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Research Article
Crude Protein Yield, Total Digestible Nutrients and Tannin Content of Jack Bean (*Canavalia ensiformis*) at Various Growth Stages in Blora, Central Java, Indonesia

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Abstract
Background and Objective: Indigenous legumes of Indonesia, such as jack bean (*Canavalia ensiformis*), offer promising potential as alternative sources of energy and protein in ruminant nutrition. This study aimed to determine the crude protein yield, total digestible nutrient yield and total tannin of jack bean (*Canavalia ensiformis*) plant at different growth stages cultivated in alluvial soil at Blora, Central Java, Indonesia. **Materials and Methods:** This study used a Completely Randomized Block Design (RCBD) with four replications in every stage. Sixteen jack bean seeds were sown in 1×1 m^2 plots. The following parameters observed were crude protein (CP) yield, Total Digestible Nutrient (TDN) yield and total tannins, which were measured at three distinct growth stages, the vegetative, flowering and pod setting stages before harvesting. Data were analyzed using Analysis of Variance (ANOVA) and continued with Duncan's Multiple Range Test ($p<0.05$). **Results:** The growth stage had a significant effect ($p<0.05$) on the CP yield of leaves and stems. On the other hand, highly significant differences ($p<0.01$) were observed in the CP yield of stems, TDN yield of leaves, stems and total (leaves+stems) and total tannins. The CP yield of leaves and total yield increased significantly with advancing growth stages. Additionally, the CP yield of stems and TDN yield of leaves, stems and total yield increased very significantly with advancing growth stages. However, total tannins showed a highly significant decrease as the growth stage progressed. **Conclusion:** Harvesting at the pod-setting stage is the optimal stage for achieving the highest CP and TDN yields. On the other hand, total tannin content across all growth stages of jack bean plants is within safe levels for ruminant consumption.

Key words: Legume forage, local bean, nutrient, secondary metabolite, sustainability

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Feed security has become a hot topic in Indonesia to support the government's program in meeting the population's protein needs to achieve the Golden Indonesia 2045 target. This makes it necessary to increase feed production to support and enhance the productivity of ruminants as one of the instruments for producing sustainable protein food. Feed security, particularly for ruminants, can be achieved by increasing the biomass production of grasses and legumes. Although grasses have the advantage of high fresh biomass production, reaching up to 61.5 ton/ha in elephant grass cultivar Gama Umami and Rhodes grass (*Chloris gayana* Kunth.), the protein content, which is crucial for livestock productivity, is still categorized as low[1,2]. This issue must be addressed to produce forage that meets the quality and quantity requirements for ruminant feed on a national scale. One effort to address this is by supplementing legume plants as forage rich in protein content.

Legume plants are widely distributed in Indonesia, but their benefits remain largely unexplored, including the jack bean (*Canavalia ensiformis*). The jack bean is a plant with good environmental adaptability, capable of surviving under various conditions. It is drought-resistant and pest-tolerant, which are important factors in selecting feed plants for tropical areas like Indonesia. Additionally, jack bean has applications in food production, pharmaceuticals, livestock feed and green manure[3]. The jack bean can grow in areas with an annual rainfall range of 800-2000 mm, altitudes between 0-1800 m above sea level and various soil conditions. It thrives even in low-nutrient soil, high erosion levels, waterlogging and soil pH ranging from acidic to alkaline[4,5].

Biomass production is a critical factor in the selection of feed plants. Jack bean has high biomass production and nutrient content, making it a potential alternative feed for ruminants. It can produce around 21.81 ton/ha of fresh biomass and 11.29 ton/ha of dry matter in tropical environments. Studies show that jack bean forage contains 25.50% crude protein and has a high dry matter digestibility of 57.83% at six months of age under tropical conditions[6]. Various efforts have been made to increase biomass production, such as intercropping soybeans and grasses, particularly to improve crude protein and TDN yields[7,8]. This makes jack bean forage a highly potential feed crop for ruminants.

Like other legume plants, jack bean contains antinutritional factors that can limit its use as a feed crop. Jack beans contain antinutrients such as trypsin inhibitors, phytates, tannins and saponins[9]. However, studies on the antinutritional content, particularly tannins, in jack bean forage are still limited. Tannins in legume plants can affect feed consumption in livestock due to their bitter taste, reducing feed intake. Additionally, the tannin content in legume plants used as feed can lower dry matter digestibility in ruminants[10]. This issue needs attention, considering the high potential of jack bean as a feed crop with high biomass production and protein content.

