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Evaluation of nutritional values of tree-forage legume leaves from Gunungkidul District, Indonesia

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Abstract. *Darma ING, Jayanegara A, Sofyan A, Budiartilaconi E, Ridla M, Herdian H. 2023. Evaluation of nutritional values of tree-forage legume leaves from Gunungkidul District, Indonesia. Biodiversitas 24: 2733-2744.* This study aimed to assess the nutritional value of tree legume leaves found in Gunungkidul District. A completely randomized design with 19 treatments (legume species) and six replications (nylon bags) was employed. The study evaluated the chemical composition, *in sacco* dry and organic matter degradation characteristics, and their correlation with the chemical composition. The results indicated variations in the crude protein (CP) content (12.44% to 26.9%), crude fiber (CF) content (16.13% to 27.38%), total digestible nutrients (TDN) (71.10% to 88.17%), and total phenol content (2.47% to 21.87%) on a dry matter basis. Among the legume species, *Indigofera tinctoria* L. exhibited the highest soluble fraction (*a*) values for dry matter (42.89±1.15%) and organic matter (40.32±1.37%). Additionally, *I. tinctoria* displayed the highest potential degraded fraction values (*a+b*) for dry matter (88.33±0.90%) and organic matter (88.12±0.95%). The effective degradation of dry matter (EDDM) and organic matter (EDOM) were 64.57±0.48% and 63.78±0.68%, respectively. The correlation coefficients (r) between ash, CP, TDN, CF, total phenol, and EDDM and EDOM values were 0.54, 0.58, 0.53, -0.73, -0.53, and 0.52, 0.55, 0.52, -0.72, -0.56 respectively. In conclusion, tree-forage legume leaves from Gunungkidul District exhibited varying nutritional values, with *I. tinctoria* showing the highest values for effective dry and organic matter degradation compared to other tree legumes. The ash content, CP, and TDN demonstrated positive correlations with the EDDM and EDOM values, whereas the CF content and total phenol exhibited negative correlations with the EDDM and EDOM values of tree legume leaves.

Keywords: Chemical composition, rumen degradation characteristics, tree-legume leaves

INTRODUCTION

Ruminants have an essential role in the agricultural system in Gunungkidul District. Large ruminants (cattle and buffaloes) are a source of energy to cultivate the land, and manure has produced a source of fertilizer for crops. In addition, ruminants are a form of farmer's life savings that can be sold anytime when experiencing financial problems (Cahyaningsih et al. 2022). However, the scarcity of feed ingredients, especially during the dry season, is one of the factors that hinder its development. The soil, dominated mainly by calcareous rocks and the difficulty of water sources during the dry season, decreases the availability and quality of existing forage. In addition, the livestock rearing system is still small-scale with a low level of capital, making purchasing farmers low to commercial feed ingredients on the market. This situation makes the productivity of ruminants in Gunungkidul Regency during the dry season lower than in the rainy season. Therefore, efforts are needed to obtain feed sources and ingredients available throughout the year at low prices to support the development of a ruminant livestock system in Gunungkidul Regency. One of them is by utilizing the leaves of the tree legume plant.

Katsande et al. (2018) stated that the leaves of tree legumes are a source of feed with relatively high nutritional value and are available throughout the year to develop small-scale ruminants in tropical climates. Further explained by Castro-Montoya and Dickhoefer (2020), the natural ability of leguminous plants to symbiotic with rhizobium to bind nitrogen from the atmosphere and use it in protein synthesis of plant tissues makes legume plants have a higher protein content than grasses. Leng (1997) revealed that the proteins contained in the leaves of leguminous plants can be divided into two categories. Soluble dan insoluble proteins are nitrogen sources to increase the activity of rumen microbes and bypass proteins, a source of amino acids for livestock production. Furthermore, it was stated by Simbaya (2002) apart from being a source of protein; legumes also contain essential minerals needed by livestock, such as sulfur, calcium, copper, and iron.

In addition to containing nutritious compounds for livestock, tropical legumes are also reported to contain plant secondary metabolite compounds in varying concentrations that can be beneficial and detrimental to livestock (Wina 2010; Fluck et al. 2013). For example, polyphenols, mainly lignin and tannins, are two secondary metabolite compounds widely in leguminous plants in varying concentrations (Jayanegara et al. 2020). These polyphenol

compounds have a varied influence on the utilization of nutrients by livestock, such as feed intake, degradation of feed ingredients in the rumen, and the process of protein digestion in the small intestine (Jayanegara et al. 2012; Addisu 2017; Attia et al. 2018; Formato et al. 2022).

The availability of adequate information about the nutritional value of tree leguminous leaves is one of the keys to the success of its concentration as feed for ruminants. When assessing a feedstuff's nutritional value, one crucial aspect is its digestibility or degradability coefficient. The potential nutritional value of a feed ingredient, in general, can be observed from its chemical composition (Guadayoa et al. 2019). However, its actual value is determined by the amount that can be digested and absorbed in the digestive tract (McDonald et al. 2010). Since it is known that the rumen is the main digestive organ in ruminants, which is responsible for the forage digestion process, the characteristics of forage degradation in the rumen are a critical aspect that needs to be considered in its use in the arrangement of ruminant rations (Ahn et al. 1989; Belachew et al. 2013; Omar and Abdallah 2017). Bowen et al. (2008) and Mohamed and Chaudhry (2008) stated that the degradation characteristics of feed ingredients in the rumen are needed to estimate the nutrient needs for microbial protein synthesis and bypass nutrients for livestock production.

Several techniques have been developed to measure the characteristics of the degradation of feed ingredients in the rumen. *In sacco* method developed by Ørskov et al. (1980) is widely used as a standard method to measure the characteristics of forage degradation in the rumen because it mimics the actual processes occurring in the rumen (Edmunds et al. 2012). In addition, this method is simple, does not require large samples and animals, and is obtained to simultaneously measure the nutritional degradation characteristics of different feed samples (Corrêa et al. 2012). In addition, the degradation characteristics produced from this method can be used as predictors of dry matter (DM) intake, apparent digestibility of DM intake, and the growth rate of animals (Kibont and Ørskov 1993; Shem et al. 1995). Based on the studies mentioned above, the current study aims to examine the nutritional value (chemical composition and *in sacco* degradation characteristics) of the leaves of the tree leguminous plant that grows in Gunungkidul District, Yogyakarta Special Region, Indonesia.

MATERIALS AND METHODS

Experimental location

This research was conducted at the animal nutrition laboratory of the Indonesian National Research and Innovation Agency in Gunungkidul, Yogyakarta Special Region. This area has a tropical climate with two seasons: dry and rainy. The dry season ranges for five months, and the rainy season ranges for seven months, with the average number of rainy days per year being 115 days. The daily minimum temperature ranges from 18.00°C to 22.20°C, and the daily maximum temperature ranges from 32.1°C to 33.6°C. Annual rainfall is 2,457.00 mm with a minimum humidity value of 41.0% to 62.0% and maximum humidity ranging from 95.0% to 99.0%. The Gunungkidul District area has a height that varies between 0-700 m above sea level and is dominated by karst-hilly areas (Central Bureau of Statistics of Gunungkidul District 2022).

Collection and preparation of forage sample

Forage collection is carried out from January to July 2022. The 19 tree legumes were collected, and they are a conventional forage feed source for ruminants in the Gunungkidul Regency. The 19 legumes consist of *Leucaena leucocephala* (Lam.) de Wit, *Sesbania grandiflora* (L.) Poir., *Delonix regia* (Bojer ex Hook.) Raf., *Gliricidia sepium* (Jacq.) Kunth , *Indigofera tinctoria* L., *Bauhinia purpurea* L., *Caesalpinia sappan* L. (*C. sa*ppa), *Acacia mang*ium Willd., *Acacia villosa* (Sw.) Willd., *Dalbergia latifolia* Roxb., *Pterocarpus ind*icus Willd., *Senna siamea* (Lam.) H.S.Irwin & Barneby, *Samanea saman* (Jacq.) Merr., *Albizia chinensis* (Osbeck) Merr., *Tamarindus indica* L., *Cassia javanica* L., *Parkia speciosa* Hassk., *Calliandra calothyrsus* Meisn. and *Zapoteca tetragona* (Willd.) H.M.Hern.

