

***In Vitro* Nutrients Digestibility of the Combination *Tithonia diversifolia* and Napier Grass (*Pennisetum purpureum*)**

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ABSTRACT

The objective of this study was to evaluate the effects of the combination *Tithonia diversifolia* and napier grass (*Pennisetum purpureum*) on *in vitro* digestibility. This research was carried out using a randomized block design with 4 treatments of the level combination of *Tithonia diversifolia* and napier grass and 4 replications. The following treatments were performed: T1 = 20% *Tithonia diversifolia* + 80% napier grass; T2 = 40% *Tithonia diversifolia* + 60% napier grass; T3 = 60% *Tithonia diversifolia* + 40% napier grass; T4 = 80% *Tithonia diversifolia* + 20% napier grass. The data were subjected to an analysis of variance (ANOVA), and differences between the treatment means were tested using Duncan's multiple range test (DMRT). The parameters measured were as follows: dry matter digestibility (%), organic matter digestibility (%), crude protein digestibility (%), neutral detergent fiber (NDF) digestibility (%) and Acid Detergen Fiber (ADF) digestibility (%). The results revealed that dry matter digestibility, organic matter digestibility, crude protein digestibility, NDF digestibility and ADF digestibility were significantly ($P < 0.05$) increased by the combination in T1. The combination of 20% *Tithonia diversifolia* + 80% napier grass resulted in the highest *in vitro* nutrients digestibility.

Keywords: *In vitro*, *Tithonia diversifolia*, *Pennisetum purpureum*, Digestibility

INTRODUCTION

The *Tithonia diversifolia* plant is a shrub that has potential to be used as alternative animal feed. *Tithonia diversifolia* has spread in Indonesia, especially in West Sumatra, which grows and is often found on the sides of the road and in the rice fields considered as bush, harbinger and obstructing the view that has been wasted and some of them are utilizing as a compost, natural pesticides, but not many used as animal feed, especially ruminant animal. *Tithonia diversifolia* cultivated in West Sumatra can produce as much as 30 tons of fresh material or 6 tons of dry matter per year with a land area of about 1/5 ha. If grown as a hedge, *Tithonia diversifolia* can produce 27 kg of dry weight of each harvested from three harvests for one year (Hakim, 2001).

Tithonia diversifolia is a type of flowering plant with a golden yellow color that comes out at the end of the rainy season with a similar appearance to the sunflower. In addition to fast growth also has a good nutritional content. The leaves and flowers of the *Tithonia diversifolia* plant have high productivity and good nutritional content. Nutrient content of whole plant (leaf + stem) of *Tithonia diversifolia* was dry matter 18.4%, crude protein 19.4%, crude fat 5.8%, crude fiber 19.4% while only leaf part contains crude protein 25.9% and crude fiber 14.5% (Adrizal and Montesqrit, 2013). Fasuyi et al., (2010) reported that *Tithonia diversifolia* also contains many antinutrition substances such as phytic acid,

tannins, saponins, oxalates, alkaloids and flavonoids that are limiting factors in their use in rations.

Several studies on *Tithonia diversifolia*, supplementation to the ration mixture do not have a negative effect on ruminants' productivity and digestion during the doses of excessive level in livestock rations. This study aims to find the best level of *Tithonia diversifolia* combined with napier grass and to show its effect on *invitro*.nutrients digestibility.

MATERIALS AND METHODS

This research was carried out in nutrition laboratory of animal science Faculty, Andalas university, Padang. The rumen fluid is taken from the rumen of the goat. *Tithonia diversifolia* is taken around the town of Padang City and Napier grasses were picked up from the Farm of Faculty of Animal Science, Andalas University Padang. The chemical composition of *Tithonia diversifolia* and Napier grass was presented in Table 1.

