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Predicting the nutritional value of tropical forages by Near Infrared Reflectance Spectroscopy (NIRS)

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Abstract – The aim was to improve multivariate calibration curves for near-infrared reflectance spectroscopy (NIRS) for the measurement and prediction of nutritive value of tropical forages. Samples from different species, years of harvest and management techniques were used for spectral readings and chemical reference analyses. For the development of partial least squares (PLS) regressions, between 1,435 and 2,080 samples were used as the calibration set and between 713 and 1,080 samples as the external validation set per property. The calibrations were chosen based on the best Q-Value (Büchi, Switzerland) which encompasses, among other factors, the coefficients of determination, standard errors of calibration, standard error of prediction, consistency, validation bias and correlation between predicted and reference data. The predicted and reference results had similar means and standard deviations for the characteristics studied. The Q-Value obtained varied between 0.57 and 0.82. The magnitude of these values suggests that the models can be used for routine analysis of forages nutritional value, replacing or complementing chemical analysis, in plant breeding programs and research projects on animal nutrition, pasture management and production systems.

Index terms: digestibility, grass, laboratory, multivariate analysis, nondestructive methods.

Introduction

Grasslands are the primary land use in Brazil, and the Brazilian cattle, from cow-calf to finishing, is essentially grass-fed on extensive pasture systems (PEREIRA et al., 2024). Brazil has more than 158 million hectares of pastures, which support around 172 million heads of cattle (OMOTE et al., 2021). This is a result of an important genetic improvement work that has been carried out in the past decades (OMOTE et al., 2021; JANK et al., 2013). In this context, chemical analyses applied to evaluate the nutritional value of forage plants play a fundamental role in the genetic improvement process of tropical forages as well as in the scientific research being conducted to establish improved animal feeding practices (ATHAYDE et al., 2020). Several of the wet chemical analyses (WC) are already well standardized and widespread (MEDEIROS et al., 2015; NOGUEIRA & SOUZA, 2005);

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however, they are usually laborious, time-consuming (NOGUEIRA & SOUZA, 2005) and risky to human health and to the environment, due to the handling of various toxic substances (ATHAYDE et al., 2020; TIBOLA et al., 2018; NOGUEIRA & SOUZA, 2005).

Near-infrared reflectance spectroscopy (NIRS) is a recognized solution for performing large scale chemical predictions. The NIRS method stands out for being a precise, non-destructive method with low operational costs, which allows several analyses to be carried out simultaneously and quickly, with the additional advantage of requiring only a small sample volume (ATHAYDE et al., 2020; TIBOLA et al., 2018). Due to its attributes, NIRS is suitable for large scale phenotyping in scientific research and plant breeding programs.

Despite its advantages, NIRS requires specific knowledge and work on multivariate calibrations. For this, large datasets with million data points need to be managed, imposing complexity. In this sense, chemometrics presents several tools to deal with such issues, such as partial least squares (PLS) regression, Savitzky-Golay first derivative, normalization, and smoothing, which allows for the calibration procedure, aiming for precision and accuracy of estimates (ATHAYDE et al., 2020; TIBOLA et al., 2018; FERREIRA, 2015).

The objective of this work was to describe the calibration procedures, as well as their results, for determining the nutritional value of tropical forage samples, applied in research projects and plant breeding programs at Embrapa Beef Cattle.

Material and Methods

Samples

A set of 1,026 samples was selected, representing forage cultivars researched at Embrapa Beef Cattle, collected on 23 timepoints between 2015 and 2020, including species of the genus Brachiaria and Panicum. The samples went through standard procedures prior analyses, which included drying in an oven at 65ºC for 48 hours and Willey grinding with 1 mm sieves (NOGUEIRA & SOUZA, 2005). After processing, the samples were sent to WC analysis.

Chemical analyses

Samples were submitted to chemical analyses, according to methods compiled by NOGUEIRA & SOUZA (2005):

- Crude protein (CP)
- Neutral detergent fiber (NDF)
- Acid detergent fiber (ADF)
- In vitro organic matter digestibility (IVOMD)
- Acid detergent lignin (ADL)
- Cellulose (CEL)
- Silica (SIL)
- Ash

The results obtained by WC were used as reference values for NIRS readings.