The collected forage is the edible portion as feed for ruminants (leaves and tinder twigs). Approximately 5-6 kg of fresh parts of each sample is taken from 5-8 trees at different locations. After collecting, they are dried in an oven at 60°C for 24 hours. Furthermore, dried samples are ground using a hummer mill and passed a 1 mm sieve to analyze the chemical composition. The rest are ground, sifted with 3 mm diameter sieve holes, and used to analyze rumen degradation characteristics.

Analysis of chemical composition

Proximate analysis was carried out based on standard procedures AOAC (1990). The dry matter (DM) and ash content were analyzed sequentially. Dry matter analysis was performed by heating the sample at 105ºC overnight, and the ash content was determined by burning the sample in a muffle furnace at 550°C for 6 hours. The Kjeldahl digestion block method analyzed the total nitrogen sample using KjelDigester K-446/K-449 (Buchi, Labortechnik AG, Switzerland). Furthermore, the crude protein (CP) content is calculated by multiplying the total nitrogen sample by 6.25. The soxhlet extraction method uses petroleum benzene solvent to determine ether extract (EE). The crude fiber (CF) content was determined by sequentially boiling the sample using each solution of H_2SO_4 1.25% and NaOH 1.25% and then burning the insoluble residues in a muffle furnace at 550ºC for 6 hours. The loss of organic matter after burning is determined as crude fiber. Nitrogen-free extract samples were calculated based on mathematical equations according to Harris (1970) by subtracting 100 from the content of the total amount of ash, CP, EE, and CF. Furthermore, the content of digestible nutrients (TDN) from each sample was also calculated, according to Harris et al. (1972).

The determination of total phenol (TP) used the Folin-Ciocalteu method, according to Makkar (2003). In the extraction step of phenol compounds from the sample, as much as 100 mg of the sample was put into a 15 mL

centrifuge plastic tube, and added 5 mL of aqueous acetone (700:300 v/v) solution, vortexed, and subjected to ultrasonic treatment for 20 minutes at room temperature (i.e., 28°C) in an ultrasonic water bath (DELTA D68H, ultrasonic cleaner, 48 kHz-60W, Taiwan). After that, the centrifuge tube is cooled at 4°C for 15 minutes and then centrifuged for 10 min at 3,000 × g using an ordinary clinical centrifuge (Tabletop centrifuge, PLC-03, Taiwan). The formed supernatants are collected and used for total phenol analysis. The extraction procedure for the same samples was performed for two repetitions, and each supernatant was analyzed for the total phenol dan total tannin content (Jayanegara et al. 2015). For the total phenol analysis, taken between 0.02 and 0.05 mL of supernatant (depending on the concentration of TP in the plant sample) was put into the test tube and made up the volume of 0.5 mL with distilled water addition. Next is added 0.25mL of Folin-Ciocalteu 1 N reagent and 1.25 mL of 20% sodium carbonate. Vortex and incubated in a dark place at room temperature for 40 minutes. Furthermore, 0.02 mL of solution was taken into a microplate well flat bottom (Asahi Glass Co.Ltd., Japan) (Wong-Paz et al. 2014). The absorbance was read at a wavelength of 725 nm using a spectrophotometer (Thermo Scientific Multiskan Go, ThermoFisher Scientific, Waltham, MA, USA). Total phenol was calibrated using a standard curve of tannic acid solution (Sigma-Aldrich Chemie GmbH, Steinheim, Germany), and the values were expressed as tannic acid equivalents.

Analysis of rumen degradation characteristics

Two adult Ongole Cross Breed bulls with an average body weight of 406±32 kg were used to analyze rumen degradation. Both cattle have fitted a permanent rumen cannula with a diameter of 15 cm. The animals used in this study have been reported and passed the ethical clearance as experimental animals accessed by The Ethical Commission of Integrated Research and Testing Laboratory Gadjah Mada University (Certificate#:00004/04 /LPPT/IV/2022). Each of the animals is placed in an individual stall. For one week before and during the study, the animal was fed by maintenance ration composed of 60% Napier grass and 40% concentrates (wheat bran, molasses, lysin, methionine, mineral mix, and salt). Feed is given twice daily at 07.00 and 15.00 in equal proportions, and drinking water is provided *ad libitum*. Analysis of sample disappearance in the rumen was carried out based on the nylon bag technique, according to ørskov et al. (1980). The nylon bag used has an inner dimension size of 6×12 cm with an average porosity of bag size of 54 μm. Approximately 5 g of sample were placed in a nylon bag, then tied tightly using a rubber band and a 40 cm long nylon thread, linked to anti-rust metal ballast. Furthermore, all the bags were incubated into the rumen 30 min before morning feeding for 6, 12, 24, 48, and 72 hours. At one time of observation, three bags were repeated for one type of sample for each animal. Thus, there were six bags for one sample type in two animals at one observation time. At the end of incubation, the bag is pulled from inside the rumen and washed with running tap water until the outside of the bag is clean of attached feed particles. Further, the bags were cleaned by washing machine with the continues flow water for 8 min. The residual samples in bags were dried in an oven at 60ºC for 72 hours. Then the residues are analyzed for the content of DM and OM following

AOAC (1990). Nutrient losses from each sample due to the washing process are estimated using bags plus samples with zero (0) hours of incubation. These bags are not incubated into the rumen but are washed, dried, and analyzed DM and OM the same way as incubated bags.

The disappearance of nutrients during the time of incubation is calculated based on the equation of Osuji et al. (1993):

Disappearance nutrients of sample =

$$\frac{((WA-WB)\times(Y))-((WC-WB)\times(Z))}{(WA-WB)\times(Y)}\times 100$$

Where:
WA : weight of original sample + nylon bag incubated (g)
WB : weight of empty nylon bag (g)
Y : nutrients content of original sample incubated (%)
WC : residues sample weight after incubation + nylon bag (g)
Z : nutrients content of sample residues (%)

The nutrient degradation constant of each legume is calculated by fitting the disappearance of nutrients of each sample into an exponential equation based on Ørskov and McDonald (1979) using the *Neway* software version 5.0 program (Chen 1994):

$$p = a + b\,(1\text{-}e^{-ct})$$

Where:
p : the actual nutrient degradation after the time (t) incubation (%)
a : the soluble fraction and rapid degradation from nutrients (%)
b : the insoluble fraction and slow degradation from nutrients (%)
c : the rate constant of degradation from b fraction (%/hours)
t : time of incubation (hour)
e : the natural logarithm's base

The potential degradability, total undegraded fractions, and effective degradability from the nutrient of each sample were calculated by the equation:

$$PD = a + b$$
$$U = 100 - (a + b)$$
$$ED = a + ((b \times c)/(c + kp))$$

Where:
PD : the potential degradability from nutrients (%)
U : the total non-degradation fraction in the rumen from nutrients (%)
ED : the effective degradability from each sample nutrient (%)
a : the soluble fraction and rapid degradation from nutrients (%)
b : the insoluble fraction and slow degradation from nutrients (%)

c : the constant degradation rate from b fraction (%/hour)

kp : constant turnover rate of the rumen digesta due to microbial degradation activity. In this study, the *kp* used was 6%

Experimental design and statistical analysis

This study was arranged on a Completely Randomized Design with 19 treatments (different tree legume leaves) and six replications (nylon bag). The differences between treatment effects were analyzed by analysis of variance (ANOVA) followed by Duncan's Multiple Range post hoc procedure Steel et al. (1997) if there were any significant results. In addition, the Pearson correlation coefficient was measured to evaluate the correlation between the sample chemical compounds and the sample degradability parameters before linear regression calculation. Therefore, to conduct the statistical calculation, CoStat statistical software version 16 was used (Cohort 2008).

