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Pengaruh Rasio *Tithonia diversifolia* dan *Panicum maximum* terhadap Kualitas Fermentasi, Komposisi Kimia, dan Kecernaan *In Vitro* Silase

The Effect of the Tithonia diversifolia and Panicum maximum Ratio on Fermentation Quality, Chemical Composition, and In Vitro Digestibility of Silage

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ABSTRAK

Penelitian ini bertujuan untuk mengevaluasi pengaruh perbedaan rasio campuran *Tithonia diversifolia* dan *Panicum maximum* terhadap kualitas fermentasi, komposisi kimia, dan kecernaan silase secara in vitro. Penelitian menggunakan RAL dengan lima rasio perlakuan (0:100, 25:75, 50:50, 75:25, dan 100:0, Tithonia:Panicum), masing-masing diulang tiga kali. Parameter yang diamati meliputi kualitas fermentasi (pH, NH_3, dan populasi BAL) komposisi kimia (BK, PK, SK, dan LK) serta kecernaan in vitro. Analisis korelasi Pearson digunakan untuk mengetahui hubungan antara komposisi kimia dan tingkat kecernaan silase. Hasil menunjukkan bahwa rasio campuran hijauan berpengaruh nyata ($P < 0.05$) terhadap pH, NH_3, BK, PK, SK, serta KCBK, KCPK dan KCSK, namun tidak berpengaruh nyata terhadap populasi BAL, LK dan KCLK. Perlakuan dengan rasio *Tithonia diversifolia* dan *Panicum maximum* 25:75 dan 75:25 (T2 dan T3) menghasilkan silase dengan pH terendah (3.92 dan 3.88), konsentrasi NH_3 terendah (3.05 dan 2.70 mM), PK tertinggi (15.9 dan 16.5%), serta KCBK tertinggi (51.2 dan 53.0%). Analisis korelasi Pearson menunjukkan hubungan positif sangat kuat antara SK terhadap KCSK ($r = 0.98$). Sebaliknya, LK menunjukkan korelasi negatif terhadap KCLK ($r = –0,85$). Temuan ini mengindikasikan bahwa rasio campuran hijauan yang seimbang (T2 dan T3) menghasilkan silase dengan kualitas fermentasi dan kecernaan terbaik.

KEYWORDS:
Chemical composition
Fermentation quality
In vitro digestibility
Panicum maximum
Silage
Tithonia diversifolia

ABSTRACT

This study aimed to evaluate the effect of different ratios of Tithonia diversifolia and Panicum maximum mixtures on fermentation quality, chemical composition, and in vitro digestibility of silage. The study used RAL with five treatment ratios (0:100, 25:75, 50:50, 75:25, and 100:0, Tithonia: Panicum), each repeated three times. Parameters observed included fermentation quality (pH, NH_3, and LAB population), chemical composition (DM, CP, CF, and EE), and in vitro digestibility. Pearson correlation analysis determined the relationship between chemical composition and silage digestibility. Results showed that the ratio of forage mixture had a significant effect ($P < 0.05$) on pH, NH_3, DM, CP, CF, IVDMD, IVCPD, and

IVCFD, but no significant impact on LAB population, EE, and IVEED. Treatments with the ratio of Tithonia diversifolia and Panicum maximum 25:75 and 75:25 (T2 and T3) produced silage with the lowest pH (3.92 and 3.88), lowest NH_3 concentration (3.05 and 2.70 mM), highest CP (15.9 and 16.5%), and highest IVDMD (51.2 and 53.0%). Pearson correlation analysis showed a strong positive relationship between CF and IVCFD (r = 0.98). In contrast, EE negatively correlated with IVEED (r = -0.85). These findings indicated that a balanced forage mix ratio (T2 and T3) produced the best fermentation quality and digestibility of silage.

1. Introduction

The fluctuating availability of forage throughout the year is a significant challenge in providing feed for ruminants, particularly during the dry season. One widely implemented strategic solution is forage preservation technology through the silage process. This process relies on anaerobic conditions to preserve the nutritional value of fresh forage through the activity of fermentative microorganisms, particularly Lactic Acid Bacteria (LAB), which lower the pH and inhibit the growth of spoilage microbes (Septian et al., 2024). The success of silage fermentation is greatly influenced by the characteristics of the raw materials, including dry matter content, the availability of water-soluble carbohydrates, and the protein-to-fiber ratio.

Panicum maximum, a tropical grass commonly used forage in Indonesia, is known for its high biomass productivity and good tolerance to tropical environments (Fanindi et al., 2020). However, its crude protein content is relatively low, and its crude fiber content is quite high, potentially reducing digestibility and nutrient conversion efficiency when used alone in silage formulations (Pazla et al., 2024).

