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Quality evaluation of tithonia (*Tithonia diversifolia*) with fermentation using *Lactobacillus plantarum* and *Aspergillus ficuum* at different incubation times

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Abstract. *Pazla R, Jamarun N, Zain M, Yanti G, Chandra RH. 2021. Quality evaluation of tithonia (*Tithonia diversifolia*) with fermentation using* Lactobacillus plantarum *and* Aspergillus ficuum *at different incubation times. Biodiversitas 22: 3936-3942.* This research aimed to evaluate the nutritional quality of tithonia (*Tithonia diversifolia*) fermented using *Lactobacillus plantarum* and *Aspergillus ficuum* with different incubation times on nutritional content, digestibility, phytase enzyme activity, and characteristics of rumen fluid. The research used an experimental method with a factorial completely randomized design to evaluate the nutritional content and phytase enzyme activity after fermentation (stage 1). A randomized block design was used to evaluate in vitro digestibility and rumen fluid characteristics (stage 2). For factorial completely randomized design, factor A is the type of microbe *(L. plantarum* and *A. ficuum*), then factor B is the incubation time (3,5,7 days). Parameters observed were the nutritional content of dry matter (DM), organic matter (OM), crude protein (CP), crude fiber (CF), and phytase enzyme activity. For the Randomized Block Design, the research treatments were A = *A. ficuum* + 5 days of incubation, B = *A. ficuum* + 7 days of incubation, C = *L. plantarum* + 3 days of incubation, D = *L. plantarum* + 5 days of incubation. Parameters measured were the digestibility of dry matter(DMD), organic matter (OMD), crude protein (CPD), crude fiber (CFD), rumen pH, VFA production, and NH_3 rumen fluid. The results showed that there was an interaction between the type of microbe and incubation time. The treatment had a significantly different effect ($P<0.05$) on the content of OM, CP, CF and phytase enzyme activity, but no interaction with the content of DM treatment. In the digestibility, the results showed that the effect was not significantly different ($P>0.05$) on DMD, OMD, CPD but had a significant effect ($P<0.05$) on CFD. The treatments also had no significant effect ($P>0.05$) on VFA but were significantly different ($P<0.05$) on NH_3. From this study, it can be concluded that fermented tithonia using *A. ficuum* with an incubation period of 7 days could improve the quality of tithonia. It can be seen from the content of CP (31.02%), CF (16.52%), phytase enzyme activity (37.36 U/ml), DMD (66.86%), OMD (67.36%), CFD (81.01%), CPD (70.37%), VFA production 135 mM and NH_3 concentration 14.31 mg/100 ml, and pH value 6.72 which is suitable for rumen microbial growth.

Keywords: *Aspergillus ficuum*, digestibility, enzyme activity, fermentation, *L. plantarum*, *Tithonia diversifolia*

INTRODUCTION

Forage is the primary source of feed for ruminants. Forage feed serves to meet the needs of livestock both for basic life, growth, reproduction, and production. Ruminant livestock that experiences a shortage of forage feed ingredients will be stunted in the growth process. Various efforts to increase livestock production to meet animal protein sources' needs will be challenging if the availability of forage is not proportional to the needs and existing livestock population. One of the efforts to overcome the shortage of forage feed ingredients is to look for alternative feeds with high nutritional content, high production, and easy to adapt. One type of plant that can be used is tithonia (*Tithonia diversifolia*).

Tithonia can grow along roads, lakesides, on the edge of rice fields, and vacant land not used by the community (Arief et al. 2019; Arief et al. 2020). This plant has large roots, many branches, a soft trunk and overgrows so that in a short time, it can form a dense bush. Hafis (2019) stated that tithonia plants harvested six times a year can produce 4.10 tons/ha-10.20 tons/ha dry biomass production or 24.00-46.80 tons/ha/year fresh production. Tithonia as animal feed has not been widely used; this is because there is not much information about the use of these plants as animal feed. Also, tithonia is often considered a weed so that it is thrown away, even though it has a fairly good nutritional content. The nutritional content of whole tithonia (leaves + stems) are dry matter (DM), (25.57%), organic matter (OM), (84.01%), crude protein (CP), (22.98%), and crude fiber (CF), (18.17%) (Jamarun et al. 2017a). Therefore, tithonia has good potential to be used and developed as forage feed because it has good nutritional content and high productivity.

