

# DEGRADATION CHARACTERISTICS OF CRUDE PROTEIN AND CRUDE FIBER OF LEGUME FORAGES IN THE RUMEN OF GOAT

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➤ Supporting Information

**ABSTRACT:** The nutritional value of a feedstuff depends not only on its chemical composition but also on the capacity of ruminal microbes to colonize and degrade it. This study compared the in sacco degradation kinetics of four legume forages (*Moringa oleifera*, *Leucaena leucocephala*, *Indigofera* and *Gliricidia sepium*) using three rumen fistulated goats in a 4×3 completely randomized design (CRD). Seventy-two nylon bags (10 × 5 cm, 40–50 µm pore size) containing 5 g of each forage (ground to 2 mm) were incubated for 4, 8, 12, 24, 48, or 72 hours (12 bags per time point). The study determined the soluble fraction (a), potentially degradable fraction (b), total degradable fraction (a+b), degradation rate constant of fraction b (c), lag time (Lt), degradation effectiveness (DE), and rumen undegradable protein (RUP). The results of CP degradation revealed no significant differences among forages in fractions a, b, or a + b, but fraction c, Lt, DE, and RUP differed significantly. The degradation rate (c, h<sup>-1</sup>) of crude protein ranked as *Moringa* (0.17) > *Leucaena* (0.09) = *Indigofera* (0.09) > *Gliricidia* (0.03), while Lt was shortest for *Moringa* (3.60 h) and longest for *Gliricidia* (11.96 h). *Moringa* and *Indigofera* exhibited the highest DE and lowest RUP of all treatments. Similar trends were observed for crude fiber: *Moringa* showed the greatest DE (26.72% Lt) compared to *Leucaena* (18.76 h Lt). In conclusion, all four legumes were efficiently degraded in the goat rumen, through the rate and extent of degradation varied markedly among species, reflecting differences in their biochemical composition and structural carbohydrates.

**Keywords:** Crude Fiber, Crude Protein, Degradation Characteristics, Goat, Legumes

## INTRODUCTION

Feed is one of the main factors influencing livestock production and its economic efficiency. Forage is often provided as a combination of grasses and legumes to complement the nutritional requirements of ruminants (Phelan et al., 2015; Richards et al., 2021). Primary forages comprise grasses and legumes, which supply essential nutrients such as crude protein and crude fiber (Katoch et al. 2022).

A quality feed ingredient, depends not only on its nutritional composition but also on the capacity of rumen microbes to adapt and degrade it; degradation efficiency—particularly of lignin—strongly influences overall digestibility (Suhartanto et al, 2000; Humer and Zebeli, 2017). Feed degradation refers to the fraction of feed that is solubilized and fermented by rumen microbes, thereby supplying nutrients to the host animal (Orskov and McDonald, 2009).

Evaluation of feed ingredient degradation in ruminants can be done by the in sacco method, where ground feed is enclosed in nylon bags and incubated in the rumen for defined intervals to assess degradation kinetics (Reis et al., 2017). Feed degradation value can be predicted from the in sacco feed degradation characteristic value (Akhirany et al. 2013; Babangida et al., 2021). The in sacco method allows precise determination of the time-dependent degradation rate of the feedstuffs (Wati et al., 2012) and enables direct measurement of ruminal degradability under physiological conditions (Harfiah, 2009). Thus, the quality of a feed ingredient can be determined from the nutritional content it has, so it is very important to know the quality of protein and crude fiber content in *Moringa oleifera*, *Leucaena leucocephala*, *Indigofera* and *Gliricidia sepium*. This study therefore aimed to characterize the in sacco degradation kinetics—rate, extent, and lag time—of these four legume forages.

The selected forages including *Moringa oleifera*, *Leucaena leucocephala*, *Indigofera* and *Gliricidia sepium* offer advantages over grasses, notably higher crude protein content and improved nutritional value, which can enhance growth, production, and reproductive performance in ruminants.

## MATERIALS AND METHODS

### Ethical approval

Experimental procedures on live animals were conducted in compliance with animal welfare principles and were approved by the Health Research Ethics Committee of Hasanuddin University Makassar: Approval No. 150/UN4.6.4.5.31/PP36/2024 prior to the commencement of the study.

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### Experimental animals and diets

The study was conducted from April to May 2024 at Hasanuddin University in Makassar, Indonesia. The method used was a 4×3 Completely Randomized Design (CRD) involving three rumen-fistulated goats (n=3) aged 1.5-2.0 years, with an average body weight of 21-25 kg. The animals received the same diet, *Moringa oleifera*, *Leucaena leucocephala*, *Indigofera* and *Gliricidia sepium*, and bran, each offered to satisfy 3% of the initial body weight (BW) on a dry-matter basis, provided twice daily (morning and evening), with water available ad libitum throughout the study.

