

# NUTRIENT CONTENT, FIBER FRACTIONS, AND RUMEN FERMENTATION IN VITRO OF MULATO GRASS GROWN WITH DIFFERENT CUTTING AGES IN LOWLAND WEST JAVA, INDONESIA

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## Abstract

One of the major challenges in ruminant production in Indonesia is ensuring the availability of high-quality and adequate forage. Identifying forage varieties with good nutritive value that adapt well to local climate conditions is crucial. This study evaluated the nutrient composition, fiber fractions, and in vitro rumen fermentation of Mulato grass harvested at different cutting ages (30, 40, and 50 days) in the lowlands of West Java, Indonesia. The nutrient content, analyzed using proximate and fiber fraction analysis, showed no significant differences across cutting ages except for ash content, which was lower at 40 days. Mulato grass cut at 30 days had lower acid detergent fiber (ADF), neutral detergent fiber (NDF), acid detergent lignin (ADL), and hemicellulose levels, while its cellulose content was higher compared to grass cut at 40 or 50 days. Rumen fermentation results indicated that grass cut at 30 days had higher in vitro dry matter digestibility (IVDMD), organic matter digestibility (IVOMD), and total volatile fatty acids (TVFA). These findings suggest that Mulato grass harvested at 30 days offers better digestibility and rumen fermentation outcomes, making it a suitable forage choice for ruminants in the lowlands of West Java, Indonesia.

**Keywords:** fiber fraction, in vitro characteristics, mulato grass, nutrient content.

## KANDUNGAN GIZI, FRAKSI SERAT, DAN FERMENTASI RUMEN SECARA IN VITRO PADA RUMPUT MULATO YANG DITANAM DENGAN USIA POTONGAN YANG BERBEDA DI DATARAN RENDAH JAWA BARAT, INDONESIA

### Abstrak

Salah satu tantangan utama dalam produksi ruminansia di Indonesia adalah memastikan ketersediaan pakan hijauan yang berkualitas tinggi dan memadai. Identifikasi varietas hijauan dengan nilai nutrisi yang baik dan kemampuan adaptasi yang optimal terhadap kondisi iklim lokal sangatlah penting. Penelitian ini mengevaluasi komposisi nutrisi, fraksi serat, dan fermentasi rumen secara in vitro dari rumput Mulato yang dipanen pada umur potong berbeda (30, 40, dan 50 hari) di dataran rendah Jawa Barat, Indonesia. Analisis komposisi nutrisi yang dilakukan melalui analisis proksimat dan fraksi serat menunjukkan tidak adanya perbedaan signifikan pada umur potong kecuali pada kandungan abu, yang lebih rendah pada umur potong 40 hari. Rumput Mulato yang dipanen pada umur 30 hari memiliki kadar acid detergent fiber (ADF), neutral detergent fiber (NDF), acid detergent lignin (ADL), dan hemiselulosa yang lebih rendah, sementara kandungan selulosanya lebih tinggi dibandingkan dengan rumput yang dipanen pada umur 40 atau 50 hari. Hasil fermentasi rumen menunjukkan bahwa rumput yang dipanen pada umur 30 hari memiliki pencernaan bahan kering in vitro (IVDMD), pencernaan bahan organik in vitro (IVOMD), dan total asam lemak volatil (TVFA) yang lebih tinggi. Temuan ini menunjukkan bahwa rumput Mulato yang dipanen pada umur 30 hari memberikan pencernaan dan hasil fermentasi rumen yang lebih baik, sehingga menjadi pilihan hijauan yang sesuai untuk ruminansia di dataran rendah Jawa Barat, Indonesia.

**Kata kunci:** Fraksi serat, Karakteristik in vitro, Rumput Mulato, Kandungan nutrisi.

## INTRODUCTION

One of the main problems in ruminant production in Indonesia is the quality and quantity of forages to be utilized by ruminants (Dahlanuddin et al., 2014; Pengelly et al.,

2003; Rusdy, 2016). This low quality and quantity of forages are caused by the low genetic potential of the forage plant itself and the tropical climatic characteristics. Climatic characteristics in tropical regions consist of an

irregular distribution of rainfall due to the existence of two distinct seasons of the year, rainy and dry, resulting in qualitative and quantitative variations in the availability of forages (Sampaio et al., 2010). The low quality of forages is caused mainly by their low availability level of crude protein (CP) and neutral detergent fiber (NDF), which is essential for animal metabolism and performance (Sampaio et al., 2010).

