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Estimating nutrient composition and polyphenol concentration using Near-Infrared Spectroscopy (NIRS) in tropical forages

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Abstract. *Parastiwi HA, Lestari NSH, Yanza YR, Niderkorn V, Ridwan R, Jayanegara A. 2023. Estimating nutrient composition and polyphenol concentration using Near-Infrared Spectroscopy (NIRS) in tropical forages. Biodiversitas 24: 6652-6660.* This study aimed to evaluate the accuracy and precision of Near-Infrared spectroscopy (NIRS) in determining nutrient composition and total phenol concentration in tropical forages. A total of 48 tropical forages from 33 species were subjected to measurements using conventional methods and NIRS equipment for rapid determination. The measured variables included Dry Matter (DM), ash, Crude Protein (CP), Ether Extract (EE), Crude Fiber (CF), and Total Phenolic (TP). The values obtained from NIRS were then statistically evaluated to obtain their coefficient of determination (R^2), Standard Error (SE), and Root Mean Square Error (RMSE). Each tropical forage was assessed with three scanning repetitions, where two were conducted to calibrate NIRS determination, and another was performed to validate the NIRS results. All values obtained from the measured samples using both methods in this study were statistically analyzed through Partial Least Square (PLS) regression model. The results showed that the accuracy of NIRS for estimating nutrient content and total phenolic among different tropical forages was varied. NIRS was precise and accurate for estimating crude protein and total phenolic contents of tropical forages but showed lower accuracy for estimating EE content.

Keywords: Estimation, NIRS, nutrient composition, polyphenols, tropical forages

Abbreviations: CP: Crude Protein; CF: Crude Fiber; DM: Dry Matter; EE: Ether Extract; TP: Total Phenols; n.d.: not determined; NIRS: Near Infrared Spectroscopy; R^2: Coefficient of determination; SE: Standard Error; RMSE: Root Mean Square Error; St. dev: Standard deviation

INTRODUCTION

Forage is the primary source of feed for fulfilling the nutrient requirements of ruminants, including grasses, legumes, and herbs. In addition, this feed plays an essential role in providing dietary fiber, energy, protein, as well as essential vitamins and minerals (Zhang et al. 2020). Several studies have shown that the quality of forages can be assessed using their nutritive value, particularly Crude Protein (CP), Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), Acid Detergent Lignin (ADL), and Water-Soluble Carbohydrates (WSC) (Küchenmeister et al. 2013; Brown et al. 2017). A widely explored method for quality determination is proximate analysis, comprising various chemical constituents that include Dry Matter (DM), ash, CP, Ether Extract (EE), Crude Fiber (CF), and Nitrogen-Free Extract (NFE). However, this method has been reported to have several limitations, including its time-consuming and labor-intensive nature (Parrini et al. 2017). Despite these limitations, the use of samples subjected to proximate analysis is recommended due to the satisfactory results, making them valuable references in evaluating feed quality, formulating animal rations, and various animal feeding studies.

Several forages that thrive in tropical climates are known to contain significant amounts of plant secondary metabolites. The main secondary metabolites are polyphenols, including phenolic acids, flavonoids, tannins, stilbenes, and lignans (Cömert and Gökmen 2018; Fayique and Thomas 2018). These polyphenols serve as a defense mechanism for plants against pathogens, fungi, UV radiation, and herbivores (Dini and Grumetto 2022). For ruminants, supplementation with specific amounts of these compounds has shown promising results in reducing

methane emissions and can influence digestibility in the rumen (Jayanegara et al. 2013, 2020; Yanza et al. 2018, 2021). Polyphenols can inhibit rumen methanogen growth and shift-free hydrogen pathways to propionate production instead of methane (Seradj et al. 2014; Yanza et al. 2022). Other beneficial effects of polyphenols in the diets of ruminants include their ability to maintain animal health by alleviating the nematode population in the gastrointestinal tract and decelerating the biohydrogenation rate of Polyunsaturated Fatty Acids (PUFA). This deceleration leads to higher concentrations of PUFA in animal products (Niderkorn and Jayanegara 2021).