### Experimental design

This study evaluated four leguminous forages in the rumen of goats over incubation periods of 4, 8, 12, 24, 48, and 72 hours, to determine their degradation kinetics and nutritional quality. The experiment employed a 4 × 3 completely randomized design (CRD) with four treatments and three replicates. Each goat received four nylon bags—one per forage species (*Moringa oleifera*, *Leucaena leucocephala*, *Indigofera*, and *Gliricidia sepium*) resulting in 12 nylon bags per incubation time. A total of 72 nylon bags were used across six incubation periods (4, 8, 12, 24, 48, and 72 hours). Each bag measured 10×5 cm and had a porosity of 40-50 µm. For each incubation time, four bags were placed in the rumen of each fistulated goat. The feed ingredients tested were as follows: L1, *Moringa* (*Moringa oleifera*); L2, *Leucaena* (*Leucaena leucocephala*); L3, *Indigofera*; and L4, *Gliricidia* (*Gliricidia sepium*), all harvested 70 days after the previous cutting. The legume foliage was oven-dried at 60 °C for five days, ground to a particle size of approximately 2 mm, and then subjected to proximate analysis (AOAC, 1995). Five grams of each ground feed sample were placed into individual nylon bags and incubated in the rumen of goats for the designated times. After incubation, the bags were removed, drained, and dried in an oven at 60 °C for 48 hours. The overall trial duration was approximately 14 days, during which each animal received a diet consisting of 70% elephant grass, 20% legume, and 10% rice bran.

The crude protein content of the feed samples, both before and after incubation, was determined by proximate analysis using the Kjeldahl method (AOAC, 2001) at the Feed Chemistry Laboratory, Faculty of Animal Husbandry, Hasanuddin University. The loss of crude protein recovered from each nylon bag after incubation reflects ruminal degradation and is used to calculate feed protein degradation by forage type and incubation time. Degraded crude protein (CP) in each sample is calculated by comparing its initial and final CP contents. The percentage losses of crude protein and crude fiber (CF) are calculated as follows: % CP Loss = (% CP Initial × Initial Sample Weight) - (% CP Final × Final Sample Weight), while the formula for calculating the percentage of crude fiber (CF) is % CF Loss = (% CF Initial × Initial Sample Weight) - (% CF Final × Final Sample Weight).

Furthermore, the crude protein and crude fiber lost during the incubation period were used to measure the value of Y by calculating the values of a, b, c and a+b which were entered into the exponential equation according to Ørskov and McDonald (1979) as follows:

$$Y = a + b(1 - e^{-ct})$$

The characteristic values of CP, CF, and ED degradation in feed can be calculated by the following formula:

$$DE = a + [(bxc)] / [(c+k)]$$

which Y = Feed degradation by rumen microbes at time t (incubation time); DE = degradation effectiveness, a = soluble feed fraction; b = feed fraction with potential for degradation; c = Degradation rate of fraction (b); a + b = Total degradation potential, including material that escapes the bag without being degraded; K = constant 0.05/hour, (Srakaew et al., 2021). Proteins that is degraded in the rumen perfectly is called rumen degradable protein (RDP), and proteins that cannot be degraded are called Rumen Undegradable Protein (RUP) of each sample calculated by the following equation: RUP = 100% - RDP (Terefe et al., 2022). Degradation curves and patterns of feed degradation in the goat rumen, determined by the in sacco method, were analyzed using the Nway program (Ismartoyo, 2011).

### Statistical analysis

The data were analyzed as a 4 × 3 completely randomized design (CRD) with four treatments and three replicates. Each incubation period (4, 8, 12, 24, 48, and 72 h) employed 12 nylon bags, for a total of 72 bags. Degradation characteristics of the legumes were evaluated by generating degradation curves using SPSS Version 16.0 and Microsoft Excel 2010. Significant differences among treatments were determined by Duncan's multiple range test (Gaspersz, 1991).

## RESULTS AND DISCUSSION

### Feed nutrient content

The feed provided to the animals was analyzed for nutrient content at the Feed Chemistry Laboratory, Faculty of Animal Husbandry, Hasanuddin University. Table 1 presents the nutrient composition of the diets used in this study. High-quality feed contains a complete spectrum of nutrients to satisfy goats' requirements for maintenance, growth, and production (Roy and Rana, 2024). Feed quality is largely determined by its protein and energy contents (Ullah-Khan et al., 2019; Rouillé et al., 2023), and a balanced nutrient profile promotes optimal livestock performance.

**Table 1 - Feed nutrient content**

Nutrient content	<i>Moringa oleifera</i>	<i>Leucaena leucocephala</i>	<i>Indigofera</i>	<i>Gliricidia sepium</i>
Dry matter (%)	16.14	21.4	24.67	23.54
Organic matter (%)	83.28	85.23	86.65	87.29
Crude protein (%)	32.70	25.63	29.50	22.79
Crude fiber (%)	13.95	20.52	20.06	23.43
Crude fat (%)	3.03	2.25	2.22	2.12
Ash (%)	16.72	14.77	13.35	12.71
NFE (%)	33.60	36.83	34.86	38.94
NDF (%)	23.38	39.00	30.99	43.48
ADF (%)	15.13	33.75	23.74	35.42
Cellulose (%)	10.93	14.61	16.52	15.72
Hemicellulose (%)	8.25	5.25	7.25	8.06
Lignin (%)	3.88	18.98	7.11	19.52

Feed Chemistry Laboratory Analysis Results, Faculty of Animal Husbandry, Hasanuddin University 2024; NDF: Neutral detergent fiber, ADF: Acid detergent fiber, NFE: Nitrogen free Extract.

