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Comparison of Chemical Composition, *In Vitro* Digestibility, and Near Infrared Reflectance Spectroscopy in Estimating *In Situ* Rumen Degradable Protein of Tropical Foliage

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ABSTRACT

Tropical foliage is an alternative source for protein enrichment in the dairy ration. However, due to the expensive, laborious, and time-consuming data-gathering method, its degradability database for inclusion in ration formulation is still lacking. This study aims to estimate tropical foliage's *in situ* protein degradability (RDP) using chemical compositions, *in vitro* digestibilities, and near infrared reflectance spectroscopy (NIRS) methods. The study used one hundred ten tropical foliage samples and observed chemical composition, *in vitro* dry and organic matter digestibility, and *in situ* degradation characteristics variables. NIRS spectra were collected to calibrate and validate the *in situ* degradation characteristics. Correlations were made prior to regression analysis. The results showed that tropical foliage varied in ash (3.02%-18.3%), crude protein (CP) (11.6%-30.7%), crude fiber (CF) (10.2%-29.8%), neutral detergent fiber (NDF) (31.0%-58.2%), acid detergent fiber (ADF) (18.7%-44.1%), dry matter digestibility (DMD) (23.9%-73.2%), organic matter digestibility (OMD) (25.6%-73.9%), and *in situ* RDP (21.0%-75.4%). The foliage was highly degraded (RDP > 60%) except for *Calliandra calothyrsus* (59%). *In situ* RDP significantly correlated with ash, CP, CF, DMD, and OMD with coefficient correlations (r) of 0.43, 0.60, -0.33, 0.74, and 0.76, respectively. Estimation of RDP using chemical composition and *in vitro* digestibility followed the equation: RDP (%)= 0.69 + 2.122 CP (%) with R^2= 0.41 (p<0.01) and RDP (%)= 0.162 ash + 1.270 CP -0.104 CF + 0.489 IVOMD, with R^2= 0.68, p<0.01). Calibration of NIRS using *in situ* RDP resulted in a regression coefficient (R^2) of 0.78. It is concluded that RDP tropical foliage can be estimated more accurately using NIRS compared to *in vitro* digestibility and chemical composition.

Keywords: in situ; in vitro; near-infrared reflectance spectroscopy; rumen degradable protein; tropical foliage

INTRODUCTION

The guidelines of protein requirement for ruminants have been shifted from a simple crude protein (CP) model into a more complex model, such as ammonia requirement for rumen microbe and amino acid absorbed in the intestine (Schwab, 2017). Protein requirements in ruminants such as dairy cattle aim to fulfill the requirements of the host and microbes in the rumen. Rumen microbes can survive up to 80% on RDP (Despal, 2005), while host animals require microbial and feed protein that escapes rumen degradation (RUP). Balancing between RDP and RUP produces the best dairy cattle performance. A 60:40 ratio of RDP to RUP has been reported to produce the most efficient microbial protein synthesis and feed digestion for tropical dairy ration (Rosmalia *et al.*, 2022b). In a high-producing cow, however, the protein requirement is higher with a higher RUP proportion (Santos *et al.*, 1998).

Protein in dairy cattle is supplied from forage and concentrate with a ratio of 50:50 (Riestanti *et al.*, 2021). In high-producing cows, the ratio of concentrate is higher due to the low-quality tropical fiber feed used (Despal *et al.*, 2020). High concentrate proportion in ration reduced milk fat component (Despal *et al.*, 2021c). Therefore, the utilization of tropical leguminous foliage can be an alternative to improve fiber feed quality in the dairy ration. Some foliage commonly found in tropical countries are *Moringa oleifera, Indigofera zollingeriana, Gliricidia sepium,*

Leucaena leucocephala, *Calliandra calothyrsus*, *Bauhinia purpurea* L., *Piper aduncum* L., *Pterocarpus indicus*, and *Calopogonium mucunoides* (Rahmat *et al.,* 2021). The foliage with rich protein in its leaf, especially legumes, has been developed for animal feed (Santamaría-fernández & Lübeck, 2020). Although the protein has been promising, the absence of anti-nutrients in foliage is rare. Some examples of anti-nutrients are lectins, oxalates, goitrogens, phytoestrogens, phytates, and tannins (Petroski & Minich, 2020). To a certain degree, anti-nutrient may benefit ruminants, such as increasing rumen undegradable protein (RUP) (McAllister *et al.,* 1994) and reducing methane production (Jayanegara *et al.,* 2019). However, the high concentrations of the anti-nutrients consumed by the animal cause dangerous symptoms, even death. Therefore, the percentage used of foliage in dairy ration should be limited.

The RDP content in the feeds varies and can differ among the regions. In tropical countries, primarily during the dry season, the forage contains low crude protein (CP) and RDP (Soliva *et al.,* 2015). Rahmat *et al*. (2021) reported different RDP percentages in tropical foliage, which need to adjust in ration formulation. The high existence of anti-nutrient in tropical foliage due to the land marginality reduced the foliage utility for dairy cattle due to its depressive effect on protein digestion (Yacout, 2016), resulting in lower ammonia production (lower RDP) in the rumen. Regardless of its disadvantages, foliage can become an animal protein feed source.

Formulation of dairy cattle ration using RDP information constraint the required RDP database. So far, a limited feed database is available for RDP information on tropical foliage. The RDP measurement can be done *in vivo*, *in vitro* (Putri *et al.,* 2021), or *in situ* (Rahmat *et al.,* 2021; Rosmalia *et al.,* 2021). However, the methods are laborious, expensive, and timely. The most frequently used RDP *in situ* method required serial observation and protein analysis to build a model to predict RDP according to the passage rate (Ørskov & Mcdonald, 1979). Although the disposal of imported bags used in the *in situ* method can be replaced using reusable local fabric (Despal *et al.,* 2022b), serial observation is required to build the RDP prediction model. The observations required a lot of sample and protein analysis. Therefore, a rapid, simple, and low-cost procedure must be developed to attain the RDP information. An accurate estimation of the RDP using existing data such as chemical composition and *in vitro* digestibility also helped to provide the RDP database (Schwab, 2017).