NIRS analyses and chemometrics

The samples were analyzed on two FT-NIR Büchi NIRFlex 500 instruments, with data collected using the NIRS Operator software (Büchi, Switzerland), using standard ring cup mini cuvettes with quartz bottoms (Foss, Denmark and Aquartzo, Brazil), according to the manufacturer's instructions. Samples were read between 4,000 and 9,000 cm^{-1}.

The spectra obtained were processed using the NIRCal® chemometrics software, version 1.6 (Büchi, Switzerland), according to the manufacturer's instructions. The spectra were aligned from curves generated on a Foss 5000 instrument (Foss, Denmark), which was previously used for NIRS analyzes, and imported into NIRCal® software, version 1.6 (Büchi, Switzerland), where it received the appropriate treatments to make the spectra compatible.

The initial alignment was improved with readings on both NIRFlex 500, and the calibration curve obtained at the end of the process was independent of the calibration curves previously used on the Foss 5000. Among the available methods, only Partial Least Square (PLS) was used for all parameters.

Results and Discussion

Chemical analyzes showed the following standard deviations (SD):

- CP: 0.1936
- NDF: 0.8137
- ADF: 0.7188
- IVOMD: 2.4171
- ADL: 0.1815
- CEL: 0.6563
- SIL: 0.3119
- Ash: 0.1632

It should be noted that IVMOD presented a relatively high SD, but this is characteristic of the methodology, because it depends on ruminal fluid collected from different animals, at different times of the year, which leads to differences in the microbiota and consequently to differences in the biochemistry properties of rumen fluid (NOGUEIRA & SOUZA, 2005).

The samples analyzed by NIRS presented the following results presented in Table 1.

Regarding regression, in addition to mean-centered PLS, the following methods were used, all available in NIRCal®, and with the following amounts of PC (main components):

- CP: MSC full, First Derivative BCAP; PC: 6 primaries, 6 secondaries
- NDF: Normalization per Unit Length, First Taylor Derivative 3 points; PC: 6 primaries, 6 secondaries
- ADF: Normalization by Unit Length, Savitzky-Golay First Derivative 9 points, Smoothing 3 points; PC: 4 primaries, 3 secondaries
- IVOMD: MSC full, Savitzky-Golay First Derivative 9 points, Smoothing 3 points; PC: 8 primaries, 8 secondaries
- ADL: MSC full, Savitzky-Golay First Derivative 9 points, Smoothing 3 points; PC: 9 primaries, 9 secondaries
- CEL: MSC full, First Taylor Derivative 3 points; PC: 6 primaries, 6 secondaries
- SIL: MSC full, First Taylor Derivative 3 points; PC: 8 primaries, 8 secondaries
- Ash: Normalization by Unit Length, First Taylor Derivative 3 points; PC: 7 primaries, 7 secondaries

All calibration curves passed the graphic heteroscedasticity test (COOK & WEISBERG, 1983).

The calibration curves obtained with the NIRFlex 500 were quite satisfactory, with Q-Value above 0.60 (value recommended by Büchi to indicate an efficient calibration), with the exception of IVMOD which, as previously mentioned, is a method that presents great variability also in WC analyses.

Table 1. NIRS results.

Item	Samples (n)			RMSE		r		Consistency		Validation bias	SD of WC	Q-Value
	Calibration	Validation	Total	Calibration	Validation	Calibration	Validation	Büchi NIRFlex 500	Foss 5000			
IVOMD	2,016	996	3,012	4.12	4.05	0.93	0.93	101.76	84.07	0.01	2.42	0.58
CP	2,031	1,017	3,048	0.76	0.75	0.98	0.98	100.80	83.03	0.02	0.19	0.82
NDF	2,036	1,014	3,050	2.57	2.54	0.91	0.91	101.9	80.97	0.02	0.81	0.60
ADF	1,946	971	2,917	2.22	2.24	0.95	0.94	99.48	85.26	0.01	0.72	0.75
ADL	1,601	803	2,404	0.36	0.38	0.91	0.91	94.85	70.80	0.01	0.18	0.68
Ash	2,080	1,030	3,110	1.01	1.01	0.85	0.85	99.79	87.83	0.00	0.16	0.65
Cellulose	1,729	864	2,593	1.33	1.29	0.95	0.96	103.33	91.35	-0.06	0.66	0.72
Sílica	1,435	713	2,148	0.64	0.65	0.87	0.87	98.84	88.30	0.01	0.31	0.68

RMSE: root mean square error.

r: correlation coefficient.

