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SCIENTIFIC NOTE

Prediction of the physical-chemical composition of tropical grasses through NIR spectroscopy

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ABSTRACT

The use of near infrared spectroscopy (NIRS) as an alternative to the techniques commonly employed in the study of forage composition needs to be explored. The objective was to construct calibration curves to predict the physical-chemical composition of tropical grasses (*Brachiaria brizantha* (Hochst. ex A. Rich.) Stapf 'Marandu', 'Piatã'; *B. decumbens* Stapf, *Panicum maximum* Jacq. 'Colonião'), by NIRS and compare two multivariate regression method. Forage samples were analyzed for crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), ash, ether extract (EE), lignin, and moisture. The values obtained by the Official Methods of Analysis of Association of Official Agricultural Chemists (AOAC) were reference values for the creation of multivariate calibration models. The samples were scanned on the NIRS. The multivariate calibration models were created by the partial least squares (PLS) method and by the multiple linear regression (MLR) method. The predictive capacity of the models was evaluated by the correlation coefficient (R) and parameters of the mean squared deviation (RMSE). When the MLR was used, only the prediction model of ash (R = 0.82) of the *P. maximum*, EE (R = 0.87) and moisture (R = 0.90) of 'Piatã' showed approximate predictive capacity, for the other components R indicated good prediction. After the validation of the models developed by the PLS regression method, the CP (0.78-0.91), NDF (0.88-0.95), lignin (0.85-0.91), and moisture (0.79-0.96) predictions presented good results. The NIRS technique can be used to determine the physical-chemical composition of tropical grasses. The MLR multivariate regression method as well as PLS can be used to predict the physical-chemical composition of tropical grasses.

Key words: *Brachiaria*, C4 forage, forage composition, multiple linear regression, multivariate statistics, near infrared spectroscopy.

INTRODUCTION

The use of pastures in the feeding of bovine is made taking into account the aspects related to the growth and development of the plants, factors directly linked to its nutritional value (Conrado et al., 2021). The *Brachiaria* ssp. stand out among the forages more used in the formation of pastures in Brazil. Grass of the genus *Brachiaria* ssp. occupy 80% to 90% of the areas of pastures in Brazil (Silva et al., 2020). Despite its good nutritional characteristics and adaptability, *Brachiaria* forage quality depends heavily on environmental factors and soil fertility, which requires a constant evaluation of the nutritional value (Monrroy et al., 2017).

To quantify forage quality analytically, some parameters such as crude protein (CP), neutral detergent insoluble fiber (NDF), acid detergent insoluble fiber (ADF), lignin, ethereal extract (EE), mineral matter (MM), humidity, among others are used (Arzani et al., 2015). The traditional chemical analyses are laborious and costly, require the use of a large number of reagents of high cost and can represent risks to human health to those who manipulate them and their eliminated residues are potentially causing damage to the environment.

The use of near infrared spectroscopy (NIRS) as an alternative to the techniques commonly employed in the study of forage composition needs to be explored; compared to traditional chemical methods offers a series of advantages. It is a physical, non-destructive method that requires minimal sample preparation. In contrast to traditional chemical analysis, no reagents are required and no residues are produced in its processing (Fernández-Cabanás et al., 2023).

The NIRS generates a large set of spectral data that does not readily evidence the desired information, requiring the use of multivariate chemometric techniques that help quantify the nutrients of interest that are involved in the complex interactions of sample components (Xu et al., 2019). In order to apply NIRS technology it is essential to develop calibration models. Therefore, the choice of the mathematical model that best applies to the set of variables, will define the accuracy of the calibrated model, facilitating its validation (Paternostre et al., 2021).

In order to correlate spectral information with reference data, two of the most widely used multivariate calibration methods are multiple linear regression (MLR) and partial least square (PLS). The objective was to construct calibration models to predict the physical-chemical composition of forages *Brachiaria brizantha* 'Marandu' and 'Piatã', *B. decumbens*, *Panicum maximum* 'Colonião' by NIRS and compare two multivariate regression methods (MLR and PLS).