**Table 2 - The average percentage ( $\pm$ SEM) of Crude Protein degradation at each incubation period**

Incubation period (Hours)	<i>Moringa oleifera</i> (%)	<i>Leucaena leucocephala</i> (%)	<i>Indigofera</i> (%)	<i>Gliricidia sepium</i> (%)	P-values
4	37.71 $\pm$ 3.94	6.33 $\pm$ 3.25	33.77 $\pm$ 8.82	16.72 $\pm$ 1.03	NS
8	58.93 $\pm$ 4.03 <sup>a</sup>	18.79 $\pm$ 6.03 <sup>b</sup>	47.37 $\pm$ 2.64 <sup>a</sup>	22.69 $\pm$ 2.27 <sup>b</sup>	P < 0.001
12	69.07 $\pm$ 1.79 <sup>a</sup>	38.32 $\pm$ 11.89 <sup>b</sup>	53.96 $\pm$ 4.00 <sup>a</sup>	27.87 $\pm$ 0.79 <sup>b</sup>	P < 0.001
24	73.16 $\pm$ 1.38 <sup>a</sup>	54.80 $\pm$ 4.67 <sup>b</sup>	65.57 $\pm$ 1.64 <sup>ab</sup>	47.60 $\pm$ 3.36 <sup>c</sup>	P < 0.001
48	77.45 $\pm$ 1.41 <sup>a</sup>	62.41 $\pm$ 1.57 <sup>b</sup>	68.62 $\pm$ 2.03 <sup>ab</sup>	63.42 $\pm$ 4.42 <sup>b</sup>	P < 0.001
72	86.32 $\pm$ 2.41 <sup>a</sup>	71.20 $\pm$ 2.17 <sup>b</sup>	71.99 $\pm$ 3.23 <sup>a</sup>	68.15 $\pm$ 5.46 <sup>b</sup>	P < 0.001

a,b,c,d: Means in the same row with different superscripts differ significantly (P<0.05); NS: not significant; SEM: Standard error of the mean.

### Crude protein degradation

The quality of a feed ingredient is reflected in its nutritional composition, particularly its crude protein content, which supports livestock productivity. In ruminants, the evaluation of feed ingredients extends beyond protein concentration to include fermentability and resistance to degradation in the rumen (Iommelli et al., 2022).

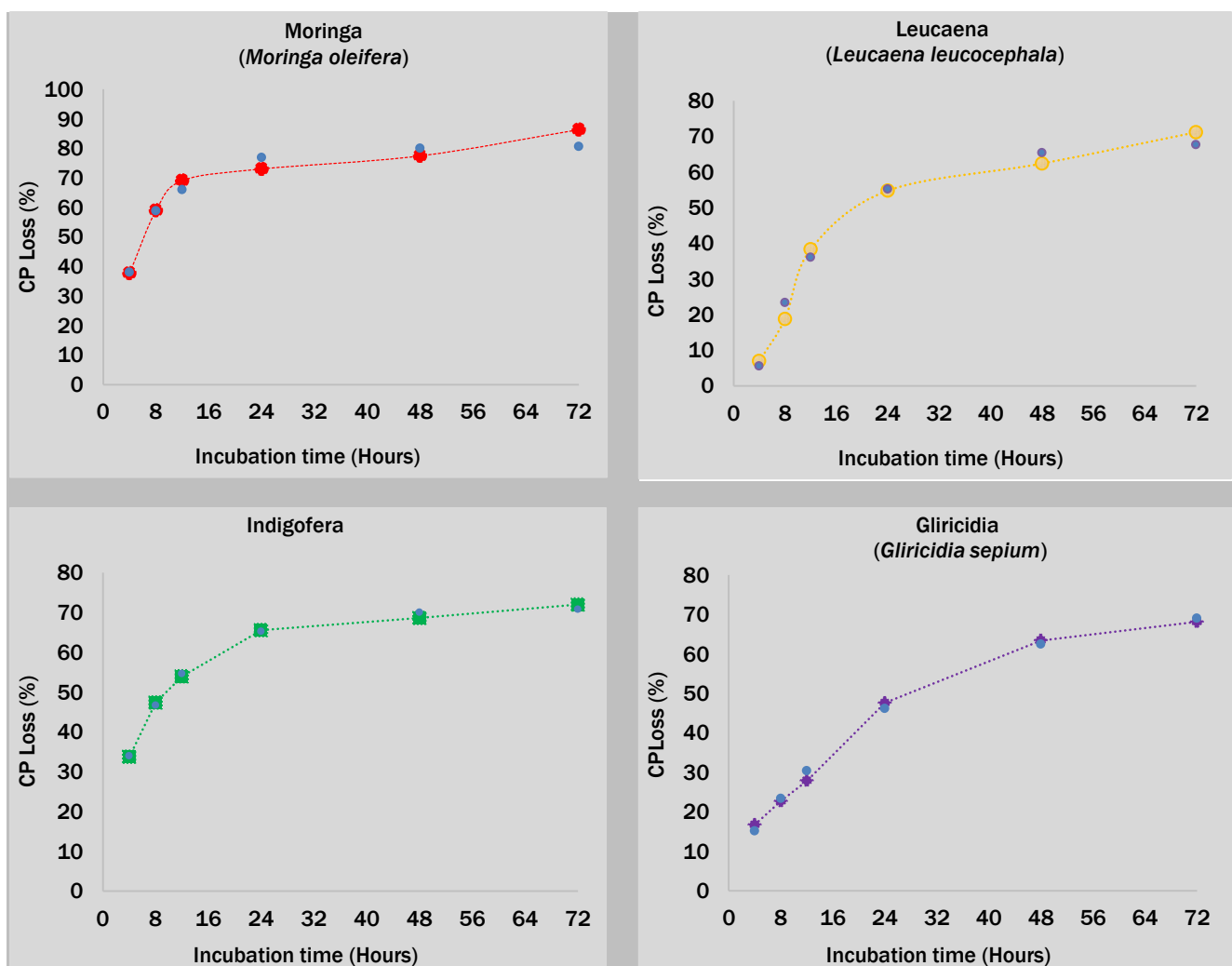
Protein degradation kinetics are therefore critical for assessing the nutritional value of dietary proteins. Using the in sacco method, crude protein degradation in the rumen is quantified by placing feed samples in nylon bags and incubating them for 4, 8, 12, 24, 48, and 72 hours. The resulting percentages of crude protein degradation are presented in Table 2.