Various methods have been explored to quantify polyphenols, including spectrophotometry (Csepregi et al. 2022), gas chromatography and mass spectrometry (GC-MS) (Lingwan and Masakapalli 2022), Ultra-Performance Liquid Chromatography (UPLC) (Cendrowski et al. 2017), and High-Performance Liquid Chromatography (HPLC) (Mizzi et al. 2020). Despite the effectiveness of these methods, there is still a need for simplified methods with accurate and efficient measurements. Near-Infrared Reflectance (NIR) spectroscopy is a rapid method for estimating plant chemical compounds with several benefits, including non-destructiveness, environmental friendliness, economic efficiency, rapid results, and effortless methods. Previous studies have used NIRS to estimate the chemical composition and digestibility of silage (Dias et al. 2023; Zicarelli et al. 2023), forage chemical composition and nutritive values, and chemical composition of feces (Andueza et al. 2017). Another study also used this method for estimating the chemical composition of other feed sources and predicting their potential nutrient digestibility in ruminants and other livestock animals (Nieto-Ortega et al. 2022; Pepeta et al. 2022). Although chemical analysis using NIRS has shown accurate estimation in monogastric cereal feed with >0.97 predicted correlation values (Nieto-Ortega et al. 2022), there is still a significant literature gap. The majority of associated studies have predominantly focused on temperate forages, underscoring the need for further investigations. Therefore, this study aimed to determine reliable NIRS calibrations for estimating proximate values and total phenol concentrations in tropical forages.

MATERIALS AND METHODS

Sample collection

A total of 48 forage samples from 33 tropical species were collected from the Field Laboratory of Agrostology, Department of Nutrition and Feed Technology, Faculty of Animal Science, IPB University, and around the Dramaga region in Bogor, West Java, Indonesia (average elevation 265 m a.s.l.; temperature 21-34°C, humidity 71-85%, precipitation 3-24%). The collected tropical forages were categorized as grass, legumes, and non-grass or non-legumes, where a whole part of each comprised the edible part (Table 1). Sample preparation was performed according to Parrini et al. (2017). Furthermore, approximately 3 kg of each was weighed and dried in an oven for 72 h. Each forage was ground, filtered through a 1 mm sieve, packaged in a plastic clip, and stored at room temperature. All sample preparations, chemical analyses, and NIRS were performed at the Laboratory of Genomic and Environment, National Research and Innovation Agency (BRIN), Bogor, West Java, Indonesia.

Chemical composition analysis and phenolic measurement

The chemical composition of each tropical forage was analyzed following AOAC (2005) for DM (method no. 934.01), ash (method no. 942.05), crude protein (CP; using a Tecator Digestor Auto TM, FOSS Analytical, Sweden; method no. 976.05), ether extract (EE, using a Soxhlet extractor Soxtec TM 2050, FOSS Analytical, Sweden; method no. 973.18), and Crude Fiber composition (CF; using Fibertec TM 2010 and FOSS Analytical, Sweden). Polyphenol concentrations in each tropical forage were measured following the protocols of Makkar (2003) using a UV-vis spectrophotometer (Shimadzu UV-1800, Shimadzu Corporation, Kyoto, Japan) at a wavelength of 725 nm.

NIRS application on estimating forage chemical composition

Each dried tropical forage was ground, homogenized, and analyzed using spectrum radiation. Approximately 25 g of each sample was placed on a Petri dish and scanned using Buchi NIRFlex N500 Fourier Transform Near Infrared (FT-NIR) spectroscopy with a wavelength of 1000-2500 nm. Furthermore, the difference between high and low spectral peaks showed characteristic differences in the chemical components of forage (Yang et al. 2017). The procedure was performed at room temperature (20°C). The 48 samples were scanned three times each, where two scanning processes were considered as calibration values and another scanning process was considered as a validation value. The observed parameters in this study included DM, CF, CP, EE, Ash, and total polyphenols. The spectral value of each parameter was denoted by R as the reflectance value. Each parameter model estimation was measured using NIRCal V5.5 (Build 3000) software, which had already been integrated with the NIRS device. Spectral data were automatically divided into the NIRS software database of the calibration and validation subsets, following the integrated algorithm within the software.

Data management and statistical analysis

All collected data from the NIRS were statistically analyzed to determine the accuracy and efficiency of the model. The statistical variables of the model were the coefficient of calibration (R^2C) and validation (R^2V), Standard Error of calibration (SE_C), Standard Error of validation (SE_V), Standard Error of Prediction (SEP), and Root Mean Square Error of Prediction (RMSEP) (Shenk and Westerhaus 1991).

The mathematical formula used for the RMSEP model validation was as follows:

$$\text{RMSEP} = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(Y_i - \hat{Y}_i)^2}$$

Where:
Yi : Variable value of the i-th validation response
Ŷi : Estimated value of the i-validation
N : Number of observations

Partial Least Square (PLS) regression analysis was used to obtain correlation values between the spectra results and conventional measured data of chemical analysis. A high Residual Predictive Deviation (RPD) value could show a good result (Lobos et al. 2013). The RPD value was calculated as: RPD = SD / SEP.