MATERIALS AND METHODS

Collection and sampling

Forage samples were monthly collected for a period of 1 yr in the Ribeirão do Largo, Bahia, Brazil. The experimental area (15º09'07" S, 40º15'32" W, 709 m a.s.l.) is characterized by a humid tropical climate, with 800 mm average annual precipitation and 27 ºC average annual temperature. Ten samples of each forage were collected monthly totalizing 120 samples of each forage: *Brachiaria brizantha* (Hochst. ex A. Rich.) Stapf 'Marandu' and 'Piatã', *B. decumbens* Stapf and *Panicum maximum* Jacq. 'Colonião'. Forages of the genus *Brachiaria* were cut at 5 cm from the soil level and the *Panicum* at 20 cm from the soil. The forage mass (natural matter) inside the square was measured by means of a digital scale. The forage samples were dried in a forced air circulation oven (60 °C) and ground at 1 mm in a Wiley mill (R-TE-680, Tecnal, Piracicaba, Brazil).

Chemical analysis

Analyzed variables were crude protein levels (CP) (method nr 654.01, Official Methods of Analysis of Association of Official Agricultural Chemists [AOAC]); acid detergent fiber (ADF) (method INCT-CA F-004/1; Instituto Nacional de Ciência e Tecnologia em Ciência Animal [INCT-CA]); corrections for protein and ashes (ADFcp), (method INCT-CA N-005/1 and INCT-CA M-003/1); neutral detergent fiber (NDF) (method INCT-CA F-002/1); and corrections for protein and ashes (NDFcp), (method INCT-CA N-004/1 and INCT-CA M-002/1); mineral matter (ash) (MM) (method nr 942.05); ethereal extract (EE) (method INCT-CA G-004/1); lignin Klason (H_2SO_4 720 g kg; method nr 920.39) and moisture (method nr 934.01) (Latimer Jr., 2016; Detmann et al., 2021). These values obtained by the traditional methods of analysis were used as reference values for the creation of multivariate calibration models (Table 1).

Table 1. Chemical composition and standard deviation of forage evaluated by the reference method. CP: Crude protein; ADF: acid detergent insoluble fiber; NDF: neutral detergent insoluble fiber; EE: ethereal extract; LIG: lignin.

	CP	ADF	NDF	ASH	EE	LIG	Moisture
	Chemical composition (g kg^{-1})						
Marandu	97.10	371.10	661.80	87.20	18.70	55.50	101.80
Piatã	75.40	395.10	687.80	69.60	14.60	41.30	97.70
Decumbens	78.80	401.30	722.10	68.30	17.60	56.80	106.30
Colonião	95.90	434.10	668.90	66.40	15.20	56.40	100.20
	Standard deviation						
Marandu	12.40	43.70	48.60	12.80	2.60	12.60	8.01
Piatã	13.90	22.01	32.70	6.40	1.80	4.01	8.50
Decumbens	6.01	20.50	22.60	9.60	1.50	10.90	8.40
Colonião	13.40	27.40	42.60	6.40	1.40	10.01	6.40

Obtaining spectra

The samples were scanned in a near infrared reflectance spectrometer (NIRS) (SpectraStar 2500 XL, Unity Scientific, Brookfield, Connecticut, USA). About 20 g forage sample were used for measurement in the spectrometer. The model used operates in the wavelength range of 700-2500 nm, 1 nm increase.

Statistical analyses

Outlier samples were taken from the data set obtained by the traditional chemical quantification methods (reference data) and the spectral data set.

From the dataset of each forage two subsets were selected, one for calibration, with 70% of the samples and the other with 30% of the samples for external validation of the model. The method of separation of the data set was based on the Kennard-Stone algorithm (Kennard and Stone, 1969). This pre-treatment of the data was used for the models elaborated by partial least squares (PLS) and multiple linear regression (MLR).

Multivariate calibration

In order to estimate the parameters of interest in forages from the NIRS, it was necessary to construct multivariate calibration models, whose function is to relate the instrumental responses, in this case spectra, of each of the samples (called matrix X) with the reference value of the samples obtained by means of the standard methods (called matrix Y).

The calibration models were created by regression by PLS and MLR methods. To create the calibration models by PLS, we used all spectral scans (1100 to 2500 nm) totaling 1401 measurements of absorbance and to prepare the calibration models by MLR were selected 16 peaks (1203, 1349, 1478, 1541, 1595, 1701, 1734, 1781, 1940, 2076, 2118, 2172, 2275, 2319, 2360 and 2500 nm).