Table 2 shows that the four legume types differed (P < 0.05) at each incubation time, likely due to variations in their structural characteristics, protein content, and fiber composition. After 4 h, *Moringa* exhibited the highest crude protein degradation (37.71 %), followed by *Indigofera* (33.77 %), *Gliricidia* (16.72 %), and *Leucaena* (6.33 %). Suhartanto et al. (2000) reported that feed degradation by rumen microbes is influenced by the nutrient composition of the substrate—particularly lignin content—which affects overall digestibility. At 8, 12, 24, 48, and 72 h, degradation values also differed significantly (P < 0.05) among the feeds. Duncan's multiple range test revealed that, after 72 h, *Moringa* again showed the highest degradation (86.32 %), followed by *Indigofera* (71.99 %), *Leucaena* (71.20 %), and *Gliricidia* (68.15 %). These findings are consistent with Akhirany et al. (2013), who observed peak degradation of fibrous forages at 72 h. Throughout the 8–48 h incubations, *Moringa* and *Indigofera* consistently degraded more rapidly than *Leucaena* and *Gliricidia*. As shown in Table 1, *Leucaena* and *Gliricidia* have lower crude protein contents (25.63 % and 22.79 %, respectively) compared to *Moringa* (32.70 %) and *Indigofera* (29.50 %), which likely contributed to their reduced degradation values. Hartadi et al. (2008) noted that differences in the potentially soluble fraction and the degradation rate of the potentially degradable fraction are affected by feed nutrient composition, rumen residence time, and substrate availability for microbial activity.

### Crude protein degradation curve

Figure 1 presents the in sacco crude protein degradation curves for the four legumes at incubation times of 4, 8, 12, 24, 48, and 72 hours. The degradation kinetics demonstrates a progressive increase in crude protein loss with longer

incubation. The most pronounced increase occurs between 4 and 12 hours, after which degradation rates begin to plateau between 24 and 72 hours as the available substrate in the rumen diminishes (Jiang et al., 2020). Low measured crude protein can result from microbial breakdown: rumen microbes hydrolyze feed proteins into amino acids, which are further deaminated into ammonia and other small compounds, thus reducing the recoverable protein fraction (Pranoto et al., 2013). Microbial proteolytic activity therefore lowers the crude protein content over time. Our results indicate that longer rumen incubation times correspond to higher crude protein degradation, reflecting progressive substrate utilization by rumen microbes. Among the four legumes tested, Moringa exhibited the highest degradation curve, while Gliricidia showed the lowest, consistent with its comparatively lower initial protein content. This observation aligns with Puastuti et al. (2015), who reported that feeds with higher protein concentrations are degraded more rapidly by rumen microbes.



**Figure 1** - Crude protein (CP) degradation curves of Moringa (*Moringa oleifera*), Leucaena (*Leucaena leucocephala*), Indigofera, Gliricidia (*Gliricidia sepium*).