The obstacle to using tithonia for animal feed is anti-nutritional substances such as phytic acid, tannins, saponins, oxalate, alkaloids, and flavonoids (Aye 2016). These limiting factors, both substances directly contained in feed ingredients or through metabolic products in livestock, can interfere with the use of feed. Also, it can affect the health and production of animals through mechanisms of decreasing nutrient intake, digestive and

absorption disorders and causing other adverse side effects. Oluwasola and Dairo (2016) stated that the most antinutrient content in tithonia was phytic acid, 79.2 mg/100gr. The high phytic acid content in tithonia causes a bitter taste, so it is not liked by livestock. Phytic acid in a material can also interfere with mineral absorption because phytic acid has chelating agent properties that can bind minerals so that the biological availability of these minerals decreases. Phytic acid can also bind to proteins and carbohydrates so that the digestion and absorption of these nutrients are disturbed (Selle et al. 2021).

Various processing methods can be used to reduce anti-nutritional substances in feed ingredients, one of which is through fermentation technology. Fermentation is the process of breaking down organic compounds into simple ones involving microorganisms. The fermentation process can increase food substances such as protein and energy and break down complex components into simple components (Jamarun et al. 2017b). Fermentation can also increase the nutritional value of low-quality ingredients and function in preserving feed ingredients, and one way to remove antinutrients or toxins contained in feed ingredients.

Microbes that can be used for fermentation in tithonia are *Lactobacillus plantarum* bacteria and *Aspergillus ficuum* mold. These microbes produce a phytase enzyme (Myo-inositol hexakisphosphate phosphohydrolase) which can hydrolyze phytic acid (myo-inositol hexakisphosphate) into inorganic monophosphate. Also, it can hydrolyze low myo-inositol and some into free Myo-inositol. Therefore, the nutrients bound by phytic acid can be utilized. Sumenger et al. (2013) reported that *L. plantarum* could produce high intracellular and extracellular phytase enzymes. Susana et al. (2000) also said that *A. ficuum* had high activity and production of phytase enzymes from 60 isolates collected. Fermentation applications of *A. ficuum* and *L. plantarum* to reduce the phytic acid content in tithonia have not been carried out. The selection of these microbes in this study is because these microbes are relatively safe, not pathogenic, and have been widely applied in fermentation.

Fermentation time is also one of the factors that must be considered in making fermented feed. The fermentation that is too short resulted in limited opportunities for microorganisms to grow. The substrate components that can be remodelled into cell mass will also be small; for this reason, proper fermentation time is needed so that microorganisms have more opportunities to grow and reproduce. The ability of microorganisms to grow and develop will affect the quality of a feed ingredient physically, chemically, and biologically. Therefore, by knowing the adequate time for the development of microorganisms, we can get the best fermentation results in terms of quality. This study aimed to evaluate the quality of fermented tithonia using *L. plantarum* and *A. ficuum* with different fermentation times in vitro.

MATERIALS AND METHODS

Sample collection and nutrient analysis

Tithonia was taken in the Koto Lalang agricultural land area, Kuranji, Padang City, West Sumatra Province. For the fermentation process, In-vitro treatment and chemical analysis were carried out at the Feed Industry and Technology Laboratory, Ruminant Nutrition Laboratory, and Biotechnology Laboratory from Andalas University, Padang, Indonesia. Proximate analysis was carried out based on the (AOAC 2010) method. In vitro test using the method of Tilley and Terry (1963). Phytase enzyme activity follows the Alltech method with some modifications (Shieh and Ware 1968).

Rejuvenation of *Aspergillus ficuum* and *Lactobacillus plantarum*

The rejuvenation of *A. ficuum* was carried out on potato dextrose agar sloping media, incubated at 30 °C for seven days. In contrast, *L. plantarum* was grown on MRS Broth media at 37°C for 48 hours. The MRS Broth media was weighed as much as 5.52 grams and then added 100 ml of distilled water, heated until it was clear on a hot plate. Cover with cotton and aluminum foil, then sterilize using an autoclave at 121°C for 30 minutes. Then cool and inoculate with 1 ml of the bacterial parent. Incubate in an incubator at 37 °C.