RESULTS AND DISCUSSION

Chemical composition

The chemical composition in a dry matter basis of the samples used in the study is presented in Table 1. The results showed that the chemical composition of legume plants used in this study varied. The dry matter content, which is a representation of components that can be mandated by livestock-, ranges from 24.85% (in *I. tinctoria*) to 38.76% (in *B. purpurea*). Ash content ranges from 5.17% (in *P. speciosa*) to 13.70% (in *I. tinctoria*). The content of Extract ether (EE) ranges from 1.39% (in *C. calothyrsus*) to 4.20 % (in *B. purpurea*). The crude fiber (CF) content, a carbohydrate described in the plant cell wall, varies from 14.33% (in *S. grandiflora*) to 28. 38% (in *P. speciosa*). In contrast, nitrogen-free extract (NFE), which is a representation of carbohydrates of cell contents, ranges from 71.10% (in *D. latifolia*) to 88.17% (in *G. sepium*). In general, differences formerly cultivation chemical composition between plant species can be caused by various factors, including the chemical and nutrient content of the soil where plants grow (Evci and Karsli 2018), the stage of plant growth and the parts of the plant (Castro-Montoya and Dickhoefer 2020).

The content of total digestible nutrients (TDN) of legume samples used in this study ranged from 71.10% (in *D. latifolia*) to 88.17% (in *G. sepium*). Total digestible nutrients are the potential energy utilizable of feedstuffs, which are very significant values to deliver adequate energy supply for animals. Variations in TDN values between the samples can be caused by differences in the composition values of organic materials (EE, CF, NFE, and CP) in each sample (Jayanegara et al. 2019).

One of the essential benefits of using legumes as feed for ruminants is as a protein source. Legumes are a plant that can bind nitrogen from their growing environment by symbiosis with *Rhizobium* bacteria and utilizing it as a nitrogen source for cell protein synthesis (Todd et al. 2005). The crude protein content of legume samples used in this study ranged from 12.44% (in *P. speciosa*) to 26.29% (in *L. leucocephala*). The results obtained in this study are lower than the research report of Rahmat et al. (2021), which states that tropical legumes have an average crude protein content of 19.88%. The difference in the value of the protein produced can be caused by various factors, including differences in the environment in which plants grow (Dal Pizzol et al. 2017; Riede et al. 2018), harvesting time (Ascencio-Rojas et al. 2020) and parts of the plant used as testing samples (Valente et al. 2016).

Table 1. Chemical composition of tree-forages legume from Gunungkidul District

Legume		Chemical composition (%DM)							
Scientific name	**Local name**	**DM[1]**	**Ash**	**CP**	**EE**	**CF**	**NFE**	**TDN**	**TP**
Leucaena leucocephala	Manding	26.19	8.06	26.29	2.09	16.24	55.33	78.50	17.07
Sesbania grandiflora	Turi	28.71	8.22	24.92	2.23	14.33	59.21	83.77	2.67
Delonix regia	Flamboyan	27.68	6.50	21.72	1.86	14.49	64.64	86.80	13.08
Gliricidia sepium	Gamal	16.84	8.82	24.90	3.53	18.31	54.51	88.17	2.96
Indigofera tinctoria	Tarum	24.85	13.70	24.09	3.03	14.94	54.14	78.17	3.25
Bauhinia purpurea	Tayuman	38.76	11.05	17.69	4.20	26.19	48.67	75.69	3.34
Caesalpinia sappa	Secang	34.24	7.28	15.36	2.12	19.83	63.21	82.46	5.85
Acacia mangium	Mangium	30.02	5.52	14.31	2.85	28.22	58.25	77.58	17.18
Acacia villosa	Villosa	29.54	5.15	18.22	1.75	26.59	54.74	71.86	17.78
Dalbergia latifolia	Sonokeling	37.52	9.19	14.99	1.83	25.51	56.71	71.10	15.87
Pterocarpus indicus	Sonokembang	33.76	5.53	15.44	1.83	22.17	62.25	78.33	21.87
Senna siamea	Johar	35.77	7.90	13.72	1.87	23.98	60.44	74.28	5.97
Samanea saman	Trembesi	35.20	11.15	13.65	1.64	18.06	64.20	77.84	4.28
Albizia chinensis	Sengong	38.33	8.70	16.55	1.64	16.13	67.18	84.05	18.10
Tamarindus indica	Asam jawa	34.04	11.15	13.65	1.64	18.06	64.20	77.84	9.36
Cassia javanica	Trembalo	37.30	5.52	13.43	1.90	22.78	63.86	78.74	16.94
Parkia speciosa	Petai	37.48	5.17	12.44	2.54	28.38	60.44	76.30	12.44
Calliandra calothyrsus	Kaliandra merah	34.22	6.77	18.03	1.39	21.14	61.22	75.79	21.77
Zapoteca tetragona	Kaliandra putih	25.59	9.50	17.87	1.66	20.08	58.84	74.69	2.47

Note: [1]DM: Dry Matter; CP: Crude Protein; EE: Extract Ether; CF: Crude Fiber; NFE: Nitrogen Free-Extract; TDN: Total Digestible Nutrients; TP: Total Phenol

The ruminant protein requirements in rations can be expressed as crude protein and/or nitrogen. Feed proteins in the microbial rumen will be degraded with the final products as peptides, amino acids, and ammonia that rumen microbes will use for protein synthesis in their bodies (Bach et al. 2005). In general, the need for crude protein to support normal rumen microbial activity is around 6% to 8%, while for livestock needs, it is about 7% to 20%. The requirements are influenced by gender, physiological status, and livestock species (Huston and Pinchak 1991). In this study, the CP content of legume leaves was higher than the requirements of rumen microbes for normal activities and livestock needs. So based on the CP content, the legumes tested in this study have the potential to be used as feed for ruminants.

Phenolic compounds are one of the bioactive plant components. They are secondary metabolites plants produce for self-defense against predators, diseases, and environmental stress (Wink 1988). In this study, the total phenol content from legume samples varied between 2.47% (in *Z. tetragona*) and 21.87% (in *P. indicus*). The variations in the total phenol sample used in this study can be caused by differences in plant species that cause metabolic differences in cells (Hadacek 2002). Furthermore, it is explained by Rezende et al. (2015) that differences in the growing environment also cause differences in the total content of plant phenols. Ampofo et al. (2020) stated that increasing temperatures in the growing environment are reported to increase the accumulation of phenol compounds, and plants that grow at poor soil fertility have a higher content of phenol compounds than plants that grow on soils with nutrient adequacy (Wright et al. 2010).

Rumen dry matter degradation of tropical tree legume leaves

In sacco ruminal dry matter disappearances

The *in sacco* ruminal dry matter (DM) disappearances of legume samples used in the study are presented in Table 2. The loss of dry matter during incubation time varies and shows a noticeable difference ($P<0.05$) between legume species. *Indigofera tinctoria* produced the highest DM loss at 0-hour incubation with a value of 43.22±1.14%, while *A.mangium* produced the lowest value with a value of 9.80±0.30%. At the 6-hour incubation, the highest value was also produced by *I. tinctoria* with a value of 55.40±1.53%, and *C. calothyrsus* produced the lowest value of 19.22±0.62%. At 12-hour incubation, the highest DM loss was produced by *S. grandiflora* with a value of 70.37±1.82% and the lowest value of *P. speciosa* with a value of 18.80±0.58%. In 24-hour incubation, *S. grandiflora* and *I. tinctoria* produced the highest DM loss with values of 78.26± 1.25% and 78.04±1.04%, respectively, while *P. speciosa* generated the lowest DM loss values with a value of 20.38±0.20%. In the 48-hour incubation, the highest DM loss values were produced by *S. grandiflora* (82.77±0.86%), and *I. tinctoria* (83.52±1.51%), and the lowest value was generated by *P. speciosa* with 22.12±0.58%. Until the end of the incubation time (72-hour), the highest DM loss value was generated by *I. tinctoria*, with a value of 88.05±0.68%. In contrast, the lowest value of DM loss was generated by *P. speciosa* at 25.32±0.75%.

