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REGULAR ARTICLE

# Comparative Nutritional Profiles and Fermentation Characteristics of Selected Tropical Legumes Subjected to Intensive Coppicing

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**Abstract:** This study evaluates the nutritional profiles and fermentation characteristics of three selected tropical legumes, *Flemingia macrophylla*, *Indigofera zollingeriana*, and *Gliricidia sepium*, from fresh states through post-ensiled fermentation under a repeated coppicing regime. A Randomized Complete Block Design was employed across four three-month coppicing periods. Samples were evaluated for DM, CP, EE, ash, NDF, ADF, GE, and silage pH and temperature. Forages were ensiled for 21 days with a 5% molasses additive. The analysis was done through ANOVA and Linear Mixed Model using R software. Post hoc Tukey's HSD for significant differences ($\alpha = 0.05$). Repeated coppicing reduced pre-ensiled DM across all species ($p<0.0001$), reflecting progressive succulence in late-period regrowths. Increasing defoliation increased CP content in *I. zollingeriana* ($p=0.0121$) and *G. sepium* ($p<0.0001$) through maximization of leaf-to-stem ratios, while *F. macrophylla* had low initial CP levels and accumulated NDF ($p=0.0035$). In the post-ensiling period, *G. sepium* maintained a very stable pH level of 4.30 in its silage in the third phase ($p<0.0001$), which indicates almost perfect homofermentation. *Indigofera zollingeriana* had moderate silage acidification (pH 5.56-6.64) and underwent considerable proteolysis (CP loss: 10.50%), although acid hydrolysis of NDF brought down NDF content to 28.65%. The pH value in *F. macrophylla* did not show any steep drop (4.75-6.11) because of high buffering capacity. However, it was completely stabilized in terms of temperature to avoid aerobic spoilage. *Gliricidia sepium* produced the most chemically stable and easily digestible silage, thereby making it an ideal forage for tropical ruminants. A coppicing period of 90 days, before ensiling with wilting at a dry matter level of 30%, and addition of 5% molasses are suggested.

**Keywords:** coppicing; fermentation; forage legumes; *Flemingia macrophylla; Indigofera zollingeriana*; *Gliricidia sepium*

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## 1. Introduction

Feeding strategies for ruminants in year-round tropical production have inherent limitations owing to the seasonality of changes in forage quality. During long dry periods, there is a quick maturation of native grasses, which leads to increased lignification and decreased CP levels, ultimately resulting in reduced voluntary intake and reduced digestibility of nutrients. The use of tropical legumes in basal diets offers the advantage of drought resistance, high biomass yield, and provision of amino acids due to their ability to fix nitrogen symbiotically. Some good options for such species in this regard are *F. macrophylla*, *I. zollingeriana*, and *G. sepium*. *Flemingia macrophylla* grows well on acid aluminum-toxic soil conditions and gives a high yield of fresh biomass per hectare per annum. It does have relatively high levels of condensed tannins (6–11%) and saponins, which might result in an increase in structural fibers, but the positive aspect of using this plant is its ability to reduce enteric methane production. *I. zollingeriana* has an excellent leaf-to-stem ratio, fast re-sprouting after defoliation, and high CP levels, giving high dry matter per hectare per annum. *Gliricidia sepium* is a multipurpose leguminous fodder tree renowned for its adaptability across wide tropical geographic ranges, excellent symbiotic nitrogen-fixing capacity, and high leaf crude protein content (20–30% under optimized management), though its utilization involves consideration of anti-nutritional factors like coumarins (Silva et al., 2024; Tahuk et al., 2022).

Although possessing many agronomical advantages, the continuous conservation of these protein-rich legumes via anaerobic ensiling poses major biochemical obstacles. For effective fermentation in silage-making, epiphytic lactic acid bacteria must quickly metabolize water-soluble carbohydrates into lactic acid to reduce the silo pH to less than 4.5 to arrest the cellular respiration process and block the activity of putrefying bacteria like Clostridia and Enterobacteria (McDonald et al., 1991). But legumes create a double whammy for the silo fermentation process: the presence of very little WSC and extremely high natural buffering ability due to the abundance of organic acids, mineral cations, and ammonia formation.

Thus, the biomass of legumes exhibits a strong resistance to sudden reductions in pH, which typically result in large amounts of nitrogen gas loss, extreme reduction in the dry mass of the crop, and the creation of an undesirable and poisonous silage high in butyric acid (Figueiredo et al., 2025). Repeated coppicing increases tissue succulence (lowering dry matter), reduces water-soluble carbohydrates, and alters the inherent buffering capacity through increased protein concentration, directly influencing subsequent fermentation outcomes. This issue is overcome through the use of additional WSC sources, such as molasses, that would activate the homofermentation pathways of lactic acid bacteria (LAB) to surpass the buffer threshold of the legume (Zi et al., 2022). Moreover, the agronomic practices involving the timing and frequency of coppicing greatly impact the physio-morphological state of the plant before ensiling.

Therefore, the primary objectives of this study are: (1) to assess the impact of successive coppicing periods on the pre-ensiling nutritional composition of the selected legumes; (2) to assess the impact of successive coppicing periods on post-ensiling fermentation quality; and (3) to identify which of the selected legumes will be most suitable for long-term forage conservation under tropical conditions.