Titonia fermentation process

One hundred grams of tithonia flour was put into a plastic bag and added with distilled water until the water content reached 60% and then homogenized. Sterilize in an autoclave for 30 minutes at a temperature of 121°C. After the medium has cooled, 10% of the sample weight has been inoculated with microbes and 5 ml for *L. plantarum*, then stored in a sterile room. Then, samples were harvested after 3,5 and 7 days and weighed fresh, then in the oven to dry at 60°C. If it is dry, the medium is stored and ready for the next stage, namely proximate analysis and In-vitro digestibility testing.

Research design

Stage 1; the research method used to evaluate the chemical composition of post-fermentation tithonia, is an experimental method with a randomized completely design with a factorial pattern of 2 x 3 with three replications. The parameters measured were dry matter, organic matter, crude protein, crude fiber, and phytase enzyme activity. The research treatment is as follows:

The first factor is the addition of microorganisms (A):

A1 = Tithonia + *L. plantarum*

A2 = Tithonia + *A. ficuum*

The second factor is the length of fermentation (B):

B1 = 3 days

B2 = 5 days

B3 = 7 days

Stage 2; the experimental design used in the in vitro test was a randomized block design with four treatment combinations and three groups (rumen fluid collection) as replication. The treatment in this second stage was based

on the results of the best nutritional value (4 best treatments) in stage one. The parameters measured were dry matter digestibility (DMD), organic matter digestibility (OMD), crude protein digestibility (CPD), crude fiber digestibility (CFD), total VFA concentration, NH_3 concentration, and rumen fluid pH.

The treatments tested are:

A: Fermented tithonia with *A. ficuum* for five days

B: Fermented tithonia with *A. ficuum* for seven days

C: Fermented tithonia with *L. plantarum* for three days

D: Fermented tithonia with *L. plantarum* for five days

Data were analyzed using analysis of variance (ANOVA) according to (Steel and Torrie 2002). If the study of variance results shows a significant effect, further tests are carried out with Duncan's Multiple Range Test.

RESULT AND DISCUSSION

Dry matter content of fermented tithonia

The dry matter content of fermented tithonia with different microbes and incubation time for each treatment is presented in Table 1.

The analysis of variance showed no interaction between the treatment of different types of microbes and incubation time on the DM content of fermented tithonia. However, every microbial treatment and incubation time showed a significant effect (P<0.05) on DM content. DMRT test results showed that the DM content of the A1 (*L. plantarum*) treatment showed a significant impact (P<0.05) with the A2 (*A. ficuum*) treatment. Incubation time in treatment B1 (3 days incubation) showed significantly different results (P<0.05) with treatment B2 (5 days incubation) and treatment B3 (7 days incubation), while treatment B2 (5 days incubation) showed similar results, not significantly different (P>0.05) with treatment B3 (7 days incubation).

The difference DM content in treatments A1 and A2 was associated with the type and ability of each different microbe in fermenting a material. Bacteria have a faster growth at optimum conditions when compared to molds, so that the ability to digest substrate is also more extraordinary. Bacteria as inoculum in the fermentation process takes less time than molds; bacteria have a more straightforward cell structure. So most bacteria have a shorter generation time when compared to molds whose cell structure is more complicated and the generation time is quite long (Saylor and Casale 2020).

The high DM content in treatments B1 was influenced by the short fermentation time. According to Mirnawati et al. (2010), the short fermentation time resulted in the substrate decomposition process not being optimal, so that the water content was low, and DM was still high. During the fermentation process, the substrate undergoes a decomposition process that causes changes in water content. Changes in DM occur due to evaporation, substrate hydrolysis, or metabolic water production.

Organic matter content of fermented tithonia

The organic matter content of fermented tithonia with different microbes and incubation time for each treatment is presented in Table 1.