Table 2. The disappearance of dry matter (DM) of tree-forage legumes at various time incubation in the rumen (%)

Legume	Incubation time (hours)					
	0	6	12	24	48	72
Leucaena leucocephala	18.12±0.40$^{i1)}$	34.59±1.41^{e}	50.03±1.14^{d}	67.26±1.68^{c}	78.13±0.92^{b}	80.63±0.60^{c}
Sesbania grandiflora	23.56±0.53^{e}	41.35±1.50^{b}	70.37±1.82^{a}	78.26±1.25^{a}	82.77±0.86^{a}	85.67±0.46^{b}
Delonix regia	28.14±1.02^{c}	37.10±0.55^{d}	42.06±0.48^{g}	51.51±0.71^{f}	69.27±0.40^{e}	74.98±0.61^{f}
Gliricidia sepium	32.18±0.70^{b}	39.10±0.79^{c}	44.0±1.50^{f}	59.99±2.56^{d}	72.63±1.02^{d}	76.88±1.13^{de}
Indigofera tinctoria	43.22±1.14^{a}	55.40±1.53^{a}	63.62±1.68^{b}	78.04±1.04^{a}	83.52±1.51^{a}	88.05±0.68^{a}
Bauhinia purpurea	20.94±0.60^{g}	32.26±1.08^{f}	42.31±2.19^{g}	50.52±1.33^{f}	55.30±0.73^{i}	59.37±1.75^{i}
Caesalpinia sappa	22.80±2.02^{ef}	40.53±1.72^{bc}	59.29±0.69^{c}	71.32±0.59^{b}	74.25±0.71^{c}	77.86±1.30^{d}
Acacia mangium	9.80±0.30^{n}	20.60±0.90^{k}	23.05±1.07^{m}	25.60±1.00^{j}	28.48±0.36^{n}	30.87±1.76^{o}
Acacia villosa	17.41±0.44^{ij}	23.65±0.23^{ij}	25.16±0.27^{kl}	30.55±0.52^{i}	36.11±0.43^{l}	41.33±0.36^{m}
Dalbergia latifolia	17.50±0.55^{ij}	24.14±0.51^{i}	26.43±0.47^{k}	30.01±0.66^{i}	34.19±1.28^{m}	39.28±0.53^{n}
Pterocarpus indicus	16.83±1.27^{j}	26.36±1.02^{h}	29.27±0.62^{j}	32.21±1.45^{h}	36.47±1.19^{l}	39.42±0.40^{n}
Senna siamea	19.29±0.60^{h}	34.33±0.80^{e}	38.36±1,01^{h}	50.08±1.97^{f}	59.96±0.62^{h}	63.20±1.28^{h}
Samanea saman	24.70±0.54^{d}	34.56±0.75^{e}	38.01±0.82^{h}	50.64±2.12^{f}	55.80±1.29^{i}	60.42±1.39^{i}
Albizia chinensis	22.53±0.21^{f}	29.26±0.8^{1g}	33.67±0.57^{i}	38.53±0.95^{g}	47.90±0.48^{j}	51.49±1.03^{j}
Tamarindus indica	28.31±0.41^{c}	41.26±1.25^{b}	47.19±1.57^{e}	56.82±1.31^{e}	63.95±1.87^{g}	70.35±0.78^{g}
Cassia javanica	22.15±0,42^{f}	31.15±0.89^{f}	38.88±1.92^{h}	56.39±1.67^{e}	67.10±2.33^{f}	75.88±0.68^{ef}
Parkia speciosa	12.02±0.04^{m}	16.88±0.52^{m}	18.80±0.58^{n}	20.38±0.20^{k}	22.12±0.58^{o}	25.32±0.75^{p}
Calliandra calothyrsus	13.05±0.04^{l}	19.22±0.62^{l}	24.52±0.28^{l}	30.48±0.21^{i}	36.90±0.70^{l}	43.22±0.68^{l}
Zapoteca tetragona	14.70±0.37^{k}	22.80±0.59^{j}	26.08±0.47^{k}	33.38±1.09^{h}	42.01±0.91^{k}	47.02±0.96^{k}

Note: $^{1)}$Different superscripts within the same column in each parameter indicate significant differences ($P<0.05$)

Indigofera and *Sesbania* always have a higher dry matter loss than other legumes at each incubation time. The same results were also reported by Balgees et al. (2013), Rahmat et al. (2021), and Orden et al. (2017), which showed that *Indigofera* and *Sesbania* had a higher dry matter disappearance compared to other types of tropical legumes.

In general, variations in the DM loss value in each sample can be caused by many factors. Belachew et al. (2013) stated that differences in the chemical composition of the DM constituents of each plant species would provide differences in the microbial degradation rate to feed DM when incubated in the rumen. For incubation at 0 hours, the dry matter degradation rate is more due to mechanical influences when processing feed ingredients, causing an uneven distribution of coarse and fine particles in bags (Welch 1986; Singh et al. 1989; Hackmann et al. 2010).

In sacco *degradation characteristics and effective degradation of dry matter*

The *in sacco* degradation characteristics and effective degradation of dry matter of tropical legumes used in this study are presented in Table 3. The table shows that DM degradation characteristics and effective degradation vary (P<0.05) among species. For example, *I. tinctoria* produced the highest value of the soluble fraction (*a*) from DM with a value of 42.89±1.15% and the lowest fraction of *a* was produced by *P. speciosa* with a value of 12.73±0.25. The high value of fraction *a* from DM in *I. tinctoria* theoretically indicates that its species has the highest components of soluble fractions from dry matter that can be rapidly degraded by microbe compared to the other species tested. Vice versa in *P. speciosa* legumes. According to Ørskov et al. (1980), the high value of soluble fractions can be caused by two factors: the feed ingredients contain high soluble fraction components and a more generous fine particle size lost during the washing process.

The highest insoluble fraction but the potential to be degraded (*b* fraction) from DM was obtained in *L. leucocephala* with values of 64.86±0.68% (P<0.05). In contrast, the lowest *b* fraction from DM was obtained in *P. speciosa* with values of 11.80±1.14%. The value of fraction *b* from DM represented a fraction of forage dry matter that is insoluble but can be slowly degraded by rumen microbes. In addition, many factors can influence the high or low value of the fraction of b in forage. According to Belachew et al. (2013), one of the factors affecting insoluble fraction from DM in forage is the presence of secondary metabolites of plants, namely condensed tannin (CT). It was further reported by González et al. (2002), Gupta et al. (2011), and Orden et al. (2017) that the CT content in plant leaves caused a decrease in leaf degradation and the increased value of insoluble fraction from the plant leaves.

The highest degradation rate of *b* fraction (*c*) from dry matter is produced by *A. mangium* with a value of 0.10±0.01. h^{-1}, while *D. regia* generates the lowest value of 0.02±0.0. h^{-1}. The value of *c* from DM describes the degradation rate of insoluble components from dry matter. The *b* value from dry matter is strongly influenced by the constituent components of the dry matter, such as the presence of limiting factors such as lignin and the presence of secondary plant metabolic such as tannins (Alam et al. 2007).