As a complement, *Tithonia diversifolia* is gaining increasing research due to its high protein content and bioactive potential as a natural feed additive. This forage contains secondary metabolites such as tannins, saponins, and flavonoids, which can act as antioxidants and antimicrobials. However, in high concentrations, they can inhibit the activity of fermentative microorganisms and rumen microbes (Olmo-González et al., 2022). Therefore, a balanced mixture of Tithonia and Panicum is essential for optimizing nutrient balance and silage fermentation.

Several previous studies have discussed the benefits of Tithonia in its fresh form and as an additive. However, studies on the effect of varying the proportions of Tithonia and Panicum in silage formulations on fermentative quality, chemical composition, and in vitro digestibility are still scarce. This information is crucial for developing efficient

and nutritionally valuable silage formulation strategies based on local forage sources. No comprehensive study has systematically evaluated how different Tithonia-to-Panicum ratios affect the overall silage quality, particularly in tropical forage systems.

This study aimed to evaluate the effect of different ratios of *Tithonia diversifolia* and *Panicum maximum* on fermentation quality, chemical composition, and in vitro silage digestibility. It is hoped that the results will contribute to the utilization of local forages to provide high-quality silage feed for ruminants in tropical regions.

2. Materials and Methods

2.1. Research Materials

Fresh *Tithonia diversifolia* leaves and *Panicum maximum* grass were used as the main ingredients in silage production, with molasses as an additive. The forages were collected from the areas of Payakumbuh and Lima Puluh Kota located at altitudes ranging from approximately 450 to 750 meters above sea level. The forages were wild plants that grew naturally in the area. The silage process was carried out using a digital scale, a forage cutter, and a plastic or tarp as a flat silo. Fermentation quality was then evaluated using a digital pH meter, a steam distillation apparatus, a Conway unit, and an incubator. Chemical composition tests were conducted using a 105°C oven, a 550°C furnace, a Kjeldahl apparatus, a Soxhlet apparatus, and a fiber digester. In vitro digestibility tests used an anaerobic incubator, fermentation tubes, a centrifuge, and a CO_2 pump, with fresh rumen fluid as the microbial source.

2.2. Experimental Design

This study was designed using a Completely Randomized Design (CRD) method to evaluate the effect of the forage mixture ratios of *Tithonia diversifolia* and *Panicum maximum* on silage quality. There were five treatments, each replicated three times, for 15 experimental units. The treatments varied in the fresh weight ratio of Tithonia and Panicum as follows:

T0 = 0% Tithonia and 100% Panicum
T1 = 25% Tithonia and 75% Panicum
T2 = 50% Tithonia and 50% Panicum
T3 = 75% Tithonia and 25% Panicum
T4 = 100% Tithonia and 0% Panicum

2.3. Ensilage Stages

The silage-making process is standardized to ensure uniformity of treatment. *Tithonia diversifolia* leaves and *Panicum maximum* grass are harvested during the vegetative phase, chopped to a size of approximately 3–5 cm, and then weighed according to the treatment ratios (0:100, 25:75, 50:50, 75:25, and 100:0), with each unit weighing 5 kg fresh. Prior to ensiling, the moisture content of *Tithonia diversifolia* and *Panicum maximum* was measured at 89.3% (Desyrakhmawati et al., 2015) and 77.9% (Nahak T. Ba et al., 2015), respectively. Molasses is added at 4% of the total fresh weight (200 g/5 kg), dissolved in water (1:1), and sprayed evenly using a manual sprayer (Yitbarek & Tamir, 2014). Mixing is carried out on a tarp with a thickness of approximately 5 cm. The material is then gradually transferred into a 5–6 kg plastic silo, compacted to create anaerobic conditions. The silo is tightly closed and weighted to prevent oxygen entry. Fermentation lasted 21 days at a temperature of 24-27°C and a humidity of 55-65% (Mudhita et al., 2024). After fermentation, the silage was taken from the center of the silo as a sample for quality analysis.

2.4. Parameter Observations

The parameters observed in this study included fermentative quality, chemical composition, and in vitro digestibility evaluation. Fermentation quality testing measured pH, ammonia/NH_3 concentration (mM) (General Laboratory Procedure, 1966), and lactic acid bacteria (LAB) populations (log cfu/g) (Modification of Mugabe *et al.,* 2019). Next, a chemical composition analysis of the silage was performed to determine dry matter/DM (%), crude protein/CP (%DM), crude fiber/CF (%DM), and ether extract /EE (%DM) (AOAC, 2005). In addition, an in vitro digestibility evaluation was conducted, including dry matter digestibility/IVDMD (%), crude protein digestibility/IVCPD (%), crude fiber digestibility/IVCFD (%), and ether extract digestibility/IVEED (%) (Tilley & Terry, 1963). All parameters were analyzed according to standard laboratory procedures to ensure data accuracy and consistency.