Near-infrared reflectance spectroscopy (NIRS) has been used in estimating the nutrient composition of feed (Agustiyani *et al.,* 2021), feed digestibility (Zahera *et al.,* 2022), milk component (Oktavianti *et al.,* 2022), and milk's fatty acid health index (Despal *et al.,* 2021a). The use of NIRS for RDP determination has been attempted (Tremblay *et al.,* 1996; Hsu *et al.,* 1998; Flis, 2005; Belanche *et al.,* 2013), but most of the feeds used were temperate forages which were less accurate to be used in estimating the RDP of tropical foliage (Despal *et al.,* 2020). Another reason for the urgency of the development of the RDP database is based on the regulation of the Indonesian government on the obligation of RDP information in ruminant feeds. Therefore, this study is aimed to compare the accuracy estimation of *in situ* degradation of tropical foliage protein in the rumen (RDP) using chemical composition, *in vitro* digestibility, and near-infrared reflectance spectroscopy (NIRS) method.

MATERIALS AND METHODS

Sample Preparations

This research was conducted at the Laboratory of Dairy Nutrition, Faculty of Animal Science, IPB University, Indonesia. The samples of tropical foliage were collected from the various regions in West Java, Indonesia, including Bandung, Bogor, and Sumedang Regency, with a total of 110 samples. The legumes were *M. oleifera* (n= 3), *I. zollingeriana* (n= 5), *G. sepium* (n= 26), *L. leucocephala* (n= 28), *C. calothyrsus* (n= 29), *B. purpurea* L. (n= 9), *P. aduncum* L. (n= 3), *P. indicus* (n= 5), and *C. mucunoides* (n= 2). The fresh foliage samples were collected from a matured tree. Approximately 3 kg of each edible portion (leaves with petioles plus the fraction of the stem, which completely snapped on bending) were collected. The edible portion was determined based on the tenderness of the stem. After being weighed, the foliage samples were chopped and dried using the sun drying method and then placed in the oven at a temperature of 60 ^{o}C for 48 h. After that, the dried samples were ground and sieved through a 1-mm screen before analysis.

Chemical Analysis and *In Vitro* Digestibility

Chemical analysis was conducted using proximate analysis (DM, ash, CP, and CF) according to AOAC (2015), while analysis of NDF and ADF was conducted according to AOCS (2005). *In vitro* dry matter digestibility (IVDMD) and organic matter digestibility (IVOMD)) were measured according to Tilley & Terry (1963). The DM of tropical foliage was measured after drying the sample in a 105 ^{o}C Eyela NDO 400 oven (made in Japan) for 48 h. Ash content was measured after incinerating the sample in a 500 ^{o}C Naberthem N50 muffle oven (made in Germany) for six hours. Gerhart Kjeldahl system (made in Germany) was used for CP analysis. NDF and ADF were also analyzed using the Ankom 200 fiber technique but with different solutions. The neutral detergent solution was used for NDF, while the acid detergent solution was used for ADF. The IVDMD and IVOMD were measured after anaerobic incubation of the sample using buffered rumen liquid, followed by aerobic incubation using HCl-pepsin in a 39 ^{o}C water bath (Tilley & Terry, 1963).

In Situ Digestibility Method

The three ruminal fistulated male Frisian Holstein cattle aged 2-4 years old with an average body weight of 519±80.61 kg were used in this study. The cattle were fed twice daily in the morning and afternoon with composition diets of 60% Napier grass and 40% concentrate mix on a dry matter basis. The ration contained 61% TDN,

11.6% CP, 0.39% Ca, and 0.33% P. The nylon bags used in this study were self-produced using Abutai material (Despal *et al.,* 2022b), comparable to ANKOM nylon bags. A 5 g of foliage samples were put in the nylon bag, incubating in the rumen for 6, 9, 12, 24, 48, and 72 h. Three bags per sample were incubated to generate sufficient residue for subsequent analysis. The nylon bag with 0 h was filled with the samples without incubating in the rumen. After incubation, the nylon bags were cleaned to remove contamination of feed particles, digesta, rumen fluid, or microbes stuck on the bag. After cleaning, the nylon bag was dried in the oven at a temperature of 60 ^{o}C for a week. Then the bags were weighed using the digital scale laboratory type OHAUS PA214C (made in the USA). The residual samples in the nylon bag were collected to determine the DM, OM, and CP residues, according to AOAC (2015). The ruminal degradability of DM, OM, and CP or the kinetic variables were estimated using the equation according to Ørskov & Mcdonald (1979). The CP calculation used in determining RDP uses a similar method as Rosmalia *et al*. (2021) mention.

Calibration and Validation of Near-Infrared Reflectance Spectroscopy

The foliage spectrum was collected using Buchi NIRFlex N-500 Solids Cell (made in Switzerland). The steps for running the NIRS tool followed a similar procedure used by Despal *et al.* (2021b). The tool was warmed up, and the system suitability test (SST) was run for about 15 minutes to ensure the tool was ready. The spectrum of the foliage was collected after scanning all 110 samples with approximately 25-50 g of each using the NIRS. The sample was put in a petri dish, and the dish was put in the dish holder. Each sample was examined in triplicates. After the spectrum collection, the chemo-metric data from the laboratory analysis were inputted into the NIRS ware Management Console (Despal *et al.,* 2021a). The calibration and validation models were carried out using NIRCal V5.6 using Partial Least Squares (PLS) regression and validation set. The values of standard error calibration (SEC), standard error of prediction (SEP), the highest calibration and validation coefficient of regression (R^2C and R^2V), and residual predictive deviation (RPD) were used as the reference for determining the best model. External validation was conducted using ten independent sample sets. T-test was conducted to examine the difference between data resulting from NIRS and *in situ* kinetic degradation. The standard error of laboratory (SEL) and standard error of prediction (SEP) was compared to get the prediction error relative (PRL). An external calibration was considered a success if the SEP closed to SEL.