Table 3. Degradation fractions (a, b, a+b, U, and c) and effective degradability (ED) of dry matter (DM) of tree forages legume (%) at ruminal outflow rates (kp) of 0.06 h^{-1}

Legumes	a[1)]	b	$a+b$	U	c	ED
	%				h^{-1}	kp=0.06 h^{-1}
Leucaena leucocephala	17.23±0.42^{ij}[2)]	64.86±0.68^{a}	82.10±0.44^{c}	17.91±0.44^{m}	0.06±0.00^{cd}	49.19±0.63^{d}
Sesbania grandiflora	21.56±0.43^{f}	63.78±0.38^{a}	85.33±0.68^{b}	14.67±0.68^{n}	0.09±0.00^{b}	60.04±0.60^{b}
Delonix regia	28.63±0.56^{c}	59.03±1.45^{b}	87.66±1.52^{a}	12.11±1.52^{o}	0.02±0.03^{h}	44.59±0.25^{f}
Gliricidia sepium	31.12±0.83^{b}	53.06±2.64^{d}	84.17±2.77^{b}	15.83±2.77^{n}	0.03±0.00^{g}	48.76±0.73^{d}
Indigofera tinctoria	42.89±1.15^{a}	45.44±1.75^{e}	88.33±0.90^{a}	11.67±0.90^{o}	0.06±0.00^{de}	64.57±0.48^{a}
Bauhinia purpurea	20.84±0.58^{fg}	37.74±1.27^{g}	58.58±1.36^{h}	41.42±1.36^{h}	0.07±0.01^{c}	40.43±0.72^{h}
Caesalpinia sappa	21.70±1.73^{f}	55.53±1.15^{c}	77.24±0.95^{d}	22.77±0.95^{l}	0.08±0.01^{b}	54.22±0.36^{c}
Acacia mangium	10.46±0.40^{n}	18.73±0.53^{l}	29.20±0.84^{n}	70.82±0.84^{b}	0.10±0.01^{a}	22.12±0.48^{n}
Acacia villosa	18.418±0.30^{hi}	26.89±1.10^{j}	45.31±1.33^{k}	54.69±1.33^{e}	0.03±0.00^{gh}	26.28±0.21^{l}
Dalbergia latifolia	18.81±0.51^{h}	21.97±1.86^{k}	40.78±2.20^{l}	59.23±2.20^{d}	0.03±0.01^{g}	26.35±0.37^{l}
Pterocarpus indicus	17.81±1.15^{ij}	20.43±1.04^{k}	38.24±0.95^{m}	61.76±0.95^{c}	0.07±0.01^{c}	28.49±0.64^{j}
Senna siamea	20.46±0.73^{g}	43.83±1.05^{e}	64.30±0.97^{f}	35.72±0.97^{j}	0.06±0.00^{ef}	40.03±0.59^{h}
Samanea saman	24.87±0.64^{d}	36.63±1.05^{gh}	61.50±1.56^{g}	38.50±1.56^{i}	0.05±0.01^{f}	40.43±0.24^{h}
Albizia chinensis	23.19±0.30^{e}	31.87±1.67^{i}	55.06±1.61^{i}	44.94±1.61^{g}	0.03±0.00^{g}	33.90±0.24^{i}
Tamarindus indica	29.40±0.48^{c}	40.73±1.67^{f}	70.13±1.58^{e}	29.87±1.58^{k}	0.05±0.00^{ef}	47.4±0.42^{e}
Cassia javanica	21.33±0.56^{f}	60.28±1.78^{b}	81.60±2.03^{c}	18.40±2.03^{m}	0.03±0.00^{g}	42.32±0.90^{g}
Parkia speciosa	12.73±0.25^{m}	11.80±1.14^{m}	24.52±1.26^{o}	75.48±1.26^{a}	0.05±0.02^{def}	18.20±0.31^{o}
Calliandra calothyrsus	13.65±0.28^{l}	32.23±1.33^{i}	45.88±1.30^{k}	54.13±1.30^{e}	0.03±0.00^{g}	24.53±0.10^{m}
Zapoteca tetragona	15.55±0.32^{k}	35.25±1.71^{h}	50.80±1.90^{j}	49.20±1.90^{f}	0.03±0.00^{g}	27.33±0.50^{k}

Note: [1)] *a*: soluble fraction and rapid degradation, *b*: insoluble fraction and slow degradation, *a+b*: potential degradability fraction, *c*: rate constant of degradation from b fraction, *U*: non-degradation fraction, *ED*: effective degradability, *kp*: constant turnover rate of the rumen digesta due to microbial degradation activity. [2)] Different superscripts within the same column in each parameter indicate significant differences (P<0.05)

The highest value of potential degradability fraction (*a+b*) from dry matter legume tested in this study was produced by *S. grandiflora* and *D. regia* with values of 88.33±0.90 and 87.66±1.52, respectively. It can be because both legumes have higher *a* and *b* values than the other. This situation also causes these two legumes to have a lower total non-degradation fraction (U) DM value than the other legumes (12.11±1.52% and 11.67±0.90%, respectively).

The calculation of effective degradability (ED) from dry matter (DM) of tree forage legume leaves at ruminal outflow rates (*kp*) of 0.06 h^{-1} showed that *I. tinctoria* has the highest EDDM value compared to other types of legumes, namely 64.57±0.48%. The same result was reported by Rahmat et al. 2021, which found that *Indigofera* had a higher EDDM value than the other tropical legume.

Rumen organic matter degradation characteristics of tropical tree legume leaves

In sacco *ruminal organic matter disappearances*

The results of the calculation of disappearances of organic matter are presented in Table 4. The loss of organic matter during incubation has the same pattern as the loss of dry matter in the rumen. In general, *P. speciosa* always produces the lowest organic matter loss compared to other legume samples, namely at 0, 6, 12, 24, 48, and 72 incubation, respectively are 10.19±0.17%, 16.01±0.55%, 17.78±0.55%, 19.67±0.29%, 21.49±0.49%, and 24.52±1.18%, respectively. On the other hand, *Indigofera tinctoria* produced the highest value of OM loss at 0, 6, 24, 48, and 72 incubation times with values of 40.57±1.23%, 54.42±1.43%, 78.04±0.88%, 83.99±1.53%, and 87.74±0.75%, respectively. But at 12 hours incubation, *S. grandiflora* gave the highest OM loss compared to other sample types, namely 69.23±1.85%.

Similarly, the pattern of OM loss and DM loss of legumes in the present study is likely because most legume dry matter is a component of organic matter (characterized by the low ash content of dry matter, Table 1). The same pattern of dry and organic matter loss during incubation in the rumen was also reported by Ascencio-Rojas et al. 2019, who conducted a study on the *in sacco* degradation of six tropical plant leaves.

In sacco *degradation characteristics and effective degradation of organic matter*

The results of characteristic calculations and the effective degradation of organic matter are presented in Table 5. Table 5 shows the characteristics of degradation among species of legumes vary. The highest value of the soluble fraction (*a*) from organic matter is produced by *I. tinctoria* with a value of 40.32±1.37%. The lowest value was produced by *A. mangium*, which is 7.79±0.33%. The high value of soluble fraction in *Indigofera* shows its legume has a higher potential as a source of nutrients (energy and protein) that can be rapidly degraded in the rumen than other legumes; including this legume in a total mixed ration has been demonstrated to enhance the overall digestibility of the ration (Suharlina et al. 2016). The value of soluble fraction from the organic matter of *Indigofera* in the present study was higher than reported by Rahmat et al. (2021). These can be due to differences in the plants' environment and the age of *Indigofera* plants used as samples. Mwangi et al. (2022) stated that differences in plant age would give differences in the value of soluble chemical components. Therefore, with the increase of plant maturity, the value of the rumen's soluble and rapidly degraded fraction will decrease due to the increasing number of insoluble fractions such as plant cell wall components.