Table 1. The chemical composition of *Tithonia diversifolia* and Napier grass

Composition (%)	Feed ingredients	
	Napier Grass	<i>Tithonia diversifolia</i>
Dry Matter	21.23	25.57
Crude Protein	10.88	22.98
Crude Fiber	32.77	18.17
Fat	2.48	4.71
Ash	10.54	15.99
NDF	66.57	61.12
ADF	41.71	40.15
Cellulose	34.18	34.59
Lignin	6.29	4.57

Source: Nutritional Analysis of Ruminant Nutrition Laboratory, Faculty of Animal Science Andalas University (2017)

Tithonia diversifolia and Napier grass were combined in 4 treatments with 4 replications were T1 = 20% *Tithonia diversifolia* + 80% Napier grass, T2 = 40% *Tithonia diversifolia* + 60% Napier grass, T3 = 60% *Tithonia diversifolia* + 40% Napier grass and T4 = 80% *Tithonia diversifolia* + 20% Napier grass. The design used was a randomized block design. Differences between treatments mean were tested with Duncan Multiple Range Test (DMRT) at 5% level.

Parameters measured were dry matter, organic matter, crude protein, NDF and ADF digestibility using *in vitro* method (Tilley and Terry, 1963). Incubation for 48 hours at 39°C. After incubation for 48 hours the fermentation activity was stopped by immersion with ice water to stop microbial activity and continued by the separation between the supernatant and the residue. The precipitate of the supernatant mixture and the residue was filtered with the paper of whatman no 41 and dried in 60°C oven, then analyzed the digestibility of nutrients and fiber fraction of the materials.

RESULTS AND DISCUSSION

The *in-vitro* digestibility of combination of *Tithonia diversifolia* with napier grass are presented in Table 2.

Table 2. The *in-vitro* nutrients digestibility of the combination of *Tithonia diversifolia* with napier grass

Digestibility (%)	Treatments			
	T1	T2	T3	T4
Dry Matter	58.30 ^a ±0.53	56.59 ^b ±0.68	49.98 ^c ±0.33	48.04 ^d ±0.26
Organic Matter	57.85 ^a ±1.34	55.76 ^a ±1.21	49.17 ^b ±0.40	47.17 ^b ±0.37
Crude Protein	66.14 ^a ±1.41	54.12 ^b ±0.75	47.30 ^c ±0.62	42.40 ^d ±1.53
NDF	57.28 ^a ±0.86	53.29 ^{ab} ±0.64	46.98 ^{bc} ±3.39	41.88 ^c ±3.59
ADF	48.73 ^a ±2.32	44.26 ^a ±1.66	37.84 ^b ±3.75	35.46 ^b ±4.32

Means in the same row with different a,b letters are significant at $p < 0.05$,

The addition of *Tithonia diversifolia* levels from the treatment of 20% Tithonia (T1), T2 : 40% Tithonia, T3 : 60% Tithonia and T4 : 80% Tithonia treatment, indicated the lower all nutrients digestibility. The growth of microbial and metabolism activity in the rumen less optimal with the addition of *Tithonia diversifolia*, because this plant contains some antinutrition factors such as phytic acid, tannin, oxalate, saponin, alkaloids and flavonoids that can inhibit the digestion process in the rumen (Fasuyi *et al.*, 2010). This antinutrition has a negative effect on livestock depending on the amount of doses given.

Phytic acid compounds are anti-nutritional substances that in *Tithonia diversifolia* amount of 79.2 mg / 100g (Oluwasola and Dayro, 2016). In contrast to monogastric animals, ruminant has microbes in the rumen that can produce fitase enzyme from bacteria *Actinobacillus sp* and *Bacillus pumilus* (Lamid, 2012). The enzyme has capable to breaking the Phosphor bond with phytate, so that P can be absorbed and used as a mineral source of phosphorus (P) for livestock. Phytate in the rumen can also form complex bonds with Zn to form Zn-phytate complexes. This complex is easily to be degraded by enzyme and release Zn slowly to be used for rumen microbial growth and finally for the growth of animal (Hernaman *et al.*, 2007).