SD of WC: standard deviation of wet chemistry analyses.

Q-Value: Quality reference value of the calibration curve, generated by the NIRCal software.

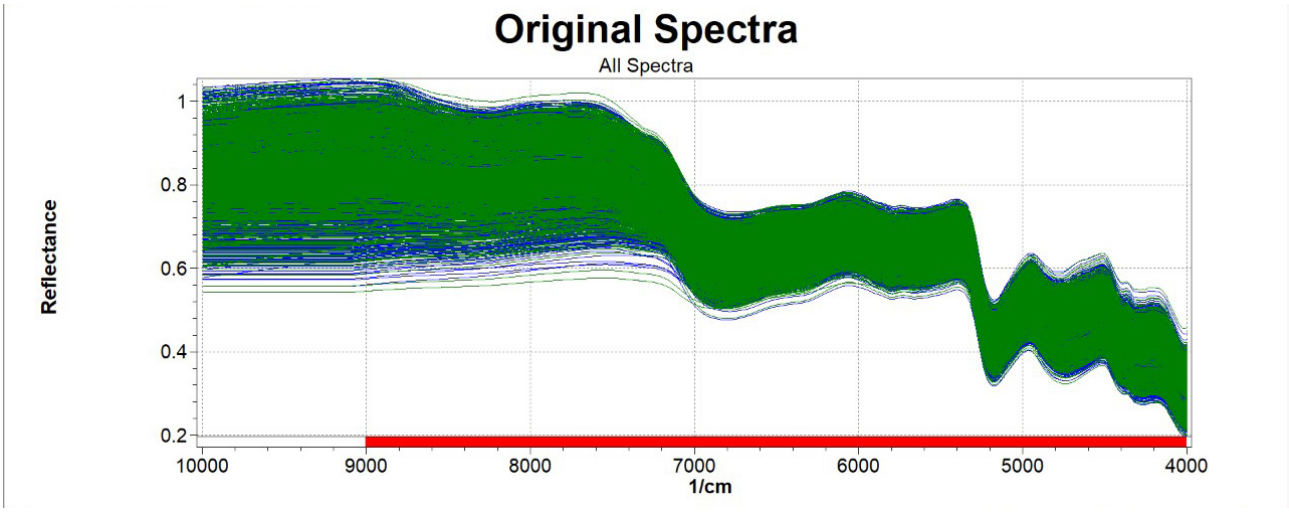

Figure 1. Original spectra for IVOMD.