The biomass production and tannin content of jack bean plants can be influenced by cutting time at the appropriate growth stage. Harvesting plants at the vegetative stage can reduce biomass production, but delaying harvesting can reduce forage quality. The dry matter and crude protein production of soybean plants decreased as the growth stage advanced[11,12]. Conversely, dry matter and crude fiber content increased with later growth stages. The older plant age reduced crude protein production while increasing prussic acid content in the leaves of *Crotalaria juncea* L.[13].

Studies on growth stages to optimize the quality and quantity of jack bean forage are still very limited. Therefore, further research is necessary to explore jack bean's crude protein production, TDN and total tannin content at different growth stages to enable its application as ruminant feed in areas with similar environmental conditions in Indonesia. The main objective of this study was to determine the crude protein production, TDN and total tannin content of jack beans at different growth stages, using it as an alternative protein source feed for ruminants.

MATERIALS AND METHODS

Plant material and experimental site: Jack bean (*Canavalia ensiformis*) accession seeds were used as plant material for this study. The study was carried out at an elevation of 100 m above sea level in Megeri Village, Kradenan District, Blora Regency, Central Java (7°21'40"S and 111°26'36"E) (Fig. 1). Laboratory analysis of nutrient and total tannin was conducted from December, 2023 to February, 2024 at the Faculty of Animal Science, Universitas Gadjah Mada, Sleman, Special Region of Yogyakarta (7°46'09.8"S and 110°23'11.4"E). Most of the land in Blora Regency is dry land. The soil type is characterized as alluvial with a slightly alkaline pH of 7.59. Jack beans can grow well in the soil pH from acid to alkaline[3]. The C-organic, total-N and available P soil is the low category, while the available K is high (Table 1). The climate of Blora Regency is tropical, with relatively high temperatures in the dry season and sufficient rainfall in the rainy season[6]. Precipitation and air temperature data for the location in 2023 (Fig. 2) were obtained from the Ministry of Public Works and People's Housing (PUPR) Bengawan Solo Data for Ngawi Regency, East Java.

Table 1: Soil characteristics and physical properties in the experimental site

Soil parameter[1,2]	Value
pH (H_2O)	7.9
C Organic (%)	0.50
N Total (%)	0.12
Available P (ppm)	1.33
Available K (Me %)	0.72

[1]C: Carbon, N: Nitrogen, P: Phosphorus, K: Potassium; [2]Analysis at Soil Laboratory, Faculty of Agriculture, Universitas Gadjah Mada, Indonesia

Fig. 1: Experimental site of *Canavalia ensiformis* research in Blora, Central Java, Indonesia

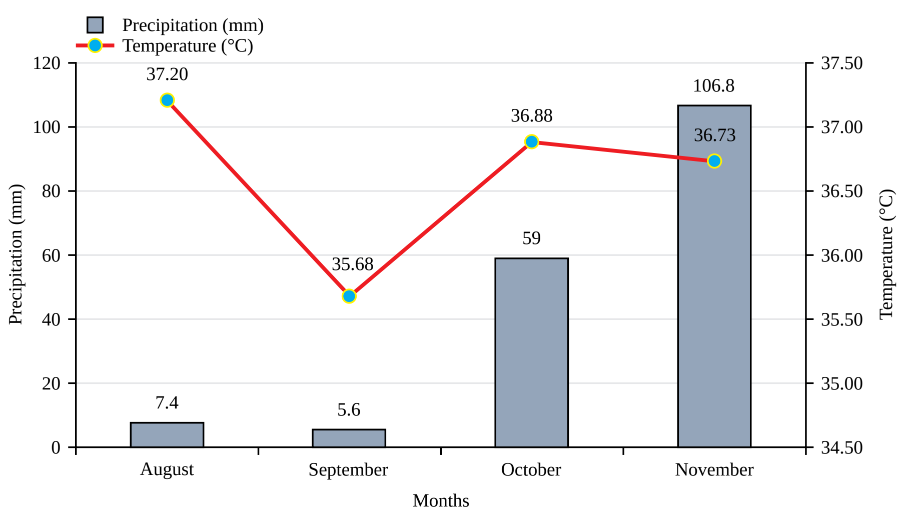

Fig. 2: Monthly precipitation and average temperatures during experimental cultivation of 2023