Table 4. The disappearance of organic matter (OM) of tree forage legumes at various time incubation in the rumen (%)

Legumes	Incubation time (hours)					
	0	6	12	24	48	72
Leucaena leucocephala	15.44±0.771$^{i1)}$	31.37±2.04^{f}	47.54±2.08^{d}	67.08±1.49^{c}	79.25±0.98^{c}	81.71±0.73^{c}
Sesbania grandiflora	20.80±0.69^{e}	39.21±1.55^{c}	69.23±1.85^{a}	77.30±1.14^{a}	81.94±0.95^{b}	85.09±0.50^{b}
Delonix regia	26.78±1.08^{c}	35.28±0.56^{d}	40.80±0,52^{g}	50.20±0.67^{g}	69.10±0.40^{f}	74.62±0.39^{f}
Gliricidia sepium	30.89±0.90^{b}	38.88±0.82^{c}	42.58±1.46^{f}	58.81±2.42^{d}	72.12±0.75^{e}	76.35±1.16^{e}
Indigofera tinctoria	40.57±1.23^{a}	54.42±1.43^{a}	62.83±1.89^{b}	78.04±0.88^{a}	83.99±1.53^{a}	87.74±0.75^{a}
Bauhinia purpurea	19.03±0.93^{f}	30.33±1.52^{f}	40.98±2.15^{g}	48.62±1.69^{g}	53.38±0.81^{k}	57.90±1.99^{j}
Caesalpinia sappa	22.82±1.98^{d}	41.45±1.15^{b}	60.05±0.88^{c}	72.45±0.49^{b}	75.47±0.71^{d}	78.39±1.31^{d}
Acacia mangium	6.85±0.22^{l}	18.39±1.14^{l}	21.04±1.16^{n}	23.67±0.87^{k}	27.26±0.54^{p}	30.58±1.78^{p}
Acacia villosa	15.84±0.51^{hi}	22.15±0.17^{j}	23.59±0.20^{m}	29.37±0.53^{j}	35.06±0.51^{o}	40.96±0.93^{n}
Dalbergia latifolia	17.22±0.65^{g}	24.47±0.53^{i}	26.43±0.46^{l}	30.50±0.71^{j}	34.85±1.34^{o}	39.95±0.56^{no}
Pterocarpus indicus	16.49±1.39^{gh}	26.00±1.09^{h}	29.17±0.67^{k}	32.29±1.41^{i}	36.91±0.88^{n}	39.42±0.60^{o}
Senna siamea	18.67±0.35^{f}	34.23±0.69^{de}	38.44±0.92^{hi}	49.99±1.92^{g}	60.40±0.88^{i}	63.44±1.10^{h}
Samanea saman	22.91±0.80^{d}	33.58±0.76^{e}	37.23±1.02^{i}	50.24±2.01^{g}	55.30±1.44^{j}	60.33±1.46^{i}
Albizia chinensis	20.64±0.21^{e}	27.74±1.23^{g}	32.56±0.68^{j}	37.86±1.04^{h}	48.03±0.92^{l}	51.85±1.32^{k}
Tamarindus indica	27.21±0.50^{c}	38.86±1.36^{c}	44.83±1.82^{e}	55.08±1.35^{f}	63.66±1.60^{h}	69.28±0.86^{g}
Cassia javanica	21.50±0.48^{e}	30.95±0.88^{f}	39.07±2.32^{h}	56.75±1.91^{e}	67.85±2.08^{g}	75.88±0.68^{e}
Parkia speciosa	10.19±0.17^{k}	16.01±0.55^{m}	17.78±0.55^{o}	19.67±0.29^{l}	21.49±0.49^{q}	24.52±1.18^{q}
Calliandra calothyrsus	11.50±0.23^{j}	18.55±0.66^{l}	23.36±0.62^{m}	29.85±0.39^{j}	37.28±0.52^{n}	43.16±0.64^{m}
Zapoteca tetragona	12.42±0.36^{j}	20.03±0.65^{k}	24.02±0.37^{m}	30.74±1.45^{j}	40.62±1.24^{m}	45.35±1.18^{l}

Note: $^{1)}$Different superscripts within the same column in each parameter indicate significant differences (P<0.05)

Table 5. Degradation fractions (a, b, a+b, U, and c) and effective degradability (ED) of organic matter (OM) of tree forages legume (%) at ruminal outflow rates (kp) of 0.06 h-1

Legumes	*a*[1)]	*b*	*a* + *b*	*U*	*c*	*ED*
	——————————%——————————				% h^{-1}	*kp*=0.06 h^{-1}
Leucaena leucocephala	14.12±0.74$^{k2)}$	69.86±0,51^{a}	83.98±0.93^{b}	16.02±0.93^{m}	0.06±0.00^{de}	47.48±0.67^{d}
Sesbania grandiflora	18.74±0.55^{i}	65.91±0.75^{b}	84.65±0.66^{b}	15.36±0.66^{m}	0.09±0.00^{a}	58.52±0.57^{b}
Delonix regia	26.85±0.64^{d}	61.69±0.28^{c}	88.54±2.70^{a}	11.46±2.70^{n}	0.02±0.00^{j}	43.29±0.35^{f}
Gliricidia sepium	29.83±0.93^{b}	54.50±2.41^{d}	84.32±2.88^{b}	15.68±2.88^{m}	0.03±0.00^{hi}	47.61±0.74^{d}
Indigofera tinctoria	40.32±1.37^{a}	47.81±1.84^{e}	88.12±0.95^{a}	11.88±0.95^{n}	0.06±0.00^{cd}	63.78±0.68^{a}
Bauhinia purpurea	18.97±0.91^{i}	37.91±1.70^{h}	56.86±1.42^{h}	43.12±1.42^{g}	0.07±0.01^{b}	38.68±0.77^{i}
Caesalpinia sappa	21.78±1.75^{f}	56.29±1.11^{d}	78.07±0.91^{d}	21.93±0.91^{k}	0.09±0.01^{a}	55.02±0.27^{c}
Acacia mangium	7.79±0.33^{n}	20.72±0.86^{k}	28.51±1.13^{m}	71.49±1.13^{b}	0.09±0.01^{a}	20.05±0.65^{o}
Acacia villosa	16.91±0.34^{j}	29.51±2.31^{j}	46.42±2.52^{j}	53.58±2.52^{e}	0.02±0.00^{ij}	24.88±0.21^{m}
Dalbergia latifolia	18.58±0.62^{i}	22.61±1.39^{k}	41.19±1.93^{k}	58.81±1.93^{d}	0.03±0.01^{h}	26.59±0.30^{l}
Pterocarpus indicus	17.44±1.31^{j}	21.06±1.08^{k}	38.50±0.80^{l}	61.51±0.80^{c}	0.07±0.01^{b}	28.36±0.64^{k}
Senna siamea	19.93±0.54^{h}	44.61±1.14^{f}	64.54±0.93^{f}	35.46± 0.93^{i}	0.05±0.00^{ef}	39.96±0.57^{h}
Samanea saman	23.19±0.78^{e}	37.91±0.76^{h}	61.10±1.33^{g}	38.90±1.33^{h}	0.05±0.01^{f}	39.59±0.14^{h}
Albizia chinensis	21.29±0.37^{fg}	34.54±2.28^{i}	55.83±2.01^{h}	44.17±2.01^{g}	0.03±0.00^{hij}	32.84±0.30^{j}
Tamarindus indica	27.99±0.55^{c}	42.39±1.39^{g}	70.38±1.45^{e}	29.62±1.45^{j}	0.04±0.00^{fg}	45.60±0.52^{e}
Cassia javanica	20.63±0.62^{gh}	60.45±1.76^{c}	81.08±2.06^{c}	18.92±2.06^{l}	0.04±0.00^{gh}	42.40±0.91^{g}
Parkia speciosa	10.92±0.30^{m}	12.46±0.74^{l}	23.38±0.81^{n}	76.62±0.81^{a}	0.06±0.01^{bc}	17.26±0.32^{p}
Calliandra calothyrsus	12.39±0.34^{l}	33.4±1.29^{i}	46.42±1.20^{j}	53.58±1.20^{e}	0.03±0.00^{hij}	24.05±0.24^{n}
Zapoteca tetragona	12.95±0.38^{l}	37.48±2.28^{h}	49.86±2.43^{i}	50.14±2.43^{f}	0.03±0.00^{hij}	24.71±0.58^{m}

Note: [1)] *a*: soluble fraction and rapid degradation; *b*: insoluble fraction and slow degradation; *a+b*; potential degradability fraction; c: rate constant of degradation from b fraction; *U*: non-degradation fraction; *ED*: effective degradability; *kp*: constant turnover rate of the rumen digesta due to microbial degradation activity. [2)] Different superscripts within the same column in each parameter indicate significant differences (P<0.05)

L. leucocephala produced the highest *b* fraction from organic matter with a value of 69.86±0.51% compared to other legumes. It is likely due to the higher content and binding activity of condensed tannins (CT) in the constituent organic matter of Leucaena compared to other legumes, thus increasing the insoluble fraction in *Leucaena* (Tan et al. 2011). However, Gaviria-Uribe et al. (2022) reported that Leucaena also contains other bioactive compounds such as mimosine, alkaloids, saponins, and steroids that contribute to the decreased soluble fraction of Leucaena species.