Research Design and Data Analysis

This study used a combination of field explorative and laboratory research types. The different chemical contents and *in vitro* digestibility data between the foliage were compared using analysis of variance (ANOVA) followed by Tukey's test (Steel & Torrie, 1996). *In situ* degradation kinetics data of the foliage were analyzed using descriptive statistics. The theoretical degradation of DM, OM, and CP was calculated using a regression model developed from the serial data (0, 6, 9, 12, 24, 48, and 72 h) observations. Correlations between chemical and *in vitro* data were made prior to regression analysis to find the determinant. NIRS databases were calibrated and validated using partial least squares (PLS) from NIR Cal V5.6 from Buchi (Despal *et al.,* 2021a). The accuracy of chemical, *in vitro*, and NIRS in determining the *in situ* RDP was compared using the determination coefficient (R^2). Data analysis was conducted using SPSS version 20.

RESULTS

Chemical Compositions and *In Vitro* Digestibility of Tropical Foliage

The chemical compositions and *in vitro* digestibility of the tropical foliage are shown in Table 1. The table shows that tropical foliage chemical compositions and *in vitro* digestibility vary between and within species. *P. aduncum* contained the highest ash, while *I. zollingeriana* contained the highest CP. The CF and fiber fractions were at the highest in *C. mucunoides*. A combination of higher CP and low CF resulted in the highest IVDMD and IVOMD in *M. oleifera*. All tropical foliage contained CP of more than 20%, except *C. mucunoides*, therefore, can be used as protein sources for dairy cattle. The modest CF content (10.2%-29.7%) in most tropical foliage was a good source of fiber with a high proportion of NDF, which was digested fiber. The IVDMD and IVOMD values of the tropical foliage varied greatly. The CP and CF contents in the foliage determine the IVDMD and IVOMD of the foliage.

In Situ Dry Matter, Organic Matter, and Crude Protein Degradability

DM, OM, and CP degradability. Dry matter degradation of tropical foliage at different incubation times is shown in Figure 1a. It shows that the total DM disappearance increases linearly with the increased rumen's incubation time. The patterns of the DM disappearances were different among the foliage. Within 6 h, the rate of disappearance is faster than later. *P. aduncum* showed a smaller portion of disappearance at the first 6 h but then continuously degraded at the decline speed up to 72 h. *M. oleifera* and *I. zollingeriana* were degraded faster since the beginning of incubation and higher than the other foliage in every incubation time. After 72 h incubation, more than 80% DM of *M. oleifera*, *I. zollingeriana*, and *P. aduncum* were degraded, while the others were less than 73%. *C. calothyrsus* and *C. mucunoides* degradation were <54% after 72 h incubation. After incubation in the rumen, the degradation of foliage organic matter (OM) followed a similar pattern as DM disappearances (Figure 1b).

Degradations of foliage protein in the rumen after incubation are shown in Figure 1c. CP degradation was slightly different from the patterns of DM and OM degradation. The most rapid degradation of CP was still at

Table 1. Chemical composition and *in vitro* digestibility of tropical foliage (Average and standard error mean (SEM))

Foliage	N	Chemical composition							
		DM	Ash	CP	CF	NDF	ADF	IVDMD	IVOMD
Moringa oleifera	3	91.37±0.32	10.97±0.09^{ab}	25.03±1.20^{bc}	15.18±1.50^{ab}	23.02±1.30^{a}	36.14±2.28^{a}	66.31±0.95^{e}	68.57±0.37^{c}
Indigofera zollingeriana	5	90.04±0.96	9.10±0.20^{ab}	29.76±0.56^{c}	14.17±0.77^{ab}	25.85±1.41^{a}	38.39±2.84^{ab}	63.56±4.23^{e}	70.11±1.50^{c}
Gliricidia sepium	26	90.32±0.22	11.27±0.87^{b}	24.23±0.58^{bc}	16.22±0.68^{abc}	31.33±0.95^{abc}	42.81±0.74^{ab}	54.84±0.98^{cde}	59.19±0.68^{bc}
Leucaena leucocephala	28	90.42±0.37	8.13±0.23^{ab}	23.19±0.93^{bc}	21.31±0.72^{bc}	26.27±1.08^{ab}	41.10±0.70^{ab}	46.35±1.17^{bc}	49.89±1.02^{b}
Calliandra calothyrsus	29	90.37±0.35	6.32±0.24^{a}	20.66±0.67^{ab}	19.92±0.90^{bc}	28.20±1.05^{a}	39.80±0.93^{b}	28.54±1.49^{a}	31.44±1.49^{a}
Bauhinia purpurea L.	9	91.03±0.42	8.63±0.40^{ab}	21.52±0.78^{ab}	21.11±1.09^{bc}	32.62±1.70^{bcd}	48.99±1.68^{ab}	48.60±1.26^{bcd}	51.24±1.24^{b}
Piper aduncum L.	3	91.48±0.67	17.81±0.24^{c}	23.17±3.36^{bc}	11.62±0.62^{a}	36.84±5.43^{a}	37.97±2.34^{bc}	63.54±0.69^{de}	61.67±0.47^{c}
Pterocarpus indicus	5	90.45±0.30	6.89±0.55^{ab}	21.65±1.19^{ab}	21.60±1.82^{bc}	45.25±6.26^{cd}	50.59±1.16^{c}	44.47±1.74^{b}	47.24±2.08^{b}
Calopogonium mucunoides	2	90.83±0.18	7.05±0.49^{ab}	15.02±0.73^{a}	23.74±0.82^{c}	60.52±1.37^{d}	54.62±0.63^{d}	48.61±5.14^{bcd}	50.56±5.35^{b}

Note: N= number of sample; DM= dry matter; CP= crude protein; CF= crude fiber; NDF= neutral detergent fiber; ADF= acid detergent fiber; IVDMD= *in vitro* dry matter digestibility; IVOMD= *in vitro* organic matter digestibility; Means with different superscripts within the same column differ significantly ($p<0.05$).