Climatic condition: Figure 2 shows the precipitation in April, 2024, coinciding with the planting period, recorded the highest value (5.73 mm) compared to other months during the observation and harvesting of jack bean plants (*Canavalia ensiformis*) in May, June and July, 2024 (0.00, 1.58 and 0.44 mm, respectively). On the other hand, the average air temperature in February, 2024 (34.25°C) was relatively lower than in May and June (34.78 and 36.34°C, respectively) but slightly higher than in July (34.22°C). The agroclimatic conditions during planting were extreme and not ideal for plant growth. Jack bean can grow well with a monthly precipitation of 58.33-333.33 mm[14] and within a temperature range of 20-30°C[15].

Experimental design and cultivation: The research area employed a randomized block design with three treatments and four replications in every stage. Each replication consisted of plots measuring 1 m^2 (1×1 m) with a total of 12 plots and a spacing of 0.5 m between plots, comprising three growth stages: Vegetative (V), flowering (F) and pod-setting (P). Planting was done by making holes in the soil approximately 2-3 cm deep[16], with one jack bean seed planted per hole at a spacing of 25×25 cm, in 4 rows, resulting in a plot density of 16 seeds/m^2. Plant maintenance included watering and weeding, with watering conducted once a day at 05:00 am if there was no rainfall and weeding done weekly.

Measurement of cutting and yield: The plant sample cutting was performed 14 cm from the soil surface in different plant growth stages. The cut plants were then weighed using a hanging scale to obtain fresh weight. After weighing the total fresh weight (leaves and stems), the separation between leaves and stems was conducted to weigh the fresh weight of each part separately[11]. Total fresh, fresh leaf and fresh stem weight is converted into ton/ha. The determination of CP yield is carried out by multiplying the fresh biomass production with the CP values of the leaves, stems and total, which have been previously analyzed. A similar method is applied to determine the TDN yield, where the previously calculated TDN values are multiplied by the fresh biomass production of leaves, stems and total. The CP and TDN yield values are then converted into ton/ha.

Laboratory analysis: The chemical analysis of nutrient content includes dry matter (DM), ash, crude protein (CP), crude fiber (CF) and ether extract (EE), was performed[17]. Nitrogen-free extract (NFE) and total digestible nutrient (TDN) content are calculated[18,19]. The DM is measured by drying the samples in an oven at 105°C to completely remove moisture, leaving only the dry matter. Ash content is analyzed by incinerating the sample in a furnace at 600°C for 4 hrs. The CP (calculated as N×6.25) is assessed using the Kjeldahl method, which includes digestion, distillation and titration steps. The CF is determined using solvents of strong acids and bases, while EE is measured using the Soxhlet extraction technique with petroleum ether (60-80°C) as the solvent. The Folin-Ciocalteu method is applied to analyze total tannin content.

Statistical analysis: The statistical analysis was used to compare the results of CP yield, TDN yield and total tannin among different growth stages. The data were statistically analyzed using Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) if significant differences were found (at a significance level of 95%) using SPSS 23.0 (SPSS Inc USA).

RESULTS AND DISCUSSION

Crude protein yield: The analysis results showed that the growth stages of jack bean plants had a significant effect ($p<0.05$) on leaf and total crude protein (CP) production and a highly significant effect ($p<0.01$) on the stem. Leaf CP yield showed an increasing trend during the flowering stage but decreased during the pod-setting stage. On the other hand, the stem CP yield of jack beans showed an increasing trend during the flowering and pod-setting stages. Similarly, total CP yield also showed an increasing trend during the flowering and pod-setting stages, with the highest total yield observed in the pod-setting stage.

The leaf CP yield differed from other plant parts, such as stems and total yield, which peaked during the pod-setting stage. The high leaf CP yield during the flowering stage may be attributed to the accumulation of metabolic products from the vegetative stage. Krawutschke *et al.*[20] stated that crude protein production in plants might increase as a result of a phenomenon termed "system growth" characterizing the vegetative growth stage. During this stage, plants use their nutrients primarily for growth and protein accumulation occurs only after the vegetative stage. Consequently, protein production peaks during the flowering stage.