RESULTS AND DISCUSSION

Chemical composition of tropical forages

The chemical composition of various tropical forage samples is presented in Table 1. The observed parameters included DM, ash, CP, EE, CF, and TP. The moisture content among the samples showed a high DM proportion, ranging from 89.65% (*Acacia villosa* (Sw.) Willd.) to 98.74% (*Setaria splendida* Stapf). The ash proportion varied from 4.77% (*Acacia villosa*) to 11.43% (*Panicum maximum* var. Trichoglume Robyns), while the fat content expressed as EE was between 0.45% to 4.67% (*Stylosanthes hamata* (L.) Taub.).

A wide range of values of tropical forages were shown by CP, ranging from 6.72% (*Stylosanthes hamata*) to 25.26% (*Indigofera zollingeriana* Miq.). Meanwhile, for the CF proportion, the lowest and the highest measured contents expressed by the similar species, *S. hamata*, was from approximately 16.08% to 46.05%. A wide range proportion was also shown by total phenol parameters from 0.57% to 16.68%, where the lowest TP value was represented by *S. hamata* and the highest TP value was observed in *Calliandra calothyrsus* Meisn.

Different tropical forage species had varying chemical and bioactive contents. The majority of the scanned tropical forages were from the Fabaceae family, while the data showed diversity in chemical composition across different species among tropical forages. The results showed that higher DM and CF contents were found in the Poaceae family. Grass was a plant belonging to the Poaceae family. The majority of the respected plants were composed of solid materials, particularly lignocellulose molecules, rather than edible cellulose molecules. Consequently, the solid lignin bond structure could be indestructible and remain a residual compound material, even when appropriately dried (Bell et al. 2018).

Chemical analysis showed higher protein and total phenol content in the Fabaceae family. This family of plants comprises legumes that are naturally engaged in N fixation and have a symbiotic relationship with soil bacteria (Wang et al. 2018). High levels of phenolic compounds in legumes are attributed to their natural biosynthesis pathways of isoflavonoids and lignans in protecting themselves from their role as defense mechanisms against pathogens (Mazur et al. 1998; Tungmunnithum et al. 2021). Consequently, high levels of phenols and protein compounds have been found in legume species rather than in other tropical plant species.

NIRS for estimating the chemical composition of tropical forages

In this study, the chemical composition of tropical forages and total phenols showed varied results for the calibration and validation subsets for the different parameters, as shown in Table 2. The CF value showed the highest range value, which deviated by 7.42 %, followed by the CP and TP content, which deviated at 4.89% and 4.41% in the calibration subset, respectively. Furthermore, the PLS regression model showed a favorable calibration result for DM, ash, CP, and TP, demonstrated by their slight SE (SEP: 1.43, 1.12, 1.15, and 1.13) and high R^2 values (R^2V: 0.68, 0.66, 0.94, 0.82) (Table 3). The CP parameter exhibited the best calibration and validation with R^2>0.90. The results showed that CF and EE were demonstrated by their low R^2 values in both calibration and validation, while the Total Phenolic (TP) content had satisfactory R^2 values with an acceptable SE distribution.

Although the EE was precisely determined based on its SE, the estimated EE in tropical forages using NIRS did not show satisfactory accuracy. However, the lowest precision was observed for the CF prediction values among all determined parameters because the standard error (SEC, SEV, and SEP) for CF showed high results. Based on the results, there was an affiliation between statistical values for calibration and validation, which showed precise and accurate results, expressed by their SEP and RMSEP values (Table 4).

In this study, calibration data subsets of each generated spectral curve from various tropical forages did not show a high variation pattern in all parameters (Figure 1). This condition could deliver a positive outcome in the NIRS calibration process to predict the forage chemical content in certain environments. This was because the spectral band waves were generated through an irradiation process on scanned tropical forages. Yang et al. (2017) stated that there was a differentiation between high and low spectral peaks. This showed that the various forage types had different characteristics following their response to electromagnetic wavelengths from NIRS, thereby influencing the chemical contents. Schwanninger et al. (2011) also affirmed that the chemical content determination of plant components using the NIRS band must be assigned at 780-2,500 nm electromagnetic wavelengths or 12,800-4,000 infrared band per cm wavelengths to obtain a precise and accurate estimation. The infrared wavelength in this study was still in a well-considered range of approximately 1,000-2,500 nm or 10,000-4,000 infrared bands per cm wavelength. Therefore, infrared wavelengths were absorbed by each tropical forage. The chemical content could be predicted because of the different chemical bonds that absorbed different wavelengths (Wu et al. 2015).