## 2. Materials and Methods

### *2.1 Planting and Establishment*

The research location was situated in Baybay, Leyte, Philippines, on campus at Visayas State University (10.74° N, 124.79° E). Baybay has a type IV climate, receiving an average of 2600 mm of rain per year, with an average temperature of 27.5°C. Soil in this area is classified as clay loam. Baseline soil analysis revealed a slightly acidic pH ranging from 6.05 to 6.14, total organic matter of 1.92–2.30%, total nitrogen of 0.17–0.21%, available phosphorus of 26.20–49.20 mg/kg, and exchangeable potassium of 74.66–120.66 mg/kg. The forages *F. macrophylla* and *I. zollingeriana* were planted using seeds, while the

*G. sepium* plant was planted using stem cuttings. The planting materials were cultivated in polythene bags containing fertile soil for three months in a nursery, and were watered every day before transplanting. Preparation of the land was done in order to enhance soil conditions, and the plant materials were transplanted three months after seed planting. At the age of one year, all forages were harvested for the first time through coppicing. Four other coppices were done every three months. These harvests occurred from March 2024 to February 2025, capturing one dry season (Period 1: 228.5 mm rainfall, 74% humidity) and three wet seasons (Periods 2–4: 592.9–1,029.6 mm rainfall, 80–86% humidity).

*2.2 Ensiling Procedure*

All forages were wilted and air-dried before ensiling to achieve a dry matter content of 30%, thereby preventing excessive anaerobiosis associated with too much moisture content, promoting the growth of clostridia rather than lactic acid bacteria (FAO, 2020). The forage was then chopped into pieces of 2 to 4 cm in length to aid in compaction and promote ruminal peristalsis. An exogenous water-soluble carbohydrate, molasses, was added at 5% of the fresh weight to stimulate the growth of homofermentative LAB due to the high buffering power of legume forages (Zi et al., 2022). This 5% molasses addition was based on the weight of the wilted and air-dried biomass, and no water was added.

*2.3 Chemical Analysis*

The fresh and ensiled samples were analyzed in the Nutrition Laboratory of the Department of Animal Science, Visayas State University, using the AOAC methods for proximate and fiber analyses on a dry matter basis. Leaf-to-stem ratios were calculated using dry matter weights, and all reported post-ensiling DM values refer strictly to oven-dried silage DM, not as-fed DM. Dried samples were milled using a 1-mm mesh sieve. Dry Matter was estimated according to AOAC method 930.15; Crude Protein by AOAC method 990.03; Ether Extract by the procedure of Thiex et al. (2003); Total Ash by AOAC method 942.05; and Gross Energy by adiabatic bomb calorimetry. The contents of Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) were estimated by AOAC method based on the Sequential Detergent Procedure of Van Soest et al. (1991). Silage temperature was recorded immediately upon silo opening at day 21, measured at the Nutrition Laboratory of the Department of Animal Science, VSU.

*2.4 Experimental Design and Statistical Analysis*

The experimental design followed a Repeated Measures Randomized Complete Block Design without sub-sampling. The field layout evaluated three legume species subjected to four coppicing period treatments, replicated across four spatial blocks oriented horizontally along a field moisture gradient from a wetter to a drier area. The three-month intervals of coppice harvests made up the repeated-measures factor. The analysis was done through ANOVA and Linear Mixed Model to address the violation of the assumption of sphericity revealed by Mauchly's test using R software. Post hoc Tukey's HSD for significant differences ($\alpha = 0.05$).

## 3. Results and Discussion

*3.1 Chemical Profile of F. macrophylla Pre- and Post- Ensiling*

The nutritional analysis of fresh forage for *F. macrophylla* showed that its chemical composition was impacted by the repeated coppicing on multiple fronts (see Table 1). The content of DM fell from 38.10% in Period 1 to 27.50% in Period 4 ($p<0.0001$), indicating how the mobilization of non-structural carbohydrates stored in the roots occurred to maintain vigorous regenerative growth after each new defoliation event (Figueiredo et al., 2025; Nadir et al., 2024). Since the juvenile plants are active and consist of primary, thin-walled cells with plenty of water inside them, their DM content is bound to be lower at each defoliation cycle.

Crude protein remained unaffected by coppicing periods (p = 0.5817), remaining stable at 6.70-9.61%, indicating the biological hardiness of *F. macrophylla* symbiotic nitrogen-fixing root nodules, with continuous nitrogen fixation efficiency regardless of any defoliation severity (Mashudi et al., 2023). The relatively higher CP levels recorded in Periods 2 and 4 are indicative of the greater leaf-to-stem proportion of new regrowth biomass because of the natural tendency of young leaves to have more photosynthetic enzymes like Rubisco (Azzahra et al., 2025). However, *F. macrophylla* remains to have a lower protein content compared to other similar tropical leguminous plants (Asare, 1985).

Ether extract increased from 2.00% in Period 1 to 8.51% in Period 3, sustained throughout Period 4 (p<0.0001). Such an EE increase is attributed to the increasing dominance of leaves in harvested biomass, following the impact of intense coppicing on the formation of secondary woody stems. Epicuticular waxes and galactolipids that form part of chloroplast membranes are synthesized exclusively by leaves (Nadir et al., 2024). The ash content underwent a steep peak in Period 2 up to 7.05%, followed by steady levels of approximately 4.20-4.55%, which can be linked to the activation of mineral absorption after canopy damage (Ernakovich et al., 2014). NDF increased steadily from 56.31% to 73.70% (p=0.0035), associated with rapid synthesis of hemicellulose in newly formed cell walls (Meel et al., 2025). ADF exhibited a brief peak at 65.74% during Period 2, subsequently decreasing to 45.85% by Period 4 (p=0.0012), thus indicating the occurrence of a stress-induced temporal lignification process which later resulted in perpetual juvenility of regrowth tissues with lower lignification. On the other hand, GE values stayed constant at 4,121-4,164 cal/g (p=0.489), thus confirming that the caloric content of *F. macrophylla* is a remarkably stable property determined by the basic bonds of its carbon structure (Viennasay & Wanapat,2020). The substantially higher gross energy of *F. macrophylla* in relation to its low protein is explained by its high ether extract. Since Ether extract possesses approximately 2.25 times the caloric density of carbohydrates, this EE (%) accumulation accounts for the elevated total energy profile compared to the other legumes.