The impact of low quality and quantity of forages on the animal is high in several aspects, such as low quantity and quality of milk production (Sun et al., 2020; Wang et al., 2014), low nutrient digestibility (Sampaio et al., 2010; Souza et al., 2010), and low health robustness caused by low immunity (Bertoni et al., 2009). These impacts have resulted in low implementation of farmers and animal welfare, especially in developing tropical countries, such as Indonesia. Therefore, these impacts need to be minimized to support farmers and animal welfare.

Several researchers have been engaged in the search for higher genetic potentials as well as more tropical climatic-adaptive forage plants to solve the impact of its low quality and quantity (Benabderrahim & Elfalleh, 2021; Rahman & Kawamura, 2011; Simeão et al., 2021). One of the potential forage plants is Mulato (*Brachiaria hybrid cv. Mulato*). Mulato is reportedly adapted to infertile soils and known for its tolerance of prolonged drought and regrowth after sporadic frost (Inyang et al., 2010). Furthermore, Mulato has superior nutritive value of crude protein (CP) concentrations fluctuating between 90 and 170 g kg<sup>-1</sup> and in vitro digestibility from 550 to 620 g kg<sup>-1</sup> when compared with other warm-season grasses (Lascano et al., 2006).

Mulato grass was developed and hybridized in 1989 by the International Center for Tropical Agriculture (CIAT) in Colombia with the cultivar code CIAT 36087 (Argel et al., 2007). Mulato grass is the product of hybridization between *Brachiaria ruziziensis* and *Brachiaria decumbens* cv. Basilisk. It is reported that Mulato grows well at altitudes ranging from sea level to 1800 meters above sea level in either a humid tropical environment or a sub-humid region with 5 to 6 dry months with annual rainfall above 700 mm, which also grows well in acidic and well-drained soil (Argel et al., 2007).

Mulato grass is also reported to respond well to fertilization, especially Nitrogen. Hence, in a regular fertilization rate, the forage yield of Mulato may range from 10 to 27 ton DM/ha/year, of which 20% might be produced in the dry season (Argel et al., 2007). Another study reported that Mulato had a higher herbage accumulation compared to other strains of *Brachiaria* (i.e. Ippora), which was produced in the Amazon biome (Paraiso et al., 2019). In the Southeast Asian region, Thailand, Mulato produced 60% higher DM compared to Ruzi grass, which made Mulato an interesting strain of grass to be utilized as the main forage for dairy cows (Pizarro et al., 2013). In the nutritional aspect, Mulato's crude protein (CP) content ranges from 10% to 17%, which is influenced by harvest frequency, canopy height, soil nitrogen availability, and seasonal changes (Silva et al., 2016; Vendramini et al., 2014).

This study was conducted in Subang Regency, specifically within the Cipunagara District, where the socio-economic importance of lowland areas is pronounced in terms of animal production and agriculture (Fathurohman et al., 2023). Such regions, with their dense populations, are integral to the local economy, particularly in the agricultural sector. Research in these settings is poised to directly bolster local livelihoods, offering substantial contributions to enhancing sustainable agricultural practices (Kengo et al., 2021). Additionally, the Cipunagara District provides a fundamental baseline for comparative studies, enabling researchers to draw contrasts with other ecological zones, such as uplands, in terms of crop yields, nutritional values, and environmental adaptability (De Costa & Sangakkara, 2006; Rao & Coe, 1991; Sandar et al., 2022). This not only promises to elevate agricultural productivity but also aligns with broader national objectives of food security and rural or urban development.

To our knowledge, the study that addressed the proper time of Mulato harvest, especially in lowland areas in Tropical countries, is lacking. The aim of this study is to evaluate the nutritive value of Mulato biomass in different cutting ages of Mulato grass in West Java Province, Indonesia.