#### Degradation characteristics of crude protein

The in sacco method evaluates protein degradation by incubating feed samples directly in the rumen. This approach also characterizes degradation parameters, including the soluble fraction (a), the potentially degradable but non-soluble fraction (b), and the degradation rate of fraction b (c). The crude protein degradation characteristics for the four diets are presented in Table 3. Table 3 shows that fraction (a) did not differ significantly among the four legumes ( $P > 0.05$ ). Fraction (a) reflects the truly soluble portion of the feed that dissolves readily in the rumen and during initial washing (Wati et al., 2012). Duncan's multiple range test indicated that Moringa had the highest fraction a (39.59 %), followed by *Indigofera*, *Leucaena*, and *Gliricidia*, confirming Moringa's superior solubility and *Gliricidia*'s relative resistance. Katongole et al. (2021) noted that a high soluble CP fraction in forages is often associated with low acid detergent fiber (ADF) content. Moreover, lignin, which cannot be degraded by rumen microbes, substantially reduces cell-wall degradability (Hatfield and Kalscheur, 2020; Wang et al., 2022). Fraction b represents the potentially degradable but insoluble protein fraction. Statistical analysis indicated no significant differences in fraction b among the four legumes ( $P > 0.05$ ). This fraction is expected to consist of amino acid-rich proteins that escape ruminal degradation and are absorbed in the

intestine. Duncan's multiple range test ranked fraction b as follows: *Gliricidia* (43.45 %) > *Moringa* (41.14 %) > *Leucaena* (36.47 %) > *Indigofera* (32.82 %). La Goffe (1991, cited in Widjyobroto et al., 1995) noted that such proteins are often bound to fibrous cell-wall components, rendering them resistant to enzymatic attack. This resistance may also reflect tannin–protein complex formation, which reduces ruminal protein degradation (Min et al., 2003; Patra & Saxena, 2010). The degradation rate constant (c) quantifies the rate at which fraction b is degraded. Here, c differed significantly among legumes ( $P < 0.05$ ). *Moringa* exhibited the highest rate ( $0.17 \text{ h}^{-1}$ ), followed by *Leucaena* ( $0.09 \text{ h}^{-1}$ ), *Indigofera* ( $0.09 \text{ h}^{-1}$ ), and *Gliricidia* ( $0.03 \text{ h}^{-1}$ ). Higher c values indicate greater microbial accessibility and faster degradation, influenced by cell-wall composition and substrate availability (Van Soest, 1994; Wati et al., 2012). Noviandi et al. (2021) further emphasized that nutrient composition, incubation time, and cell-wall content modulate both the soluble fraction and the degradation rate of the potentially degradable fraction. The low c value in *Gliricidia* may result from its specific cell-wall constituents (Aye and Adegun, 2013). Lag time—the interval before the onset of measurable degradation—also differed significantly ( $P < 0.05$ ). Duncan's test identified *Gliricidia* as having the longest lag time (11.96 h), indicating that its proteins require more time to become accessible to rumen microbes.

#### Effectiveness of crude protein degradation

Feed degradation effectiveness (DE)—which integrates the soluble fraction (a), the potentially degradable fraction (b), and the degradation rate of fraction b (c)—varied significantly among the four legume species ( $P < 0.05$ ). *Moringa* exhibited the highest DE ( $64.99 \% \pm 7.80$ ), closely matching the 64.29 % reported by Sumadi et al. (2017). *Leucaena*'s DE was  $50.62 \% \pm 1.49$ , similar to values of 50.74 % (Sumadi et al., 2017) and 55.61 % (Yustisiana and Kustantinah, 2011). *Indigofera* showed a DE of  $59.72 \% \pm 1.76$ , higher than the 48.00 % observed by Syamsi et al. (2022). *Gliricidia*'s DE was  $49.83 \% \pm 0.28$ , differing from previously reported values of 66.14 % (Hadi et al., 2011) and 47.00 % (Syamsi et al., 2022). These discrepancies likely reflect differences in cell-wall composition, plant maturity, and cutting age, all of which influence forage nutrient profiles. Suhartanto et al. (2000) emphasized that feed degradability is strongly affected by nutrient composition—particularly lignin content—which constrains microbial access and digestibility in the rumen.

#### Crude fiber degradation

The in sacco method was used to assess crude fiber degradation in the rumen by incubating feed samples in nylon bags for 4, 8, 12, 24, 48, and 72 hours. Table 4 presents the percentages of crude fiber degradation obtained by this method. Analysis of variance indicated that, at 4 hours of incubation, crude fiber degradation did not differ significantly among the four legumes ( $P > 0.05$ ); the ranked order was *Gliricidia* > *Indigofera* > *Moringa* > *Leucaena*. This ranking likely reflects inherent differences in fiber content and composition. At 8, 12, 24, 48, and 72 hours, degradation values differed significantly among the legumes ( $P < 0.05$ ). After 72 hours, *Gliricidia* showed the highest degradation (54.92 %), followed by *Leucaena* (54.13 %), *Moringa* (53.41 %), and *Indigofera* (52.23 %). These losses during ruminal incubation are assumed to represent the proportion of crude fiber digestible by rumen microbes.