The predictive capacity of the models was evaluated by the correlation coefficient (R) and the parameters of the root mean square error (RMSE) described in Equations 1 and 2, respectively:

$$R = \frac{\sum_{i=1}^{n}(x_i - \bar{x}) \cdot (y_i - \bar{y})}{\sqrt{\sum_{i=1}^{n}(x_i - \bar{x})^2 \cdot \sum_{i=1}^{n} y_i - \bar{y})^2}} \quad (1)$$

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n}(y_i - y_i)^2}{n}} \quad (2)$$

where x and y are the measured values of both variables and $\bar{x}$ and $\bar{y}$ are the arithmetic means of both variables.

Value of R between 0.50 and 0.65, indicates that more than 50% of the variance in y is represented by the variance in x, a value of R between 0.66 and 0.81 indicates "approximate" forecast and an R between 0.82 and 0.90 indicates "good" forecast and R above 0.90 are considered "excellent" (Saha et al., 2017). The models were validated by external validation methods. For the partial least squares (PLS) and multiple linear regression (MLR) analyses, the SAS statistical program (SAS Institute, Cary, North Carolina, USA) was used.

RESULTS AND DISCUSSION

Multiple linear regression (MLR) multivariate calibration models

The correlation coefficients (R) (Table 2) evidence that for the CP the best prediction equations were those of *B. brizantha* 'Marandu' and *P. maximum* 'Colonião'. The R value of 0.85 for CP of temperate pasture and R value of 0.90 for CP of grasslands located in north-central Apennine were observed in similar studies using NIRS (Thulin et al., 2014; Parrini et al., 2022).

Table 2. Coefficient of correlation (R) between the experimental values and the predicted value through the multiple linear regression (MLR) and root mean-square error (RMSE) calibration models of the calibration process. CP: Crude protein; ADF: acid detergent insoluble fiber; NDF: neutral detergent insoluble fiber; EE: ethereal extract; LIG: lignin.

	CP	ADF	NDF	ASH	EE	LIG	Moisture
			MLR calibration				
Marandu	0.91	0.72	0.86	0.77	0.84	0.71	0.86
Piatã	0.79	0.87	0.89	0.79	0.69	0.82	0.78
Decumbens	0.85	0.89	0.92	0.80	0.87	0.82	0.90
Colonião	0.92	0.85	0.92	0.82	0.79	0.87	0.90
			RMSE calibration				
Marandu	0.43	1.79	1.89	0,63	0.09	0.77	0.35
Piatã	0.59	0.76	1.02	0.30	0.10	0.15	0.61
Decumbens	0.20	0.62	0.63	0.42	0.05	0.38	0.23
Colonião	0.42	1.11	1.21	0.29	0.06	0.38	0.19

The cellulose and lignin present in the cell wall of plants are part of the ADF; however, it should be noted that there may be some contaminants such as pectin, minerals and N compounds (Ferreira and Thiex, 2022). In this study ADF and NDF values were corrected for ashes and proteins, aiming to eliminate this type of contaminants. The correlation coefficient of the forage prediction models for ADF (Table 2) are similar to the correlation coefficients of 0.94 and 0.79 when analyzing grasses and forage soybean, respectively (Arzani et al., 2015; Asekova et al., 2016). The correlation coefficient of the calibration models for NDF prediction of 'Marandu', 'Piatã', *B. decumbens*, and 'Colonião' (Table 2). Calibration curve for prediction of NDF with correlation coefficient of R = 0.97 and R = 0.85 in forage studies are described in the literature (Andueza et al., 2016; Asekova et al., 2016).

Although minerals do not absorb in the near infrared region, the NIRS is able to detect ash contents in fodder, associated in complexes with organic compounds, the NIRS was efficient to predict the ash content of the analyzed materials (Table 2).

The quantification of EE of forages by the traditional methods used presents a difference between them. Some provide higher estimates than others, possibly due to increased extraction of non-fatty EE such as pigments and waxes (Barbosa et al., 2017). When observing low correlation coefficients for predicting EE in NIRS the drawback may be related to the reference data used. Only for 'Marandu' and *B. decumbens* were the efficient calibration models for EE. A further study of comparisons between traditional methods

to choose the best method to quantify EE and then use it as a reference for prediction in NIRS would be pertinent.

Regarding the calibration models to predict the lignin content of forages only 'Marandu' presented an approximate forecast. For the other forages the R indicates good prediction. In a similar study, a correlation coefficient was found for the lignin prediction model of 0.68 (Thulin et al., 2014).

