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Research Article

## Silage Formulation Based on *Tithonia diversifolia* and *Panicum maximum*: In Vitro Fermentation Evaluation for Tropical Feeding Systems

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### ABSTRACT

Ensuring year-round availability of high-quality forage is critical for improving the productivity of ruminant livestock, particularly under tropical climatic conditions where seasonal fluctuations frequently disrupt feed supply. This study evaluated the fermentative quality of silage produced from varying ratios of *Tithonia diversifolia* and *Panicum maximum* using an in vitro rumen fermentation system. A Completely Randomized Design (CRD) was applied with five treatment groups (T0 = 0% *Tithonia*, T1 = 25%, T2 = 50%, T3 = 75%, and T4 = 100%) and three replicates each. After a 21-day ensiling period, samples were assessed for pH, total gas production, ammonia nitrogen (NH<sub>3</sub>-N), and volatile fatty acids (VFA) using fresh rumen fluid from slaughtered cattle as the inoculum. Results revealed that increasing the proportion of *T. diversifolia* enhanced fermentative activity, as indicated by higher total gas and VFA production, and lower pH values. Treatment T3 (75% *Tithonia*) showed the most favorable fermentative profile, with significantly higher gas and VFA yields and NH<sub>3</sub>-N concentrations within the optimal range for microbial protein synthesis. Conversely, T4 (100% *Tithonia*) exhibited signs of fermentative inhibition, likely due to anti-nutritional factors such as tannins and saponins. In contrast, T0 (100% *Panicum*) demonstrated limited fermentability due to its high fiber and low soluble carbohydrate content. These findings suggest that a 75:25 ratio of *Tithonia diversifolia* to *Panicum maximum* optimizes rumen fermentation and may serve as a promising strategy for improving silage quality in tropical forage systems.

**Keywords:** in vitro fermentation, *Panicum maximum*, silage, *Tithonia diversifolia*

### INTRODUCTION

The stable and high-quality availability of forage is a key factor in improving the productivity of ruminant livestock. In tropical climates such as Indonesia, seasonal fluctuations often result in inconsistent forage supply, particularly during the dry season. One strategy to maintain the continuity of feed supply is through the ensiling of forage. Silage fermentation serves as a preservation method and can enhance digestibility and nutrient content when appropriate materials and techniques are used (Muchlis et al., 2023). However, the final quality of silage largely depends on the characteristics of the forage used, especially its nutrient content and chemical composition.

*Panicum maximum* is widely recognized as a superior forage due to its high biomass productivity. Nevertheless, its protein content and soluble carbohydrate levels have limitations,

both of which are essential for optimal fermentation (Dhalika et al., 2015). On the other hand, *Tithonia diversifolia*, a shrubby species, is known for its high protein (Jamarun et al., 2024; Pazla et al., 2021a), energy content, and is easily degraded by rumen microbes (Sirait & Simanihuruk, 2021). also has potential as an alternative feed (Pazla et al., 2018; Arief et al., 2018; Jamarun et al., 2020). Combining these two types of forage is a promising strategy for improving silage quality: *Panicum maximum* provides structural fiber, while *Tithonia diversifolia* contributes fermentable protein and energy. However, anti-nutritional compounds in *Tithonia diversifolia*, such as tannins and saponins, may limit its inclusion in the mix, as excessive amounts can inhibit rumen microbial activity (Yogianto et al., 2014).

To date, studies on the integration of these two forage types into silage remain limited, particularly regarding the effects of different mixing ratios on fermentative quality within an in vitro rumen system. In vitro, evaluation is a practical approach to assess microbial fermentation responses to various feed formulations before in vivo application. Therefore, this study aimed to evaluate the fermentative quality of silage made from various proportions of *Tithonia diversifolia* and *Panicum maximum*. Parameters such as pH, gas production, ammonia concentration (NH<sub>3</sub>-N), and volatile fatty acids (VFA) content were measured as key indicators of microbial activity and fermentation efficiency. The results of this research are expected to contribute to developing more efficient and applicable silage formulation strategies based on tropical forage combinations in ruminant livestock systems.

## MATERIAL AND METHODS

This study was conducted in November 2022 at the Integrated Laboratory of the Faculty of Animal Science, Universitas Andalas, Campus II Payakumbuh. The research evaluated the fermentative quality of silage produced from a combination of *Tithonia diversifolia* leaves and *Panicum maximum*, using an in vitro testing approach.

The primary materials used in this study were fresh *Tithonia diversifolia* leaves and *Panicum maximum* grass collected from forage plots surrounding the university campus. The plants were harvested at the vegetative stage, where their nutrient content is optimal. After harvesting, the forages were chopped using a forage chopper into 3–5 cm lengths to facilitate uniform mixing and fermentation.