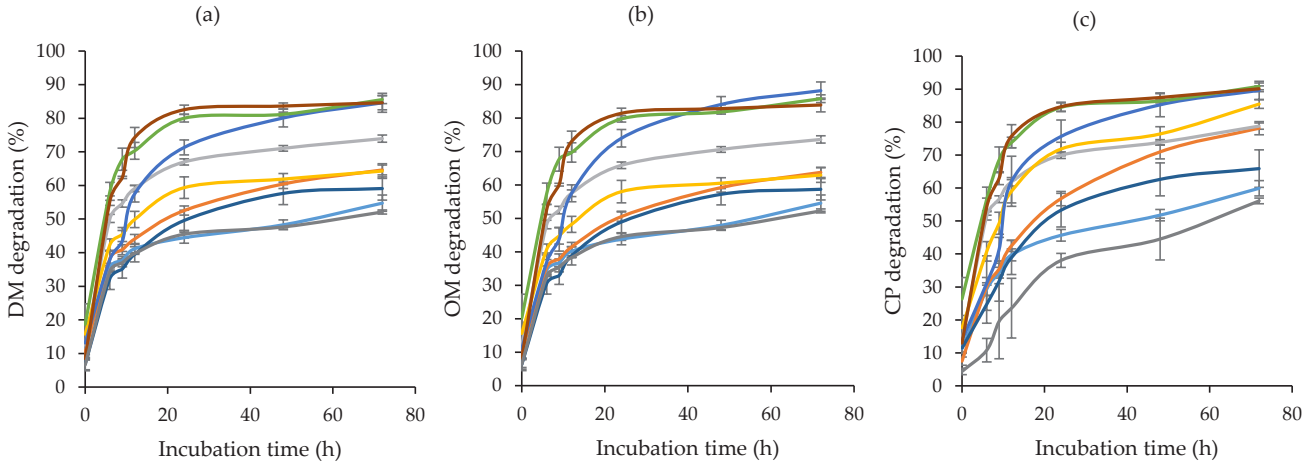

Figure 1. Degradation of dry matter (a), organic matter (b), and crude protein (c) after *in situ* incubation. Note: *Calliandra calothyrsus* (), *Leucaena leucocephala* (), *Gliricidia sepium* (), *Bauhinia purpurea* L. (), *Piper aduncum* L. (), *Indigofera zollingeriana* (), *Pterocarpus indicus* (), *Moringa oleifera* (), and *Calopogonium mucunoides* ().

the first six h incubation for most of the foliage, except for *C. mucunoides*. The CP degradations in *I. zollingeriana* and *M. oleifera* were faster and higher than in the other foliage. Although CP degradation in *P. aduncum* was slower until 24 h incubation time, it continued high after 24 h and reached similar levels to *I. zollingeriana* and *M. oleifera.* Degradations of CPs in *C. calothyrsus* and *C. mucunoides* did not reach 60% even after 72 h incubation. Most of the foliage CP was degraded >70% after 72 h incubation, except for *C. calothyrsus* and *C. mucunoides*.

Kinetics variables and effective degradations (ED) of DM, OM, and CP. The degradation kinetics of DM, OM, and CP are shown in Table 2. The soluble fraction of the foliage DM varied from 3.46% to 18.9%. The solubilities of *I. zollingeriana* and *B. purpurea* were higher than the other foliage, meanwhile the solubility of *P. falcataria* was very low. The solubility of OM followed a similar pattern to that of DM. However, protein solubility was higher than DM and OM solubilities, especially in *I. zollingeriana,* which reaches up to 26%. In contrast, CP in *C. mucunoides* was mainly insoluble.

M. oleifera and *P. aduncum* have DM and OM potentially degraded (*b* coefficient) by more than 70% (*b* coefficient). In addition to *M. oleifera* and *P. aduncum*, *L. leucocephala* CP was also potentially degraded by more than 70%. On the other hand, *P. falcataria*, *C. mucunoides*, *C. calothyrsus,* and *B. purpurea* DM, and OM were potentially degraded (*b* coefficient) by less than 50%.

The rate of DM, OM, and CP disappearances (c) varied among the foliage. The *c* coefficient of DM and OM were mainly related, while CP was slightly different. *P. aduncum* and *P. indicus* have a *c* coefficient of less than 0.1 %/h. In addition to the *P. aduncum* and *P. indicus*, *B. purpurea*, *C. mucunoides,* and *P. falcataria* also have a *c* coefficient for CP less than 0.1 %/h. Only *L. leucocephala* has a *c* coefficient of DM of more than 0.2 %/h. The *c* coefficients have not correlated with the foliage's effective degradation (ED). For example, the high c coefficient in *C. calothyrsus* produces the lowest ED value. The ED of CP, known as RDP, was more than 60% in *I. zollingeriana* and *M. oleifera,* while others were less than 60%.

Prediction of *In Situ* Degradability Using NIRS

The ability of NIRS to estimate *in situ* DM, OM, and CP degradability and their kinetics are shown in Table 3. The accuracy of prediction can be seen from the coefficient of determination (R^2) and residual predictive

deviation (RPD), both calibration and validation. The table shows that R^2 for all *in situ* degradability predictions was more than 0.6, which was accurate. RPD of more than 1.5 was found in all models except for *a* DM coefficient. Validation attempts failed to improve the prediction accuracy for *a* DM coefficient. The data suggested that *in situ* degradability can be estimated using NIRS accurately.