Although there was no significant difference in leaf CP yield between the flowering and pod-setting stages, a downward trend in CP yield was observed from the flowering to the pod-setting stage. This decline might be influenced by the crude fiber content in the leaves, which increases as the plant matures. This was consistent with the statement by Krawutschke *et al.*[20], that crude protein content has a negative correlation with crude fiber content, where crude protein content decreases as crude fiber content increases.

Dewanti *et al.*[13] also noted that crude fiber content increases in older plants due to the development of structural tissues, leading to a reduction in crude protein content. Similar results were reported by Prasojo *et al.*[11], who observed a decline in crude protein production during the pod-setting stage in the leaves of soybean plants of the Moshi Dou Gong cultivar.

Stem and total CP yields showed an increasing trend during the flowering and pod-setting stages, although there was no significant difference between these two stages (Table 2). The increase in CP yield with advancing growth stages could be attributed to biomass production. Prasojo *et al.*[21] found that the fresh biomass production of jack bean plants tended to increase with growth stages. Krga *et al.*[22] stated that crude protein production is influenced by plant biomass production. Leguminous plants, such as peas, have high crude protein content when cultivated in monoculture compared to intercropping systems. However, peas grown in monoculture showed lower crude protein production due to lower biomass compared to intercropped systems.

Furthermore, the increase in stem and total CP yields during the flowering and pod-setting stages was due to the addition of plant organs such as flowers and pods. These stages coincide with a period when nitrogen is redistributed to the seeds and is temporarily present in the vascular tissues of the stem. Nitrogen remobilization in leguminous plants is considered an adaptive mechanism to dry environmental conditions caused by water deficits[11].

Total digestible nutrient yield: The analysis results showed that the growth stages significantly affected ($p<0.01$) the TDN yield of leaves, stems and the total TDN yield of jack bean plants (Table 2). The TDN yield of leaves, stems and total TDN yield showed an increasing trend with the advancement of the growth stages of jack bean plants. The highest TDN yields for leaves, stems and total were recorded during the pod-setting stage. Conversely, the lowest yields were observed during the vegetative stage.

The TDN yield of leaves increased until the pod-setting stage, although there was no significant difference between the flowering and pod-setting stages. The increase in TDN yield of leaves during the flowering and pod-setting stages could be attributed to the nutrient content available in the leaves of jack bean plants. Kaithwas *et al.*[23] stated that TDN values are influenced by the nutrient composition of the plant, which includes crude protein, crude fat, crude fiber and non-fiber carbohydrates that are used to calculate the TDN value. Leaves are plant organs rich in nutrients such as crude protein, making leaf biomass production an essential factor affecting TDN yield. Castro-Montoya and Dickhoefer[24] noted that the number of leaves in leguminous plants (such as *Medicago sativa* and *Trifolium pratense*) influences the nutrient content of leaves, with higher leaf biomass leading to increased nutrient content. Blumenthal *et al.*[25] and Solati *et al.*[26] found that harvesting plants during the reproductive growth stage results in higher leaf biomass and nutrient content compared to the vegetative growth stage.

A similar pattern was observed in the TDN yield of stems, which increased with plant growth stages. However, there was no significant difference between the flowering and pod-setting stages. Interestingly, the TDN yield of stems was lower compared to leaves, likely due to the higher crude fiber content in the stems. Witt *et al.*[27] noted that crude fiber content in leguminous plants is influenced by the stem-to-leaf ratio, with fiber content increasing as the stem ratio rises in leguminous plant biomass. High crude fiber content in stems is due to a higher proportion of structural tissue compared to leaves[10]. Javanmard *et al.*[28] mentioned that TDN values are highly influenced by fiber content, which represents the nutrients available for livestock. The TDN values illustrate the potential energy of forage feed, which correlates with the digestibility of dry matter and organic matter. Additionally, the increase in TDN yield of stems during the flowering and pod-setting stages may be due to the increased stem biomass. Leguminous plants such as jack bean, with a climbing growth habit, tend to produce high stem biomass. Rivera *et al.*[29] found that climbing leguminous plants produce longer stems, resulting in higher stem biomass as plant growth progresses. Prasojo *et al.*[21] stated that older plants produce more stems.