2.5. Data Analysis

Data were analyzed using analysis of variance (ANOVA) based on a completely randomized design (CRD). If there is a significant difference ($P < 0.05$), further testing using Duncan's Multiple Range Test (DMRT) is performed. A Pearson correlation

analysis is also performed to determine the relationship between silage chemical composition (DM, CP, CF, and EE) and in vitro digestibility (IVDMD, IVCPD, IVCFD, and IVEED). This analysis aims to identify the relationship between nutrient content and silage digestibility (Montgomery, 2017).

3. Results and Discussion

3.1. Silage Fermentative Quality

Analysis of variance results showed that the treatment, consisting of a mixture ratio of *Tithonia diversifolia* and *Panicum maximum*, had a significant effect ($P < 0.05$) on pH and ammonia (NH_3) concentration. However, the treatment did not considerably impact the LAB population ($P > 0.05$). The average value of each fermentation quality parameter is shown in Table 1.

Table 1. Fermentation characteristics and microbial populations of various mixture ratios of *Tithonia diversifolia* and *Panicum maximum*

Parameters	Treatment				
	T0 (0%:100%)	T1 (25%:75%)	T2 (50%:50%)	T3 (75%:25%)	T4 (100%:0%)
pH	4.51 ± 0.05^{a}	4.13 ± 0.07^{b}	3.92 ± 0.06^{c}	3.88 ± 0.04^{c}	4.41 ± 0.05^{a}
NH_3 (mM)	4.10 ± 0.25^{a}	3.40 ± 0.22^{b}	3.05 ± 0.18^{c}	2.70 ± 0.20^{c}	3.60 ± 0.26^{b}
LAB (log cfu/g)	6.5 ± 0.3	6.8 ± 0.2	7.0 ± 0.3	$6.9 \pm 0,.2$	6.7 ± 0.3

Note : Data are presented as mean ± standard deviation (n = 3). Numbers followed by different superscript letters in the row indicate significant differences ($P < 0.05$) based on the DMRT test.

3.1.1. Silage pH

Differences in pH values between treatments reflect variations in fermentation at each forage mixture ratio. Increasing the proportion of *Tithonia diversifolia* in the mixture is generally followed by a decrease in pH up to a certain point. The pH value decreased from 4.51 at T0 to 4.13 at T1, 3.92 at T2, and reached its lowest point of 3.88 at T3. This is likely due to the high protein content and fermentable compounds such as water-soluble carbohydrates (WSC) in Tithonia, which encourage the growth of lactic acid bacteria (LAB) and the production of organic acids, especially lactic acid, as the main fermentation product (Osuga et al., 2024). However, in the treatment with 100% Tithonia diversifolia (T4), the pH value increased slightly compared to T3. This may be attributed to antinutritional compounds such as tannins and saponins, which are known to have antimicrobial effects (Olmo-González et al., 2022). These compounds could have inhibited the activity of fermentative microbes, including lactic acid bacteria, thereby

reducing lactic acid production and resulting in a higher final pH. In addition, the absence of Panicum maximum in this treatment might have led to an unbalanced fiber-to-sugar ratio, further affecting fermentation efficiency. Furthermore, excessively dominant Tithonia without Panicum may have a less-than-ideal balance of fiber and sugar for efficient fermentation.

In this study, the silage pH was generally within the optimal range for the ensilage process, between 3.8 and 4.2, which creates anaerobic conditions and suppresses the growth of spoilage microorganisms, thus supporting nutritional stability and quality during storage (Kung Jr. et al., 2018). However, treatments with pH values above 4.2, such as T0 and T4, increase the potential for silage quality degradation due to undesirable microbial activity. Therefore, the lowest pH was found in mixtures with a balanced ratio of Tithonia and Panicum (50:50 to 75:25). This appears to optimize the synergy between fermentation substrate availability and microbial activity without excessively containing inhibitory compounds. These conditions create an ideal anaerobic environment for lactic acid production, resulting in stable and lower pH silage.

3.1.2. Silage NH_3

The results showed that the mixture ratio of *Tithonia diversifolia* and *Panicum maximum* significantly affected the silage's ammonia (NH_3-N) content. The decrease in NH_3 levels observed with increasing proportions of Tithonia up to treatment T3 indicates that adding Tithonia to the silage formulation can suppress protein degradation during the ensiling process. This effect is likely related to the content of secondary metabolites in Tithonia, such as tannins and flavonoids, which are known to have antimicrobial properties against proteolytic bacteria (Gutierrez et al., 2015). This is important because higher NH_3 values reflect greater protein degradation, indicating less efficient fermentation (Mudhita et al., 2024).