Table 1. Chemical profile of *F. macrophylla* pre-ensiling (fresh) across four coppicing periods

| Parameters | Coppicing Period | | | | SEM | p-value |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | | |
| DM, % | 38.10a | 30.80b | 30.00b | 27.50b | 1.13 | <0.0001 |
| CP, % | 6.70 | 9.61 | 8.06 | 9.54 | 0.17 | 0.5817 |
| EE, % | 2.00b | 6.81a | 8.51a | 7.45a | 0.71 | <0.0001 |
| Ash, % | 1.24c | 7.05a | 4.55b | 4.20b | 0.30 | <0.0001 |
| ADF, % | 53.07b | 65.74a | 46.37b | 45.85b | 3.21 | 0.0012 |
| NDF, % | 56.31b | 72.02ab | 73.70a | 72.45a | 4.05 | 0.0035 |
| GE (cal/g) | 4125 | 4121 | 4129 | 4164 | 24.4 | 0.489 |

*Means within the same row with different superscripts differ significantly (Tukey's HSD, P < 0.05)*

After ensiling, DM was affected by coppicing period (p=0.0048), with Period 2 recording the highest value (80.83%). This reflects both the pre-ensiling wilting protocol and Period 2's morphological transitional state, where plants began depositing structural carbohydrates, while the lower DM in Periods 3 and 4 (67.78% and 70.04%) reflects the plant's increasing reliance on root reserves to produce succulent vegetative regrowths (Alam et al., 2026). CP was not affected by coppicing period post-ensiling (p=0.1619), though values declined markedly relative to fresh forage (4.60–11.90%). This reduction is attributable to plant-mediated protease activity and clostridial deamination: plant enzymes cleave proteins into soluble non-protein nitrogen (NPN) before anaerobic acidification is achieved, and if pH remains elevated, putrefactive clostridia further deaminate free amino acids into volatile ammonia ($NH_3$-N), which volatilizes during oven-drying (Wulandari et al., 2023).

Silage pH was not affected by coppicing period (p=0.1199), ranging from 4.75 to 6.11. A critical finding was that *F. macrophylla* consistently failed to achieve the standard preservation threshold of pH 4.5 even with 5% molasses supplementation. This is because of the high organic acid concentration and mineral cation base of the crop, which act as effective buffers to the buildup of hydrogen ions (Viennasay & Wanapat, 2020). The temperature during the ensiling process was significantly influenced by the coppicing period (p<0.0001). This is because Period 1 had higher DM content (38.10%), making it physically tough forage that could not compact easily due to the presence of air, thereby causing prolonged heat production through aerobic respiration (Borreani et al., 2018; Kung et al., 2018). On the other hand, the succulent regrowth of Periods 2-4 compacted easily due to the absence of air and consequently created anaerobic conditions.

Table 2. Chemical Profile of *F. macrophylla* Post-ensiling (Silage) Across Four Coppicing Periods

| Parameters | Coppicing Period | | | | SEM | p-value |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | | |
| DM, % | 70.42ab | 80.83a | 67.78b | 70.04b | 4.90 | 0.0048 |
| CP, % | 4.60 | 11.90 | 5.70 | 8.58 | 1.80 | 0.1619 |
| EE, % | 1.07c | 5.28ab | 3.50b | 7.39a | 0.16 | <0.0001 |
| Ash, % | 3.72c | 7.33a | 8.02ab | 7.09b | 0.50 | 0.0006 |
| ADF, % | 51.62b | 57.12ab | 60.06a | 51.93b | 2.10 | 0.005 |
| NDF, % | 57.31 | 55.60 | 59.03 | 61.73 | 1.87 | 0.1385 |
| GE (cal/g) | 4140 | 4139 | 4114 | 4132 | 34.00 | 0.3499 |
| pH | 5.23 | 6.11 | 4.75 | 5.20 | 0.34 | 0.1199 |
| Temp. | 30.61a | 30.27b | 30.23b | 30.36b | 0.04 | <0.0001 |

*Means within the same row with different superscripts differ significantly (Tukey's HSD, P < 0.05)*

### *3.2 Chemical Profile of I. zollingeriana Pre- and Post- Ensiling*

Fresh DM percentage in *I. zollingeriana* reduced significantly from 40.50% in Period 1 to 19.20% in Period 4 (p<0.0001), highlighting morphological plasticity in response to leaf removal pressure in this plant (Table 3). Due to repeated cutting, this plant is forced to grow succulent, water-containing vegetation again, and the time gap of three months ensures that no further woody growth occurs in between (Figueiredo et al., 2025; Antari et al., 2023). This very low percentage of fresh DM poses serious problems for ensiling, as stringent wilting is needed to avoid putrefaction.