Based on the results, NIRS could generate a precise and accurate regression model of each observed parameter that was adjusted to the optimally absorbed wavelengths of the scanned tropical forages. Schwanninger et al. (2011) studied the utilization of NIRS to estimate wood components and confirmed that the spectra generated were influenced by several factors, including particle size,

surface characteristics, porosity, refractive index, and density (Schwanninger et al. 2011). Each material's temperature and moisture content could affect the generated spectra (Ikoyi and Younge 2020). Diverse particle sizes caused variation in the spectral data results, while homogeneous and smaller particle sizes (0.5 mm) improved the precision of the observed results, as showed by high R^2 and lower SE. This condition could explain the similar pattern of the generated spectra curve for the different 33 tropical forage species due to their similar preservation conditions attributed to particle size, temperature, and moisture content.

Table 1. Chemical composition of the tropical forage samples

Scientific name	Family	Measured chemical content (%)					
		DM	Ash	CP	EE	CF	TP
Pterocarpus indicus	Fabaceae	93.66	6.11	17.62	2.05	30.53	5.21
Acacia villosa	Fabaceae	94.09	4.83	16.77	2.85	31.99	11.78
Leucaena leucocephala	Fabaceae	93.90	7.92	20.86	2.62	20.82	12.53
Indigofera zollingeriana	Fabaceae	89.65	9.43	25.85	n.d.	20.82	3.25
Stylosanthes scabra	Fabaceae	95.74	5.63	10.20	1.35	n.d.	3.67
Albizia falcataria	Fabaceae	93.62	6.53	15.06	2.00	28.41	5.93
Stylosanthes hamata	Fabaceae	96.53	9.49	12.50	0.91	46.05	1.69
Pueraria triloba	Fabaceae	94.34	6.79	22.28	1.66	29.26	1.35
Hibiscus tiliaceus	Malvaceae	95.73	6.79	19.11	1.44	31.10	4.53
Artocarpus heterophyllus	Moraceae	93.85	10.89	17.52	n.d.	22.96	8.43
Centrosema pubescens	Fabaceae	96.08	7.18	19.26	3.33	22.96	1.24
Pennisetum purpuphoides	Poaceae	96.28	11.04	9.74	3.30	35.87	0.62
Manihot utilisima	Euphorbiaceae	90.34	8.02	25.17	4.47	20.88	1.52
Calliandra calothyrsus	Fabaceae	93.71	5.81	21.51	3.82	19.33	12.93
Neolamarckia cadamba	Rubiaceae	91.94	7.37	16.98	3.65	19.97	2.62
Pennisetum purpureum	Poaceae	96.54	10.79	11.97	1.35	33.43	1.15
Flemingia strobilifera Linn.	Fabaceae	95.05	5.55	17.08	2.29	n.d.	6.51
Modis modium	Unknown	94.03	7.26	19.15	3.14	32.66	5.50
Bauhinia purpurea	Fabaceae	93.31	9.07	20.46	4.26	30.72	2.68
Setaria anceps	Poaceae	95.74	9.91	15.48	4.05	32.72	n.d.
Gliricidia sepium	Fabaceae	93.63	11.30	23.18	4.67	18.57	2.80
Panicum maximum	Poaceae	97.98	6.81	8.61	2.96	40.23	n.d.
Setaria splendida	Poaceae	98.74	8.79	11.45	2.72	33.11	2.41
Brachiaria decumbens	Poaceae	97.16	5.64	8.04	3.03	36.19	1.06
Euchlaena mexicana	Poaceae	n.d.	5.81	7.89	0.69	41.76	n.d.
Calopogonium mucunoides	Fabaceae	94.32	5.13	n.d.	3.16	33.68	2.95
Arachis pintoi	Fabaceae	94.99	9.31	16.30	1.36	28.90	1.77
Trichantera gigantea	Acanthaceae	95.19	n.d.	n.d.	n.d.	16.68	0.57
Senna alata	Fabaceae	94.74	n.d.	19.69	n.d.	22.61	6.90
Stylosanthes scabra	Fabaceae	93.41	5.34	10.93	1.70	44.43	3.27
Arachis pintoi	Fabaceae	94.97	9.71	17.78	1.80	27.13	2.52
Indigofera zollingeriana	Fabaceae	92.07	10.13	25.26	1.96	18.94	2.24
Gliricidia sepium	Fabaceae	93.55	7.79	20.12	3.95	20.52	3.54
Centrosema pubescens	Fabaceae	93.78	8.30	15.75	1.34	35.44	0.84
Leucaena leucocephala	Fabaceae	93.67	7.75	19.86	2.79	16.08	13.48
Brachiaria decumbens	Poaceae	93.89	8.94	11.57	1.79	29.52	1.17
Pennisetum purpureum	Poaceae	96.07	9.95	9.43	2.22	34.65	1.06
Pennisetum purpureum schumach)	Poaceae	n.d.	10.59	10.82	2.41	29.81	1.22
Bauhinia purpurea	Fabaceae	93.86	9.84	20.70	3.92	26.87	1.65
Stylosanthes hamata	Fabaceae	96.23	5.83	12.25	1.51	42.88	4.20
Modis modium	Unknown	96.03	4.77	15.12	1.98	32.79	10.97
Pennisetum purpuphoides	Poaceae	96.25	11.43	10.43	1.52	37.83	0.84
Panicum maximum var.Trichoglume	Poaceae	95.98	9.80	12.51	2.15	34.56	n.d.
Calliandra calothyrsus	Fabaceae	94.53	5.39	20.71	1.78	19.99	16.17
Stylosanthes guianensis	Fabaceae	97.22	6.18	12.84	0.45	40.24	2.34
Brachiaria humidicola	Poaceae	n.d.	n.d.	6.72	n.d.	34.35	1.21
Setaria splendida	Poaceae	95.33	9.15	15.11	1.09	30.11	1.26
Setaria anceps	Poaceae	96.69	8.37	12.60	3.71	33.10	n.d.