#### Crude fiber degradation curves

The crude fiber loss of the tested feed during incubation periods of 4, 8, 12, 24, 48, and 72 hours is presented in the corresponding table, while the degradation curves or patterns—distinguished by smooth and jagged lines—are illustrated in Figure 2. The peak of fiber degradation occurred at 72 hours of incubation, as shown in Figure 2. According to Orksov et al. (1980), the optimal rumen incubation time for fibrous feed ranges from 48 to 72 hours. This is supported by Suparjo (2010), who stated that incubation intervals of 12, 24, 48, and 72 hours are most appropriate for fibrous feeds. These findings suggest that the 24–72 hour period provides optimal conditions for rumen microbes to interact with and degrade the incubated feed substrate (Ambar and Djajanegara, 1982).

#### Degradation characteristics of crude fiber

The in sacco method characteristics feed degradation by estimating the soluble fraction (a), the potentially degradable fraction (b), and the degradation rate of fraction b (c). These parameters for the forage feeds are presented in Table 5. Fraction a represents the truly soluble cell contents. Analysis of variance indicated no significant differences in fraction (a) among the feeds ( $P > 0.05$ ), reflecting similar water-solubility profiles. Feedstuffs with low water solubility dissolve and degrade less readily in rumen fluid, corresponding to lower quality. According to Duncan's test, *Gliricidia* had the lowest mean fraction a (30.02 %), while *Moringa* had the highest (39.59 %). Regression analysis showed that fraction (a) was not significantly correlated with fraction b or c ( $P > 0.05$ ), suggesting that the soluble cell components measured in fraction a do not predict the quantity or rate of the potentially degradable fraction. Factors influencing fraction a are therefore limited to the water-soluble constituents of the plant cells (Van Soest, 1982; Lestari et al., 2012). Differences in nutrient composition among feeds also affect degradability. The high fraction (a) of streptokinase (SK) in legumes reflects their neutral detergent fiber (NDF) and hemicellulose contents. A greater hemicellulose-to-crude-fiber ratio enhances forage quality (Parakkasi, 1998). This concurs with Tillman et al. (1991), who reported that SK degradation is strongly influenced by crude fiber content and by fiber constituents such as cellulose, hemicellulose, and lignin.



**Table 3 - Degradation characteristics crude protein (average  $\pm$ SEM)**

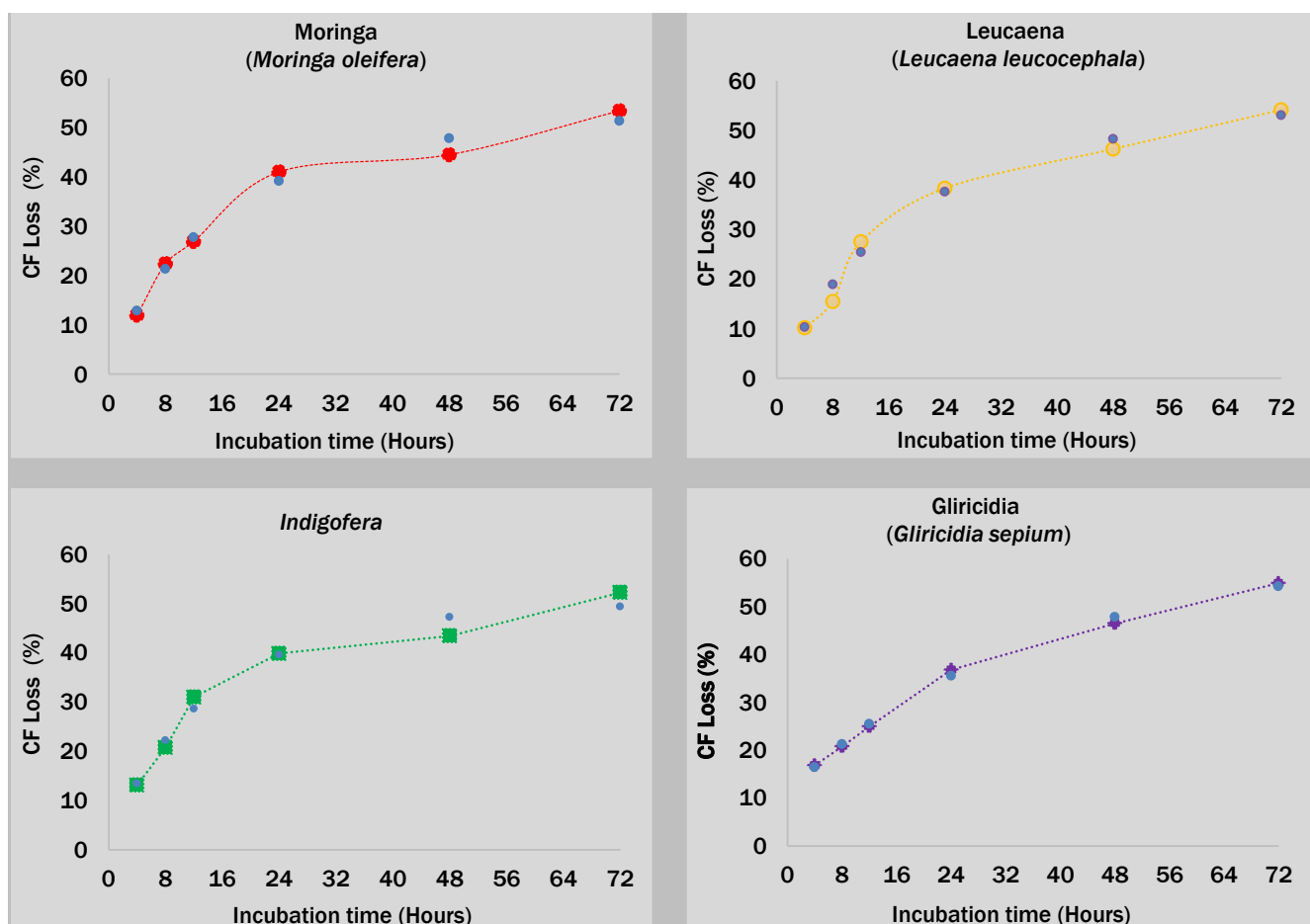
Feed	<i>Moringa oleifera</i>	<i>Leucaena leucocephala</i>	<i>Indigofera</i>	<i>Gliricidia sepium</i>	P-values
Degradation Characteristics					
a (%)	39.59 $\pm$ 0.30	32.11 $\pm$ 0.30	38.13 $\pm$ 0.30	30.02 $\pm$ 0.30	P<0.001
b (%)	41.14 $\pm$ 2.42	36.47 $\pm$ 4.81	32.82 $\pm$ 2.50	43.45 $\pm$ 6.25	NS
c (%/h <sup>-1</sup> )	0.17 $\pm$ 0.05 <sup>a</sup>	0.09 $\pm$ 0.02 <sup>ab</sup>	0.09 $\pm$ 0.03 <sup>ab</sup>	0.03 $\pm$ 0.03 <sup>b</sup>	P<0.001
a+b (%)	80.73 $\pm$ 2.42	68.89 $\pm$ 5.10	70.95 $\pm$ 2.50	73.47 $\pm$ 6.25	NS
Lt (hour)	3.60 $\pm$ 0.95 <sup>b</sup>	10.90 $\pm$ 2.05 <sup>a</sup>	6.03 $\pm$ 0.84 <sup>b</sup>	11.96 $\pm$ 1.02 <sup>a</sup>	P<0.001
DE (%)	64.99 $\pm$ 4.50 <sup>a</sup>	50.62 $\pm$ 0.86 <sup>b</sup>	59.72 $\pm$ 0.78 <sup>a</sup>	49.83 $\pm$ 0.16 <sup>b</sup>	P<0.001
RUP (%)	35.01 $\pm$ 4.50	40.28 $\pm$ 0.86	40.28 $\pm$ 0.78	50.17 $\pm$ 0.16	P<0.001