Unlike *F. macrophylla*, the CP of *I. zollingeriana* showed a directional increase with successive coppicing periods, varying from 9.45% in Period 1 to 20.54% in Period 4 (p=0.0121). This shows that *I. zollingeriana* possesses the greatest CP responsiveness among the three species studied. Continuous defoliation inhibited the growth of nutrient-deficient woody stems, hence achieving an optimal leaf-to-stem ratio, as photosynthetic protein production, such as Rubisco, occurred only in the harvested part (Azzahra et al., 2025; Nadir et al., 2024). The observed figures were comparable with those recorded by Antari et al. (2023), where CP was greater than 20% in repeatedly clipped *I. zollingeriana*, making it the forage plant with the highest amount of protein content among those tested. The ash content increased significantly from 1.65% in Period 1 to more than 9% in other periods (p<0.0001), indicating that the activation of mineral transport in the post-canopy defoliation phase is highly active (Ernakovich et al., 2014). GE exhibited a decline after Period 1 (3,888 cal/g) to a new lower plateau level (p=0.0008).

Table 3. Chemical profile of *I. zollingeriana* pre-ensiling (fresh) across four coppicing periods.

| Parameters | Coppicing Period | | | | SEM | p-value |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | | |
| DM, % | 40.50$^{a}$ | 25.80$^{b}$ | 20.80$^{bc}$ | 19.20$^{c}$ | 0.06 | <0.0001 |
| CP, % | 9.45$^{b}$ | 9.94$^{b}$ | 12.64$^{ab}$ | 20.54$^{a}$ | 0.131 | 0.0121 |
| EE, % | 5.26 | 9.09 | 9.56 | 10.13 | 1.31 | 0.058 |
| Ash, % | 1.65$^{b}$ | 9.34$^{a}$ | 10.79$^{a}$ | 10.95$^{a}$ | 0.09 | <0.0001 |
| ADF, % | 36.83$^{a}$ | 34.66$^{ab}$ | 40.98$^{a}$ | 23.45$^{b}$ | 3.74 | 0.0136 |
| NDF, % | 30.01$^{b}$ | 56.24$^{a}$ | 46.14$^{ab}$ | 49.84$^{ab}$ | 5.29 | 0.0181 |
| GE (cal/g) | 3888$^{a}$ | 3572$^{b}$ | 3439$^{b}$ | 3532$^{b}$ | 64.9 | 0.0008 |

*Means within the same row with different superscripts differ significantly (Tukey's HSD, P < 0.05)*

The post-ensiling characteristics of *I. zollingeriana* showed a vital gap between the nutritive value of the fresh material and the silage quality (Table 4). The silage pH was found to be the most crucial diagnostic parameter, which was relatively high (6.64 in Period 1 and 6.66 in Period 2) but then dropped to 5.56 only in Period 3 (p<0.0001). Such pH values are relatively high in relation to the preservation requirement for leguminous species, which should be around 4.5 (McDonald et al., 1991). Hence, the use of 5% molasses was ineffective against such a strong buffering property of the plant during the first two periods of cutting. The unusually high content of CP in fresh material resulted in an increased buffering effect due to a significant number of organic acids, basic amino acids, and ammonia obtained from the quick action of plant proteases on proteins, thus neutralizing the lactic acid produced by lactobacilli (Jayanegara et al., 2020; Mashudi et al., 2023).

Table 4. Chemical profile of *I. zollingeriana* post-ensiling (silage) across four coppicing periods.

| Parameters | Coppicing Period | | | | SEM | p-value |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | | |
| DM, % | 78.92$^{a}$ | 57.50$^{b}$ | 41.41$^{c}$ | 37.62$^{c}$ | 0.04 | <0.0001 |
| CP, % | 7.03 | 11.79 | 10.59 | 10.50 | 1.32 | 0.1437 |
| EE, % | 2.23$^{b}$ | 7.20$^{a}$ | 3.40$^{b}$ | 9.50$^{a}$ | 0.70 | 0.0002 |
| Ash, % | 6.64$^{c}$ | 11.83$^{ab}$ | 12.07$^{a}$ | 10.35$^{b}$ | 0.41 | <0.0001 |
| ADF, % | 21.79$^{b}$ | 26.54$^{ab}$ | 30.49$^{a}$ | 21.70$^{a}$ | 1.34 | 0.0021 |
| NDF, % | 25.03 | 23.84 | 24.96 | 28.65 | 1.84 | 0.3248 |
| GE (cal/g) | 3789$^{a}$ | 3594$^{bc}$ | 3453$^{c}$ | 3644$^{ab}$ | 37.8 | <0.0001 |
| pH | 6.64$^{a}$ | 6.66$^{a}$ | 5.56$^{c}$ | 5.60$^{b}$ | 0.06 | <0.0001 |
| Temp. | 30.73$^{a}$ | 30.23$^{b}$ | 30.27$^{b}$ | 30.28$^{b}$ | 0.04 | <0.0001 |

*Means within the same row with different superscripts differ significantly (Tukey's HSD, P < 0.05)*

The effects of partial acidification were observed through protein loss. In period 4, fresh protein content was recorded as 20.54%, while post-ensiled protein content remained stable at 10.50%. The protein content was reduced by about 50% of its original nitrogen content. This extensive proteolysis took place through a series of steps, where plant proteases within the cell hydrolyzed proteins into peptides and amino acids following rupture of the cells, and the high pH facilitated the conversion of these compounds to volatile ammonia by clostridial deaminases (Saez-Plaza et al., 2013; Azzahra et al., 2025). Despite this limitation, NDF collapsed dramatically from fresh peaks of 56.24% to post-ensiling values of 23.84–28.65% (p=0.3248 for the post-ensiling comparison, reflecting uniform acid hydrolysis across periods). This beneficial hemicellulose hydrolysis by fermentation-derived organic acids effectively pre-digested the structural cell wall, enhancing potential ruminal colonization. ADF also remained low (21.70–30.49%), reflecting the highly digestible cell wall profile maintained through intensive coppicing, which ruminants can access more readily.