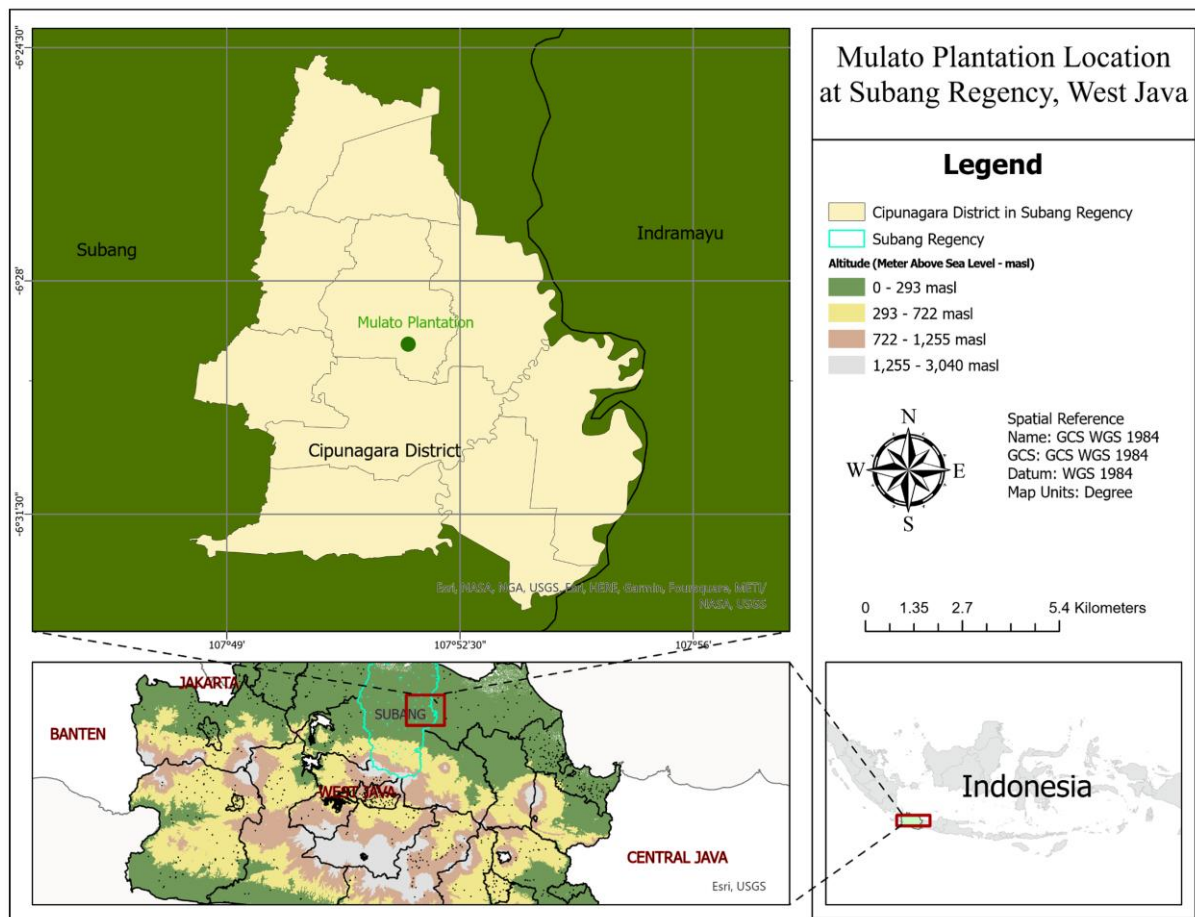
## MATERIAL AND METHODS

### *Study area and experimental design*

Our experiment was conducted in a grass land at Cipunagara District ( $6^{\circ}28'57.8''\text{S}$   $107^{\circ}51'43.2''\text{E}$ ), Subang Regency, West Java, Indonesia (Fig. 1). The climate conditions of the study area were categorized in tropical rainforest climate (Beck et al., 2018) with low altitude (39 masl; (Indonesian Statistic Bureau, 2020) and low annual rainfall (1000 mm/year; (Indonesian Statistic Bureau, 2020). The average annual ambient temperature of this area is  $28^{\circ}\text{C}$ , with an average annual relative humidity of 71.4% (Indonesian Statistic Bureau, 2020). Prior to the experiment, we analyzed the soil characteristics of the experimental site. The land of study area is an area of sedimentation from alluvial materials deposited from the upstream area. Soil that is

formed comes from the main material of conglomerate rock, which is characteristic of the sedimentation area, and clay rock. The soils are associated with tropoudults, dystropepts, and haplortox, which generally indicate low fertility potential. This soil association can be characterized by its relatively light color with reddish brown. The pH of the soil before the experiment is 5.1, while the nitrogen content is 0.16%.