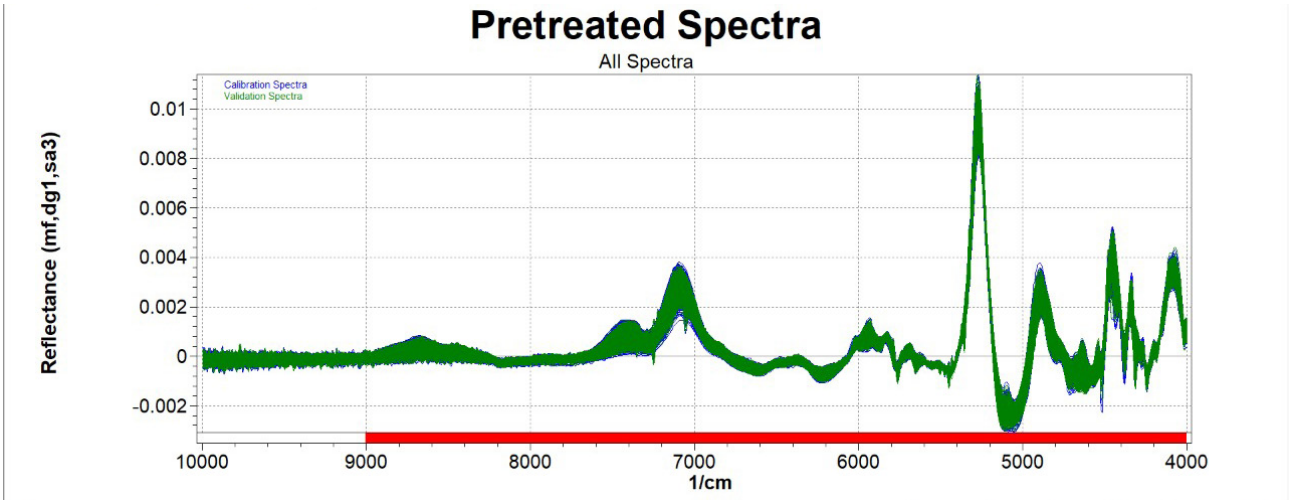

Figure 2. IVOMD spectra following chemometric processing.

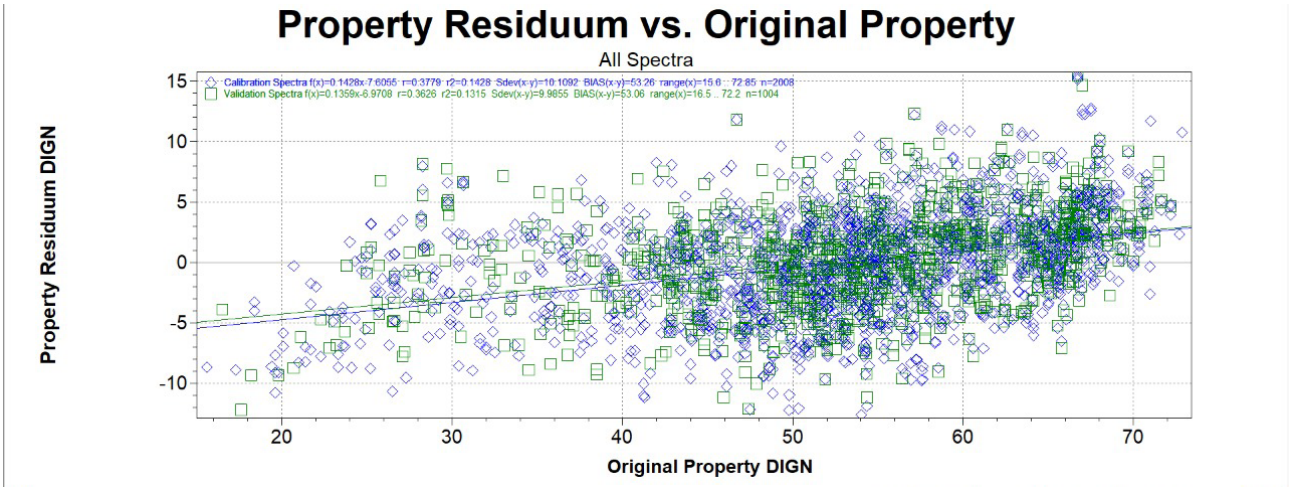

Figure 3. IVOMD residual plot showing evident homoscedasticity of the results.

The validity of the calibration curves is reinforced by the consistency values. Büchi recommends that the consistency should be as close to 100% as possible and within a range between 80% and 110%. It was observed that, for all calibration curves, the consistency values in the NIRFlex 500 ranged from 94.85 for ADL and 103.33 for cellulose, showing that the obtained curves were suitable curves for analyses of tropical forages.

In Foss 5000, the consistency values were, in all cases, considerably lower, with one value below 80%, one value above 90%, and the others between 80 and 90%. The consistency values in Foss followed the same trend as in Büchi, including the minimum and maximum values 70.80 for ADL and 91.35 for cellulose.

Conclusion

It is possible to carry out calibrations to accurately estimate the nutritional value of tropical forage samples. NIRS can be applied in routine analysis of forages nutritional value, replacing or complementing chemical analysis, in plant breeding programs and research projects on animal nutrition, pasture management and production systems.

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