a: soluble fraction, b: potential degradation fraction, a+b: total potential degradation, c: degradation rate of fraction, Lt: lag time, de: degradation effectiveness, rup: rumen undegradable protein. Different superscripts in the same row indicate significant differences (P<0.05). SEM: Standard error of mean.

**Table 4- The average percentage ( $\pm$ SEM) of Crude fiber degradation of legume forages at each incubation period**

Incubation period (Hours)	<i>Moringa oleifera</i> (%)	<i>Leucaena leucocephala</i> (%)	<i>Indigofera</i> (%)	<i>Gliricidia sepium</i> (%)	P-values
4	11.97 $\pm$ 4.517	10.19 $\pm$ 4.48	13.16 $\pm$ 3.54	17.01 $\pm$ 1.33	NS
8	22.41 $\pm$ 0.91 <sup>a</sup>	17.27 $\pm$ 4.15 <sup>ab</sup>	20.78 $\pm$ 1.87 <sup>a</sup>	20.76 $\pm$ 1.76 <sup>ab</sup>	P<0.001
12	26.94 $\pm$ 0.75 <sup>b</sup>	27.53 $\pm$ 4.47 <sup>ab</sup>	31.00 $\pm$ 4.50 <sup>a</sup>	24.92 $\pm$ 1.44 <sup>b</sup>	P<0.001
24	41.02 $\pm$ 3.57 <sup>a</sup>	38.34 $\pm$ 4.83 <sup>c</sup>	39.86 $\pm$ 2.05 <sup>b</sup>	36.79 $\pm$ 1.84 <sup>d</sup>	P<0.001
48	44.51 $\pm$ 2.43 <sup>b</sup>	46.24 $\pm$ 3.60 <sup>ab</sup>	43.44 $\pm$ 1.69 <sup>b</sup>	46.48 $\pm$ 4.20 <sup>a</sup>	P<0.001
72	53.41 $\pm$ 4.66 <sup>b</sup>	54.13 $\pm$ 0.77 <sup>a</sup>	52.23 $\pm$ 1.52 <sup>b</sup>	54.92 $\pm$ 5.20 <sup>a</sup>	P<0.001

a,b,c,d, : Means in the same row with different superscripts differ significantly (P<0.05); NS: not significant; SEM: Standard error of the mean.