However, in treatment T4 (100% Tithonia), ammonia levels increased again. This increase may be related to secondary metabolites such as tannins and saponins in Tithonia diversifolia, which are known to affect rumen and silage microbial populations. These compounds can suppress the growth of beneficial fermentative bacteria while allowing proteolytic bacteria to dominate, resulting in higher ammonia production due to increased protein degradation (Olmo-González et al., 2022; Osuga et al., 2024). Thus, an excessively high proportion of Tithonia may alter microbial balance and reduce

fermentation efficiency. Ammonia is formed from the deamination of proteins or amino acids by microbial activity, particularly by spoilage microorganisms such as proteolytic bacteria (Liu et al., 2022). Furthermore, the increased ammonia levels in this treatment may also be due to suboptimal fermentation quality due to an imbalance between the availability of fermentable substrates and the moisture content of the silage material. Therefore, a balanced forage combination of *Tithonia diversifolia* and *Panicum maximum* (especially in treatments T2 and T3) provides the most favorable fermentation conditions for minimizing ammonia formation and maintaining protein integrity in the silage. Similar findings were reported by Barboza et al. (2025), who observed elevated ammonia levels in silage containing high proportions of *Tithonia diversifolia*, suggesting that excessive protein content may lead to increased proteolysis.

3.1.3. LAB Population

Differences in the ratio of *Tithonia diversifolia* and *Panicum maximum* mixtures between treatments did not significantly affect the lactic acid bacteria (LAB) population. The provision of molasses likely influenced this as an additional energy source, which provided a uniform fermentable substrate to support LAB growth across all treatments. Furthermore, uniformly controlled fermentation conditions, such as temperature, humidity, and compaction techniques, also played a role in maintaining a stable LAB population.

The lactic acid bacteria (LAB) population in this study ranged from 6.5 to 7.0 log cfu/g, comparable to the results of Riyanti et al. (2024), who reported a LAB population of 5.33–9.16 log cfu/g in *Pennisetum purpureum* silage supplemented with *Saccharomyces cerevisiae* and *Lactobacillus plantarum*. These values indicate that the LAB population in the current study was within the optimal range for good silage fermentation. The LAB populations detected in all treatments were within adequate limits and showed no significant differences. However, significant differences in fermentation parameters such as pH and ammonia (NH_3) concentrations indicate that fermentation quality is more influenced by the effectiveness of microbial activity in fermenting the substrate than by population size. The lower pH and lower NH_3 levels in treatments with a balanced Tithonia and Panicum mixture ratio (T2 and T3) indicate that the fermentation process was more optimal. This condition was characterized by more efficient lactic acid production and lower protein degradation. Therefore, it can be concluded that

fermentation effectiveness is determined by the metabolic ability of microbes to utilize the substrate efficiently, not solely by the number of microbes present

3.2. Silage Chemical Composition

The results of the analysis of variance showed that the mixture ratio of *Tithonia diversifolia* and *Panicum maximum* forages significantly ($P < 0.05$) affected the dry matter (DM), crude protein (CP), and crude fiber (CF) content of the silage. However, no significant effect ($P > 0.05$) was found on the ether extract (EE) content. The average chemical composition of silage from each treatment is presented in Table 2.

Tabel 2. Chemical composition of various mixed ratios of *Tithonia diversifolia* and *Panicum maximum*

Parameters	Treatment				
	T0 (0%:100%)	**T1 (25%:75%)**	**T2 (50%:50%)**	**T3 (75%:25%)**	**T4 (100%:0%)**
Dry matter (%)	23.1 ± 0.4^{d}	23.8 ± 0.3^{c}	24.3 ± 0.4^{b}	24.8 ± 0.3^{a}	25.0 ± 0.5^{a}
Crude protein (%DM)	14.2 ± 0.3^{e}	15.1 ± 0.4^{d}	15.9 ± 0.3^{c}	16.5 ± 0.4^{b}	17.0 ± 0.3^{a}
Crude fiber (%DM)	27.0 ± 0.3^{a}	26.2 ± 0.4^{b}	25.5 ± 0.5^{c}	25.1 ± 0.3^{c}	25.0 ± 0.4^{c}
Ether extract (%DM)	2.71 ± 0.14	2.50 ± 0.10	2.41 ± 0.12	2.31 ± 0.09	2.19 ± 0.11

Note : Data are presented as mean ± standard deviation (n = 3). Numbers followed by different superscript letters in the row indicate significant differences ($P < 0.05$) based on the DMRT test.