The correlation coefficients of the validation data (Table 3) show the efficiency of the models for prediction of the parameters evaluated. It is observed in Table 3 that for CP the validated models present R above 0.90 for 'Marandu', 'Piatã' and 'Colonião'. This indicates the similarity between data obtained by the traditional methods and validation data obtained from the absorbed NIRS. Only *B. decumbens* presented R less than 0.90, although R = 0.85 (Table 3) suggests a good correlation and a good model.

Table 3. Correlation coefficient (R) between the experimental values and the predicted value through the multiple linear regression (MLR) and root mean-square error (RMSE) validation models of the validation process. CP: Crude protein; ADF: acid detergent insoluble fiber; NDF: neutral detergent insoluble fiber; EE: ethereal extract; LIG: lignin.

	CP	ADF	NDF	Ash	EE	LIG	Moisture
			MLR Validation				
Marandu	0.95	0.85	0.92	0.89	0.95	0.95	0.94
Piatã	0.91	0.90	0.93	0.82	0.77	0.90	0.78
Decumbens	0.85	0.92	0.93	0.91	0.91	0.89	0.97
Colonião	0.92	0.87	0.98	0.72	0.90	0.95	0.92
			RMSE Validation				
Marandu	0.24	1.77	1.59	0.46	0.07	0.32	0.20
Piatã	0.49	0.87	0.88	0.28	0.08	0.16	0.40
Decumbens	0.27	0.68	0.76	0.31	0.05	0.32	0.14
Colonião	0.18	0.95	0.75	0.38	0.05	0.37	0.18

When validated, all models for ADF prediction of forages presented R above 0.90; indicating that the models elaborated by the MLR method can be used in the NIRS to predict NDF values for all forages studied. To predict ash, the best validated model was for *B. decumbens*, which presented R = 0.91 (Table 3) and the least efficient was the model for 'Colonião' R = 0.72 (Table 3).

In relation to lignin the best described models were for 'Marandu' and 'Colonião', both presenting R = 0.95 (Table 3), considered as excellent. The models for 'Piatã' and *B. decumbens* can be considered good models.

By observing the above data, it can be stated that MLR is an efficient multivariate calibration tool for the elaboration of calibration and validation curves for the prediction of nutrients of tropical forages.

Multivariate calibration models for partial least squares (PLS)

For prediction of CP, the model of 'Marandu', 'Piatã', 'Colonião' is considered good based on the value of R. For ADF and NDF the best model was for 'Marandu', which presented R = 0.82 (Table 4). However, all models can be used, since the value of R indicates an approximate forecast.

In relation to the models for predicting ash, the model developed for 'Marandu', *B. decumbens* presented R of 0.70 and 0.69, respectively (Table 4). For EE, none of the calibrated models can be considered efficient for prediction. For lignin, the best model was that of 'Colonião' (R = 0.84) and the only model that cannot be used is the lignin prediction of 'Piatã' (0.54). For moisture all models can be used; however, the most accurate is the 'Colonião' (R = 0.80) (Table 4).

The correlation coefficients of the calibration models by the PLS method for prediction of CP of forages indicate that the developed models have the potential to predict the protein content of the forages, the least efficient prediction equation for the prediction of CP was that of *B. decumbens* (R = 0.72) (Table 4).

Table 4. Correlation coefficient (R) between the experimental values (reference values) and the predicted value by the partial least squares (PLS) model and the root mean-square error (RMSE) calibration values of the models in the calibration process. CP: Crude protein; ADF: acid detergent insoluble fiber; NDF: neutral detergent insoluble fiber; EE: ethereal extract; LIG: lignin.

Item	CP	ADF	NDF	Ash	EE	LIG	Moisture
PLS Calibration							
Marandu	0.82	0.82	0.82	0.70	0.61	0.67	0.67
Piatã	0.82	0.70	0.78	0.64	0.36	0.54	0.73
Decumbens	0.72	0.78	0.73	0.69	0.55	0.75	0.75
Colonião	0.85	0.81	0.80	0.58	0.50	0.84	0.80
RMSE Calibration							
Marandu	0.58	1.40	1.09	1.67	0.15	0.80	0.45
Piatã	0.59	1.08	1.38	0.37	0.11	0.24	0.37
Decumbens	0.28	0.89	1.17	0.53	0.09	0.46	0.40
Colonião	0.55	1.23	1.91	0.42	0.09	0.41	0.27

The ADF prediction equations for 'Marandu' were more efficient than that for 'Piatã', *B. decumbens* and 'Colonião' calibration models (Table 4). It should be noted that the values of correlation coefficients of the prediction calibration equations of the present work did not go through the bias adjustment.