The analysis of variance showed an interaction (P<0.05) between different types of microbes and incubation time on OM content of fermented tithonia. Factor A (type of microbe) had an insignificant effect (P>0.05), but factor B (incubation time) has a significant impact (P<0.05) on the OM content of fermented tithonia. The results of the DMRT test showed that the OM content in the A2B1 treatment was significantly (P<0.05) higher than the A1B1, A1B2, A1B3, and A2B3 treatments. The high and low content of OM in the treatment is also made possible by microbial activity in the fermentation process, which causes the breakdown of the substrate content, making it easier for existing microorganisms to digest organic matter. The fermentation of organic matter releases fermented products in the form of sugar, alcohol, and amino acids caused by the activity of micro-services so that changes occur that affect the nutritional value. It is following the opinion of Putra et al. (2019), which states that the fermentation process carried out by microorganisms will cause changes that affect the nutritional value in which carbohydrates are converted into alcohol, organic acids, water, and CO_2.

The decrease in OM content is due to the nutrients that have been utilized and remodelled by microbes. Microbial growth is closely related to the length of fermentation, where microbial development cycles start from the growth phase to the death phase. Mirnawati et al. (2013) added that the longer the fermentation time, the more food substances were overhauled.

Table 1. The phytochemical characteristic of fermented tithonia with different types of microbes and incubation time

Factor A (Types of microbes)	Factor B (incubation time) B1	B2	B3	Average
Dry matter				
A1	90.17	89.86	89.49	89.84 ^{B}
A2	91.76	91.26	90.12	91.05 ^{A}
Average	90.96^{a}	90.56 ^{b}	89.8 ^{b}	90.44
Organic matter				
A1	87.78^{aA}	87.51^{abA}	87.04^{bA}	87.44
A2	88.40^{aB}	87.91^{aA}	86.67^{bA}	87.66
Average	88..09^{a}	87.71 ^{a}	86.86 ^{b}	87.55
Crude protein				
A1	29.01^{aA}	27.56^{bB}	24.30^{cB}	26.96 ^{B}
A2	27.25^{cB}	29.14^{bA}	31.02^{aA}	29.14 ^{A}
Average	28.13	28.35	27.66	28.05
Crude fiber				
A1	17.95^{aB}	17.37^{abA}	17.18^{bA}	17.50
A2	18.84^{aA}	16.75^{bA}	16.52^{bA}	17.37
Average	18.40^{a}	17.06^{b}	16.85^{b}	17.44
Phytase activity				
A1	9.12^{bB}	11.71^{aB}	6.68^{cB}	9.17^{B}
A2	28.37^{cA}	36.12^{bA}	37.46^{aA}	33.98^{A}
Average	18.74^{c}	23.91^{a}	22.07^{b}	21.58

Note: Values with different superscripts in the row (lower case) and columns (capital letters) are significant (P<0.05). A1= *L. plantarum*, A2= *A. ficuum*, B1= 3 days, B2= 5 days, B3= 5 days

The crude protein content of fermented tithonia

The crude protein content of fermented tithonia with different microbes and incubation time for each treatment is presented in Table 1.

The analysis of variance showed an interaction ($P<0.05$) between different types of microbes and incubation time on OM content of fermented tithonia. Factor A (type of microbe) had an insignificant effect ($P>0.05$), but factor B (incubation time) has a significant impact ($P<0.05$) on the OM content of fermented tithonia. The results of the DMRT test showed that the OM content in the A2B1 treatment was significantly ($P<0.05$) higher than the A1B1, A1B2, A1B3, and A2B3 treatments. The high and low content of OM in the treatment is also made possible by microbial activity in the fermentation process, which causes the breakdown of the substrate content, making it easier for existing microorganisms to digest organic matter. The fermentation of organic matter releases fermented products in the form of sugar, alcohol, and amino acids caused by the activity of micro-services so that changes occur that affect the nutritional value. It is following the opinion of Putra et al. (2019), which states that the fermentation process carried out by microorganisms will cause changes that affect the nutritional value in which carbohydrates are converted into alcohol, organic acids, water, and CO_2.

The decrease in OM content is due to the nutrients that have been utilized and remodelled by microbes. Microbial growth is closely related to the length of fermentation, where microbial development cycles start from the growth phase to the death phase. Mirnawati et al. (2013) added that the longer the fermentation time, the more food substances were overhauled.

The crude fiber content of fermented tithonia

The crude fiber content of fermented tithonia with different types of microbes and incubation time for each treatment is presented in Table 1.