Table 2: Crude protein and total digestible nutrient yield of jack bean (*Canavalia ensiformis*) at each growth stage (Means±Standard Deviation)

	Growth stage[2] (DM ton/ha)		
Parameters[1]	Vegetative (V)	Flowering (R)	Pod-setting (P)
Leaf CP yield	3.39±0.43^{a}	5.28±0.47^{b}	4.92±0.58^{b}
Stem CP yield*	0.84±0.09^{a}	1.52±0.12^{a}	2.34±0.38^{b}
Total CP yield	4.20±0.51^{a}	5.93±0.51^{ab}	7.49±1.02^{b}
Leaf TDN yield*	5.45±0.70^{a}	10.53±0.94^{b}	11.82±1.40^{b}
Stem TDN yield *	3.23±0.36^{a}	6.50±0.53^{a}	11.44±1.86^{b}
Total TDN yield *	10.50±1.27^{a}	18.37±1.58^{b}	26.19±3.57^{c}

[1]CP: Crude protein, TDN: Total digestible nutrient; [2]V: 55 day after planting (DAP), R: 75 day after planting (DAP), P: 90 day after planting (DAP); [abc]Means in the same row with different superscripts differ significantly p<0.05 and *Means in the same row with different superscripts differ significantly p<0.01

Table 3: Total tannin content of jack bean (*Canavalia ensiformis*) at each growth stage (Mean±Standard Deviation)

Growth stage[1]	Total tannin (%)*
Vegetative (V)	1.60±0.18[b]
Flowering (R)	1.81±0.15[b]
Pod-setting (P)	1.17±0.31[a]

[1]V: 55 day after planting (DAP), R: 75 day after planting (DAP), P: 90 day after planting (DAP); [ab]Means in the same row with different superscripts differ significantly p<0.05 and *Means in the same row with different superscripts differ significantly p<0.01

The total TDN yield increased with plant growth stages, with the pod-setting stage showing the highest total TDN yield. This can be attributed to the higher leaf-to-stem ratio, which influences plant biomass production. Choosing the right growth stage for harvesting is crucial in determining biomass production and forage quality for ruminant feed. Similar findings were reported by other studies in leguminous plants such as *Vigna unguiculata*, *Glycine max* and *Crotalaria juncea*[9,10,30]. These studies found that the biomass production of leaves, stems and total increases at later growth stages compared to the vegetative stage. In other words, the proportion of leaves is critical in determining TDN yield. Singh and Mukhi[31] stated that TDN values correlate with leaf production, decreasing by 3.1% when leaf production drops by 10%. Efforts to improve total TDN yield in leguminous plants are necessary to enhance ruminant productivity. Darma *et al.*[32] suggested that increasing the leaf-to-stem ratio in leguminous plants and reducing indigestible fractions like stem crude fiber can positively impact the digestibility of dry matter and organic matter. This approach can mitigate factors that hinder microbial activity in the rumen, thus improving ruminant productivity.

Total tannins: The analysis of variance results indicated that the growth stages significantly affected (p<0.01) the total tannins of jack bean plants. A decrease in total tannin content was observed with advancing growth stages. As shown in Table 3, the pod-setting stage had the lowest total tannin content compared to the vegetative and flowering stages, although there was no significant difference between the vegetative and flowering stages. However, the highest total tannin content was significantly obtained during the flowering stage (p<0.01).

The total tannin analysis in this study revealed that the pod-setting stage had the lowest value. This may be due to the reduced leaf production during the pod-setting stage, as most tannins are found in plant leaves. The high tannin content in leaves serves as a defense mechanism against predators, including plants and animals. Li *et al.*[33] reported that advancing growth stages in leguminous plants such as *Onobrychis viciifolia*, *Dalea purpurea* and *Dalea candida* Michx. ex Willd leads to a reduction in tannin content. The low total tannin content during the pod-setting stage is closely related to the reduced proportion of tannin-rich leaves. The proportion of leaves decreases with advancing growth stages. Theodoridou *et al.*[34] stated that as growth stages progress, the proportion of leaves to the total plant decreases, resulting in lower tannin content in plants like sainfoin and alfalfa. Nevertheless, at the pod-setting stage, jack bean experiences an augmentation in biomass attributable to the formation of legumes. Concurrently, with the advancement of the plant age, there is an escalation in the biomass production exhibited by jack bean[35].

