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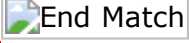

[and in vitro Fermentability of Fermented Oil Palm Fronds by Phanerochaete chrysosporium in Combination with Tithonia \(Tithonia diversifolia\) and Elephant Grass \(Pennisetum purpureum\)", Pakistan Journal of Nutrition, 2018](#)

OPEN ACCESS Pakistan Journal of Nutrition ISSN 1680-5194 DOI: 10.3923/pjn.2018.39.45 Research Article [Populations of Rumen Microbes and the In vitro Digestibility of Fermented Oil Palm Fronds in Combination with Tithonia \(Tithonia diversifolia\) and Elephant Grass \(Pennisetum purpureum\)](#)  Novirman Jamarun, Mardiati Zain, Arief and Roni Pazla Faculty of Animal Science, Andalas University, Padang, Indonesia [Abstract Objective: The aim of this research was to](#)  identify the rumen [microbial](#)  populations and [determine the in vitro](#)  nutrient [digestibility of](#)  fermented [oil palm fronds](#)  (FOPF) [by](#)  Phanerochaete [chrysosporium,](#)  which were supplemented [with Ca, P and Mn minerals](#) [and](#)  combined [with tithonia](#)  (Tithonia diversifolia) [and elephant grass](#)  (Pennisetum purpureum). [Methodology: This study used a randomized block design consisting of](#)  4 [treatment groups: T1 = 20% FOPF+](#)  80% (tithonia+elephant grass), T2 = 40% FOPF+60% (tithonia+elephant grass), T3 = 60% FOPF+40% (tithonia+elephant grass) and T4 = 80% FOPF+20% (tithonia+elephant grass). Four replicates were used per treatment. The ratio of tithonia to elephant grass was 1:4. Data were analyzed by [analysis of variance \(ANOVA\)](#)

and differences among means were tested using Duncan's multiple range tests (DMRT) with 5 and 1% confidence intervals. The

 measured variables were  total bacteria, total protozoa, dry matter digestibility (DMD), organic matter digestibility (OMD) and crude fiber digestibility (CFD). Results: The results showed that total population of bacteria, DMD, OMD and CFD

were the highest ( $p < 0.01$ ) in the T1 treatment.  Total

protozoa were lowest in T1  but the  difference with the other treatments was not significant ( $p > 0.05$ ).

Conclusion: T1 (combination of 20% FOPF and 80% tithonia+elephant grass) had the best effect on rumen microbial populations and in vitro digestibility. Key words: In vitro digestibility, FOPF, Tithonia

diversifolia, Pennisetum purpureum, microbial populations Received:

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(Tithonia diversifolia) and elephant grass (Pennisetum purpureum).

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 Competing Interest: The  authors have

declared that no competing interest exists. Data Availability: All

relevant data are within the paper and its supporting information

files. INTRODUCTION One of the primary determinants affecting the

success of a livestock business is the availability of continuous and

abundant feed ingredients. Lately, the availability of animal feed has

become increasingly limited. Factors affecting the availability of

fodder include the increasing prices of raw materials for fodder and

the decrease in land used to produce forage due to changes in land

use in favor of food production and residential areas. Therefore, the

exploration of potential new resources that can be used as alternative

feed should be a top priority. Alternative feed is expected to replace

some or all of the forage and can also reduce dependence on the

commonly used concentrated ingredients. Palm fronds are a waste

product of palm oil plantations that can be used as animal feed. Each

palm tree can produce 22 stem/pieces of palm fronds per year and

the weight of palm fronds per stem reaches 2.2 kg (after peeling for

feed) thus, each hectare can produce approximately 9 t/ha/year of

fresh palm fronds for feed, which is equivalent to 1.64 t/ha/year of

dry matter<sup>1</sup>. One factor constraining the use of palm fronds as animal

feed is the high content of crude fiber and lignin<sup>2,3</sup>. The lignin binds