The chopped forages were then weighed according to five treatment compositions based on fresh weight ratios 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*

Each treatment consisted of a total of 5 kg of fresh material. The mixing process was conducted on a plastic sheet or tarp with a forage layer approximately 5 cm thick to allow even additive distribution. Molasses was used as an additive at 4% of the total fresh weight (i.e., 200 g per 5 kg of forage) (Dhalika et al., 2021). It was first diluted with clean water in a 1:1 ratio, then sprayed evenly onto the forage mixture using a manual sprayer. The material was thoroughly mixed until a homogeneous blend was achieved.

Following mixing, the silage material was packed into fermentation containers made of plastic bags or drums with a 5–6 kg capacity. The materials were filled in layers while being compressed

at each stage to expel air and ensure anaerobic conditions. The top of each silo was sealed with plastic, tightly tied, and weighed down with a cover or weight to prevent oxygen entry. All silage units were stored in a shaded area at ambient temperature (approximately 27–30°C) for 21 days to allow optimal fermentation (Landupari et al., 2020).

After the fermentation period, silage from each treatment was sampled homogeneously from the middle section of the silo, transferred into sealed containers, and stored in a refrigerator until further analysis. The experiment was designed using a Completely Randomized Design (CRD), consisting of five treatments (T0–T4), each replicated three times, resulting in 15 experimental units.

Fermentative quality was assessed through in vitro testing using rumen fluid as the microbial inoculum. Fresh rumen fluid was collected from slaughtered cattle at the Municipal Abattoir (RPH) in Payakumbuh. The fluid was immediately collected post-slaughter and filtered through a double layer of cheesecloth to remove large particles and preserve microbial integrity (Afdal & Yurleni, 2015).

The in vitro fermentation was carried out under anaerobic conditions in a closed fermentation system in the laboratory. Parameters observed included in vitro pH, total gas production, ammonia concentration (NH<sub>3</sub>-N), and volatile fatty acids (VFA) content. Data were analyzed using analysis of variance (ANOVA) to determine the effect of treatments on each parameter. When significant differences were detected, Duncan's Multiple Range Test (DMRT) was applied at a 5% significance level. Statistical analyses were performed using SPSS version 25.

## RESULTS AND DISCUSSION

The observations of fermentative parameters of silage made from *Tithonia diversifolia* and *Panicum maximum* combinations are presented in Table 1. The parameters measured include in vitro pH, total gas production, ammonia (NH<sub>3</sub>-N) concentration, and volatile fatty acids (VFA) content.

Table 1. Mean Values of Fermentative Parameters of Silage Made from *Tithonia diversifolia* and *Panicum maximum* combinations

| Parameter                      | Treatment                |                          |                          |                          |                          |
|--------------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
|                                | T0<br>(0%:100%)          | T1<br>(25%:75%)          | T2<br>(50%:50%)          | T3<br>(75%:25%)          | T4<br>(100%:0%)          |
| pH                             | 4,70 <sup>a</sup> ± 0,05 | 4,55 <sup>b</sup> ± 0,06 | 4,40 <sup>c</sup> ± 0,04 | 4,25 <sup>d</sup> ± 0,05 | 4,30 <sup>d</sup> ± 0,06 |
| Total gas (mL)                 | 40,3 <sup>d</sup> ± 1,8  | 51,0 <sup>c</sup> ± 2,2  | 65,8 <sup>b</sup> ± 1,5  | 68,5 <sup>a</sup> ± 1,9  | 60,2 <sup>b</sup> ± 2,0  |
| NH <sub>3</sub> -N (mg/100 mL) | 5,5 <sup>e</sup> ± 0,2   | 7,1 <sup>d</sup> ± 0,3   | 9,3 <sup>c</sup> ± 0,4   | 10,6 <sup>b</sup> ± 0,5  | 12,7 <sup>a</sup> ± 0,3  |
| VFA (mmol/L)                   | 60,2 <sup>e</sup> ± 2,1  | 70,4 <sup>d</sup> ± 2,0  | 80,5 <sup>bc</sup> ± 2,3 | 83,1 <sup>ab</sup> ± 1,8 | 78,8 <sup>c</sup> ± 2,5  |

Note: According to Duncan's Multiple Range Test, different superscripts within the same row indicate significant differences ( $p < 0.05$ )

### pH

The pH value after in vitro incubation reflects the final result of microbial fermentation activity on the feed substrate (Mariani & Suryani, 2016). A decrease in pH during fermentation is caused by the accumulation of metabolic products such as volatile fatty acids (VFA) (Jamarun et al., 2019) and other organic acids. Therefore, in vitro pH is an important indicator of the fermentability of feed materials (Fitria et al., 2024).