*3.3 Chemical Profile of G. sepium Pre- and Post- Ensiling*

*Gliricidia sepium* exhibited a nutritional trajectory under repeated coppicing that was markedly distinct from the other two species, particularly in its enhanced fermentation outcomes (Table 5). Fresh DM declined from 40.90% in Period 1 to 20.00% in Period 4 (p=0.0011), reflecting physiological rejuvenation under repeated coppicing as apical dominance was broken and non-structural carbohydrates were mobilized from root reserves to generate succulent vegetative regrowths (Figueiredo et al., 2025; Nadir et al., 2024). CP increased dramatically from 8.14% in Period 1 to 19.10% in Period 4 (p<0.0001), the most decisive CP progression observed among all species. Repeated coppicing suppressed nutrient translocation into dense, nitrogen-poor woody stems, concentrating photosynthetic enzymes such as Rubisco in foliar tissues harvested at each cutting (Azzahra et al., 2025; Silva et al., 2025). This consistent trajectory of increasing CP under defoliation management confirms *G. sepium*'s responsiveness to harvest intensity as a management lever for forage quality improvement.

Table 5. Chemical profile of *G. sepium* pre-ensiling (fresh) across four coppicing periods.

| Parameters | Coppicing Period | | | | SEM | p-value |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | | |
| DM, % | 40.90$^{a}$ | 24.20$^{b}$ | 21.70$^{b}$ | 20.00$^{b}$ | 2.65 | 0.0011 |
| CP, % | 8.14$^{b}$ | 7.82$^{b}$ | 10.63$^{b}$ | 19.10$^{a}$ | 0.08 | <0.0001 |
| EE, % | 4.47$^{b}$ | 9.49$^{a}$ | 8.98$^{a}$ | 10.32$^{a}$ | 0.77 | 0.0011 |
| Ash, % | 1.32$^{b}$ | 6.63$^{a}$ | 5.79$^{a}$ | 5.52$^{a}$ | 0.49 | <0.0001 |
| ADF, % | 33.47$^{ab}$ | 39.09$^{a}$ | 40.37$^{a}$ | 26.42$^{b}$ | 2.60 | 0.0175 |
| NDF, % | 44.82 | 52.01 | 64.51 | 61.74 | 5.65 | 0.1194 |
| GE (cal/g) | 3704 | 3834 | 3787 | 3691 | 77.10 | 0.386 |

*Means within the same row with different superscripts differ significantly (Tukey's HSD, P < 0.05)*

Ether extract increased very quickly from 4.47% (Period 1) to a plateau value greater than 8.98% (Periods 2–4, p=0.0011) owing to the development of a dominant leaf composition with high levels of chloroplast galactolipids and epicuticular waxes (Abdullah & Suharlina, 2010). Ash levels were found to increase from 1.32% in Period 1 to more than 5.5% in other periods (p<0.0001) as a result of active mineralization due to canopy regrowth (Ernakovich et al., 2014). The levels of NDF were not influenced significantly by coppicing period (p=0.1194) as total wall formation did not change much since the plant continued to form a flexible hemicellulose wall structure (Antari et al., 2023). ADF level decreased to 26.42% in Period 4 (p=0.0175) as expected, as intensive coppicing increasingly inhibited lignin synthesis in tissues, thereby preserving tissues in an ever-young stage, which is highly desirable for animal digestion (Ath-Thifa et al., 2024). The GE levels were not influenced significantly either (p=0.386)

The post-ensiling performance of *G. sepium* demonstrated the most superior fermentation outcomes among all species evaluated, with silage pH achieving 4.30 in Period 3 and 4.35 in Period 4 — the only silage values to breach the critical 4.5 preservation threshold across the entire study (Table 6, p<0.0001). This progressive acidification improvement across coppicing cycles was directly linked to the plant's increasing succulence (declining DM) in later periods, which enhanced substrate availability and LAB colonization efficiency. The 5% molasses additive supplied sufficient exogenous WSC for epiphytic LAB to generate a decisive lactic acid surge that completely overcame the species' buffering capacity

(Luo et al., 2021; Yang et al., 2025). The achievement of pH below 4.5 represents a qualitative inflection point: at this acidity, plant proteases are irreversibly denatured, and putrefactive Clostridia are completely inhibited, creating a biochemically stable environment for long-term nitrogen preservation (McDonald et al., 1991). CP conservation in *G. sepium* silage further validated fermentation superiority. The silage CP values stayed constant at 8.30–9.27% during all periods ($p = 0.8904$), unlike *I. zollingeriana*, which underwent up to 50% loss in CP content. These constant values indicate that the sharp pH drop in Periods 3 and 4 was enough to denature protein-degrading enzymes and suppress activity of Clostridia bacteria until any nitrogen degradation happened (Yang et al., 2025). Despite seeming low compared to fresh *G. sepium* (19.10% in Period 4), these values reflect effective biochemical silage sealing, where nitrogen was not lost as ammonia gas but stored in a stable, ruminally digestible compound (Yang et al., 2025). Post-ensiled ADF reached a maximum value of 44.96% in Period 2 and later reduced to lower levels in Periods 3 and 4 ($p = 0.005$). This trend continued due to the inability of anaerobic LAB to break down the complex lignocellulosic material (Azzahra et al., 2025). GE stayed constant at 3,866–3,924 cal/g in post-ensiled material ($p = 0.422$), showing the high efficiency of energy conservation using a homofermentative fermentation mechanism enhanced by molasses addition (Luo et al., 2021).