External validation of the model using an independent sample set is shown in Table 4. The t-test between laboratory and NIRS data resulted in an insignificantly different for all *in situ* degradability variables. The data suggested that *in situ* degradability data generated from NIRS were similar to the laboratory data generated using in situ procedures developed by Ørskov & McDonald (1979). PRL less than two was found in the

Table 2. Dry matter, organic matter, and crude protein *in situ* kinetics variables of tropical foliage (n= 112)

Kinetic variables	Tropical foliage								
	Gliricidia sepium	*Leucaena leucocephala*	*Calliandra calothyrsus*	*Bauhinia purpurea* L.	*Piper aduncum* L.	*Indigofera zollingeriana*	*Pterocarpus indicus*	*Moringa oleifera*	*Calopogonium mucunoides*
DM									
a (%)	13.2± 6.77	11.5±8.33	12.2±6.84	17.6±10.2	12.7±5.04	18.9±13.3	8.89±4.03	8.73±0.38	7.20±1.92
b (%)	58.0± 5.20	51.4±9.09	38.8±11.9	45.8±10.9	71.3±8.02	63.7±12.9	52.8±1.11	77.1±2.23	41.5±2.84
a+b (%)	71.2±4.69	62.9±7.53	51.0±14.2	63.4±5.62	84.0±4.91	82.6±1.63	61.7±3.89	85.8±2.60	48.7±0.92
C (%/h)	0.15±0.04	0.24±0.77	0.15±0.08	0.12±0.05	0.07±0.02	0.16±0.02	0.09±0.04	0.16±0.01	0.15±0.00
ED (%)	55.7±5.14	45.4±7.82	38.3±6.62	49.3±4.61	54.8±2.00	67.0±3.96	42.0±3.48	66.8±2.16	38.2±0.01
OM									
a (%)	11.8±6.52	11.1±8.18	12.0±7.30	17.3±10.3	10.4±8.22	20.4±15.7	7.84±3.96	8.03±0.12	7.07±1.82
b (%)	58.7±5.32	51.8±10.3	40.4±16.8	44.8±10.7	77.8±11.1	62.6±15.0	53.6±0.93	77.2±2.90	41.6±3.00
a+b (%)	70.5±4.93	62.8±8.39	52.4±19.6	62.1±5.65	88.2±4.13	83.0±1.55	61.4±3.21	85.2±2.79	48.6±1.18
C (%/h)	0.14±0.04	0.16±0.43	0.14±0.08	0.12±0.06	0.07±0.02	0.15±0.02	0.09±0.04	0.15±0.01	0.14±0.01
ED (%)	54.1±5.56	42.5±7.16	37.6±6.63	48.1±4.61	55.7±1.49	67.0±4.70	41.0±3.54	65.4±2.26	37.2±0.07
CP									
a (%)	15.4±10.6	8.24±6.92	11.3±6.50	18.9±10.2	10.6±5.52	26.0±14.3	10.3±4.53	14.6±4.18	2.72±0.90
b (%)	59.6±10.3	78.5±32.5	48.5±17.2	65.3±6.62	80.7±3.69	62.7±14.6	63.2±6.47	75.3±3.79	60.9±12.6
a+b (%)	75.0±4.45	83.6±37.0	59.8±17.6	84.2±5.47	91.4±3.13	88.6±1.35	73.5±4.32	89.9±2.02	63.6±11.7
C (%/h)	0.14±0.04	0.15±0.48	0.15±0.38	0.09±0.04	0.07±0.02	0.12±0.02	0.07±0.03	0.13±0.03	0.04±0.03
ED or RDP (%)	58.5±5.86	45.3±8.19	37.0±8.45	58.3±6.18	56.0±8.04	70.1±4.43	43.9±7.33	68.2±2.51	26.5±7.76

Note: a= soluble fraction, b= potentially degradable fraction, a+b = summary of a and b values c= rate of degradation, ED= effective degradation, DM= dry matter, OM= organic matter, CP= crude protein, RDP= rumen degradable protein.

Table 3. FT-NIRS calibration using data generated from the conventional method

Variables	Calibration								Validation							
	N	AVG	Range		SD	SEC	R^2C	RPD	N	AVG	Range		SD	SEP	R^2V	RPD
			Min	Max							Min	Max				
DM																
a	126	9.810	3.186	17.863	3.025	2.287	0.636	1.322	63	9.737	3.428	17.070	3.026	2.365	0.623	1.280
b	150	49.888	28.927	72.754	10.287	3.942	0.872	2.610	75	49.947	28.213	73.427	10.225	4.099	0.859	2.495
c	98	0.119	0.051	0.170	0.033	0.014	0.844	2.329	49	0.119	0.055	0.168	0.033	0.015	0.826	2.205
a+b	190	60.574	40.668	87.782	10.413	4.666	0.833	2.232	95	60.916	40.710	87.986	10.337	4.703	0.831	2.198
ED	174	46.096	29.747	62.271	7.938	3.589	0.830	2.212	87	46.220	29.805	62.711	7.963	3.596	0.831	2.214
OM																
a	116	10.223	3.876	24.462	4.844	2.658	0.769	1.822	58	10.428	4.595	22.945	4.710	2.842	0.729	1.658
b	106	52.121	31.522	65.941	9.002	2.255	0.941	3.992	53	52.508	31.690	65.805	9.053	2.342	0.940	3.865
c	88	0.117	0.051	0.234	0.046	0.022	0.808	2.054	44	0.115	0.042	0.213	0.041	0.028	0.677	1.492
a+b	102	62.192	37.913	86.975	11.483	2.344	0.960	4.900	51	62.417	38.602	87.071	11.525	2.370	0.959	4.864
ED	146	44.923	27.448	69.636	9.353	2.481	0.934	3.770	73	44.863	27.125	69.860	9.384	2.570	0.932	3.651
CP																
a	118	9.600	0.956	18.987	4.270	2.426	0.756	1.760	59	9.631	1.440	18.809	4.200	2.541	0.730	1.653
b	68	59.244	32.155	84.618	13.036	3.022	0.949	4.314	34	59.147	32.783	83.153	12.874	3.222	0.941	3.996
c	138	0.088	0.017	0.157	0.032	0.013	0.859	2.464	69	0.087	0.013	0.153	0.031	0.014	0.828	2.217
a+b	110	74.343	46.984	95.456	11.166	4.490	0.861	2.487	55	74.466	47.607	94.928	11.106	4.626	0.849	2.401
ED or RDP	170	46.449	23.427	63.816	9.569	5.068	0.781	1.888	85	46.524	23.926	63.368	9.553	5.100	0.777	1.873