The calibration developed models for the prediction of NDF of 'Marandu' and 'Colonião' were more expressive than those of 'Piatã' and *B. decumbens*, according to the correlation coefficients (Table 4). The correlation coefficients of the lignin prediction models were R = 0.67; R = 0.54; R = 0.75 and R = 0.85 (Table 4) for 'Marandu', 'Piatã', *B. decumbens*, 'Colonião', respectively. The best prediction equation for lignin was for 'Colonião'.

The RMSE of 1.71 was used to calibrate models to estimate *B. brizantha* NDF in Panama (Monrroy et al., 2017), values close to those found in this work for NDF (1.09, 1.38, 1.17, 1.91) and ADF (1.40, 1.08, 0.89, 1.25).

The correlation coefficients in the validation of the models (Table 5) were higher than the correlation coefficients of the calibration (Table 4), for all parameters analyzed. For CP the best model was for 'Marandu' (R = 0.91). The models for 'Piatã' and 'Colonião' are considered good (R = 0.84 and R = 0.89, respectively).

Models considered excellent were those elaborated for prediction of NDF of 'Piatã' (R = 0.94) and 'Colonião' (R = 0.95). It can be considered a good model for 'Marandu' (R = 0.89) and *B. decumbens* (R = 0.88), (Table 5).

The predictive potential of the models developed by the PLS method are well evidenced when analyzing the validation correlation values of the methods, the RMSEV values (Table 5).

The RMSEV is the root mean square error of validation. The lower the RMSEV value the better the prediction model. The RMSEV values for prediction of CP of forages of 1.3 (Thulin et al., 2014) and 0.78 (Monrroy et al., 2017) are higher than the values observed in this study (0.33, 0.65, 0.36 and 0.48). The RMSEV for *Brachiaria* ADF prediction of 1.47 (Monrroy et al., 2017) is similar to that of 'Marandu' (1.66) and 'Colonião' (1.33), but superior to RMSEV for 'Piatã' (0.97) and *B. decumbens* (0.72). Regarding the validation models for NDF prediction of forages, the RMSEV found in the present study (Table 5) was lower than that reported by Monrroy et al. (2017) of 1.85.

Table 5. Correlation coefficient (R) between the experimental values (reference value) and the predicted value by the partial least squares (PLS) model and root mean-square error (RMSE) validation by PLS in the validation process. CP: Crude protein; ADF: acid detergent insoluble fiber; NDF: neutral detergent insoluble fiber; EE: ethereal extract; LIG: lignin.

	CP	ADF	NDF	Ash	EE	LIG	Moisture
			PLS Validation				
Marandu	0.91	0.85	0.89	0.86	0.84	0.85	0.90
Piatã	0.84	0.87	0.94	0.80	0.52	0.87	0.79
Decumbens	0.78	0.91	0.88	0.95	0.54	0.85	0.96
Colonião	0.89	0.71	0.95	0.61	0.70	0.91	0.88
			RMSE Validation				
Marandu	0.33	1.66	1.59	0.50	0.14	0.57	0.30
Piatã	0.65	0.97	0.88	0.31	0.10	0.18	0.30
Decumbens	0.36	0.72	0.76	0.20	0.11	0.37	0.18
Colonião	0.48	1.33	1.05	0.45	0.08	0.39	0.22

CONCLUSIONS

Near infrared spectroscopy (NIRS) can be used to determine crude protein, acid detergent fiber, neutral detergent fiber, ash, ether extract, lignin, and moisture content in tropical grasses. The multiple linear regression (MLR) method as well as the partial least squares (PLS) can be used to determine the physical-chemical composition of tropical grasses. However, the MLR method was more efficient in this work because it presented higher correlation coefficients.

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Author contribution
Conceptualization: R.R.S. Methodology: L.S.S. Software: M.M.S.P., M.M.L. Validation, F.F.S. Formal analysis: L.V.S. Investigation: M.M.S.P.; A.B.P.; J.W.D.S. Resources: R.R.S. Data curation: M.M.S.P.; L.S.S.; L.V.S. Writing-original draft: M.M.S.P.; R.R.S. Writing-review & editing: D.M.L.-Jr. Visualization: D.M.L.-Jr. Supervision: R.R.S.; F.F.S. Project administration: R.R.S. Funding acquisition: R.R.S. All co-authors reviewed the final version and approved the manuscript before submission.

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