3.2.1. Dry Matter (%)

The dry matter content of silage increased with the increasing proportion of *Tithonia diversifolia* in the forage mixture (T0-T4). Increasing the dry matter content plays a crucial role in the success of the fermentation process. Silage with a dry matter content within the optimal range (20–35%) can support the activity of Lactic Acid Bacteria (LAB), suppress the growth of spoilage microorganisms, and reduce the risk of damage due to fluid seepage (Borreani et al., 2018). Silage that is too wet tends to result in unstable fermentation, while material that is too dry can inhibit the activity of fermentative microbes (Griswold et al., 2009).

The higher dry matter values in treatments T2 (50:50), T3 (75:25), and T4 (100:0) indicate that formulations with a sufficiently high proportion of Tithonia can produce silage with better nutrient density. Differences in dry matter content between treatments suggest that the composition of the forage mixture significantly determines the physical quality of the silage. Although Tithonia is known to have a high water content, these results indicate that under certain conditions and processing, its use in silage formulations can still produce material with optimal dry matter content.

3.2.2. Crude Protein (%DM)

The increasing proportion of *Tithonia diversifolia* in the silage mixture from treatments T0 to T4 showed a trend towards increasing crude protein content. This indicates that *Tithonia diversifolia* has a higher protein content than *Panicum maximum*. Therefore, the greater its proportion in the mixture (T1 to T4), the greater its contribution to the silage's CP value. Tithonia leaves are rich in protein and non-protein nitrogen compounds, making them a potential forage to increase the nitrogen content in mixed silages (Pazla et al., 2024).

Treatments T2 and T3 produced relatively high CP levels, but still within the range that supports stable fermentation, as indicated by the low NH_3 levels in both treatments. This suggests that adding Tithonia increases protein content and protects the protein from degradation during the silage process (Fasuyi & Ibitayo, 2021). Conversely, at T0 (100% Panicum), the lowest CP levels were found, reflecting the low protein content of Panicum maxima when used as a sole forage. The highest CP levels were achieved in the T4 treatment (100% Tithonia). Still, this condition requires further study due to the potential presence of antinutritional compounds that may inhibit optimal protein utilization by rumen microbes. Overall, the increase in CP from T0 to T4 indicates that *Tithonia diversifolia* can be used as a high-protein forage supplement in silage formulations. Treatments T2 and T3 appear to provide an optimal balance between protein content and fermentation quality, making them the most promising formulations for enhancing the nutritional value of ruminant silage.

3.2.3. Crude Fiber (%DM)

Observations showed decreased crude fiber (CF) content as the proportion of *Tithonia diversifolia* in the forage mixture increased from T0 to T4. Treatment T0, which used only Panicum maximum, produced the highest CF content, while treatments T3 and T4, which had higher proportions of Tithonia, showed lower CF levels. This indicates that *Tithonia diversifolia* has a relatively lower crude fiber content than Panicum maximum, so increasing its contribution to the mixture leads to decreased CF levels in the silage (Oluwasola & Dairo, 2016).

Panicum maximum is a source of structural fiber that can support rumen movement and maintain digestive health. Still, its use as a sole ingredient, as in T0, tends to produce silage with excessively high fiber content, which negatively impacts digestibility and

nutrient utilization (Idowu et al., 2020). Conversely, mixtures with Tithonia, such as in T2 and T3, can reduce crude fiber content to levels more suitable for ruminant livestock needs, without eliminating the structural benefits of Panicum. Although the reduced CF content in T4 indicates potential for improved digestibility, 100% Tithonia formulations still require caution due to the potential presence of antinutritional compounds such as tannins and saponins, which can reduce the effectiveness of fermentation and nutrient utilization (Olmo-González et al., 2022). Therefore, forage combinations such as those in T2 and T3 produce silage with high protein content and more moderate fiber content, which supports better nutritional quality and digestibility of the silage.

3.2.4. Eher extract (%DM)

Differences in the proportions of Tithonia and Panicum within the range of T0 to T4 did not significantly affect the total lipid fraction in the silage yield. Both Tithonia and Panicum are generally low-fat forages, so changes in their proportions are not sufficient to substantially impact eher extract (EE) content. Biologically, eher extract content in forage crops is influenced by plant type, harvest age, and plant part used (Ali et al., 2021). However, the lipid fraction in forages does not play a significant role in fermentation because it is not the primary substrate for fermentative microorganisms. Overall, the stability of eher extract values across all treatments (T0-T4) indicates that this parameter is insensitive to Tithonia and Panicum composition changes. Therefore, eher extract content is not a primary parameter in evaluating the effects of treatments on silage nutritional quality, especially when using low-lipid forages, as in this study.