Table 6. Chemical profile of *G. sepium* post-ensiling (silage) across four coppicing periods

| Parameters | Coppicing Period | | | | SEM | p-value |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | | |
| DM, % | 82.00$^{a}$ | 33.33$^{b}$ | 44.45$^{b}$ | 45.02$^{b}$ | 2.70 | <0.0001 |
| CP, % | 8.30 | 9.04 | 8.82 | 9.27 | 1.08 | 0.8904 |
| EE, % | 3.13$^{b}$ | 8.88$^{a}$ | 6.29$^{a}$ | 8.64$^{a}$ | 0.62 | 0.0005 |
| Ash, % | 7.05$^{b}$ | 12.55$^{a}$ | 12.93$^{a}$ | 12.84$^{a}$ | 0.37 | <0.0001 |
| ADF, % | 28.33$^{b}$ | 44.96$^{a}$ | 38.46$^{ab}$ | 31.24$^{b}$ | 2.82 | 0.005 |
| NDF, % | 41.60 | 40.20 | 47.60 | 45.97 | 0.05 | 0.0576 |
| GE (cal/g) | 3915 | 3924 | 3871 | 3866 | 29 | 0.4223 |
| pH | 6.46$^{a}$ | 5.73$^{b}$ | 4.30$^{d}$ | 4.35$^{c}$ | 0.0054 | <0.0001 |
| Temp. | 30.73$^{a}$ | 30.21$^{b}$ | 30.20$^{b}$ | 30.27$^{b}$ | 0.00112 | <0.0001 |

*Means within the same row with different superscripts differ significantly (Tukey's HSD, $P < 0.05$)*

### *3.4 Comparative Fermentation Dynamics and Species Suitability*

Three species were distinguished in their fermentation characteristics, which, however, appeared to follow the same mechanism. *Gliricidia sepium* proved to have the most beneficial fermentation profile characterized by a gradual CP accumulation during coppicing, followed by an effective fermentation acidification process; thus, it became the only species that achieved the preservation pH level below 4.5 during fermentation. A lesser buffering load and hence the better action of molasses addition in comparison with *I. zollingeriana*, both having relatively high levels of CP accumulation at the late periods of growth, may point to *G. sepium*'s leaf tissue susceptibility to acidification. The case of *I. zollingeriana* may seem contradictory in terms of its agronomics (as an excellent high-CP fresh feed up to 20.54% of CP by Period 4) and biochemistry (as a difficult silage crop). Its poor fermentation process and fast proteolysis led to a loss of about 50% of CP. In terms of fresh-cut supplementation or wilted feeding for a limited period of time, *I. Zollingeriana* cannot be surpassed. But for long-term preservation as silage, the natural buffering nature requires further management measures beyond just adding 5% molasses, which has been used in this study. *Flemingia macrophylla* fills an important niche that is unique among the three plants. Despite the high tannin levels that prevent acid formation during fermentation, the ability of tannins to buffer protein digestion and act as a protein bypass (thus preventing proteolysis in the rumen) is highly valued, along with the reduction in enteric methane production (Viennasay &

Wanapat, 2020). Due to the accumulation of NDF (>72%) and high buffering capacity that prevents acid production in silages, this plant is not suitable for pure silage preparation but is highly valued as a complementary forage.

## 4. Conclusion

The process of coppicing thrice over a period of three months had a profound effect on the nutritional and fermentation traits of the tropical legumes examined by lowering lignin concentration in the biomass and enhancing forage digestibility. The legume that stood out amongst the rest in terms of fermentability, nitrogen conservation, lower fiber proportions, and consistent energy levels, when mixed with 5% molasses, was *G. sepium*. The one species that recorded the highest amount of crude protein when subjected to coppicing, but whose fermentation process resulted in low-quality silage and substantial protein loss, was *I. zollingeriana*. *F. macrophylla* did not have great potential in terms of silage, due to its high fiber concentration and inability to be acidified effectively, even though its nutritive value, adaptability, and ability to conserve proteins with tannins make it a great forage crop.

According to the results presented above, it can be said that species utilization should correlate with the goal of production, whereby the use of *I. zollingeriana* for protein enrichment, *G. sepium* for production of silage and long-term storage of forage material, and *F. macrophylla* for degraded land rehabilitation and feed for animals with low requirements should be encouraged. It can also be suggested to use a 90-day interval of coppicing for maximum leaf yields, minimum lignification, and highest forage quality suitable for ensiling. Furthermore, it can be advised to include 5% of molasses to promote lactic acid fermentation, decrease pH, and reduce nitrogen losses. Pre-ensiling wilting of at least 30% of dry matter is also necessary to improve the fermentation process and prevent undesired microbial activity. Farmers can rely on *G. sepium* to produce a chemically stable silage containing 8.82–9.27% preserved crude protein, as a supplemental source of protein for animals, per day, while optimizing annual dry matter harvests. The future direction of further studies is supposed to include improvement of *I. zollingeriana* fermentation properties through higher inclusion of molasses and/or microbial inoculants; measurement of condensed tannin content of *F. macrophylla* silages; determination of in vivo digestibility and effects on livestock performance by silaging *G. sepium*. Limitations of the current study included that no quantifiable tannin analysis was conducted, and animal growth performance data; these findings require further empirical validation before broad application across diverse tropical ecosystems.