Note: DM= dry matter, OM= organic matter, CP= crude protein, RDP= rumen degradable protein, RUP= rumen undegradable protein, a= soluble fraction, b= potentially degradable fraction, a+b= summary of a and b values c= rate of degradation, ED= effective degradation, N= total number of observations, AVG= average, SD= standard of deviation, Min= minimum value, Max= maximum value, SEC= standard error of calibration, R2C= determination coefficient of calibration, RPD= residual predictive deviation, SEP= standard error of validation, R^2V= determination coefficient of validation, NIRS= near infrared reflectance spectroscopy.

a+b, *c*, and ED coefficients of DM and OM degradability. However, only the c coefficient was found in CP degradability kinetics less than 2. The data suggested that there is still a possibility to improve the prediction accuracy if the substrate being modeled can be selected more accurately.

Prediction of *In Situ* Degradability Using Chemical Composition and *In Vitro* Digestibility

The correlation of *in situ* degradation and kinetics with chemical composition and *in vitro* digestibility is shown in Table 5. The table shows that the *a* and *c* coefficients of DM and OM degradability were not correlated with the chemical composition. The *b* and *a+b* DM and OM degradability coefficients correlate with Ash, CP, and CF but not with ADF and NDF. For CP degradability, the *a* coefficient was correlated with CP, while the ED or RDP was correlated with ash, CP, and CF. The ash and CP were positively correlated with *in situ* degradation kinetics, while CF was negatively correlated.

In vitro digestibility correlated more with *in situ* degradability than with chemical compositions. Almost all DM degradability kinetics were correlated with the *in vitro* digestibility, except for the *a* and *c* coefficients (Table 6). *In vitro* digestibility was positively correlated with the *in situ* degradation kinetics. The regression model to predict the *in situ* degradability kinetics based on the correlated variables is shown in Table 6.

The table shows that the *in situ* degradation kinetics estimation using chemical composition resulted in a coefficient determination of less than 0.5. Estimation using *in vitro* digestibility produced R^2>0.5, except for the *b* and *a+b* coefficient of OM degradability. The best estimation of ED of CP, or RDP, was using a combination of chemical composition and *in vitro* digestibility, which produced R^2>0.67.

DISCUSSION

Chemical Composition, *In Vitro* Digestibility, and *In Situ* Degradation of Tropical Foliage

Variations of chemical compositions and *in vitro* digestibility of tropical foliage influence their roles in ruminant nutrition (Rosmalia *et al.,* 2021). Forage relative value (RFV) or forage unit for lactation (UFL) explained the positive influence of protein and digestibility and the negative influence of fiber for ruminants (Despal *et al.,* 2021c). Tropical foliage contains high CP (>20%), which can be used as a leafy protein source for a ruminant. *M. Oleifera* and *I. zollingeriana* contained CP >25%. The high CP in *I. zollingeriana* and *M. oleifera* was also reported by Putri *et al.* (2019) and Kakengi (2005). The CP contents of *G. sepium* and *L. leucocephala* found in this study were comparable (Putri *et al.,* 2019). The ADF contents in the foliage used in this study were slightly higher than those reported by Putri *et al.* (2019). The age of harvested (Despal *et al.,* 2017), plant cell wall components (Raffrenato *et al.,* 2017), and drying method influence the fiber fraction in a plant (Alomar *et al.,* 2003).

Foliage with high ash, CF, NDF, and ADF reduced the foliage digestibility, therefore, was less available for a ruminant. Although CP content in the foliage was high (CP >20%), their contribution to ruminant nutrition became less (Rahmat *et al.,* 2021) due to its low digestibility. The IVDMD and IVOMD of more than 60% were only found in *M. oleifera, I. zollingeriana,* and *P. aduncum*

Table 4. External validation of the developed NIRS models using the independent tropical foliage sample set

In situ degradation kinetic variables	Laboratory data		NIRS		T-Test	SEL	SEP	PRL (SEP/SEL)
	AVG	SD	AVG	SD				
DM								
a	10.921	4.099	10.512	2.683	0.508	1.963	4.221	2.151
b	48.770	12.405	50.011	12.304	0.105	2.133	5.016	2.352
c	0.138	0.027	0.145	0.034	0.255	0.026	0.034	1.289
a+b	76.637	7.858	74.785	14.789	0.325	7.782	10.141	1.303
ED	48.408	8.245	47.375	7.321	0.109	4.127	3.922	0.950
OM								
a	9.812	5.053	10.856	4.894	0.067	1.530	3.155	2.061
b	48.830	11.544	49.588	10.778	0.364	2.104	4.501	2.139
c	0.119	0.074	0.107	0.051	0.466	0.038	0.088	2.326
a+b	65.062	9.714	64.788	10.433	0.672	1.550	2.849	1.837
ED	50.153	9.068	49.454	8.185	0.314	1.633	3.138	1.921
CP								
a	11.951	6.839	13.191	3.803	0.225	1.966	4.811	2.446
b	59.689	10.782	58.222	10.940	0.191	2.511	5.942	2.367
c	0.092	0.041	0.085	0.023	0.170	0.013	0.026	1.924
a+b	76.253	15.873	74.948	13.105	0.329	2.742	5.909	2.155
ED or RDP	47.630	9.825	45.585	7.140	0.106	2.764	6.724	2.432

Note: DM= dry matter, OM= organic matter, CP= crude protein, RDP= rumen degradable protein, RUP= rumen undegradable protein, a= soluble fraction, b= potentially degradable fraction, a+b= summary of a and b values c= rate of degradation, ED= effective degradation, AVG= average, NIRS= near infrared reflectance spectroscopy, SD= standard of deviation, SEL= standard error of laboratory, SEP= standard error of prediction, PRL= prediction error relative.