In this study, the pH values varied significantly among treatments, indicating different fermentation environments. Lower pH values in treatments with higher proportions of *Tithonia diversifolia* (particularly T3 and T4) indicate the accumulation of fermentation acids, suggesting active microbial fermentation. This supports the idea that *Tithonia*, which contains soluble carbohydrates and readily degradable protein, provides more fermentable substrates for rumen microbes, leading to increased VFA production and pH reduction (Rahayu et al., 2018).

However, while T4 (100% *Tithonia*) also had a low pH, it was slightly higher than T3. This suggests that a complete substitution with *Tithonia* may not result in a linear increase in fermentative activity. Anti-nutritional compounds such as tannins and saponins in *Tithonia diversifolia* may inhibit certain microbial groups, particularly cellulolytic bacteria, reducing fermentation efficiency despite the abundance of substrates (Yogianto et al., 2014).

Conversely, T0 (100% *Panicum*) showed the highest pH, indicating low fermentation activity due to limited fermentable substrates and the high content of crude fiber and lignin, which are resistant to microbial degradation. As a result, fewer VFAs were produced, and the pH remained higher (Baga et al., 2023).

Overall, the pH values support the notion that balanced combinations of forages, such as in T2 and T3, create optimal fermentation environments for rumen microbial activity. These conditions imply that silage with a balanced nutrient profile and controlled anti-nutritional factors promotes fermentation and digestion in ruminant systems more effectively.

### Total Gas Production

Total gas production during in vitro fermentation is a key indicator of rumen microbial activity in degrading organic matter and converting it into end products such as VFA, carbon dioxide (CO<sub>2</sub>), and methane (CH<sub>4</sub>). Higher gas production generally reflects greater availability of fermentable substrates and microbial efficiency (Addawiyyah et al., 2021).

In this study, gas production increased with higher proportions of *Tithonia diversifolia* in the silage formulation, peaking at T3 (75% *Tithonia*). This indicates that *Tithonia* contributed soluble carbohydrates and protein that supported microbial fermentation. The T3 treatment produced the highest total gas (68.5 mL at 72 hours), reflecting an optimal balance between energy and fiber for microbial growth. Figure 1 shows the gas production pattern during incubation.

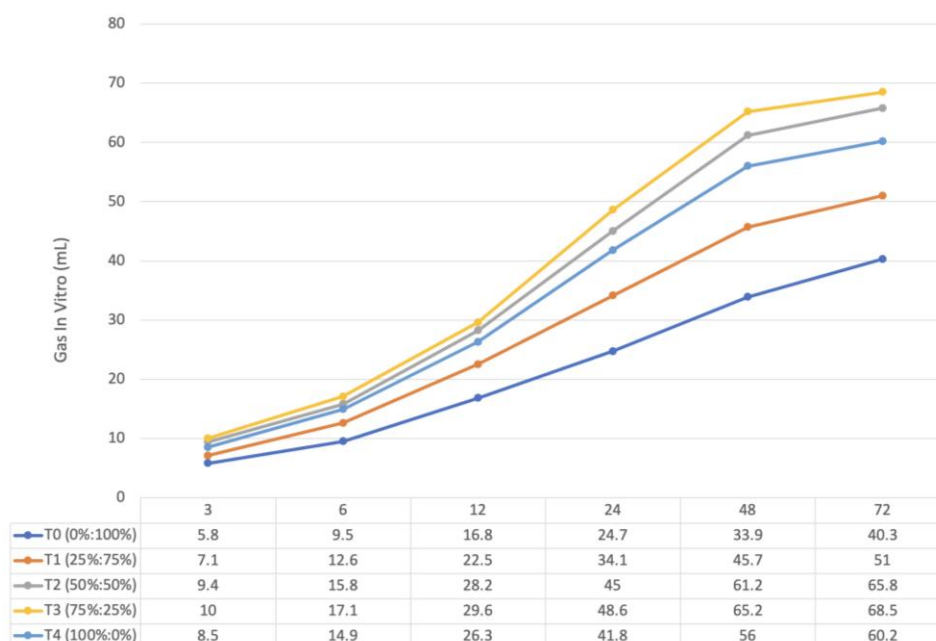


Figure 1. In Vitro Gas Production (mL) of Silage from *Tithonia diversifolia* and *Panicum maximum* combinations

T3 consistently showed the highest gas accumulation throughout the incubation period, especially between 12 and 48 hours, the most active fermentation phase. While T4 used 100% *Tithonia*, its total gas was lower than T3. This may be due to the suppressive effects of secondary metabolites like tannins and saponins on microbial fermentation, particularly in the late fermentation phase (Yogianto et al., 2014). This is also supported by the slower gas accumulation from 48 to 72 hours.

