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Research Paper

Nutrients Composition of Tithonia diversifolia Fermented by Lactobacillus plantarum and Aspergillus ficuum at Different Fermentation Time

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ABSTRACT **:** *This study aims to obtain the best nutrients composition of Tithonia diversifolia which is fermented using two microorganisms Lactobacillus plantarum and Aspergillus ficuum for 3, 5 and 7 days fermentation times. This research conducted at laboratory of Nutrition Technology using completely randomized design (CRD) in 2 x 3 factorial with 3 replications. Research parameters were dry matter, crude protein and crude fiber content. The results of the analysis showed that not significant different (P> 0.05) on dry matter content, but significantly different (P <0.05) on crude fiber and crude protein content for each treatment. Based on the results of the study it was concluded that the best treatment was obtained at fermentation of Tithonia diversifolia using Aspergillus ficuum with incubation time of 7 days, that indicated dry matter content: 90.12%, crude protein content: 31, 02% and crude fiber content: 16.52%.*

KEYWORDS**:** *Tithonia diversifolia, Lactobacillus plantarum, Aspergillus ficuum, Fermentation, Nutrient content*

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I. INTRODUCTION

Ruminants used forage for their living such as for growth, production and reproduction. Feed deficiency gave a bad effect for livestock, the growth process of livestock will be hampered and reducing productivity. To meet the livestock nutrients requirement, the feed must have good nutritional content, high production and always available continuously. One of plant that can be used to be feed for ruminants were *Tithonia diversifolia*. *Tithonia diversifolia* is a weed of the *Asteraceae* family from Mexico and has yellow flowers that are very similar to sunflowers, so it is known as the Mexican sunflower [1]. The utilization of *Tithonia diversifolia* has not been maximized due to a lack of knowledge of the use of these plants for animal feed and also *Tithonia diversifolia* is considered a weed, so that it is just thrown away. *Tithonia diversifolia* contain crude protein and crude fiber of 22.98% and 18.17 respectively [2]. The use of *Tithonia diversifolia* as animal feed is hampered by the presence of anti-nutritional substances. Anti-nutritional substances contained in *Tithonia diversifolia* are phytic acid, tannins, saponins, oxalates, alkaloids and flavonoids [3]. The most anti-nutritional substance in tithonia was phytic acid, which was 79.2 mg / 100gr [4]. The high phytic acid content in *Tithonia diversifolia* causes a bitter taste so it is less preferred by livestock. Various kinds of processing methods have been used to increase or maintain the value of that material, one of which is fermentation. Fermentation is the process of break down organic compounds into simple ones that involve microorganisms. The fermentation process can increase the availability of food substances such as protein and metabolic energy and can break down complex components into simple components [5]. Fermentation can also increase the nutritional value of low-quality forages as well as function in the preservation of feed ingredients and a way to remove anti-nutritional or toxic substances contained in feed forages. In this study, using two different types of microorganisms in fermentation process are *Aspergillus ficuum* and *Lactobacillus plantarum*. The selection of these microbes in this study because these microbes are relatively safe, not pathogenic microbes and have been widely applied in fermentation processes. In addition, this research want to see the comparison of these two microbes in fermenting of *Tithonia diversifolia* in terms of nutritional content which includes dry matter, crude protein and crude fiber content.

II. MATERIALS AND METHODS

The materials used in this experiment were *Tithonia diversifolia*, aquades. Microorganisms for fermentation: *Lactobacillus plantarum*, and *Aspergillus ficuum*. MRS Broth media, Potato Dextrose Agar (PDA) medium. The equipment was used in this experiment are a set of tools for fermentation, and a set of tools for proximate analysis, autoclave, analytical scales, and hot plates. This research was conducted at Biotechnology Laboratory and Feed Technology Laboratory, Faculty of Animal Science, Andalas University, Padang. The research design used a completely randomized design (CRD), in 2x3 factorial with 2 factors (2 species of microbes and 3 fermentation times) with 3 replications for each treatment. Sample that had been mashed was then fermented with A1 (*Lactobacillus plantarum*) and A2 (*Aspergillus ficuum*) treatments with fermentation times of B1 (3 days), B2 (5 days), B3 (7 days). This research was conducted to determine the best microorganisms and fermentation time of *Tithonia diversifolia*. The parameters measured in this study were nutritional content which are dry matter content using oven at 110^0 C, crude protein content using the Kjeldahl method, and crude fiber content using the Soxhlet method

III. RESULTS AND DISCUSSION

Dry matter content.
Effect of Treatment on Dry Matter content was shown in Table 1.

Table 1. Average dry matter content of fermented Tithonia

factor A	Factor B (fermentation time)			averages
species of microbes	B1 (3 days)	B2 (5 days)	B3 (7 days)	
A1 (L.*Plantarum*)	89.17	89.86	89.49	89.84^{a}
A2 (*A.Ficuum*)	91.76	91.26	90.12	91.05^{b}
Average	90.96^{b}	90.56^{ab}	89.8^{a}	90.44

Different superscripts in the same row are significantly different (P <0.05)

In dry matter content changes, there was no interaction between the treatment of microbial spesies and different fermentation times of Tithonia (P> 0.05). However, each species of microbial treatment and fermentation time showed was significant different (P <0.05) on the dry matter content of *Tithonia diversifolia*. The DMRT test results showed that the dry matter content in the treatment of the species of microbe A1 (*lactobacillus plantarum*) showed a significant effect (P <0.05) with A2 treatment (*Aspergillus ficuum*). The length of fermentation in treatment B1 (3 days of incubation) showed not significantly different results (P> 0.05) with treatment B2 (5 days of incubation), but significantly different (P <0.05) with treatment B3 (7 days of incubation), while B2 treatment (5 days of incubation) showed not significantly different (P> 0.05).