Table 5. The correlation coefficient (R) between *in situ* degradation kinetics of dry matter, organic matter, and crude protein with chemical composition and *in vitro* digestibility

In situ degradation kinetics	DM	Ash	CP	CF	ADF	NDF	IVOMD	IVDMD
DM								
a	0.236	0.097	0.044	-0.013	-0.084	0.032	0.097	0.123
b	0.045	0.437	0.285	-0.376	-0.014	0.045	0.728	0.727
a+b	0.181	0.480	0.302	-0.372	-0.063	0.062	0.762	0.777
c	0.147	0.001	-0.022	0.055	0.151	0.006	0.041	0.043
ED	0.183	0.486	0.431	-0.312	0.000	0.046	0.755	0.789
OM								
a	0.209	-0.090	0.045	0.007	-0.071	0.036	0.090	0.087
b	0.071	0.445	0.204	-0.363	-0.045	0.027	0.636	0.637
a+b	0.183	0.387	0.225	-0.352	-0.082	0.046	0.672	0.672
c	0.150	-0.01	-0.007	0.058	0.144	-0.001	0.027	0.029
ED	0.183	0.397	0.436	-0.335	0.002	0.043	0.752	0.766
CP								
a	0.116	0.184	0.315	-0.121	-0.220	0.028	0.181	0.227
b	-0.057	0.155	0.037	-0.045	0.091	0.042	0.328	0.312
a+b	-0.038	0.224	0.207	-0.074	0.025	0.046	0.379	0.378
c	0.082	-0.009	0.019	-0.007	0.034	-0.068	-0.008	-0.001
ED(RDP)	0.221	0.426	0.603	-0.332	-0.139	0.065	0.737	0.757

Note: DM= dry matter; CP= crude protein; CF= crude fiber; NDF= neutral detergent fiber; ADF= Acid detergent fiber; IVDMD= *in vitro* dry matter digestibility; IVOMD= *in vitro* organic matter digestibility, OM= organic matter, RDP= rumen degradable protein, a= soluble fraction, b= potentially degradable fraction, a+b= summary of a and b values c= rate of degradation, ED= effective degradation.

Table 6. Estimation of *in situ* degradation kinetics using chemical composition and *in vitro* digestibility

In situ degradability	Y	Intercept	Ash	CP	CF	IVDMD	IVOMD	R^2
DM	b	31.39	2.145	0.519	-0.542			0.34
	a+b	41.32	2.371	0.519	-0.521			0.38
	ED	17.31	1.710	0.893	-0.217			0.42
	b	16.26				0.516	0.219	0.52
	a+b	25.61				0.237	0.543	0.57
	ED	16.66				-0.230	0.847	0.61
OM	b	39.52	2.303	0.227	-0.663			0.28
	a+b	49.38	2.342	0.288	-0.632			0.28
	ED	18.05	1.703	0.866	-0.288			0.43
	b	18.20				0.653	0.063	0.40
	a+b	27.81				0.445	0.309	0.42
	ED	15.96				-0.128	0.740	0.59
CP	a	-6.19		0.816				0.13
	ED/RDP	0.69		2.122				0.41
	ED/RDP	13.31				0.718		0.55
	ED/RDP	-4.71	0.162	1.270	-0.104	0.067	0.450	0.68
	ED/RDP	-3.60	0.197	1.332	-0.108		0.489	0.67

Note: Y= degradation kinetics, DM= dry matter, CP= crude protein, CF= crude fiber, IVDMD= *in vitro* dry matter digestibility, IVOMD= *in vitro* organic matter digestibility, OM= organic matter, RDP= rumen degradable protein, a= soluble fraction, b= potentially degradable fraction, a+b= summary of a and b values, ED= effective degradation, R^2= coefficient of determination.

L due to high protein but low fiber in the foliage. Other factors that influenced the high IVDMD and IVOMD in the foliage were the low anti-nutrient content. Adding tannin extract to the foliage has been proven to reduce IVDMD and IVOMD significantly (Jayanegara *et al.*, 2019). *C. calothyrsus*, which naturally contained high tannin (18.9%) (Tiemann *et al.*, 2010), produced the lowest IVDMD and IVOMD in this study. Although CP in *C. calothyrsus* was high and the ash and CF were low, its IVDMD is only 28.5%, meaning more than 72% of the foliage consumed will be excreted via feces and unavailable for a ruminant. The anti-nutrient that directly or indirectly suppressed nutrient digestion, especially protein digestion and utilization, existed in tropical foliage in significant amounts such as protease inhibitors, lectin, saponin, and polyphenolic compounds. Phenolic compounds form complexed hydrophobic and hydrogen bonds with protein that resisted microbial digestion

(Ikhwanti *et al.,* 2020), lowered rumen ammonia concentration (Jayanegara *et al.,* 2019), and led to an increasing the ruminal escape protein value of the foliage.

According to Tiemann *et al.* (2010), tannin content in *C. calothyrsus* was 18.9%. It was higher than those in *L. leucocephala* and Flemingia, which only contained 9.9% and 7.4% of condensed tannin, respectively. Besides tannin, saponin also has a significant setback for foliage utilization for a ruminant. Saponins are a heterogenous group of glycosides occurring naturally in many plants. Its biological effects in the rumen varied due to the structural differences in their sapogenin fractions (Yacout, 2016). Furthermore, Yacout (2016) explained that saponin formed foam, inhibiting microbial fermentation and rumen microbial synthesis. Saponin concentrations in *G. sepium, C. calothyrsus, M. oleifera,* and *L. leucocephala* have been reported as much as 8.23%DM, 8.33%DM, 7.19%DM, and 4.54%DM, respectively (Susanti & Marhaeniyanto, 2014).