In mid-June 2020, Mulato II plant was established as monoculture in 3 field plots ( $25\text{ m} \times 25\text{ m}$ ) blocked by three different cutting age which is **30, 40, and 50** days, and each treatment has six replicates. The sowing rate was 18 kg/ha and row spacing was  $50 \times 50\text{ cm}$ . Watering frequency was twice daily (0700 and 1700 hrs).



**Figure 1.** Location of study area in Cipunagara District, Subang Regency, West Java Province, Indonesia ( $6^{\circ}28'57.8''\text{S}$   $107^{\circ}51'43.2''\text{E}$ ), which is categorized as a tropical rainforest climate based on the Koppen climate classification (Beck et al., 2018).

## Measurements

The measured parameters were the Mulato grass's nutrient content (proximate analysis), fiber fraction analysis (van Soest protocol), and in vitro analysis. The measured nutrient fractions using proximate analysis were the moisture (water content), ash (minerals), crude protein (CP), ether extract (EE), crude fiber (CF), and nitrogen-free extract (NFE). The fiber fraction analysis using the van Soest method comprises the determination of acid detergent fiber (ADF), neutral detergent fiber (NDF) contents, cellulose, hemicellulose, and acid detergent lignin (ADL) contents (P. J. Van Soest et al., 2020).

The grass stems were chopped into small pieces, dried at 60°C, and milled to determine the cellulose, hemicellulose, and ADL content in accordance with Van Soest and Robertson's methods (P. J. Van Soest et al., 1991, 2020; P. Van Soest & Robertson, 1979), which is commonly known as Van Soest fibre analysis. After digesting samples using chemical reagents, this approach detects neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL). First acquired through digestion in a neutral detergent solution, NDF comprises primarily of cellulose, hemicellulose, and lignin. After processing NDF with an acid detergent, ADF is formed, which consists primarily of cellulose and lignin. ADL was produced by removing cellulose from ADF using H<sub>2</sub>SO<sub>4</sub>. The cellulose content is determined by subtracting ADF from ADL, while the hemicellulose content is determined by subtracting NDF from ADF. The lignin content is represented by ADL. The in vitro analyses included dry matter digestibility, organic matter digestibility, and total volatile fatty acid (VFA) synthesis. The IVDMD and IVOMD concentrations were determined using Tilley and Terry's method (Tilley & Terry, 1963), with an adjustment to use an artificial rumen, as described in a previous work (Epifanio et al., 2019).

## Statistical analysis

Data were analysed using analysis of variance (ANOVA) in GLM procedure in SAS Statistics (Ver. 9.4, Cary, NC, USA) to determine the effects of different cutting ages on Mulato grass nutrient content, fiber

fractions, and in vitro characteristics. Therefore, the statistical model included cutting age as a fixed effect. Duncan's Multiple Range Test was carried out for subsequent comparison of means. The data were expressed as least square means (LSM) of the respective parameter with the pooled standard error of means (SEM). The difference is considered significant at  $p < 0.05$ . To determine the correlation between fibrous nutritional parameters and in vitro digestibility parameters, the Pearson's correlation analyses were used with the PROC CORR procedure in SAS Statistics (Ver. 9.4, Cary, NC, USA), with their p-value determinations.

## RESULTS

### *Nutrient Content of Mulato II Grass at Different Cutting Ages*

The proximate analysis and fiber fraction contents of Mulato II grass harvested at 30 (S30), 40 (S40), and 50 (S50) days are presented in Table 1. The ash content was significantly higher in grass harvested at 30 days (9.59%) compared to those harvested at 40 days (9.07%) and 50 days (7.87%) ( $P = 0.0072$ ), indicating a higher mineral content at the earlier cutting age. There were no significant differences in dry matter (DM), crude protein, crude fiber, crude fat, nitrogen-free extract (NFE), phosphorus (P), and nitrogen content among the different cutting ages ( $P > 0.05$ ).

Significant differences ( $P < 0.0001$ ) were observed among the cutting ages for fiber fractions, including acid detergent fiber (ADF), neutral detergent fiber (NDF), acid detergent lignin (ADL), hemicellulose, and cellulose. The ADF content increased with cutting age, from 30.79% at S30 to 41.60% at S50. Similarly, NDF content rose from 57.07% at S30 to 63.00% at S50, and ADL content increased from 4.57% at S30 to 5.97% at S50. Hemicellulose content was higher at S50 (26.28%) compared to S30 (21.40%) and S40 (20.25%). In contrast, cellulose content was higher at S30 (35.63%) and S40 (35.16%) than at S50 (26.22%).