The analysis of variance showed an interaction ($P<0.05$) between the types of microbes and the length of incubation time on the CF content of fermented tithonia. Factor A (type of microbe) had an insignificant effect ($P>0.05$), but factor B (incubation time) had a significant effect ($P<0.05$) on the CF content of fermented tithonia. The results of the DMRT test showed that the CF content in the A2B1 treatment was significantly ($P<0.05$) higher than the A1B1, A1B2, A1B3, A2B2, A2B3 treatments. Table 1 shows that the lowest decrease in CF content was found in the A2B3 treatment (*A. ficuum* and incubation time of 7 days) of 16.52%. The low content of CF in the A2B3 treatment was due to the *A. ficuum* producing cellulase enzymes. The cellulase enzymes could work optimally in reducing crude fiber content. Cellulase enzyme breaks beta-1,4 glycosidic bonds in cellulose, cyclodextrin, cellobiose, and other cellulose derivatives (Mingardon et al. 2011; Alami et al. 2017).

The longer the fermentation process, the lower the CF content; this is due to the increased opportunity for *A. ficuum* to degrade CF from the treatment substrate. Maulana (2019) reported that soy milk dregs fermented with *A. ficuum* could reduce CF content by up to 10.29%. The content of CF is still high due to the short fermentation time; the microbes have not thrived and evenly, so the enzymes produced to degrade CF have not worked optimally in reducing the CF content.

Phytase enzyme activity of fermented tithonia

The phytase enzyme activity of fermented tithonia with different microbes and incubation time for each treatment is presented in Table 1.

The analysis of variance showed that there was an interaction ($P<0.05$) between the type of microbe and the length of incubation time on the activity of the fermented tithonia phytase enzyme. Factor A (type of microbe) and factor B (incubation time) also had a significant effect ($P<0.05$) on the activity of the fermented tithonia phytase enzyme. The results of the DMRT test showed that the movement of the phytase enzyme in the A2B3 treatment was significantly ($P<0.05$) higher than the other treatments. The increased activity of the phytase enzyme is due to *A. ficuum* reaching the optimum conditions for its growth, the better the development of the mold, the more enzymes produced, so that the enzyme activity will be higher. The lowest enzyme activity was found in the A1B3 treatment, this was due to cell death which resulted in decreased enzyme activity. After reaching the optimum point, microbial growth will slowly decrease because it begins to enter the death phase so that the activity of the phytase enzyme begins to fall.

According to Maryanti (2015), the growth phase begins with the lag phase (adaptation phase), where microbes adjust to changes in the media and their environment. The phase occurs shortly after inoculation, where the cells have not experienced growth, and the number is still relatively constant. Next is the log phase (growth phase). As the name implies, this phase is a phase of cell growth characterized by a significant increase in the number of cells because the cell division process occurs optimally. This phase is the best in determining the optimal time of inoculation of a cell. The third phase is the stationary phase, in which cells will no longer grow and remain relatively constant. The condition is due to reduced nutrients and increased waste in the growth medium. This phase continues until it enters the death phase, which means the number of cells decreases drastically. Cells begin to die because the concentration of nutrients is deficient and causes cell growth to be inhibited.

Nutritional digestibility of fermented tithonia

Digestibility of dry matter, organic matter, crude protein, and crude fiber from fermented tithonia are presented in Table 2.

The analysis of variance showed that fermented tithonia with different types of microbes and incubation time had no significant effect ($P>0.05$) on DMD of fermented tithonia. This is because the CF content of the treatment is relatively the same. Digestibility is closely related to the chemical composition of the material, especially the CF content. CF content in treatments A, B, C and D respectively 16.75; 16.52; 17.95 and 17.37%. The DMD

value in each treatment ranged from 62.21 to 66.86. DMD is higher than the study of Susanti et al. (2020), where DMD in the combination of fermented tithonia and sugarcane shoots was 59.15%, and Jamarun et al. (2019), which obtained DMD in fresh tithonia of 58.56%.