Increasing the proportion of Tithonia in the mixture tends to increase DM and CP levels, reflecting the higher nutrient contribution of this crop, particularly in terms of nitrogen and functional dry matter content. On the other hand, *Panicum maximum*, known as a forage with a higher fiber structure, contributed significantly to the higher CF levels in treatments dominated by Panicum, such as T0 and T1. The decrease in CF that occurred with increasing proportions of Tithonia indicates that a balanced mixture can optimize digestibility without compromising fiber structure. Meanwhile, the low and relatively stable EE levels in all treatments suggest that these two forages are not the primary lipid sources, and changes in their proportions do not significantly impact the silage fat fraction. Overall, the appropriate combination of Tithonia and Panicum, as in T2 and T3,

can produce silage with a good nutritional balance with high protein, moderate fiber, and proper moisture content, making it a quality silage formulation strategy for ruminant feed.

3.3. In Vitro Digestibility of Silage

The results of the analysis of variance showed that variations in the composition of the *Tithonia diversifolia* and *Panicum maximum* mixtures significantly affected ($P < 0.05$) dry matter digestibility (IVDMD) crude protein digestibility (IVCPD), crude fiber digestibility (IVCFD). Still, they did not significantly affect ($P > 0.05$) on ether extract digestibility (IVEED). The average digestibility values for each treatment are shown in Table 3.

Tabel 3. In vitro digestibility of various mixed ratios of *Tithonia diversifolia* and *Panicum maximum.*

Parameters	Treatment				
	T0 (0%:100%)	T1 (25%:75%)	T0 (0%:100%)	T3 (75%:25%)	T0 (0%:100%)
Dry matter digestibility (%)	$46,1 \pm 1,2^{d}$	$48,3 \pm 1,4^{c}$	$51,2 \pm 1,3^{b}$	$53,0 \pm 1,1^{a}$	$50,5 \pm 1,5^{b}$
Crude protein digestibility (%)	$44,8 \pm 1,3^{d}$	$46,2 \pm 1,5^{c}$	$48,7 \pm 1,2^{b}$	$51,0 \pm 1,4^{a}$	$47,5 \pm 1,6^{b}$
Crude fiber digestibility (%)	$36,9 \pm 1,1^{a}$	$35,8 \pm 1,3^{b}$	$34,5 \pm 1,2^{c}$	$33,2 \pm 1,0^{d}$	$33,0 \pm 1,3^{d}$
Ether extract digestibility (%)	$47,3 \pm 0,9$	$48,0 \pm 1,1$	$48,8 \pm 1,0$	$49,0 \pm 1,2$	$48,6 \pm 1,3$

Note : Data are presented as mean ± standard deviation (n = 3). Numbers followed by different superscript letters in the row indicate significant differences ($P < 0.05$) based on the DMRT test.

3.3.1. Dry Matter Digestibility (%)

Variations in the proportions of *Tithonia diversifolia* and *Panicum maximum* forage mixtures significantly affected the silage's in vitro dry matter digestibility (IVDMD). These results reflect differences in the ability of rumen microbes to break down the nutrient matrix of silage from each treatment. The significant increase in dry matter digestibility in treatments T2 and T3 compared to T0 indicates that the addition of Tithonia to the silage formulation enhanced dry matter degradation in the rumen. This can be attributed to the high content of more easily digestible nutrients in Tithonia diversifolia, such as non-structural carbohydrates and soluble proteins, which serve as energy sources and nitrogen for rumen microbes (Pazla et al., 2024). Furthermore, the decrease in crude fiber recorded in both treatments (Table 2) also contributed to increased dry matter digestibility (IVDMD), given that a high fiber fraction generally inhibits ruminal fermentation (Almaeda et al., 2022).

Interestingly, the IVDMD value in the T4 (100% Tithonia) treatment decreased slightly compared to T2 and T3, although it was still higher than T0. This indicates that

using Tithonia alone does not provide maximum efficiency in dry matter utilization. This phenomenon is related to antinutritional compounds such as tannins and saponins in higher concentrations in T4, which can inhibit rumen microbial activity (Olmo-González et al., 2022). Therefore, although Tithonia has the potential to be a high nutrient source, its presence needs to be balanced with other forages such as Panicum to optimize nutrient utilization by rumen microbes. Overall, a balanced forage mixture formulation of Tithonia and Panicum, as in T2 and T3, has produced silage with better dry matter digestibility.

3.3.2. Crude Protein Digestibility (%)

The crude protein digestibility (IVCPD) of silage showed a significant increase with increasing proportion of *Tithonia diversifolia* in the forage mixture from T0 to T3. This increase in oil protein digestibility indicates that the protein contained in Tithonia is quantitatively higher and more readily degraded by rumen microbes compared to protein derived from *Panicum maximum*. This is supported by the characteristics of Tithonia protein, which is relatively more soluble in rumen fluid and ferments more quickly, resulting in higher digestibility (Pazla et al., 2021).