The highest value of *c* fraction from organic matter is produced by *S. grandiflora*, *A. mangium*, and *C. sappan*, with a 9%/hour. The phenomenon shows that the three types of legumes have a faster degradation rate of *b* fraction from organic matter than other legumes. This may be due to the content of secondary metabolites, especially condensed tannins of all three types of legumes, which have a lower concentration and binding activity to the components of *b* fraction from organic matter constituents. So there is a degradation rate b organic matter is faster than the other legumes. In addition, another possibility is caused by the *b* fraction of organic matter of the three legumes is indeed composed of components that can be digested faster by rumen microbes than other legumes. DePeters et al. (1997) stated that the cell wall component, as the main constituent of *b* fraction from organic matter in plants, can have a degradation rate that varies between plant species. For example, the arabinose and mannose cell walls will be more easily degraded than the upper covenant in xylose and uronic acid.

The calculation of organic matter's potential degradation (a+b) value shows that *I. tinctoria* and *D. regia* have the highest value *a+b* fraction compared to other legumes, namely 88.54±2.70% and 88.12±0.95%, respectively. This is because the two legumes have the highest values of *a* and *b* fractions compared to the other legumes. The high potential degraded value of both legumes resulted in the low value of the non-degraded (*U*) fractions, namely 11.46±2.70% and 11.88±0.95%, respectively. The opposite is true of *P. speciosa*, which has the lowest potential degradation value, and the non-degraded fraction value in the rumen are high, with values of 23.38±0.81% and 76.62±0.81%, respectively.

The estimation of the effective degradation value from organic matter (EDOM) of each legume sample tested at a ruminal outflow rate (*kp*) of 6%/h generally showed a noticeable difference (P<0.05). The occurrence of differences in the EDOM values of each legume used in the study can be caused by different chemical compositions (Van Soest 1994; Braga et al. 2018; Castro-Montoya and Dickhoefer 2020). *Indigofera* has the highest EDOM value compared to other legumes, namely with values of 87.74±0.75%, and legume *P. speciosa* has the lowest EDOM value compared to others, with a value of 17.26±0.32. The EDOM of *Indigofera* values generated in the study is now higher than those reported in previous studies by Rahmat et al. (2021).

Indigofera has a higher EDOM value than other legumes, likely due to its lower crude fiber content than other legumes, and vice versa in *P. speciosa* (Table 1). Crude fiber represents the total content of plant cell walls which contains chemical compounds that can inhibit the degradation process of feed ingredients in the rumen, such as lignin and silica. Fluck et al. (2013) and Valente et al. (2016) state that increasing cell wall content in plants will decrease the digestibility value of these plants for livestock.

Correlation between chemical composition and *in sacco* degradation parameters of tropical tree legume leaves observed

The results of calculating the value of the linear correlation coefficient of the relationship of chemical composition within *in sacco* degradation parameters of dry matter and organic matter of leaves of tree legume plants are presented sequentially in Tables 6 and Table 7, respectively. Each chemical composition has a different relationship with each observed degradation parameter. The difference in the value of the regression coefficient of each chemical composition with the measured degradation parameters indicates it.

Ash as a representation of total mineral content in forage has a high and significant positive correlation with a fraction of dry matter and organic matter, EDDM, and EDOM values of tree legume plant leaves with each correlation coefficient value of 0.68; $P<0.01$, 0.64; $P<0.01$), 0.54; $P<0.05$ and 0.52; $P<0.05$. Whereas with other fractions: fractions *b*, *a+b*, *U*, and *c*, ash has a non-significant low correlation ($P>0.05$) with correlation coefficient values of 0.19, 0.40, -0.40, and 0.2, respectively, for dry matter degradation parameters and 0.19, 0.39, -0.39 and -0.02 for organic matter degradation parameters. The data indicate that increasing ash content in legume leaves will increase the value of the dissolved fraction of dry matter and organic matter from the leaves of legume plants and increase the degradation value in the rumen. Topps (1992) states that legume leaves contain macro minerals (calcium, sulfur, sodium, magnesium, potassium, and sulfur) with high solubility values. These minerals are needed by livestock for life and production and support the growth of rumen microbes to degrade feed ingredients in the rumen (Suyitman et al. 2020).

Table 6. Pearson correlations coefficient (r) of the relationship of chemical composition (% DM basis) with *in sacco* dry matter degradation parameters

Chemical composition [1]	Dry matter degradation parameter [2]					
	a	***b***	***a + b***	***U***	**c**	***ED DM kp*=0.06 h^{-1}**
Ash	0.68**[3]	0.19^{ns}[4]	0.40^{ns}	-0.40^{ns}	0.02^{ns}	0.54*
CP	0.41^{ns}	0.60**	0.62**	- 0.62**	0.01^{ns}	0.58**
EE	0.24^{ns}	0.05^{ns}	0.13^{ns}	-0.13^{ns}	0.36^{ns}	0.25^{ns}
CF	-0.64**	-0.69**	-0.77***	0.77***	0.12^{ns}	-0.73***
NFE	-0.05^{ns}	0.03^{ns}	0.01^{ns}	-0.01^{ns}	-0.19^{ns}	-0.08^{ns}
TDN	0.43^{ns}	0.57*	0.60**	-0.60**	0.02^{ns}	0.53*
TP	-0.49*	-0.37^{ns}	-0.46*	0.46*	-0.21^{ns}	-0.57*

Note: [1] CP: Crude Protein; EE: Extract Ether; CF: Crude Fiber; NFE: Nitrogen Free-Extract; TDN: Total Digestible Nutrients; TP: Total Phenol. [2] *a*: soluble fraction and rapid degradation; *b*: insoluble fraction and slow degradation; *a+b*: potential degradability fraction; *c*: rate constant of degradation from b fraction; *U*: non-degradation fraction; EDDM: effective degradability of dry matter; *kp*: constant turnover rate of the rumen digesta due to microbial degradation activity. [3] Level of significance: *: P<0.05; **: P<0.01; ***: P<0.001. [4] ns: non significant: P>0.05

Table 7. Pearson correlations coefficient (r) of the relationship of chemical composition (% DM basis) with *in sacco* organic matter degradation parameters

Chemical composition [1]	Organic matter degradation parameter [2]					
	a	***b***	***a + b***	***U***	**c**	***ED OM kp*=0.06 h^{-1}**
Ash	0.64**	0.19^{ns}	0.39^{ns}	-0.39^{ns}	-0.02^{ns}	0.52*
CP	0.34^{ns}	0.63**	0.62**	-0.62**	0.01^{ns}	0.55*
EE	0.21^{ns}	0.04^{ns}	0.11^{ns}	-0.11^{ns}	0.38^{ns}	0.23^{ns}
CF	-0.60**	-0.71***	-0.78***	0.78***	0.09^{ns}	-0.72***
NFE	-0.01^{ns}	0.02^{ns}	0.02^{ns}	-0.02^{ns}	-0.14^{ns}	-0.06^{ns}
TDN	0.41^{ns}	0.56*	0.60**	-0.60**	0.09^{ns}	0.52*
TP	-0.45^{ns}	-0.35^{ns}	-0.44^{ns}	0.44^{ns}	-0.22^{ns}	-0.56*