## 5. Competing Interests

The authors declare no conflicts of interest regarding the publication of this paper.

## 6. Authors' Contributions

All authors contributed significantly to the work reported in this manuscript. Manuel D. Gacutan Jr. conceived and designed the study and performed the data analysis. Richmond M. Joromat executed the data collection. Richmond M. Joromat, Oscar B. Posas, Jade Dhapnee Z. Compendio, and Manuel D. Gacutan Jr. conducted the literature review, developed the data visualization, and provided critical revisions during the manuscript drafting process. All authors read, approved the final manuscript, and agree to be accountable for all aspects of the work.

## 7. Use of Artificial Intelligence (AI)-Assisted Technology

The authors declare that no artificial intelligence (AI) tools were used in the generation, analysis, or writing of this manuscript. All aspects of the research, including data collection, interpretation, and manuscript preparation, were carried out entirely by the authors without the assistance of AI-based technologies.

## 8. References


Abdullah, L., & Suharlina, A. (2010). Herbage yield and quality of two vegetative parts of Indigofera at different times of first regrowth defoliation. *Media Peternakan*, *33*(1), 44–49. DOI: 10.5398/medpet.v33i1.1246

Alam, M. A., Miah, M., Sultana, N., Sarker, M. I. A., & Deb, G.K. (2026). Optimizing Yield and Nutritional Quality in Napier Grass (cv Pennisetum purpureum) Based on Harvest Interval. Advances in Animal and Veterinary Sciences. Adv. Anim. Vet. Sci., 14(1):113-125. DOI | https://dx.doi.org/10.17582/journal.aavs/2026/14.1.113.125

Antari, R., Ginting, S. P., Anggraeny, Y. N., & McLennan, S. R. (2023). The potential role of I. zollingeriana as a high-quality forage for cattle in Indonesia. Tropical Grasslands-Forrajes Tropicales, 11(3), 183–197. https://doi.org/10.17138/TGFT(11)183-197

Asare, E. O. (1985). Effect of frequency and height of defoliation on forage yield and crude protein content of *F. macrophylla* (flemingia). *Proceedings of the XV International Grasslands Congress*, Kyoto, Japan, 164–165.

Ath-Thifa, B. N., Sari, L. A., Despal, Adrizal, Anzhany, D., & Sukria, H. A. (2024). Tropical forage NIRS database: Tropical dairy cattle forage accuracy detection using NIRS dry and wet calibrations. *International Journal of Dairy Science*, *19*(1), 27–34. https://doi.org/10.3923/ijds.2024.27.34

Azzahra, F. R., Susanto, I., Nahrowi, N., Rohmatussolihat, R., Fidriyanto, R., Widyastuti, Y., Yanza, Y. R., Niderkorn, V., Ridwan, R., & Jayanegara, A. (2025). Synergistic effects of *Lactiplantibacillus plantarum and Acacia mangium* leaf tannin extract on the fermentation quality, digestibility, and metabolomic profile of Indigofera silage. Veterinary World, 18(11), 3571–3593. https://doi.org/10.14202/vetworld.2025.3571-3593

Borreani G, Tabacco E, Schmidt RJ, Holmes BJ, & Muck RE. Silage review: Factors affecting dry matter and quality losses in silages. J Dairy Sci. 2018 May;101(5):3952-3979. doi: 10.3168/jds.2017-13837. PMID: 29685272.

Ernakovich, J. G., Hopping, K. A., Berdanier, A. B., Simpson, R. T., Kachergis, E. J., Steltzer, H., & Wallenstein, M. D. (2014). Predicted responses of arctic and alpine ecosystems to altered seasonality under climate change. *Global Change Biology*, *20*(10), 3256–3269. https://doi.org/10.1111/gcb.12568

Food and Agriculture Organization of the United Nations (FAO). 2020. Tips for smallholder silage-making by Jurgen Draiijer. Family Farming Knowledge Platform. http://www.fao.org/3/ca9942en/CA9942EN.pdf

Figueiredo, J. R. O., Pereira Filho, J. M., Feitosa, J. F. F., Cândido, M. J. D., Gilles, S., Bakke, O. A., Maranhão, S. R., Cavalcante, A. C. R., Edvan, R. L., & Bezerra, L. R. (2025). Modeling herbaceous biomass and assessing degradation risk in the Caatinga biome using Monte Carlo simulation. *Sustainability*, *17*(16), 7267. https://doi.org/10.3390/su17167267

Jayanegara, A., Wardiman, B., Kondo, M., & Ridla, M. (2020). Fermentative quality of silage as affected by protein level in the ensiled material: A meta-analysis. *IOP Conference Series: Earth and Environmental Science*, *462*(1), 012001. https://doi.org/10.1088/1755-1315/462/1/012001

Kung, L. Jr., Shaver, R.D., Grant, R.J. & Schmidt, R.J. (2018). Silage review: Interpretation of chemical, microbial, and organoleptic components of silages. Journal of Dairy Science Volume 101, Issue 5, May 2018, Pages 4020-4033. https://doi.org/10.3168/jds.2017-13909