The analysis of variance showed that tithonia fermentation with different types of microbes and incubation time gave no significant effect (P>0.05) on the OMD. The value of OMD in this study ranged from 66.03 to 67.36%. This value is not much different from the DMD of fermented tithonia. The difference is not the OMD. After all, the OMD is very closely related to the DMD because some dry matter consists of organic matter. The OMD pattern followed the DMD pattern (Pazla et al. 2018a; Arief et al. 2021; Jamarun et al. 2021). The value of OMD in this study was higher than the study of Jamarun et al. (2019), which obtained a digestibility value of organic matter of 55.46% in tithonia without fermentation.

The analysis of variance showed that fermented tithonia with different types of microbes and incubation time had no significant effect (P>0.05) on the CPD of fermented tithonia. These results illustrate that the ability of rumen microbes to utilize protein is relatively the same. Besides, digestibility is closely related to the chemical composition of the ingredients. If the design in feed ingredients is relatively the same, the digestibility produced will also be pretty similar. The CPD material is also directly proportional to the crude protein content of the material in the feed.

In addition, another factor that caused the CPD not significantly different in the treatment was the crude fiber content of the material between treatments which was relatively the same. CPD values in this study ranged from 67.78 to 70.38. This result is higher than the study of Susanti et al. (2020), which obtained a CPD value of 55.45% in the combination of sugarcane top and fermented tithonia.

The analysis of variance showed that fermented tithonia with different types of microbes and incubation time had a very significant effect (P<0.05) on the CFD. DMRT further test results showed that treatment A was significantly different (P < 0.05) with treatment B, C, and D. Treatment B was quite different (P < 0.05) with treatment C and D. Treatment C was not quite different (P>0.05) with treatment D. The average of CFD in this study ranged from 71.52-81.01%. Fermentation in tithonia can increase CFD. It is because fermentation can stretch the bonds of fiber fraction components to make rumen microbes work more optimally.

The highest CFD value in this study was found in treatment B, which was 81.01%. The increase in CFD in treatment B was due to the crude fiber content of the material in treatment B, which was lower than the other treatments with 16.52%, so that rumen microbial activity in digesting crude fiber was more optimal. According to Yanti et al. (2021) and Jamarun et al. (2018), feed ingredients with low crude fiber will generally be easier to digest because microbes easily penetrate the cell walls of these materials. On the contrary, the higher the crude fiber content contained in a feed ingredient, the cell walls will be thicker and resistant to fiber-digesting microbes. It can result in a decrease in the digestibility of the feed ingredients. It can be seen from the lowest CFD found in treatment C (71.52%), with a crude fiber content of 17.95% higher than treatments A, B, and D.

Rumen fluid characteristics of fermented tithonia

Rumen fluid characteristics from fermented tithonia are presented in Table 3.

The analysis of variance showed that tithonia fermentation with different types of microbes and incubation time gave no significant effect (P>0.05) on the pH of the rumen fluid. The pH value of the rumen fluid in this study ranged from 6.72 to 6.85, and the pH value was still in a condition that was quite optimal for rumen microbial growth. Jamarun and Zain (2013) stated that the optimal rumen pH for digestive activity in the rumen ranged from 6.0 to 7.0. Rumen fluid pH less than 6.0 or above 7.0 can inhibit rumen microbial activity so that the ability to degrade feed decreases.

Changes in rumen pH related to the production of volatile fatty acid (VFA) produced in Table 3 show that the higher the production of VFA produced, the lower the rumen pH. VFAs contain organic acids such as acetic, propionate, butyrate, which make the atmosphere in the rumen acidic. Bhatia and Yang (2017) stated that the higher the value of VFA, the more other organic acids (acetic, propionic, butyric, isobutyrate, and isovalerate) were produced the pH of the rumen fluid would below. However, based on the results, the rumen pH was not significantly different from each treatment A, B, C, and D. It was influenced by the provision of Mc Dougall buffer (artificial saliva), which played a role in maintaining pH. According to Van Soest et al. (1991), the condition of rumen pH remains constant due to the buffering capacity from saliva because it contains a lot of bicarbonate and phosphate and the absorption system of VFA through the rumen wall.

The degree of acidity in the rumen is affected by the type of feed given but generally remains at a constant pH. Stable rumen pH in this study created a familiar atmosphere in the rumen and is suitable for rumen microbes in carrying out their activities. Jamarun et al. (2017c) stated that rumen environmental conditions have a close relationship with the pH of the rumen fluid because the high and low pH in the rumen will affect the rumen microbial activity.