The T3 treatment showed the highest crude protein digestibility (IVCPD), reflecting that this ratio creates the most optimal conditions for protein degradation by rumen microbes. This mixture likely provides a balance between sufficient nitrogen content and the physical structure of the forage that supports microbial colonization in the in vitro system. Meanwhile, although T4 (100% Tithonia) also has a high protein content, its crude protein digestibility decreased slightly compared to T3. This can be explained by the potential presence of antinutritional compounds such as tannins and saponins naturally present in Tithonia, which can bind proteins or inhibit the enzymatic activity of rumen microbes (Olmo-González et al., 2022). The presence of these compounds can cause some proteins to be inefficiently degraded, thus reducing their digestibility. Overall, these results reinforce the idea that *Tithonia diversifolia* acts as a forage protein source that not only increases the total nitrogen content in silage but also enhances protein digestibility by rumen microbes, mainly when used in moderate mixture proportions such as in treatments T2 and T3. This formulation can be considered in strategies to improve feed quality.

3.3.3. Crude Fiber Digestibility (%)

Differences in the proportion of forage in silage formulations significantly contribute to variations in the resulting crude fiber digestibility (IVCFD). The pattern of decreasing crude fiber digestibility from treatments T0 to T4 indicates that the higher the proportion of Tithonia in the formulation, the lower the digestibility of the fiber fraction. However, Tithonia has a lower crude fiber content than Panicum; its high proportions, such as in T3 and T4, result in lower crude fiber digestibility than the other treatments.

The decrease in crude fiber digestibility (IVCFD) in treatments T3 and T4 is influenced by antinutritional compounds such as tannins and saponins found in Tithonia. These compounds have a high affinity for plant proteins and cell walls, forming insoluble complexes that inhibit the activity of cellulolytic enzymes during in vitro rumen fermentation (Wahyuni et al., 2014). As a result, despite quantitatively lower fiber content, its digestibility decreases due to cellulose and hemicellulose degradation inhibition. Therefore, it can be concluded that the proportion of forage in silage formulation affects not only total fiber content but also the quality of its degradation. The T2 treatment is a promising option for increasing fiber utilization efficiency without sacrificing fermentative and other nutritional aspects.

3.3.4. Ether Extract Digestibility (%)

The ether extract digestibility (IVEED) of various *Tithonia diversifolia* and *Panicum maximum* mixture treatments did not significantly affect the in vitro rumen microbial digestion of the lipid fraction in silage. The crude fat fraction in forage is generally composed of triglycerides and free fatty acids, which are not the primary substrates for rumen fermentation, thus limiting its role in fermentation dynamics and overall digestibility (Rahmawati et al., 2021). Under these conditions, lipid hydrolysis by lipase enzymes in the rumen system occurred consistently across all treatments, as indicated by stable IVEED values from T0 to T4. Thus, although increasing the proportion of Tithonia in treatments such as T3 and T4 increased dry matter and crude protein levels, this did not necessarily increase the digestibility of ether extract fraction. This suggests that in vitro ether extract digestibility (IVEED) systems is more influenced by intrinsic factors of the lipid components rather than variations in forage composition.

In vitro digestibility showed different responses to variations in the mixture ratio of *Tithonia diversifolia* and *Panicum maximum* in silage formulations. Dry matter (DM) and

crude protein (CP) digestibility increased significantly with the addition of Tithonia up to a ratio of 75% (T3), reflecting the high content of easily digestible nutrients in Tithonia, such as non-structural carbohydrates and soluble protein. However, crude fiber (CF) digestibility decreased with increasing proportion of Tithonia, likely due to the effects of antinutrients such as tannins and saponins, which inhibit cellulolytic enzyme activity. Meanwhile, ether extract digestibility (IVEED) tended to be stable across all treatments, indicating that the lipid fraction was not significantly affected by variations in forage composition, due to its limited role in rumen fermentation. Therefore, a balanced forage mixture formulation, as in treatments T2 and T3, was considered optimal in increasing the efficiency of primary nutrient digestion without compromising the fermentative quality of the silage.

3.4. Pearson's Correlation between Chemical Composition and In Vitro Digestibility of Silage

The results of the Pearson correlation analysis showed a variable relationship between the chemical composition of silage and the in vitro digestibility at various mixture ratios of *Tithonia diversifolia* and *Panicum maximum*. Statistically significant correlations were found for several parameters, indicating that silage nutrient quality directly influences rumen microbial digestibility (Mahyuddin, 2008). The correlation coefficient results are presented in **Figure 1**.