Note: [1] CP: Crude Protein; EE: Extract Ether; CF: Crude Fiber; NFE: Nitrogen Free-Extract; TDN: Total Digestible Nutrients; TP: Total Phenol. [2] *a*: soluble fraction and rapid degradation; *b*: insoluble fraction and slow degradation; *a+b*: potential degradability fraction; *c*: rate constant of degradation from b fraction; *U*: non-degradation fraction; EDOM: effective degradability of organic matter; *kp*: constant turnover rate of the rumen digesta due to microbial degradation activity. [3] Level of significance: *: P<0.05; **: P<0.01; ***: P<0.001. [4] ns: non significant: P>0.05

In general, the crude protein content has positively correlated with the fractions *a*, *b*, *a+b*, *c*, and the effective degradation values of dry and organic matter. However, it has negatively correlated with the fraction *U* of dry matter and organic matter in the leaves of the tree legume plants tested. With a correlation coefficient value of each sequentially is 0.41; *P*>0.05, 0.60; *P*<0.01, 0.62; *P*<0.01, -0.62; *P*<0.01, 0.01; *P*>0.05, and 0.58; *P*<0.01 for dry matter degradation parameter, and 0.34; *P*>0.05, 0.63; *P*<0.01, 0.62; *P*<0.01, -0.62; *P*<0.01, 0.01; *P*>0.05, 0.55; *P*<0.5 for organic matter degradation parameters. That indicated that an increase in the crude protein content in the leaves of legume plants, in general, will increase the value of the fractions *a*, *b*, *a+b*, *c* and effective degradation and decrease the value of the *U* fraction, which cannot have degraded in the rumen. This result aligns with the research reported by Tolera et al. (1997) and Foskolos et al. (2015), which stated that crude protein has a positive correlation with degradation parameters and a negative correlation with non-degraded fractions in forage.

Extract ether (EE) is generally positively correlated with the value of a, b, a+b fractions and effective degradability and negatively associated with the value of U fraction from dry and organic matter of leaves of tree legume plants. However, the correlation value statistically did not show a noticeable effect (*P*>0.05). It has likely due to the low EE content of legume leaf samples (Table 1), so it does not show a significant correlation in degradation parameters *in sacco.*

The crude fiber content has generally highly correlated with *in sacco* degradation parameters of dry matter and organic matter of tree legume plant leaves. Crude fiber has a correlation coefficient value of -0.64; *P*<0.01 and -0.60; *P*<0.01 with *a* fraction of dry and organic matter respectively, -0.69; *P*<0.01 and -0.71; *P*<0.001 with *b* fraction of dry and organic matter respectively, -0.77 and -0.78; *P*<0.001 with a+b fraction of dry and organic matter respectively, 0.77 and 0.78; *P*<0.001 with *U* fraction of dry and organic matter respectively, as well as -0.73 and -0.72; *P*<0.001 each with EDDM and EDOM of tree legume leaves. The values of the correlation coefficient indicate that an increase in CF levels will result in a decrease of a,b, a+b fractions, and EDDM and EDOM of tree legume leaves but will increase the value of fractions that cannot be degraded (U) from legume leaves in the rumen. Van Soest (1994) and Cherney (2000) stated that crude fiber, as a description of plant cell walls, contains digestibility-limiting components, one of which is lignin compounds, so an increase in CF value in forage will reduce the digestibility value of forage.

Nitrogen-free extract (NFE) in the proximate analysis system describes the carbohydrates cell content or plant nonstructural carbohydrates. Nonstructural carbohydrates are an essential energy source that can be fermented by rumen microbes quickly (Pelletier et al. 2010). Shewmaker et al. (2006) stated that nonstructural carbohydrates were positively correlated with the digestibility of ruminant feed. The higher the content in the forage, the more digestibility value will increase. However, in this study, the NFE value gives a correlation value that is opposite to the negative correlation coefficient values ranging from -0.19 to 0.03; P>0.05 for *in sacco* dry matter degradation parameters and -0.14 - 0.02; P>0.05 for *in sacco* organic matter degradation parameters. Increasing the NFE value will decrease the *in sacco* degradation parameter values of dry and organic matter from legume leaves. Cherney (2000) and Hutanen et al. (2006) stated that NFE, as a representation of the carbohydrate content of plant cells, has an inconsistent effect on forage digestibility in ruminants. In the process of determining, about 30% of plant cell wall components counted as NFE, namely hemicellulose, and lignin..

Total digestible nutrients (TDN), as an illustration of the potential energy content of feed ingredients, is generally positively correlated with the values of the parameters *a*, *b*, *a+b*, *c* and the effective degradation of both dry matter and organic matter, but negatively correlated with the value of the *U* fraction of dry matter and organic matter of tree legume leaves tested. With sequential correlation coefficient values, each is 0.43; *P*>0.05, 0.57; *P*<0.05, 0.60; *P*<0.01, 0.02; *P*>0.05, 0.53; *P*<0.05 and -0.60; *P*<0.01 for dry matter degradation parameter, 0.41; *P*>0.05; 0.56; *P*<0.05; 0.60; *P*<0.01, 0.09; *P*>0.05, 0.52; *P*<0.05 and -0.60; *P*<0.01 for organic matter degradation parameters. That shows that increasing the TDN value in the leaves of tree leguminous plants will increase the values of the fractions a, b, a+b, and c and the effective degradation of dry matter and organic matter and decrease the value of the fraction that can not be degraded in the rumen of leguminous tree leaves. It is possible because energy is one of the limiting factors in microbial degradation activity in the rumen. Therefore, increasing the potential energy contained in a feed ingredient will also increase the degradation of feed ingredients in the rumen. Lu et al. (2019) stated that one way to increase microbial activity and protein synthesis in the rumen is to increase energy intake in feed.

In general, the content of total phenol compounds (TP) negatively correlates with parameters a**,** b**,** a+b, and ***c*** and the effective *in sacco* degradation value of dry matter and organic matter from legume tree leaves tested. Meanwhile, the total phenolic compounds positively correlated with the U fraction of dry and organic matter. Each value of the correlation coefficient is as follows: -0.49; *P*<0.05 and -0.45; *P*>0.05 with a fraction value of dry matter and organic matter, -0.37; *P*>0.05 and -0.35; *P*>0.05 with b fraction of dry and organic matter, -0.46; *P*>0.05 and -0.44; *P*>0.05 with fraction a+b of dry and organic matter, -0.21; *P*>0.05 and -0.22; *P*>0.05 with c fraction of dry matter and organic matter, -0.57; *P*<0.05 and -0. 56; *P*<0.05 with the effective degradation value of the dry and organic matter, 0.46; *P*<0.05 and 0.44; *P*<0.05 with the value of the U fraction of dry and organic matter. The correlation coefficient values indicate that the higher the total phenolic compound content in the leaves of tree legume plants, the lower the value of the fractions a, b, a+b, c, and the effective degradation of dry matter and organic matter from the leaves of tree leguminosae plants in the rumen, but will increase the value of the rumen's fraction can not be degraded in the rumen (U fraction). The results of the current study are in line with the results reported by Tolera et al. (1997), who reported that the total content of phenolic

compounds in forage had a negative correlation with the degradation parameters values (a, b, a+b, c and effective degradation) of forage in the rumen.

Tree legume plants that grow in Gunungkidul regency have varying nutritional values (chemical composition and *in sacco* degradation characteristics) of leaves. For example, *Indigofera tinctoria* leaves have a higher value of the soluble fraction, potential degradation, and effective degradation value of dry matter and organic matter than other legumes. The content of ash, crude protein, and total digestible nutrients in leguminous tree leaves was positively correlated with the effective values of degradation of dry matter and organic matter. In contrast, the contents of crude fiber and total phenol were negatively correlated with the effective values of degradation of dry matter and organic matter in tree leguminous leaves grown on Gunungkidul Regency. This research can be used as basic data by breeders in utilizing the leaves of tree leguminous plants as a source of feed ingredients in ruminant livestock rations so that their utilization can be more optimal to increase livestock productivity.

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