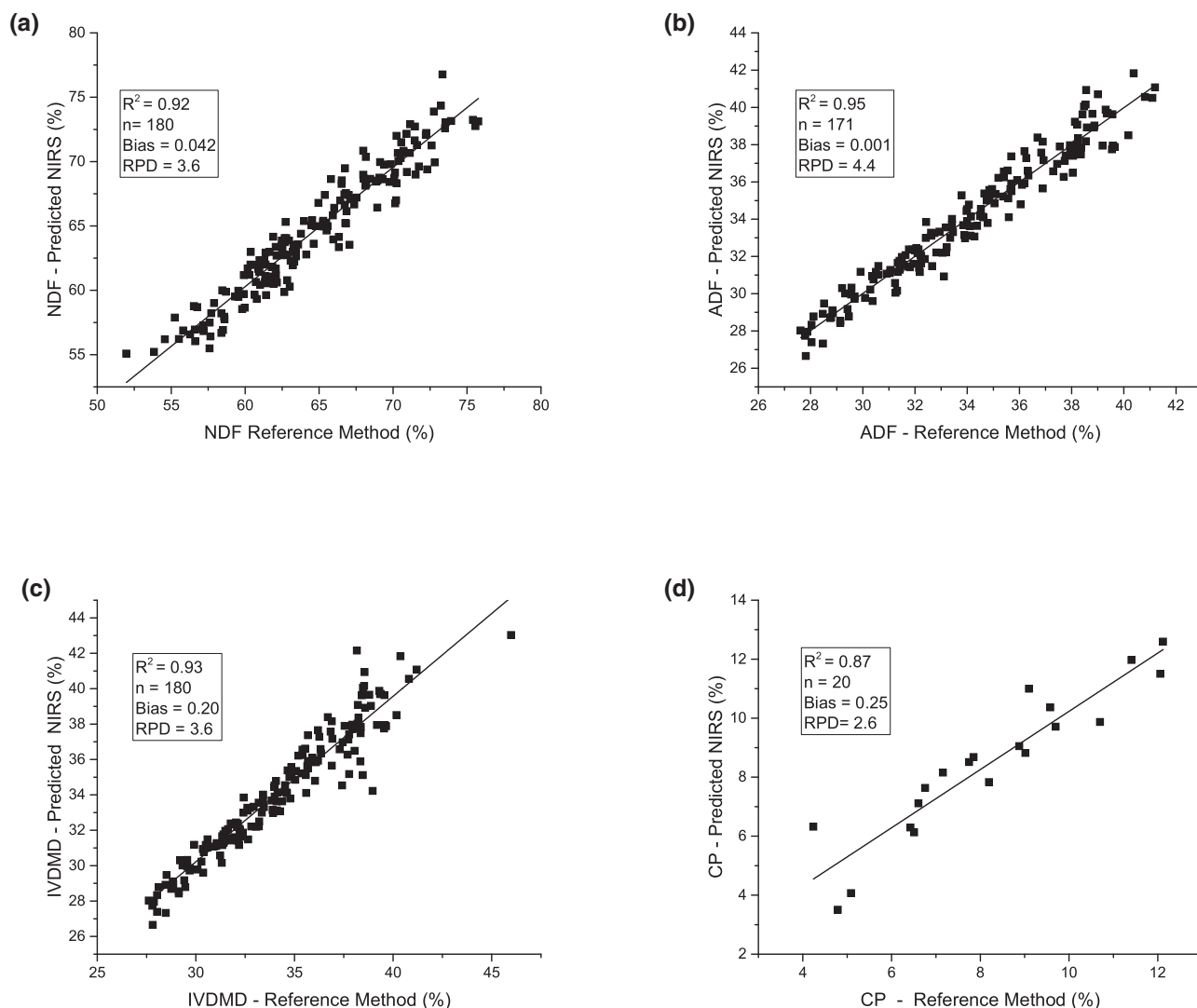


FIGURE 1 Scatter plots of near infrared reflectance spectroscopy (NIRS) predicted values vs. reference methods for external validation samples in parameters of (a) neutral detergent fiber (NDF), (b) acid detergent fiber (ADF), (c) in vitro dry matter digestibility (IVDMD), and (d) crude protein (CP) in *Brachiaria humidicola* (BH) hybrids

maintained an adequate adjustment for the independent samples set. The lower R^2 for CP can be explained by the smaller size of the external validation subset ($n = 20$). The SEP describes the error between reference data (wet chemistry) and the prediction made by NIRS for an independent set of samples and it should be as low as possible (Fearn, 2002). The SEP values obtained for the NDF, ADF, IVDMD, and CP prediction equations were 1.41, 0.79, 1.55, and 0.91%, respectively. The bias observed was lower than the values obtained for SEP of the selected models. All together, the SEP and bias were low.

The RPD measures the strength of the relationship between the values of a constituent and the error of the results predicted by NIRS (Williams, 2014). The RPD results obtained were 3.6, 4.4, and 3.6 for NDF, ADF, and

IVDMD, and 2.6 for the CP model. The RPD values below 1.9 are considered very poor and not recommended for forage tests, values of 2.0–2.4 are sufficient for rough screening, values between 2.5 and 2.9 offer a fair selection potential and values more than 3.0 are excellent. Thus, the chemometric equations for NDF, ADF, and IVDMD can be used to make reliable quantitative predictions. The CP model should be used with caution, because of the low RPD obtained.