Luo, R., Zhang, Y., Wang, F., Liu, K., Huang, G., Zheng, N., & Wang, J. (2021). Effects of sugar cane molasses addition on the fermentation quality, microbial community, and tastes of alfalfa silage. *Animals*, *11*(2), 355. https://doi.org/10.3390/ani11020355

Mashudi, M., Ndaru, W. A., Septian, P. H., Kusmartono, K., & Kartika, A. D. (2023). Roles of tree and shrub legume leaves as protein sources for cattle raised by small farmers in Bangkalan Regency. *BIO Web of Conferences*, *81*, 00046. https://doi.org/10.1051/bioconf/20238100046

McDonald, P., Henderson, A. R., & Heron, S. J. E. (1991). *The Biochemistry of Silage*. 2nd Edition, Chalcomb Publ., 3 Marlow, 40 p. Marlow, Bucks, UK: Chalcombe Publications, (1991), pp. 340, £49.50, ISBN 0-948617-225. Experimental Agriculture. 1992;28(1):125-125. doi:10.1017/S0014479700023115

Meel, M. S., Garg, S., & Gautam, L. (2025). Growth-stage dependent nutritional dynamics of super Napier grass. *International Journal of Agriculture and Food Science*, *7*(5), 262–266. https://doi.org/10.33545/2664844X.2025.v7.i5d.406

Nadir, M., Syahir, S., Munasirah, A. L., Warni, I., Sari, N., Satriani, A., Sulistiawati, M., & Anugrah, M. (2024). Exploration of nutritional value of Indigofera shoot leaves based on different ages. *International Journal of Chemical and Biochemical Sciences*, *25*(19), 773–778. Doi # https://doi.org/10.62877/91-IJCBS-24-25-19-91

Saez-Plaza, P., Michałowski, T., Navas, M. J., Asuero, A. G., & Wybraniec, S. (2013). An overview of the Kjeldahl method of nitrogen determination. Part I and Part II. *Critical Reviews in Analytical Chemistry*, *43*(4), 178–272. https://doi.org/10.1080/10408347.2012.751787

Silva, C. d. S., Araújo, G. G. L. d., Santos, E. M., Oliveira, J. S. d., Silva, T. G. F. d., Araújo, C. d. A., Novaes, J. J. d. S., de Macedo, A., de Araújo, J. S., Lima, D. O., Santos, F. N. d. S., Campos, F. S., & Gois, G. C. (2025). Fermentative characteristics, nutritional aspects, aerobic stability, and microbial populations of total mixed ration silages based on relocated sorghum silage and cactus pear for sheep diets. *Agronomy*, *15*(2), 506. https://doi.org/10.3390/agronomy15020506

Silva, P. H. F. d., Medeiros, G. R. d., Carvalho, C. B. d. M., Cavalcante, I. T. R., Santos, S. G. C. G. d., Neves, R. d. S., Ribeiro, N. L., Costa, J. H. S., & Sales-Silva, T. B. (2024). The nutritional value of gliricidia in different fed forms: a systematic review. *Ciência Rural*, *54*(11), e20230475. https://doi.org/10.1590/0103-8478cr20230475

Tahuk, P. K., Nahak, O. R. T. B., Bira, G. F., Manek, A., Manek, D., Purnama, T. Y., Bubun, E., & Subay, S. (2022). The effect of using Gliricidia sepium leaves as a source of protein in the complete feed on the performance of fattened male Bali cattle in West Timor, Indonesia. *Advances in Animal and Veterinary Sciences*, *10*(6), 1339–1349. DOI | https://dx.doi.org/10.17582/journal.aavs/2022/10.6.1339.1349

Thiex, N. J., Anderson, S., & Gildemeister, B. (2003). Crude fat, diethyl ether extraction, in feed, cereal grain, and forage (Randall/Soxtec/submersion method): Collaborative study. *Journal of AOAC International*, *86*(5), 888–898. DOI:10.1093/jaoac/86.5.888

Van Soest, P. J., Robertson, J. B., & Lewis, B. A. (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*, *74*(10), 3583–3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2

Viennasay, B., & Wanapat, M. (2020). Strategic supplementation of Flemingia silage to enhance rumen fermentation efficiency, microbial protein synthesis and methane mitigation in beef cattle. *BMC Veterinary Research*, *16*(1), 480. https://doi.org/10.1186/s12917-020-02703-x

Viennasay, B., Wanapat, M., Totakul, P., Phesatcha, B., Ampapon, T., & Cherdthong, A. (2020). Effect of F. macrophylla silage on in vitro fermentation characteristics and reduced methane production. *Animal Production Science*. https://doi.org/10.1071/AN19281

Wulandari, S., Kusuma, S. B., & Nurfitriani, R. A. (2023). Prevention of protein degradation using ensiling coffee husk. *Indonesian Journal of Animal Science*, *33*(2): 170-177. https://doi.org/10.21776/ub.jiip.2023.033.02.04

Yang, F., Fu, D., Yu, X., Lv, J., & Ma, C. (2025). Research on the spoilage characteristics of whole-plant corn silage inoculated with *Clostridium beijerinckii* SHZ-8. *Frontiers in Microbiology*, *16*, 1640283. https://doi.org/10.3389/fmicb.2025.1640283

Zi, X., Liu, Y., Chen, T., Li, M., Zhou, H., & Tang, J. (2022). Effects of sucrose, glucose and molasses on fermentation quality and bacterial community of stylo silage. *Fermentation*, *8*(5), 191. https://doi.org/10.3390/fermentation8050191