The different in dry matter content in A1 and A2 treatments were related to the species and abilities of each different microbe in fermenting a material. Bacteria grow at optimum conditions faster than fungi so that the ability to digest the substrate is also greater. Bacteria as an inoculum in the fermentation process require less time than fungi, bacteria have a simpler cell structure, so that most bacteria have a shorter generation time when compared to yeast and fungi, which have more complex cell structures and a long generation time [6].

. The higher dry matter content in treatment B1 and B2 was influenced by the short fermentation time. Short fermentation time result in the substrate decomposition process was not optimal, so that the water content was low and dry matter was still high [7]. During the fermentation process, the substrate undergoes a decomposition process which causes changes in water content. Dry matter changes occur due to evaporation, substrate hydrolysis or metabolic water production.

Effect of Treatment on Crude Protein content

Table 2. Effect of treatments on Crude protein content of fermented Tithonia

factor A	Factor B (fermentation time)			average
Species of microbes	B1 (3 days)	B2 (5 days)	B3 (7 days)	
A1 (*L.Plantarum*)	29.01^{b}	27.56^{c}	24.3^{d}	26.96^{a}
A2 (*A.Ficuum*)	27.25^{c}	29.14^{b}	31.02^{a}	29.14^{b}
average	28.13	28.35	27.66	28.05

Different superscripts in the same row are significantly different (P <0.05)

There was an interaction (P <0.05) between the species of microbes and the fermentation time of *tithonia diversifolia*. The crude protein content in the A2B3 treatment was significantly (P <0.05) higher than the A2B2, A1B1, A1B2, A2B1, A1B3, A2B2, A3B3 treatments. The higher crude protein content in A2B3 treatment (*Aspergillus ficuum* with fermentation time of 7 days) was caused by changes in inorganic nitrogen to cell protein. That the increase in protein was caused by changes in inorganic nitrogen elements into cell protein during mold growth [8]. The long fermentation time also results in more opportunities for microbes to grow so that microbes (molds) thrive and evenly, as a result the contribution of protein from the body increases and the crude protein content also increases. The high microbial population results in increased crude protein content because most microbes consist of protein. Microbes that have good growth and will be able to convert more media components into cell mass which will form proteins that come from the body itself and in the end will increase the crude protein from the material [9]. Microbes contain quite high protein 40-60% [10]. During the fermentation process, microbes release enzymes, where the enzyme is a protein and the microbe itself is also a source of single cell protein [11]

Low crude protein content was found in A1B3 treatment *(Lactobacillus plantarum* with fermentation time of 7 days). This is because the *lactobacillus plantarum* bacteria produce the protease enzyme so that it breaks down protein into amino acids. Protease enzyme breaks the peptide bonds in the protein and produces amino acids [12]. Research by Sujatmiko., B et al also said that the protein content of sorghum seed decreased along with the length of the fermentation process due to the protease activity of LAB and yeast, which initially was around 10.88 ± 0.13% down to 8.09 ± 0.53% [13]

Effect of Treatment on Crude Fiber content

Table 3. Effect of treatments on crude fiber content of fermented tithonia

factor A	Factor B (fermentation time)			average
Species of microbes	B1 (3 days)	B2 (5 days)	B3 (7 days)	
A1 (*L.Plantarum*)	17.95^{bc}	17.37^{cd}	17.18^{cd}	17.50
A2 *(A.Ficuum*)	18.84^{a}	16.75^{d}	16.52^{d}	17.37
average	18.40^{a}	17.06^{b}	16.85^{b}	17.44

Different superscripts in the same row are significantly different (P <0.05)

There was an interaction (P <0.05) between the species of microbes and the fermentation time for fermented tithonia diversifolia. The crude fiber content in treatment A2B1 was significantly (P <0.05) higher than treatment A1B1, A1B2, A1B3, A2B2, A2B3. Table 3 shown that the lowest reduction in crude fiber content was found in A2B3 treatment (*Aspergillus ficuum* with fermentation time of 7 days) of 16.52%. The low content of crude fiber in A2B3 treatment was due to the *aspergillus ficuum* mold producing cellulase enzymes where the cellulase enzymes could work optimally in reducing crude fiber content. Cellulase enzymes are enzymes that break glycosidic bonds of Beta-1,4 glucoside in cellulose, cyclodextrins, cellobiose, and other cellulose derivatives [14]. The longer the fermentation process, the crude fiber content will decrease, this is due to the increased chance of *Aspergillus ficuum* in degrading crude fiber from the treatment substrate. Research of Maulana et al reported that fermented soy milk with Aspergillus ficuum were able to reduce crude fiber content up to 10.29% [15]

IV. CONCLUSION

Based on this research, it can be concluded that the best species of microorganisms and the best fermentation time were found in A2B3 treatment (*Aspergillus ficuum* with 7 days of fermentation time). In this condition, the dry matter content was 90, 12%, crude protein content was 31.02%, and crude fiber content was 16.52%.

V. RECOMMENDATIONS

Further research needs to be carried out by making complete rations for *in-vivo*-based goat feed based on *tithonia diversifolia* leaves.

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