### *In Vitro Digestibility and Fermentation Characteristics*

The in vitro digestibility and fermentation characteristics of Mulato II grass

at different cutting ages are shown in Table 2. The in vitro dry matter digestibility (IVDMD) was significantly higher at S30 (65.17%) compared to S40 (60.85%) and S50 (53.14%) ( $P < 0.0001$ ). Similarly, the in vitro organic matter digestibility (IVOMD) was significantly higher at S30 (67.40%) than at S40 (65.06%) and S50 (57.61%) ( $P < 0.0001$ ).

Total volatile fatty acids (TVFA) production was highest at S30 ( $126.33 \text{ mmol} \cdot \text{L}^{-1} \cdot 100 \text{ mg}^{-1}$ ), decreasing significantly at S40 ( $119.50 \text{ mmol} \cdot \text{L}^{-1} \cdot 100 \text{ mg}^{-1}$ ) and S50 ( $100.67 \text{ mmol} \cdot \text{L}^{-1} \cdot 100 \text{ mg}^{-1}$ ) ( $P < 0.0001$ ). The pH levels tended to be higher at S30 (7.28) but were not significantly different among treatments ( $P = 0.0753$ ). Ammonia ( $\text{NH}_3$ )

production showed no significant differences among the cutting ages ( $P = 0.7943$ ).

### **Correlation Between Fiber Content and Digestibility Parameters**

Correlation coefficients between fibrous nutritional contents and in vitro digestibility parameters are presented in Table 3. Significant negative correlations ( $P < 0.05$ ) were found between ADF, NDF, and ADL contents and IVDMD, IVOMD, and TVFA production (Figure 2 A and B). This indicates that higher fiber contents are associated with lower digestibility and fermentation efficiency. No significant correlations were observed between fiber contents and pH or  $\text{NH}_3$  production (Figure 3).

**Table 1.** Proximate analysis and fiber fraction content of Mulato II in different cutting age in this experiment (%).

Parameter <sup>1</sup>	Cutting age			Pooled SEM	p-value	remarks
	S30	S40	S50			
<i>Proximate Analysis</i>						
DM	237.33	291.76	253.46	13.59	0.2563	
Ash	9.59 <sup>b</sup>	9.07 <sup>b</sup>	7.87 <sup>a</sup>	0.25	0.0072	sig
Crude Protein	11.09	10.70	9.26	0.47	0.2599	
Crude Fiber	28.29	28.19	28.50	0.60	0.9799	
Crude Fat	1.75	1.64	1.71	0.08	0.8724	
NFE	49.80	51.60	50.94	0.57	0.4621	
P	0.13	0.11	0.13	0.01	0.4564	
<i>Fiber Fraction</i>						
ADF	30.79 <sup>a</sup>	40.62 <sup>b</sup>	41.60 <sup>b</sup>	1.21	<.0001	sig
NDF	57.07 <sup>a</sup>	60.87 <sup>b</sup>	63.00 <sup>c</sup>	0.63	<.0001	sig
ADL	4.57 <sup>a</sup>	5.46 <sup>b</sup>	5.97 <sup>c</sup>	0.15	<.0001	sig
Hemicellulose	21.40 <sup>a</sup>	20.25 <sup>a</sup>	26.28 <sup>b</sup>	0.72	<.0001	sig
Cellulose	35.63 <sup>b</sup>	35.16 <sup>b</sup>	26.22 <sup>a</sup>	1.08	<.0001	sig
N Content	1.78	1.71	1.48	0.08	0.2599	

**Note:** DM: dry matter; NFE: Nitrogen-free extract; P: Phosphorus; ADF: acid detergent fiber; NDF: neutral detergent fiber; ADL: Acid Detergent Lignin; N: Nitrogen. Total gas production and pH

