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Submission date: 18-Feb-2020 09:39PM (UTC+0800)
Submission ID: 1259481762
File name: 4 (11).pdf (496.9K)
Word count: 2694
Character count: 14053

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Comparison of *in vitro* digestibility and rumen fluid characteristics between the tithonia (*Tithonia diversifolia*) with elephant grass (*Pennisetum purpureum*)

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Abstract. The objective of this study was to evaluate the effects of the tithonia (*Tithonia diversifolia*) and elephant grass (*Pennisetum purpureum*) on *in vitro* digestibility and rumen fluid characteristics. This research was carried out using a randomized block design with 3 treatments (the level of tithonia and elephant grass) and 5 replications. The following treatments were performed: T1 = 100% tithonia; T2 = 100% elephant grass; T3 = 50% tithonia + 50 % elephant 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 (DMD) (%), organic matter digestibility (OMD) (%), pH, volatile fatty acid (VFA) (mM) and NH_3. The results revealed that DMD, OMD and VFA were significantly ($P<0.01$) increased by the 100 % tithonia (T1), pH were lowest ($P<0.01$) in T1. However, T1 and T3 non-significantly affected ($P>0.05$) NH_3 Concentration. 100% tithonia resulted in the best *in vitro* digestibility and rumen fluid characteristics.
Keywords – *In vitro*, *Tithonia diversifolia*, *Pennisetum purpureum,* digestibility, rumen fluid characteristics.

1. Introduction

Exploration of sources of ruminant animal feed is usually needed for the main work of the world of nutritional nutrition lately. Production limitations are needed by scientists to find alternatives that can be used by farmers in their application. One of the feed commodities that can be used as alternative feed is tithonia plants. There are still few of these plants that are used for ruminant feed because these plants have been mostly used in agriculture, namely composting with sufficient content such as Nitrogen (N) and Phosphorus (P) which are quite high [1]. High N and P content is very good for ruminants. High N content includes high crude protein content, while very high P content is needed by rumen microbes for growth [2] [3]. Both of these things are needed by the rumen microbes to grow and develop. Rumen microbes are the main engine of ruminants.

There is a problem in the use of P on the tithonia because P is not available due to being bound to phytic acid. Phytic acid is the most antinutrient in tithonia [4]. In monogastric livestock the presence of phytic acid in feed ingredients is a problem because there is no enzyme that can break down phytate in the digestive tract, so that it will be a threat to livestock health. Ruminants have the advantage of monogastric in the process of phytic degradation. Rumen microbes are able to produce pytase enzymes to break P bonds in phytic acid so that p can be available to livestock and become the main mineral supporting microbial growth [5]. This study aims to evaluate the digestibility and rumen fluids characteristics of tithonia and elephant grass and evaluate the effect of the combination level of the two feed ingredients.

2. Materials and methods

For this study, Analysis of in vitro digestibility and rumen fluid fermentability was conducted in the Laboratory of Dairy Nutrition of the Bogor Agricultural Institute. Samples of T and EG were collected around the town of Padang. All samples were finely ground and mixed according to the treatment combinations. The parameters measured in this study were DM digestibility (DMD), OM digestibility (OMD), NH_3 concentration, total VFA content and pH. The chemical composition of *Tithonia diversifolia* and elephant grass is presented in Table 1.

Tabel 1. The chemical composition of *Tithonia diversifolia* and elephant grass.

Composition (%)	Feed Material	
	Elephant Grass	*Tithonia diversifolia*
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 Husbandry Andalas University (2017).

2.1. *In vitro procedure*

The *in vitro* procedure in this study followed that of [6] Rumen fluid was taken from a cow fistula at Bogor, LIPI. The fermenter tube was filled with 0,5 g of sample and 40 mL of McDougall solution was added. The tube was placed in a shaker bath at 39^0C, filled with 10 mL of rumen fluid and shaken with CO_2 for 30 second, the pH was checked (6,5-6,9) and the sample was covered with a ventilated rubber cap and fermented for 48 h. After 48 h, the rubber cap of the fermenter tube was removed and 2-3 drops of $HgCl_2$ were added to kill the microbes. The fermenter tube was centrifuged at 5.000 rpm for 15 min. The substrate was separated into a precipitate layer at the bottom and a clear supernatant at the top. The supernatant was removed, the resulting sediment was centrifuged at 5.000 rpm for 15 min and 50 mL of 0,2% pepsin-HCl solution was added. This mixture was then reincubated for 48 h without a rubber cap. The remaining digested residue was filtered using Whatman filter paper No. 41 (identified by weight) with the help of a vacuum pump. The precipitate on the filter paper was placed in a porcelain dish that was placed in a 105EC oven for 24 h. After 24 h, the porcelain cup + filter paper + residue was removed, inserted into a desiccator and weighed to determine the DM content. Furthermore, the ingredients in the cup were placed in a kiln or in an electric furnace for 6 h at 450-600^0 C and weighed to determine the amount of organic material. Residues originating from fermentation without feed ingredients were used as a control. The proximate analysis of materials and residues followed the [7] and [8] procedure.