Note: CP: Crude Protein; CF: Crude Fiber; DM: Dry Matter; EE: Ether Extract; TP: Total Phenols; n.d.: not determined

Table 2. Statistical characteristic of chemical composition (% of dry matter) from wet chemical analysis for calibration and validation of forage

Parameter	Calibration				Validation			
	Min	Max	Mean	St dev	Min	Max	Mean	St dev
DM	89.65	98.74	94.69	1.74	90.34	97.98	94.95	1.91
Ash	4.77	11.43	7.83	1.97	5.81	10.89	8.59	1.89
CP	6.72	25.85	15.65	4.89	7.89	25.26	16.60	5.43
EE	0.45	4.67	2.54	1.03	0.69	4.47	2.22	1.19
CF	16.08	46.05	29.18	7.42	16.68	42.88	32.48	8.36
TP	0.57	16.17	4.48	4.41	0.73	8.43	2.45	2.07

Note: Avg: Average; St dev: Standard deviation; DM: Dry Matter; CP: Crude Protein; EE: Ether Extract; CF: Crude Fiber; and TP: Total Phenol

Table 3. Statistical value of calibration and validation on predicting DM, ash, CP, EE, CF, and TP (%DM) obtained from PLS regression

Parameter	Calibration				Validation			
	SD	SEC	R^2C	RPD	SD	SEV	R^2V	RPD
DM	1.36	1.09	0.61	1.25	1.49	1.08	0.68	1.38
Ash	1.65	1.08	0.70	1.53	1.74	1.12	0.66	1.55
CP	4.79	0.99	0.96	4.84	5.55	1.39	0.94	3.99
EE	0.69	0.76	0.45	0.91	0.56	0.87	0.53	0.64
CF	6.71	3.18	0.82	2.11	7.05	2.89	0.89	2.44
TP	4.25	1.17	0.91	3.63	2.16	0.93	0.82	2.32

Note: SEC: Standard Error of Calibration; R2C: Coefficient determination of calibration; SEV: Standard Error of Validation; R2V: Coefficient determination of validation; SEP: Standard Error Prediction; RMSEP: Root Mean Square Error of Prediction

Table 4. Statistical analysis of validation on predicting nutrient content of tropical forages

Parameter	SEP	RMSEP	RSD	Bias	Slope
DM	1.419	1.431	1.400	-0.220	0.83
Ash	1.674	1.669	1.681	0.041	0.96
CP	1.155	1.151	1.157	0.016	0.99
EE	0.836	0.833	0.837	0.014	1.09
CF	4.740	4.811	4.727	0.914	1.08
TP	1.134	1.131	1.135	-0.065	1.03

Note: SEP: Standard Error Prediction; RMSEP: Root Mean Square Error of Prediction; RSD: Relative Standard Deviation

This study observed lower precision and accuracy in determining the DM content using NIRS (Figure 2). The low value of the prediction could be due to the non-uniform size of the sample particles, physical changes during preparation, contamination, and the wavelength absorption of the OH group from tropical forages. The calibration and validation models had dissimilar (R^2) values of 0.61 and 0.68, respectively. The DM content showed unsatisfactory estimation due to its lower R^2 and deviated SE. Furthermore, the results showed that the R^2 values of calibration and validation were lower compared to those obtained by Parrini et al. (2017) and Yang et al. (2017), achieving more than 0.90 of R^2 values for DM content.