The analysis of variance showed that tithonia fermentation with different types of microbes and incubation time gave no significant effect (P>0.05) on the production of rumen fluid VFA. It indicated that feed fermentation using *L. plantarum* and *A. ficuum* did not affect rumen microbial activity in producing VFA in the form of acetic acid, propionic acid, and butyric acid. The production of VFA in this study ranged from 116.67 mM – 135 mM; this value was still in the range of VFA concentrations under normal conditions. McDonald et al. (2010) stated that the normal range for total VFA was 70-150 mM.

Table 6. Digestibility of dry matter, organic matter, crude protein, and crude fiber from fermented tithonia

Treatments	Nutritional digestibility (%)			
	Dry matter	Organic matter	Crude Protein	Crude fiber
A	65.73	66.22	69.70	78.11[b]
B	66.86	67.36	71.50	81.01[a]
C	62.21	66.03	69.57	71.52[c]
D	66.01	66.69	67.78	73.79[c]
SE	0.32	0.36	0.62	0.69

Note: Different superscripts in the same column show significantly different effects (P<0.05)

Table 7. Rumen fluid characteristics from fermented tithonia

Treatments	Rumen fluid characteristics		
	pH	VFA (mM)	NH_3 (mg/100ml)
A	6.79	130	12.18[ab]
B	6.72	135	14.31[a]
C	6.85	116.67	10,48[b]
D	6.83	123.33	9.21[b]
SE	0.03	6.96	0.86

Note: Different superscripts in the same column show very significant different effects (P<0.05)

The insignificant difference in VFA production was also due to the relatively similar composition, feeding level, and physical form of the feed. Storm and Kristensen (2010) stated that the design of VFA in the rumen changes with differences in physical form, feed composition, level and frequency of feeding, and processing. High or low VFA production can be used as a measure of the fermentability of a feed ingredient. Jones and Murphy (1989) stated that the higher the fermentability level of the feed, the greater the VFA produced.

The analysis of variance showed that tithonia fermentation with different types of microbes and incubation time had a significantly different effect (P<0.05) on the production of rumen fluid NH_3. The results of the DMRT further test showed that treatment A was not significantly different (P>0.05) from treatments B, C, and D. However, Treatment B showed a significant difference (P<0.05) with treatments C and D. Also, treatment C showed the effect was not significantly different from treatment D.

The highest NH_3 production was found in treatment B, namely, 14.31 mg/100ml. The high output of NH_3 in treatment B indicated that the rumen microbial activity was running well. NH_3 concentration means the fermentability of feed related to protein digestibility, rumen microbial activity, and microbial population (Pazla et al. 2018b). In addition, the high production of NH_3 in treatment B was due to the increased degradation of crude protein in the rumen. NH_3 concentration was strongly influenced by protein solubility. The more soluble the protein, the easier it will be for rumen microbes to degrade it; the CPD indicates this in treatment B, which was higher than in other treatments (Table 2). It is following the opinion of Bannink et al. (2016), which states that. The higher the degradation of CP in the rumen will increase the concentration of NH_3 and vice versa

The lowest NH_3 concentration was found in treatment D, which was 9.21. The low concentration of NH_3 in treatment D was due to the low availability of N in the feed; this was indicated by the low crude protein content of the material, which was 27.56. Mayulu (2014) and Wulandari et al. (2017) stated that the lower the crude protein content of the ration, the production of NH_3 would also decrease. The NH_3 production produced in each treatment in this study ranged from 9.21 to 14.31. This value is still in the normal range for rumen microbial activity and growth. The opinion of Satter and Slyter (1973) stated that the minimum concentration of ammonia required for microbial protein synthesis is 5 mg/100ml rumen fluid. The study concluded that fermented tithonia using *A. ficuum* with an incubation period of 7 days improve the quality of tithonia. It is seen from the content of CP (31.02%), CF (16.52%), phytase enzyme activity (37.36 U/ml), DMD (66.86%), OMD (67.36%), CFD (81.01%), CPD (70.37%), VFA production 135 mM and NH_3 concentration 14.31 mg/100 ml, and pH value 6.72 which is very suitable for rumen microbial growth.

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