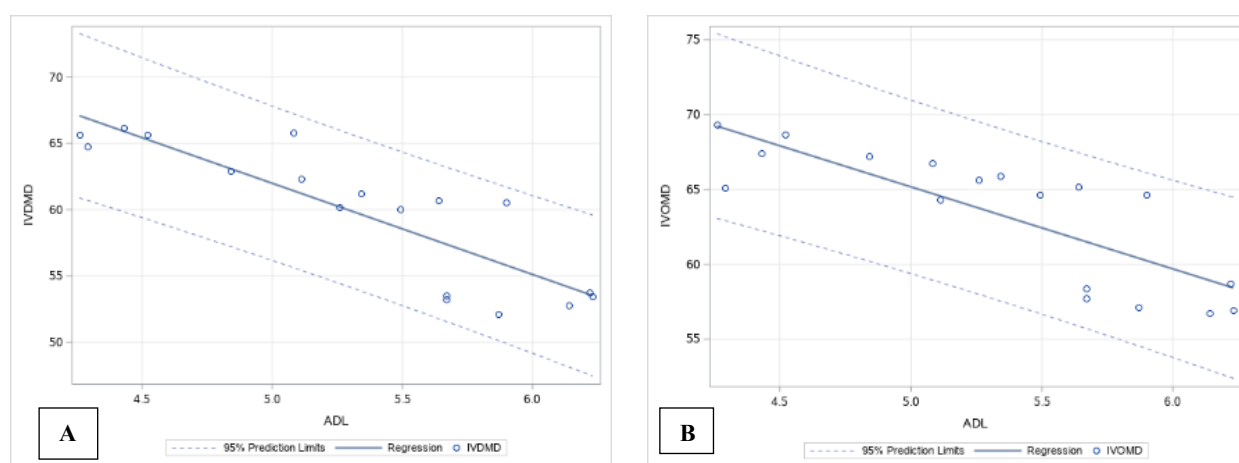
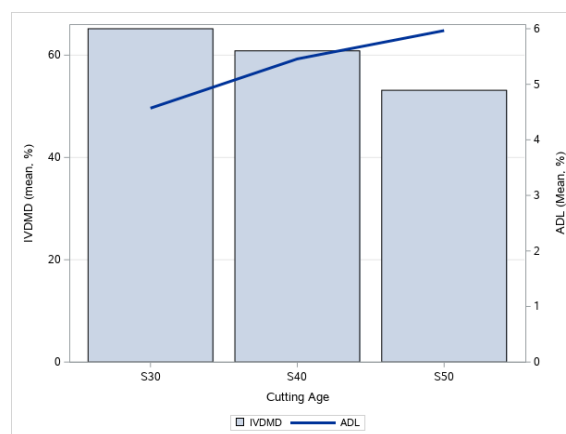
**Table 1.** Mulato II in vitro assessment characteristics in different cutting ages in this experiment (%), otherwise indicated differently).

Parameter <sup>1</sup>	S30	S40	S50	Pooled SEM	p-value	remarks
IVDMD (%)	65.17 <sup>c</sup>	60.85 <sup>b</sup>	53.14 <sup>a</sup>	1.22	<.0001	sig
IVOMD (%)	67.40 <sup>c</sup>	65.06 <sup>b</sup>	57.61 <sup>a</sup>	1.04	<.0001	sig
pH	7.28 <sup>b</sup>	7.2 <sup>a</sup>	7.22 <sup>ab</sup>	0.02	0.0753	tendency
TVFA <sup>1</sup>	126.33 <sup>c</sup>	119.50 <sup>b</sup>	100.67 <sup>a</sup>	2.80	<.0001	sig
$\text{NH}_3$	3.84	4.25	4.02	0.23	0.7943	

**Note:** IVDMD: in vitro dry matter digestibility; IVOMD: in vitro organic matter digestibility; TVFA: total volatile fatty acid ( $\text{mmol} \cdot \text{L}^{-1} / 100 \text{ mg}$  of sample).

**Table 2.** Correlation coefficients between fibrous nutritional contents and in vitro digestibility parameters.

	ADF	NDF	ADL	Hemicellulose	Cellulose
IVDMD	-0.80*	-0.88*	-0.87*	0.58	-0.77*
IVOMD	-0.72*	-0.82*	-0.81*	0.50	-0.69*
pH	0.23	0.30	0.45	-0.12	0.19
TVFA	-0.71*	-0.81*	-0.80*	0.48	-0.68*
NH <sub>3</sub>	0.15	0.04	-0.04	-0.22	0.17

\*: significant correlation ( $p < 0.05$ ).**Figure 2.** Correlation between ADL with (A) IVDMD and (B) IVOMD in this experiment.**Figure 3.** Interaction between cutting age, ADL contents, and IVDMD in this experiment.