Compared to DM, the ash content r^2 value was lower than 0.90, but the standard errors of calibration and validation were not significantly different (1.59 and 1.66). This showed that a high precision was obtained for predicting the ash content in various tropical forages. Fekadu et al. (2010) stated that NIRS could accurately predict ash content with an r^2 value of 0.86. Based on the results, it could be predicted that the ash molecules were bound to the organic matter components in the scanned forage, leading to the ability of the infrared beam to predict the observed ash content. However, Parrini et al. (2017) affirmed that the low predictive value for ash content detection was due to the absence of spectral absorption in NIRS for minerals. Low accuracy in predicting this parameter in tropical forages was disrupted because the infrared device mostly works on organic compounds.

The CP content prediction in this study was very accurate, in line with the R^2 (R^2C = 0.99, R^2V = 0.94) and SE (SEP = 1.15) values (Figure 3). The results showed that SE values between calibration and validation were likely dissimilar, but the R^2 value was greater than 0.90. According to Roberts et al. (2003), the value of the coefficient determination and standard error of CP were related to the absorption of the wavelength using NIRS on N-H organic compound groups from tropical forages. The CP content prediction in this study was not significantly different from the results of Parrini et al. (2017), with an R^2 calibration value of 0.995 and an R^2 validation of 0.977.

The spectral absorption for EE and CF content was inaccurately predicted among all the observed parameters. According to Roberts et al. (2003), calibration of fat content in forage typically yielded low results, which was attributed to a low deposition of fat in plant leaf tissue. Moreover, low-accuracy observations could be linked to insufficient aliphatic (-CH) groups in observed tropical forages (Parrini et al. 2017). The structure of fat content varied, such as saturated and unsaturated fatty acids, mono- and poly-chain fatty acids, and other triglyceride structures, including phospholipids (Pepeta et al. 2022). Problems in CF could be addressed by the various tropical forage

characteristics that contained high residual and solid lignin structures depending on the type, species, soil structure, and seasons (Bell et al. 2018), showing the possibility for low accuracy in predicting CF content in forage. Parrini et al. (2017) also stated that the fiber component had the lowest prediction precision compared to other proximate components due to its molecular structure.

Phenols are secondary metabolites in plants that contain an aromatic group linked by hydrogen bonds. In this study, the predicted TP content of tropical forage was observed precisely and accurately (Figure 4). The R^2 and SE values of the calibration and validation of TP content were likely similar. Several studies have shown that tropical forages could absorb the spectrum from NIRS based on their phenolic content, which was related to the O-H molecular groups (Ciurczak et al. 2021). However, predicting the total phenolic content in plants was related to several factors, including plant type, cultivar, environment, climatic conditions, sampling time, and degree of plant maturity (Kljusurić et al. 2016; Kagan et al. 2019). A high phenolic content was mostly detected in the *Fabaceae* family plants or legumes. Although the plants consisted of high protein and phenolic content, the relationship between these two components in legumes could vary and were influenced by various factors, such as biological, environmental, and legume cultivars (Niderkorn and Jayanegara 2021; Nicolás-García et al. 2022). Phenols are secondary metabolites with a different chemical structure from the nutrients contained in forage possessing aromatic groups (Cömert and Gökmen 2018). This showed that their absorption value was not disturbed by other chemical component functional groups.