Therefore, chemical composition information alone could not determine the nutrient availability of the ruminant feeds. Information about the degradation kinetics of feedstuffs in the rumen, especially protein degradation in the rumen (RDP), is essential to guarantee the synchronization of nutrient availability for microbial protein synthesis and to fulfill the nutrient requirement of the host animal. Degradations of DM, OM, and CP of the foliage in the rumen varied considerably among the species. The most rapid degradations occurred in the first 6 h of incubation. The pattern found in this study differed from that was reported by Despal *et al*. (2022a), which studied the degradation patterns of Napier grass, corn powder, and dairy cattle TMR. The biological mechanism of rumen microbes influences the degradation of feeds in the rumen in attacking feed particles in digesta. Factors such as the thickness of the feed surface layer and the existence of substances (such as condensed tannins and methylcellulose) that prevent microbial attachment or promote microbial detachment can inhibit degradation (McAllister *et al.,* 1994). Tropical foliage has more leafiness, less structure, and more protein, energy, and micronutrients than grasses. Despal *et al.* (2022a) reported that the degradability of Napier grass and corn powder slowed down within the first 12 h. A comparable pattern was found with the tested TMR. Different nutrient compositions in feeds produced different rates of degradation. High CF in Napier grass and the existence of linear and helical amylose and branched amylopectin in corn reduced their solubilities in cold water and produced clumping and floating in the rumen. These conditions reduce the degradation in the rumen (Cornejo-Ramírez *et al.,* 2018). The high degradability of the foliage within 6 h found in this study showed that the foliage contained a high proportion of soluble components and readily available carbohydrates (RAC) such as sugar and starch and low fiber fraction (Lascano *et al.,* 2016).

Degradation of CP in the rumen influences the availability of ammonia for microbial growth (Rosmalia *et al.,* 2022b). The rate of protein degradation in the rumen and the balance availability of energy sources from organic matter degradability improved the efficiency of microbial protein synthesis (Castro-Montoya *et al.,* 2019). The high content of CP and IVOMD in *I. zollingeriana, M. oleifera,* and *P. aduncum* produced better synchronization of ammonia and VFA availability for microbial protein synthesis, resulting in higher degradation of the foliage in the rumen found in this study (Rosmalia *et al.,* 2022).

Estimation of *In Situ* Degradation Using Chemical Composition, *In Vitro* Digestibility, and NIRS

The observation in this study reveals that estimation of the *in situ* degradation kinetics of tropical foliage using chemical composition resulted in a poor prediction when compared to *in vitro* and NIRS (R^2<0.5) (Yin, 2020). Predictors used in the models were ash, CP, and CF. The ash and CP have positive coefficients, while CF has negative ones. The model suggested that the more ash and CP contents in the foliage, the higher the degradation in the rumen. On the contrary, the higher the CF content in the foliage, the lower the rumen degradation. A positive correlation between CP and digestibility and a negative correlation between CF and digestibility have been shown in the previous study (Despal, 2010; Indah *et al.,* 2020). The higher CP in the feed, the more material could be fermented by rumen microbes to produce ammonia to support microbial growth, which leads to more enzymes produced by the microbes to digest the feeds (Rosmalia *et al.,* 2022a). The R^2 in this prediction is a relevant measurement for accuracy because it measures the error. The lower R^2 found corresponded to models with higher error, producing predictions that are less precise and less useful (Yin, 2020). Although the addition of *in vitro* data improved the R^2 to 0.68, more than 30% of the kinetics variation cannot be explained by the model (Indah *et al.,* 2020).

Estimation of degradation kinetics using chemical composition only produced R^2<0.5, which showed that more than 50% of the factors influencing the foliage degradation kinetics in the rumen could not be explained by the chemical composition alone. The IVDMD and IVOMD estimated the degradation kinetics better than the chemical composition. The R^2 found using the *in vitro* digestibility was more than 0.5. The data suggested that *in vitro* digestibility estimated the degradation kinetics better than a chemical composition. It is reasonable because the procedure inquired the IVDMD and IVOMD, which involved the rumen fermentation and enzymatic digestion, which were more relevant to the feed fermentation kinetics in the rumen (Anzhany *et al.,* 2022; Despal *et al.,* 2022a).

The ED value of CP or RDP was estimated more accurately using a combination of chemical composition and *in vitro* digestibility. In the absence of the RDP information of tropical foliage, the prediction formula 0.197 ash + 1.332 CP – 0.108 CF + 0.489 IVOMD – 3.60 can be used to gather the data with R^2= 0.67. The prediction accuracy could be increased slightly if the IVDMD was included, as shown in Table 6. The result suggested that the available data on the chemical composition and *in vitro* digestibility of tropical foliage can be used to gather the RDP data that are still lacking. The obligation

of the RDP information in ruminant feedstuffs trade within the Indonesian region can be fulfilled using this formula. However, there is a need for caution regarding the method of earning the data, and the customer should be aware that the RDP data were not measured directly.

The developed database of tropical foliage degradation kinetics in this study can be used to estimate the foliage degradability characteristics using NIRS. Calibration and validation of the database produced R^2 >0.8 for almost all of the degradation kinetics. Some variables even produced R^2>0.9. The data suggested that the developed database can accurately estimate tropical foliage's degradation kinetics. The NIRS R^2C degradation kinetics prediction found in this study was higher (R^2 up to 0.95) than R^2C for the prediction of chemical composition (Despal *et al.,* 2020; Agustiyani *et al.,* 2021; Despal *et al.,* 2021b), *in vitro* digestibility (Zahera *et al.,* 2022), and milk fatty acids (Despal *et al.,* 2021a) of the previous study (R^2<0.9). Although the R^2C of RDP found in this study was 0.78, the external validation of the model using an independent sample set showed that the degradation kinetics produced using laboratory procedures were similar to NIRS data. It is suggested that the NIRS equipped with local tropical foliage degradation kinetics can be used to accurately estimate the tropical foliage *in situ* degradation kinetics, including RDP.

CONCLUSION

Estimation of *in situ* RDP using chemical composition (0.69 + 2.122 CP with R^2= 0.41) alone is less accurate than the combination of chemical composition and *in vitro* digestibility (0.162 ash + 1.270 CP -0.104 CF + 0.489 IVOMD, with R^2= 0.68) and NIRS (R^2>0.78). Therefore, using the pre-calibrated NIRS database to produce the degradation kinetics of feeds, including the RDP information, is suggested.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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