Interestingly, the total tannin content increased during the flowering stage compared to the vegetative stage, although the difference was not significant. This increase could be attributed to the presence of flowers, which influence the total tannin content. Studies by Li *et al.*[33] and Berard *et al.*[36] showed that the flowering stage in plants like *Dalea purpurea* Vent. and *Lotus corniculatus* resulted in higher tannin content than the vegetative stage. They reported that the flowers of these plants contain high tannin levels that protect their reproductive organs. Li *et al.*[33] added that the flowering stage involves high carbon storage, which can be used to produce secondary metabolites. MacAdam and Villalba[37] noted that plants like *Trifolium repens* L., can produce tannins in their flowers. This aligns with Sinha and Kumar[38], who stated that flavan-3-ols, monomers of tannins, are found not only in leaves and stems but also in reproductive organs such as flowers. Tannins are produced in plant organelles called tannosomes located in plant vacuoles[8]. Additionally, tannins can also be found in intercellular spaces and cell walls. However, factors influencing total tannin content in leguminous plants include environmental temperature, species, growth stages and accessions[39,40].

The total tannin content in jack bean plants at all growth stages was relatively low, ranging from 1.17 to 1.81% of DM. This is supported by Yusiati *et al.*[41], who reported total tannin contents in *Leucaena leucocephala*, *Arachis hypogaea* and *Gliricidia sepium* of 6.57, 1.99 and 4.30%, respectively. Total tannin content exceeding 5% of DM consumed by livestock could have negative effects, such as reduced feed intake and dry matter digestibility[34]. Conversely, the total tannin content in jack bean plants at all growth stages remained within the safe range (below 5%), which could positively impact livestock and the environment. Positive effects include reduced methane production in the rumen of ruminants. Lagrange *et al.*[42] explained that consuming *Medicago sativa* with tannin levels of 1-4% DM lowers ruminal protein degradability, indirectly reducing NH_3 production and subsequently methane production. Adding 2.5% DM of tannin extract from *Acacia mearnsii* reduces methane production while increasing VFA production, thus enhancing ruminant productivity[36]. Lima *et al.*[2] noted that tannin content in livestock feed ranging from 0.2 to 2.3% can reduce methane production and tannins in feed interact with components like protein and fiber. Girard *et al.*[35] emphasized that the interaction between tannins and protein prevents protein degradation in the rumen (protein protection), allowing feed protein to be digested in the abomasum and absorbed in the small intestine. This benefits ruminants by optimizing feed protein utilization, positively impacting their productivity.

CONCLUSION

Harvesting jack bean plants at the pod-setting stage (90 DAP) yields the highest CP and TDN yields for leaves, stems and total biomass. On the other hand, total tannin content is highest during the flowering stage compared to the vegetative and pod-setting stages. The total tannin content across all growth stages (vegetative, flowering and pod-setting) of jack bean plants is within safe levels for ruminant consumption. Further research is recommended to evaluate the effects of different harvesting stages on the *in vivo* digestibility and rumen fermentation characteristics of jack bean as a functional feed ingredient for ruminants. Additionally, exploring processing methods such as ensiling or chemical treatments to optimize the nutritional value and reduce potential antinutritional factors could enhance its applicability in ruminant feeding systems. Moreover, assessing the agronomic performance and environmental benefits of integrating jack bean into sustainable livestock production systems would provide valuable insights for its broader adoption.

SIGNIFICANCE STATEMENT

This study highlights the potential of jack bean (*Canavalia ensiformis*) as a functional feed ingredient for ruminants, particularly in regions with alluvial soils like Blora, Central Java, Indonesia. The findings indicate that advancing growth stages significantly enhance crude protein (CP) and Total Digestible Nutrient (TDN) yields, making the pod-setting stage the optimal harvest period for maximizing nutrient availability. Additionally, the reduction in total tannin content with maturity ensures its safety for ruminant consumption. These insights contribute to the development of sustainable and locally sourced high-protein feed to support national feed security and environmental sustainability.

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