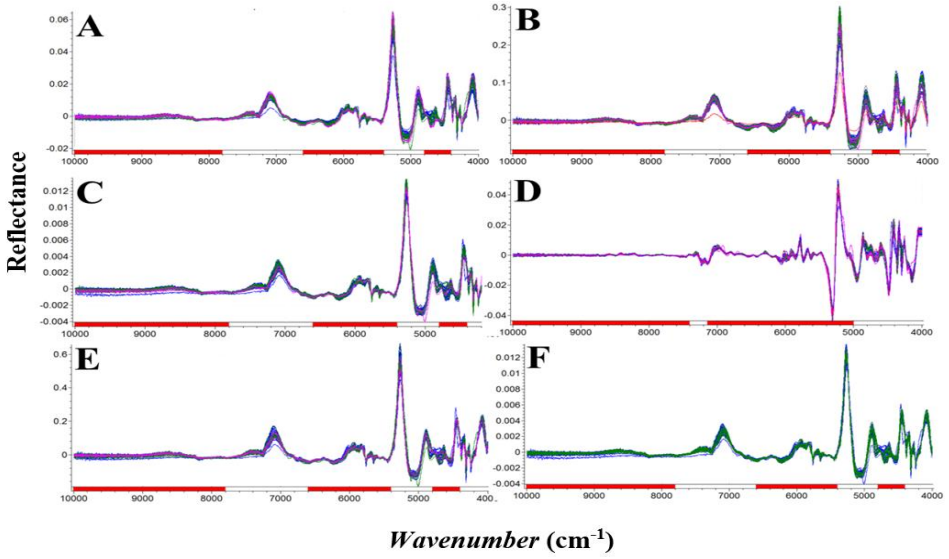

Figure 1. The relationship between wavenumber ($cm^{-1)}$ and NIRS component prediction for: A. Dry matter, B. Ash, C. Crude protein, D. Ether extract, E. Crude fiber, and F. Total polyphenols consisted in tropical forages

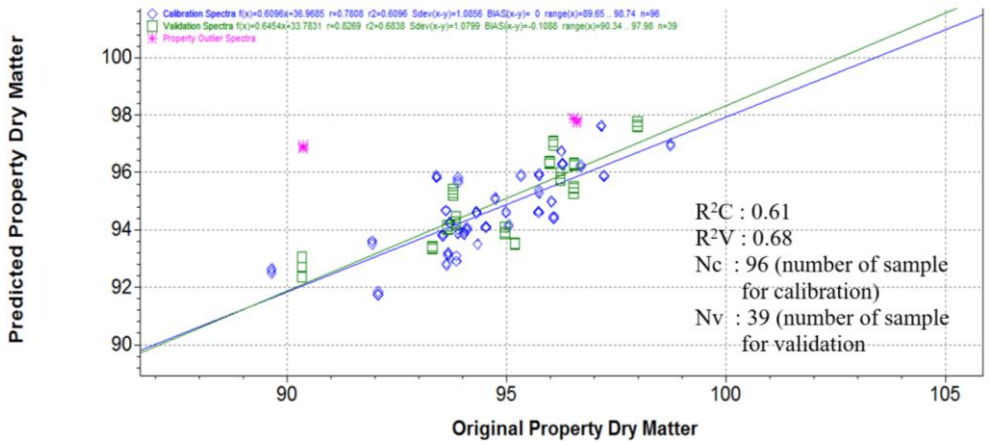

Figure 2. The relationship between conventional measurement analysis value with NIRS prediction on % DM content

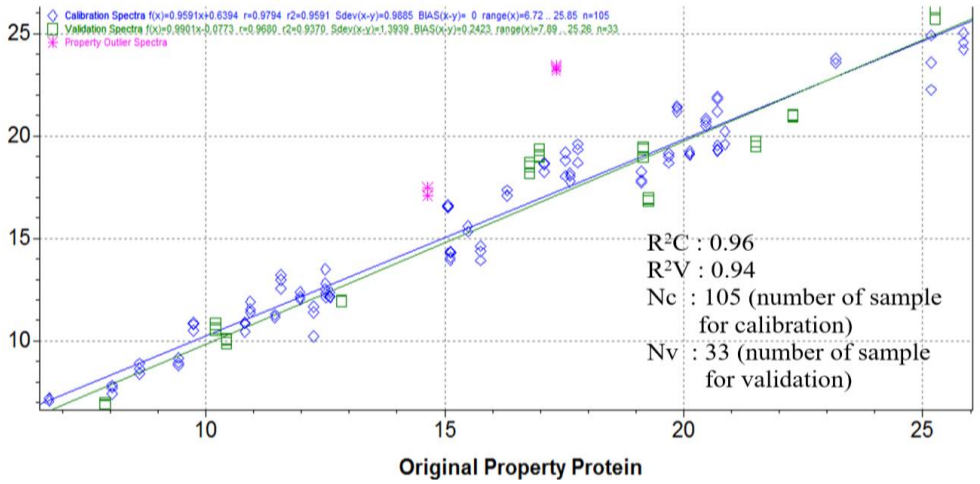

Figure 3. The relationship between conventional measurement analysis value with NIRS prediction on crude protein content (% DM) of observed tropical forages