## Discussion

This study highlights how the cutting age of Mulato II grass significantly affects its nutrient composition and in vitro digestibility, which has important implications for its use as ruminant feed in tropical lowland areas (Adams et al., 2019; Costa et al., 2016). The higher ash content observed in grass harvested

at 30 days suggests that mineral concentrations are higher at earlier growth stages (Schlegel et al., 2016). During early growth stages (germination and seedling development), mineral nutrients are especially critical. Seeds contain stored reserves of minerals, but these are often limited. As a seed germinates, the young plant must quickly begin absorbing nutrients from its environment to support rapid

cell division and the establishment of roots and shoots. For example, developing seedlings have high demands for nutrients like nitrogen (for new proteins and enzymes) and phosphorus (for ATP and nucleic acids) to fuel their intense metabolic activity. If any essential nutrient is lacking at this stage, growth can be stunted or abnormal right from the start. By contrast, during later stages (vegetative growth, flowering, and fruiting), nutrient deficiencies often manifest as specific symptoms in mature tissues (such as leaf discoloration or poor yield). These findings align with previous studies indicating that ash content tends to decrease as *Brachiaria* species mature (Wassie et al., 2018) and are consistent with observations in other forage crops such as triticale (Coblentz et al., 2018). Similarly, research by Nguyen et al. (2024) showed that harvesting Mulato II grass earlier than its typical regrowth period improves nutrient quality, including mineral content, making it more suitable as ruminant feed.

As the cutting age advances, the fiber fractions, such as acid detergent fiber (ADF), neutral detergent fiber (NDF), acid detergent lignin (ADL), and hemicellulose, show a significant increase. This pattern reflects the natural maturation process, where older grass accumulates more structural carbohydrates and lignin, leading to greater rigidity but reduced digestibility. The increase in lignin content strengthens plant cell walls and lowers nutritional value for ruminants, as they lack the necessary enzymes to break it down (Astudillo-Neira et al., 2022). Similar trends in fiber accumulation with grass maturity have been reported by Nguyen et al. (2024) and Adnew et al. (2023), suggesting that delaying harvest could result in lower forage quality. The accumulation of fiber fractions in plant cell walls during growth and development is primarily driven by the biosynthesis and deposition of cellulose, hemicellulose, pectin, and lignin, coordinated through vesicle trafficking and cytoskeletal organization (Kumar & Turner, 2015; Zhang & Zhou, 2015). Cellulose is synthesized at the plasma membrane, while hemicelluloses and pectins are produced in the Golgi and integrated into the wall matrix, contributing to extensibility and structure (Cosgrove, 1997; Lampugnani et al., 2018). Wall-loosening proteins like expansins and enzymes such as XTHs and pectinases facilitate expansion by modifying

interactions between polysaccharides (Cosgrove, 1997; DeGrave, 2022). Microtubule-guided alignment of cellulose microfibrils ensures structural orientation (Chernova & Gorshkova, 2007), while different growth modes, such as intrusive and multinet growth, influence fiber patterning and wall thickening (Mühlethaler, 1961). The cell wall integrity (CWI) sensing mechanism and hormonal signals further modulate biosynthesis and remodeling in response to developmental and environmental cues (Gigli-Bisceglia et al., 2020; Sakurai, 1991). Together, these mechanisms ensure the adaptive accumulation of fiber components essential for plant form and function.

The findings also indicate that higher ADF and NDF levels at 50 days are linked to reduced digestibility, as shown by the lower values of *in vitro* dry matter digestibility (IVDMD) and *in vitro* organic matter digestibility (IVOMD). The significant negative correlations between fiber content (ADF, NDF, ADL) and digestibility parameters (IVDMD, IVOMD, TVFA) confirm that increased fiber and lignin content negatively impact fermentation efficiency and nutrient availability (Peters et al., 2022). These results align with studies showing that high lignin levels hinder fermentation and nutrient absorption (Fukushima et al., 2015; Jung et al., 1997; Gameda & Hassen, 2015).

Furthermore, higher total volatile fatty acid (TVFA) production in grass harvested at 30 days suggests more efficient fermentation by rumen microbes (Ma et al., 2021). This can be attributed to the lower fiber content and reduced lignification, allowing better microbial access to nutrients (Gameda & Hassen, 2015). Similar findings by Nguyen et al. (2024) indicate that harvesting Mulato II grass one to two weeks earlier than usual can enhance forage quality and fermentation potential, ultimately improving feed efficiency in ruminants.