The correlations (R^2) between the reference data and NIRS predictions for the independent validation set ranged from .87 to .95 (Figure 1). Our models for NDF (Figure 1a), ADF (Figure 1b), and IVDMD (Figure 1c) are considered highly reliable. The external validation for the CP model (Figure 1d) had a lower R^2 and RPD compared to the

models for the other parameters, but these values are still positive indicators and suggest that the model is useful.

Several authors have developed specific models to measure nutritive quality of *Brachiaria* for breeding programs using NIRS. For example, Simeone et al. (2018) developed models with samples of palisade grass, ruzigrass, and signal grass from four different regions of Brazil. Molano et al. (2016) developed models with samples of BH, inter-specific *Brachiaria* hybrids and legumes like Brazilian jackbean (*Canavalia brasiliensis* Mart. ex Benth.), Brazilian centro [*Centrosema brasilianum* (L.) Benth.], Asian pigeon wings (*Clitoria ternatea* L.), and cratylia [*Cratylia argentea* (Desv.) Kuntze.] from different locations of Colombia. Both studies obtained similar results to ours, including R^2 close to 1.0 and RPD values of 3.0 slightly below 3.0. However, some models were built with groups of heterogenic samples and it is unclear whether a single global predictive model can be effectively applied to all samples derived from heterogeneous crop species (Xu, Zhao, Shi, & Wang, 2017). Effectively, it is not feasible to build a prediction equation using samples from legumes and grasses from various species, and then apply it in a breeding program with a relatively narrow genetic base due to the low prediction accuracy. In general terms, it is still necessary to carefully monitor all NIRS models' performance using the reference method (wet chemistry) and compare with the values predicted by the equations for estimating model accuracy. Routinely, we apply the reference method (i.e., wet chemistry) to at least 10% of the samples evaluated by NIRS to confirm the accuracy of the model and monitor the calibration range. If necessary, the models can be enriched to improve accuracy.

Parameters including live weight gain, milk production, and reproduction efficiency can be directly improved by increasing forage quality parameters in the diet (Boval & Dixon, 2012). In our forage grass breeding program, is very important to select superior materials with adequate nutrition levels. Therefore, we constantly evaluate the nutritional quality parameters of our hybrid-breeding selections. Rapid and inexpensive analysis techniques for evaluating these parameters are needed to enable breeders to evaluate a large number of genotypes and facilitate decisions about which selections to discard, advance, or use as parents. Thus, NIRS predictive models are very useful for forage breeders and breeders of many other crops. The objective of this study was to build specific curves for BH hybrids and generate appropriate prediction accuracies for the germplasm pool targeted in our BH-breeding program. Based on our results, NIRS seems to be a very useful tool as it is quick, reliable, environmentally friendly, and low cost. The NIRS method has reduced environmental footprint than the traditional method because no chemicals (e.g., chemical solvents and water) are needed.

The use of this technology will save time and resources and increase the number of samples that can be analyzed when estimating the protein and cell wall contents as well as digestibility for BH hybrids in the CIAT breeding program. In addition, different research groups and seed companies in Latin America and Australia will benefit from these proposed models resulting in the development and utilization of improved forage grass materials by farmers.

4 | CONCLUSION

Brachiaria humidicola is a forage grass that is a major source of animal feed in many livestock systems in the tropics, particularly for poorly drained and waterlogged environments. CIAT preserves a wide diversity of *Brachiaria* genotypes in its germplasm collection. This diversity is the founder source for germplasm selection in *Brachiaria*-breeding programs. In this context, it is critical to have a rapid and low-cost method to assess forage quality of the *Brachiaria* genotypes in the collection. The NIRS is a suitable technique for rapid, low-cost prediction of chemical composition of these forage grasses. Calibrated models for different forage quality parameters have been developed and validated and are now used for routine analyses at the forage quality laboratory of CIAT, reducing the use and exposure to chemical reagents and producing reliable results in a fraction of time and cost of traditional wet chemistry techniques.

Results obtained in this study showed that NIRS equations developed for four of the main forage quality parameters (NDF, ADF, IVDMD, and CP) are adequate to predict the values of these forage components. These chemometric models have shown to be robust, reliable, and environmentally friendly and can be used for quantitative analyses of BH hybrids. However, the model for CP must be used cautiously and the number of samples in the calibration set and external validation set should be increased to improve the predictive efficiency of the model (RPD). It is critical to carefully monitor the models' performance using the reference method (wet chemistry) and compare those results with the values predicted by NIRS equations. In the CIAT laboratory, at least 10% of the samples that were evaluated by NIRS for these four parameters are also simultaneously evaluated with wet chemistry to confirm the accuracy of the model and to enrich the calibration set. The CIAT BH-breeding program is benefitting from the development of these predictive NIRS models as the number of samples that can now be scored for NDF, ADF, CP, and IVDMD has increased and the cost of analysis has decreased.

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CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest

ORCID

Johanna Mazabel  <https://orcid.org/0000-0002-7494-4608>

Margaret Worthington  <https://orcid.org/0000-0001-8019-165X>

Valheria Castiblanco  <https://orcid.org/0000-0003-2801-2153>

Michael Peters  <https://orcid.org/0000-0003-4237-3916>

Jacobo Arango  <https://orcid.org/0000-0002-4828-9398>

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