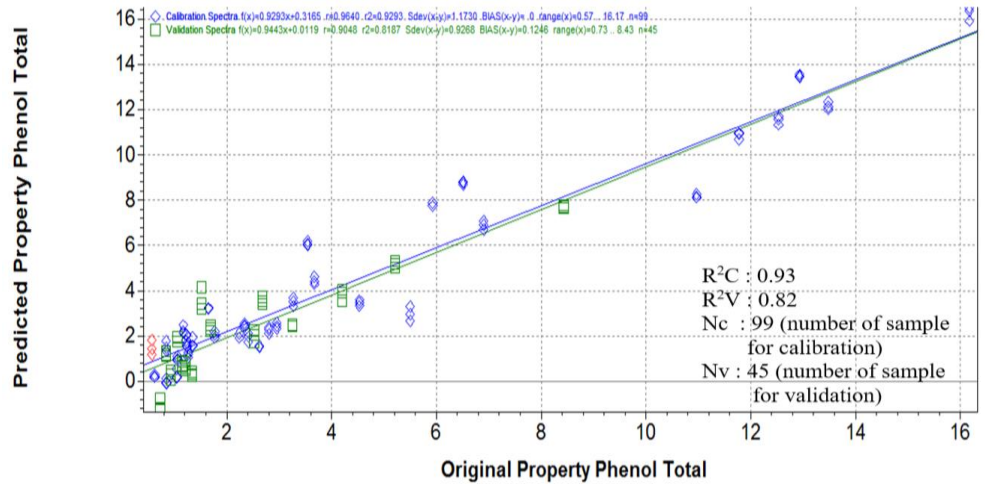

Figure 4. The relationship between conventional measurement analysis value with NIRS prediction on total phenols content (% DM) of observed tropical forages

Statistical measures, such as the coefficient of determination for validation (R^2V), Standard Error of Prediction (SEP), Ratio Performance Deviation (RPD), bias, and slope, are essential for evaluating the precision and dependability of NIRS analysis outcomes, as noted by Ikoyi and Younge (2022). Furthermore, R^2V values are classified as excellent when greater than 0.95, good between 0.9 and 0.95, moderate between 0.8 and 0.9, and acceptable between 0.7 and 0.8, according to Malley et al. (2004). Following this categorization, the CP parameter was rated as good (R^2V = 0.94), while TP and CF were in the moderate category (R^2V = 0.82 and 0.89). Furthermore, Williams (2001) suggested that R^2 values ranging from 0.55 to 0.8 were suitable for calibration screening and prediction purposes. A lower SEP value showed a more accurate prediction model in this study. The CP and TP models exhibited lower error values in comparison to CF, and this was consistent with previous studies (Parrini et al. 2017; Despal et al. 2020; Wulandari et al. 2020), which showed high accuracy in CP predictions using NIRS.

To further optimize the precision of NIRS predictions, the RPD value must be considered. Lobos et al. (2013) stated that an RPD value of at least 2.5 was required for effective NIRS predictions. Ikoyi and Younge (2022) classified RPD into various categories, including excellent, very good, acceptable, and unreliable, when RPD was >3, 2-3, 1.4-2, and <1.4. The calculated RPD values for CP and CF were exemplary (RPD = 3.99) and very good (RPD = 2.44, 2.32), while EE and DM parameters showed low RPD values, marking them as unreliable for NIRS predictive modeling. The low accuracy in these cases was caused by the use of a diverse range of forage families as samples, suggesting a need for more consistent forage classification in future predictive model development.

The bias value showed the mean deviation between NIR predicted values and laboratory results, while the

slope represented the variation in NIRS predictions per unit spectroscopy change in laboratory values, as explained by Walker (2010). Ideal predictions were characterized by bias values nearing zero and slope values approaching one (unity), as described by Malley et al. (2004). The CF parameter exhibited a relatively high bias, signaling a less satisfactory NIRS prediction model. CP and TP showed low bias values (near zero) with slopes close to one, denoting a sufficiently accurate NIRS prediction model.

The ability of NIRS to predict the chemical components of various tropical forages perfectly fits with conventional laboratory analysis of certain chemical components, such as crude protein and total phenols, considering their R^2 and SE values. Accurate laboratory data and a clear spectrum could reflect a robust regression model (Yang et al. 2017). This showed that NIRS was accurate and precise in predicting CP and TP, followed by their SEP and RMSEP values in the model prediction. Felde et al. (2007) stated that the magnitude of tolerable RMSEP value was twice the standard value of conventional measured analysis. This appropriate RMSEP value was directed to an accurate and precise NIRS predictive value.

In conclusion, the application of NIRS for predicting chemical components in tropical forages showed promising results for CP and TP contents but was not effective in predicting CF and EE contents. The proximity of the statistical values for calibration and validation further validated the calibration quality. However, the accuracy varied across parameter components due to factors, such as spectral absorption characteristics and the presence of organic and inorganic groups. The predictive accuracy of NIRS was recommended for the SEP and RMSEP values, specifically for CP and TP. The NIRS showed its potential as a predictive tool for conventional chemical component analysis, with various effects based on the characteristics of specific plant components and the quality of calibration models.

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