**Figure 2 - Crude fiber (CF) degradation curves of Moringa (*Moringa oleifera*), Leucaena (*Leucaena leucocephala*), Indigofera, Gliricidia (*Gliricidia sepium*).**

Fraction (b) represents the slowly degradable feed fraction. Statistical analysis revealed no significant differences in fraction (b) among the four forages ( $P > 0.05$ ), likely reflecting similar structural carbohydrate contents (NDF and ADF). Duncan's test ranked fraction b as follows: Gliricidia (32.11 %) > Leucaena (26.71 %) > Moringa (18.60 %) > Indigofera (12.38 %). The variability in this fraction correlates with the fiber composition of each forage (Wati et al., 2012). Chemical analysis showed the following ADF and NDF contents: Moringa, 15.13 % ADF and 23.38 % NDF; Leucaena, 33.75 % ADF and 39.00 % NDF; Indigofera, 23.74 % ADF and 30.99 % NDF; Gliricidia, 35.52 % ADF and 43.48 % NDF. As noted by Lopez et al. (2000) and Van Soest (1994), ADF and NDF levels can strongly influence forage digestibility. Moreover, fiber fractions bound by lignin resist microbial attack (Harfiah et al., 2009), and high lignin content further impedes ruminal degradation (Zhong et al., 2021).

The constant c describes the degradation rate of fraction b in the feed. Statistical analysis indicated no significant differences in c among the four legumes ( $P > 0.05$ ). The highest fiber degradation rates were observed in Moringa and Indigofera ( $0.05 \text{ h}^{-1}$ ), followed by Leucaena ( $0.04 \text{ h}^{-1}$ ), with Gliricidia exhibiting the lowest rate ( $0.02 \text{ h}^{-1}$ ). A lower degradation rate constant can correspond to higher overall in sacco digestibility, as reported by Rasjid and Ismartoyo (2014). Degradation speed is influenced by the degradability of cell-wall constituents (Van Soest et al., 1982). Variations in the parameters a, b, c, and effective degradability (ED) among the legume forages likely reflect differences in their nutrient and cell-wall compositions (Hadi et al., 2011). Gharechahi et al. (2023) noted that both inter- and intra-species differences in plants result in varying proportions of cellulose, hemicellulose, and lignin. Lag time (t) represents the period required for rumen microbes to adapt to the feed substrate. Analysis of variance indicated no significant differences in lag time among the feeds tested ( $P > 0.05$ ). However, Duncan's multiple range test showed that Moringa exhibited the longest lag time, whereas Gliricidia had the shortest.

#### Effectiveness of crude fiber degradation

Feed degradation (DE) effectiveness integrates the soluble fraction (a), the potentially degradable fraction (b), and the degradation rate of fraction b (c) to estimate the proportion of feed that is digested. Crude fiber DE did not differ significantly among the four legumes ( $P > 0.05$ ). Moringa exhibited the highest DE ( $43.98 \% \pm 2.87$ ), followed by Indigofera ( $42.39 \% \pm 1.24$ ), Leucaena ( $40.52 \% \pm 1.80$ ), and Gliricidia ( $39.37 \% \pm 3.48$ ), although these differences were not statistically significant. Feed DE is influenced by factors such as species, plant maturity, lignification level, and rumen incubation time. Moringa's superior DE likely reflects its lower crude fiber content, since cellulose and hemicellulose bound to lignin are resistant to microbial and enzymatic attack, reducing digestibility (Komar, 1984; Tillman et al., 1998). Pangestu (2005) similarly noted that fiber composition and lignin associations vary among forages, leading to differential degradation in the digestive tract. According to Mehrez and Orskov (1977), DE depends on fractions (a) and (b), the degradation rate c, and the feed passage rate. Liyama and Lam (2001) further emphasized that degradation characteristics vary with plant part, age, and lignification, reflecting intrinsic feedstuff properties.

## CONCLUSION

The degradation kinetics of crude protein in the four legumes over 4, 8, 12, 24, 48, and 72 hours of ruminal incubation are summarized as follows: Moringa ( $c = 0.17 \text{ h}^{-1}$ ; lag time = 3.60 h; DE = 64.99 %), Leucaena ( $c = 0.09 \text{ h}^{-1}$ ; lag time = 10.90 h; DE = 50.62 %), Indigofera ( $c = 0.09 \text{ h}^{-1}$ ; lag time = 6.03 h; DE = 59.72 %), and Gliricidia ( $c = 0.03 \text{ h}^{-1}$ ; lag time = 11.96 h; DE = 49.83 %). All four forages are readily degraded by rumen microbes, although they require varying adaptation periods before reaching maximal degradation. Overall, longer incubation times correspond to higher degradation extents.

## DECLARATIONS

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#### Author's contribution

M.Yahya contributed to data collection and data analysis, as well as drafting and writing the manuscript. I.Ismartoyo, and R.Islamiyati contributed to the experiments, ideas, and research design.

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#### Consent to publish

All authors agree to the publication of this manuscript.

**Data availability**

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

**Competing interests**

The authors declare no competing interests in this research and publication.

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