The *Tithonia diversifolia* level of 20% (T1) shown the highest digestibility due to the optimal phytic acid antinutrition degraded by rumen microbial fitase enzyme, so that P availability for growth and development of optimum rumen microbe. P is an essential component of microbial growth, especially maintaining the integrity of cell membranes and cell walls, components of nucleic acids and parts of high-energy molecules (ATP, ADP, etc.) (Bravo *et al.*, 2003; Rodehutsord *et al.*, 2000). The synthesis of microbial proteins will increase when P is available to sufficient quantities (Karsil and Rusel, 2001). Some feed ingredients are deficient in P minerals, so P is added to the feed for rumen microbes to grow and thereby increase digestibility (Febrina *et al.*, 2016; Jamarun *et al.*, 2017; Pazla, 2015; Zain *et al.*, 2010). Optimal growth of rumen microbes in T1 causes optimal rumen bacteria activity in degrading feed so that dry matter and organic matter digestibility shown the highest value. The dry matter digestibility in this experiment was lower compared to the results reported by Odedire and Oloidi (2014), which is 70.98% dry matter digestibility on the use of 30% *Tithonia diversifolia* mixed in concentrate for goat rations.

Tithonia diversifolia also contains many essential amino acids for microbial growth such as methionine, leucine, isoleucine and valine and also not maximally utilized because phytic acid has a bond with amino acid (Pallauf and Rimback, 1996). Decreasing protein digestibility caused by phytic acid can suppress the use of protein or amino acids by forming protein-fitat complexes that changes in protein structure. Changes in the protein structure will result in decreased protein solubility, enzyme activity and protein digestibility (Urbano *et al.*, 2000; Greiner and Konietzny, 2011). *Tithonia diversifolia* also contains tannin as antinutrition. Tannins in plants have interactions with proteins. This is stated by Nyachoti *et al.* (1997) that each tannin and protein interaction exhibits different kinetic depending on the structure of tannin, protein, pH and other compounds. At a combination level of 80% *Tithonia diversifolia* and 20% napier grass, crude protein digestion shown the lowest value. At the level of 20% *Tithonia diversifolia* and 80% napier grass showed the highest crude protein digestibility and this value continued to decrease along with the increased *Tithonia diversifolia* level. It is explained that the tannin properties can bind the protein source. Dzwela *et al.* (1997) also explained that tannins can form complex bonds with protein and cell wall carbohydrates in the rumen, resulting in reduced microbial activity in degrading dry matter. Increasing level of *Tithonia diversifolia* can form complex bonds that are difficult to remove, so that the amount of protein that can be degraded by rumen microbes become less. Tangendjaja *et al.* (1992) states that tannin has properties binding to proteins and other polymers. The tannins will form complex bonds with proteins become less digestible because at a pH above 3.5, the tannin-protein complex becomes stable. (Nolan, 1993). Hargerman (1989) also said that the tannin and protein concentration will affect on the complexity of tannin- protein.

In addition to phytic and tannin acids, *Tithonia diversifolia* also has saponin antinutrition which in certain doses gives a positive effect to the defaunation agent that caused increasing the bacterial population and ultimately improving the digestibility of feed nutrients in the rumen (suharti *et al.*, 2009). Treatment T1 are also optimum for the use of Titonia plants that containing saponin. Saponin can kill populations of rumen protozoa which ultimately also result in decreased digestibility in the rumen. This is because protozoa also produce enzymes to break down the fibers. Reduced protozoa population due to saponin will suppress the activity of fiber enzymes so that the dry matter digestibility of fiber will decrease significantly. The antinutritional effects of alkaloids, oxalates, and flavonoids are also suspected to safe limits in T1 treatment. The combination of some antinutrition in Titonia plants that endanger livestock can have a positive effect when administered at the right level.

CONCLUSIONS

The combination of 20% *Tithonia diversifolia* with 80% napier grass yields the best combination to produce the highest *in-vitro* digestibility of nutrients.

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