T0 (100% *Panicum*) produced the lowest gas (40.3 mL), reflecting the limited availability of fermentable substrates in *Panicum*, which is high in indigestible fiber and low in soluble carbohydrates. In addition, this is also due to the low protein content in T0 compared to other treatments (Pazla et al., 2021b). Treatments T1 and T2 followed a logical trend, with T2 consistently producing more gas than T1 at all time points, suggesting that increasing *Tithonia* to 50% still enhances fermentation without adverse anti-nutritional effects.

Overall, gas production data from total values (Table 1) and fermentation dynamics (Figure 1) indicate that T3 provided the most optimal fermentation condition. This supports the idea that balanced forage combinations can improve rumen fermentation efficiency and feed utilization.

### NH<sub>3</sub>-N

Ammonia concentration (NH<sub>3</sub>-N) is critical in assessing protein degradation and microbial nitrogen utilization during fermentation. Ammonia is produced through microbial deamination of dietary protein into amino acids and then into NH<sub>3</sub>, which can be reused by microbes for microbial protein synthesis. However, if ammonia exceeds microbial assimilation capacity, it accumulates in the medium (Bakhtiar et al., 2013).

In this study, increasing proportions of *Tithonia diversifolia* resulted in higher NH<sub>3</sub>-N concentrations, with the highest in T4. This indicates extensive degradation of the high protein content in *Tithonia* by rumen microbes (Putri et al., 2024). However, excessively high NH<sub>3</sub>-N, as in T4, may suggest an imbalance in the energy-to-nitrogen ratio, reducing nitrogen utilization efficiency.

T0 (100% *Panicum*) showed the lowest  $\text{NH}_3\text{-N}$  concentration, reflecting low protein content and limited available nitrogen for microbial growth, potentially limiting microbial protein synthesis (Suharti et al., 2018). T2 and T3 had moderate  $\text{NH}_3\text{-N}$  values, close to the optimal range (8–10 mg/100 mL) for microbial synthesis, suggesting that balanced forage ratios can provide sufficient nitrogen without excessive accumulation, supporting effective fermentation and microbial growth.

Elevated  $\text{NH}_3\text{-N}$  in T4 may also be linked to anti-nutritional compounds like saponins and tannins, which can disrupt microbial protein synthesis and nitrogen conversion efficiency (Yogianto et al., 2014). Thus, high  $\text{NH}_3\text{-N}$  levels do not always equate to efficient fermentation.

In summary,  $\text{NH}_3\text{-N}$  results suggest that treatments T2 and T3 provide efficient fermentation conditions, with adequate nitrogen availability supporting microbial activity and fermentation stability in the rumen. These findings support the recommendation for balanced *Tithonia*-*Panicum* combinations to achieve optimal fermentative and nutritional value.

### Volatile Fatty Acid (VFA)

VFAs are the main end products of microbial anaerobic carbohydrate fermentation in the rumen and serve as a primary energy source for ruminants (Nurwildan et al., 2022). Higher VFA concentrations indicate better substrate fermentability and efficient microbial utilization of both soluble carbohydrates and fiber components (Rahayu et al., 2018).

In this study, increasing *Tithonia diversifolia* levels generally led to increased VFA production, peaking at T3. This suggests that up to 75% inclusion of *Tithonia* enhances the availability of fermentable substrates such as soluble carbohydrates and degradable protein. Combining the more fibrous *Panicum maximum* creates synergy by balancing fast- and slow-release energy sources to maintain stable fermentation.

However, in T4 (100% *Tithonia*), VFA concentration slightly declined compared to T3, indicating that sole use of *Tithonia* may not yield the most efficient fermentation. This may be due to higher concentrations of secondary metabolites like tannins and saponins, which can inhibit microbial activity, fiber degradation, and substrate conversion into VFA (Yogianto et al., 2014).

Conversely, T0 had the lowest VFA content, confirming that *Panicum maximum* alone lacks fermentable substrates. Its high crude fiber and lignin content limit nutrient availability for rumen microbes, resulting in slower and less efficient fermentation (Wijayanti et al., 2012).

Thus, the increase in VFA production in T2 and T3 supports the notion that balanced *Tithonia*-*Panicum* combinations enhance fermentative silage quality. High VFA in this treatment is derived from carbohydrates and protein (Pazla et al., 2021b). These combinations offer optimal substrate composition to support microbial fermentation and energy yield, providing a strong rationale for using balanced forage ratios in silage-based feeding strategies.

### CONCLUSIONS

Based on the observations, it can be concluded that the forage ratio significantly influences rumen microbial fermentation activity. The formulation containing 75% *Tithonia diversifolia* and 25% *Panicum maximum* exhibited the most balanced and optimal fermentative response compared to other treatments. These findings support the initial objective of the study, which was to identify the optimal combination ratio of tropical forages capable of producing high fermentative quality silage under in vitro rumen conditions.

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