Although crude protein (CP) content decreased with advancing cutting age, the differences were not statistically significant. This trend is consistent with findings from Adnew et al. (2023) and Worku et al. (2021), who reported higher CP levels in Mulato II grass harvested at earlier stages. The decline in CP content as the plant matures is likely due to the dilution effect, where the accumulation of



structural carbohydrates reduces the relative protein concentration.

Interestingly, the study found that grass harvested at 30 days contained higher cellulose levels compared to grass harvested at 50 days. This could be attributed to the greater proportion of digestible cellulose relative to indigestible lignin in earlier growth stages. As the plant matures, lignin binds with cellulose and hemicellulose, making them less accessible to microbial enzymes (Barros et al., 2015). The higher cellulose content in younger plants contributes to better digestibility, reflected in the improved IVDMD and IVOMD values.

Changes in nutrient composition and digestibility with different cutting ages are primarily influenced by the plant's physiological development. Younger plants contain higher proportions of easily digestible cell components such as proteins, sugars, and minerals. However, as the plant matures, the proportion of structural carbohydrates and lignin increases, creating a physical barrier that limits microbial access to nutrients (Barros et al., 2015). This shift reduces the overall digestibility and nutritional value of the forage.

Harvesting Mulato II grass at 30 days is recommended to optimize its nutritional value and digestibility for ruminants. High-quality forage with elevated CP content and lower fiber fractions can enhance growth rates, milk production, and overall animal health. These findings are particularly useful for farmers in tropical lowland areas who aim to maximize forage quality and efficiency. This study provides practical recommendations regarding the optimal harvesting time to maximize the nutritional benefits of Mulato II grass in ruminant diets.

The advantages of early harvesting are further supported by Silva et al. (2016), who reported that harvesting Mulato II grass at a canopy height of 25 cm optimizes its nutritional value and grazing efficiency. Their research found that earlier harvesting resulted in higher CP content and improved digestibility, which are critical for ruminant nutrition. Similarly, Adnew et al. (2023) demonstrated that harvesting Mulato II grass at 30 days enhances its nutrient composition and *in vitro* digestibility, further supporting our findings. Their study highlighted that earlier-harvested grass had higher CP content and

lower fiber fractions compared to later harvests, leading to improved forage quality.

Our findings align with those of Nouhoun et al. (2021), who observed that the Mulato II cultivar offers superior nutritive value when harvested earlier, with higher CP content and better digestibility. Botero-Londoño et al. (2021) also found that extending the cutting interval decreased the nutritional quality of King grass, leading to lower protein levels and higher fiber content. These studies collectively emphasize the importance of early harvesting to maintain forage quality.

However, one limitation of this study is that it was conducted under controlled experimental conditions within a specific tropical lowland area. Factors such as soil fertility, climate variations, and farm management practices can influence forage nutrient composition and digestibility. Therefore, applying these findings to different regions or farming systems should be approached cautiously.

Future studies should explore field trials across diverse agroecological settings to validate the effects of cutting age on Mulato II grass. Previous research by Adnew et al. (2023) in Ethiopia and Worku et al. (2021) in the Ethiopian highlands has demonstrated that environmental conditions can have a significant impact on forage quality. Additionally, further research should investigate animal performance metrics, such as growth rates and milk production, when fed Mulato II grass harvested at different stages. Examining interactions between cutting age and other management factors, such as fertilization, irrigation, and plant spacing, could provide valuable insights to enhance forage production strategies (Botero-Londoño et al., 2021; Nouhoun et al., 2021).

## CONCLUSION

In conclusion, this study might confirm a plausible timing of Mulato II grass cutting age in Indonesia, which is 30 days of age. In this age, harvested Mulato II grass contains more protein content, although slightly lower in fibre content. Furthermore, it is interesting to note that higher IVDMD, IVOMD, and *in vitro* VFA production were higher with lower ADL content in the cutting age of 30 days,



ensuring a better digestibility when fed to the animal. However, the results of this study might only be limited to the tropical lowland area with a tropical rainforest climate. The results of this experiment might be valuable as a baseline for the farmers in the tropics regarding the best time to harvest their Mulato grass, to obtain the optimum nutritional value of the grass.

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