2.2. *Rumen fluid characteristics*

The measured rumen fluid characteristics were as follows: Ruminal pH, NH_3 –N concentration and VFA content. Fermenter tubes were each filled with 0.5 g of sample and 40 mL of buffer solution and 10 mL of fresh rumen fluid (ratio of 4:1) were added. After the tube received CO_2, the tubes were closed with a rubber ventilator. Tubes were inserted into the fermenter shaker water bath at a temperature of 39^0 C and incubated for 4 h, followed by analyses of pH, NH_3 and VFA. The ruminal pH was measured using a pH meter. The NH_3 -N concentration was measured using the micro diffusion (Conway) method and the total VFA content was measured using the steam distillation method.

3. Result and discussion

Data on digestibility values and characteristics of rumen fluid treatment are presented in Table 2.

Table 2. Data on digestibility values and characteristics of rumen fluid treatments.

Parameters	Treatments		
	T1	T2	T3
Digestibility (%)			
Dry matter (DMD)	58,56[a]	55,99[b]	57,12[c]
Organic Matter (OMD)	55,46[a]	53,99[b]	54,72[c]
Rumen Fluid Characteristics			
pH	6,78[a]	6,79[b]	6,80[c]
VFA (mM)	125,88[a]	87,53[b]	112,15[c]
NH_3 (mM)	22,48[ab]	20,41[a]	22,69[b]

Means in the same row with different letter (a,b and c) are significant (P<0,01).

Table 2 shows that the use of 100% tithonia plants (T1) was very significant (P <0.01) increasing DMD and OMD. The lowest DMD and OMD were obtained on 100% elephant grass (T2). The high level of DMD and OMD in tithonia plants is due to the tithonia nutrient content better than elephant grass. This can be seen from the level of protein, the amount of amino acids and mineral content. [9] reported that tithonia contains a lot of amino acids. [1] added that the tithonia leaves contain high levels of N, P, K, Ca and Mg minerals. The level of lignin titone is lower when compared to elephant grass. Amino acids found in tithonia are needed by rumen microbes in their growth, while mineral content such as P and Mg greatly stimulates the rumen microbes to grow and develop [10]. The development of rumen bacteria will be positively correlated with an increase in DMD and OMD [11]. Lower lignin in plants allows more bacteria to degrade feed so that DMD and OMD are higher in this treatment. [11] reported a positive correlation between decreasing lignin and increasing DMD and OMD. The combination between tithonia and elephant grass in treatment T3 is better than treatment using elephant grass (T2). This is due to the contribution of the nutrient content of the tithonia plant to be able to supply the rumen microbial needs better so that it increases DMD and OMD in this treatment.

The pH value in this study is the pH value that is still normal for the growth and development of rumen microbes. According to [12] the optimal pH range for cellulose digestion is 6,4-7. If the pH of the rumen fluid drops to below 6 it will inhibit microbial activity. Furthermore, [13] stated that pH greater than 7,1 can reduce the microbial population drastically so that the energy produced is low. Conditions for the rumen microbes to be able to perform activities optimally when the rumen pH is in normal conditions is 6-6,9 [14]. [15] reported that a mixture of several levels of elephant grass combination with different tithonia produced a normal pH value of 6,67-6,68. pH at T1 treatment showed the lowest pH value. Low pH in this treatment is due to the amount of nutrient content of the VFA produced. High VFA content will reduce pH. In accordance with the opinion of [16] that the higher the VFA value, the more other organic acids will be produced so that the rumen fluid pH will be low.

The highest VFA value (P <0.01) is found in T1. VFA describes the amount of carbohydrates degraded by rumen microbes. The growth and development of rumen microbes is thought to be very good in T1 due to a better supply of nutrients in the form of amino acids and minerals from tithonia plants. As reported by [9] that Tithonia contains a lot of essential amino acids for the growth of microbes such as methionine, leucine, isoleucine and valine. Rumen liquid VFA production can be used as a benchmark for feed fermentability, where the higher the level of feed fermentability, the greater the VFA produced [17].

The highest NH3 concentration was produced in T3 treatment but not significantly different (P> 0.05) with T1. The high content of crude protein in the tithonia and the number of amino acids contained in the tithonia will positively correlate to NH_3 concentrations. NH_3 concentration is one indicator to determine feed fermentability which is related to protein digestibility, rumen microbial activity and

population [18]. NH_3 in all three treatments is still within normal limits. [19] states that ammonia concentration to support rumen microbial growth is 6 mM – 21 mM. NH_3 and VFA which increase linearly will form more microbial proteins so that they have a positive effect on livestock digestibility and productivity.

4. Conclusion

100% tithonia resulted in the best *in vitro* digestibility and rumen fluid characteristics.

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Acknowlecgments

This research was funded by the Andalas University Profesor Grant 2018 and was part of the Ph.D. dissertation of Roni Pazla in Animal Nutrition, Faculty of Animal Science, Andalas University.

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