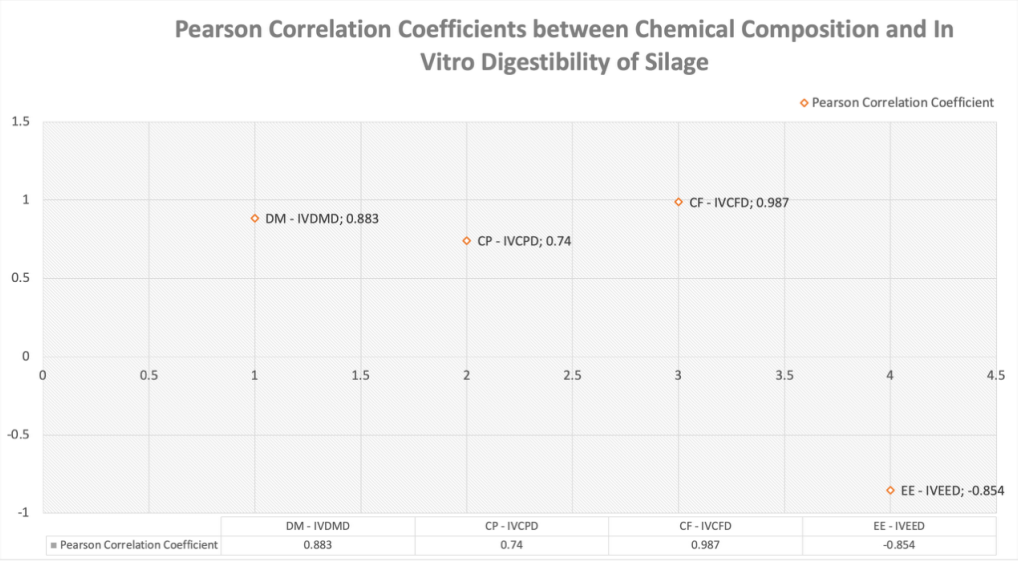

Figure 1. Graph of the Pearson correlation coefficient between chemical composition and in vitro digestibility of silage with *Tithonia diversifolia* and *Panicum maximum* Ratio

Dry matter content (DM) showed a strong positive correlation with dry matter digestibility (IVDMD) (r = 0.883; p = 0.047). These results indicate that increasing dry matter content in silage, as indicated by an increased proportion of *Tithonia diversifolia*, tends to be followed by increased dry matter digestibility. Optimal dry matter content is essential for fermentation, maintaining silage stability, and providing sufficient substrate for rumen microbes to decompose (Tanga et al., 2006). Crude protein (CP) content also showed a positive correlation with crude protein digestibility (IVCPD) (r = 0.740), although this relationship was not statistically significant (p = 0.153). Although not substantial, this correlation reflects that the increased CP content of *Tithonia diversifolia* contributes to the high value of crude protein digestibility, as also reported by Hidayatullah and Rini (2024), that protein from legumes or broadleaf plants tends to be more easily digested than from C4 grasses such as *Panicum maximum*. Crude fiber (CFR) showed a robust and significant positive correlation with crude fiber digestibility (IVCFD) (r = 0.987; p = 0.0018). An increase followed the increase in fiber in treatments dominated by *Panicum maximum* in fiber digestibility. This is because the fiber fraction contained in Panicum has a higher proportion of cellulose-hemicellulose and lower lignification than *Tithonia diversifolia*. Ether extract content (EE) showed a strong negative correlation with ether extract digestibility (IVEED) (r = –0.854), although not significant (p = 0.065). Moderate amounts of fat can increase feed efficiency, but excessive amounts can interfere with rumen fermentation and reduce the activity of fiber-digesting microbes (Suharti et al., 2018). The decrease in fat digestibility at a higher mixture ratio of Tithonia content may be associated with the lipid content or certain bioactive compounds in Tithonia that act as inhibitors against lipolytic microbial activity. In general, these results confirm that changes in chemical composition due to variations in the proportion of *Tithonia diversifolia* and *Panicum maximum* affect the quality of silage fermentation and its digestibility.

4. Conclusion

This study demonstrates that varying the ratio of *Tithonia diversifolia* and *Panicum maximum* in silage significantly influences fermentation quality, chemical composition, and in vitro nutrient digestibility. Balanced mixtures, especially T2 (50:50) and T3 (75:25), enhanced crude protein, reduced crude fiber, and improved rumen digestibility. These results highlight the potential of local forage-based silage to enhance nutrient

utilization in ruminants. Silage with 50–75% *Tithonia diversifolia* is recommended to improve forage quality in tropical regions. Further research should assess animal responses and production outcomes under practical feeding conditions.

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