strongly with cellulose and hemicellulose in plant cell walls, making it

difficult for rumen microbial enzymes to penetrate cells, which

ultimately decreases the digestibility of feed ingredients. A

fermentation technique using Phanerochaete chrysosporium

supplemented with the minerals Ca, Mn and P has successfully

degraded lignin in palm fronds, decreasing the lignin content. The

decrease in lignin content can facilitate the performance of rumen

microbes to use nutrients from feed ingredients, which ultimately increases digestibility<sup>4,5</sup>. Techniques that combine multiple forages can contribute more forage with diverse compositions to increase microbial protein synthesis as a source of protein for host animals and to reduce rumen protozoan populations<sup>6</sup>. A decline in protozoan populations can lead to increases in populations of bacteria, thereby increasing feed degradation, which ultimately increases the digestibility of feed ingredients. Tithonia (*Tithonia diversifolia*) is a potential feed material for ruminants that can be combined with fermented oil palm fronds (FOPF). Leaves of this plant contain complex amino acids and high protein and the availability is high<sup>6</sup>. Tithonia cultivated in West Sumatra can produce as much as 30 t of fresh material or 6 t of dry matter per year with a land area of approximately 1/5 ha. When grown as a hedge, tithonia can produce 27 kg of dry weight from three harvests in 1 year<sup>7</sup>. The research on tithonia as a ruminant feed material is limited because the leaves contain many anti-nutritional substances that endanger livestock such as phytic acid, tannins, saponins, oxalates, alkaloids and flavonoids<sup>8</sup>. The administration of the correct dose is the key to optimal use of tithonia as a feed ingredient. The correct dose can optimize the nutritional potential of tithonia and minimize the effect of anti-nutritional substances. Jamarun et al.<sup>9</sup> obtained the best values for in vitro dry matter, organic material and rough fiber digestibility using a 20% dose of tithonia in a combination of tithonia and elephant grass forage. Anti-nutritional content in tithonia is also suspected to affect defaunation, which decreases the number of protozoa in the microbial population leading to increase in the number of bacteria. Increasing the number of bacteria always increases the digestibility of food substances in the rumen. The aim of this study was to determine the rumen microbial populations and in vitro digestibility of oil palm fronds (OPFs) fermented by Phanerochaete chrysosporium, which were supplemented with the minerals Ca, P and Mn and combined with tithonia and elephant grass. **MATERIALS AND METHODS** For this study, the fermentation of OPFs was conducted at the Laboratory of Technology for Feed Industries, Andalas University. Analysis of in vitro digestibility and rumen microbial populations was conducted in the Laboratory of Dairy Nutrition of the Bogor Agricultural Institute. Samples of FOPF, tithonia and elephant grass 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 total protozoa, total bacteria, DMD, DMO and CFD. Fermented oil palm fronds, tithonia and elephant grass: The

End Match palm fronds used as the raw material in this study were taken from the distal two-thirds of the End Match oil palm frond (OPF). The OPF substrates were cut, dried and finely milled. The End Match oil palm frond was fermented using Phanerochaete chrysosporium, which was supplemented with 2000 ppm Ca, 2000 ppm P and 150 ppm Mn, according to the procedure End Match of Jamarun et al. End Match 5. The End Match tithonia plants used in this study had soft plant End Match parts (stems, leaves and flowers) with a 6-16 cm long trunk from the top. End Match Tithonia and End Match elephant grass were cut, dried and crushed. The ratio of End Match tithonia and End Match elephant grass in each treatment ratio was 1:4, based on Jamarun et al. End Match

9. The nutritional contents of End Match FOPFs, tithonia and End Match elephant grass are presented in Table 1. The composition of the feed ingredients and the nutrition of the treatment rations are presented in Table 2. Table 1: Nutritional content of FOPF, tithonia and elephant grass End Match Raw material -----

	Nutritional content (%)			
FOPF	Tithonia	Elephant grass	End Match	
Dry matter	72.01	25.57	21.23	10.88
Organic matter	91.34	84.01	89.46	88.96
Crude protein	8.89	22.98	10.65	9.77
Crude fiber	38.59	18.17	32.77	31.60
Lignin	18.35	4.57	6.29	8.43

Table 2: Composition of feed ingredients and nutrition of treatment rations

Treatment rations	Variables (%)				End Match				
	A	B	C	D	FOPF	20.00	40.00	60.00	80.00
Tithonia	16.00	12.00	8.00	4.00	64.00	48.00	32.00	16.00	0.00
Elephant grass	64.00	48.00	32.00	16.00	0.00	0.00	0.00	0.00	0.00
Dry matter	93.58	92.33	90.88	89.73	88.96	89.56	90.15	90.75	90.15
Organic matter	88.96	89.56	90.15	90.75	12.42	11.54	10.65	9.77	9.77
Crude protein	12.42	11.54	10.65	9.77	31.60	33.35	35.09	36.84	36.84
Crude fiber	31.60	33.35	35.09	36.84	8.43	10.91	13.39	15.87	15.87
Lignin	8.43	10.91	13.39	15.87					

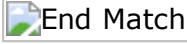



In vitro procedure: The in vitro procedure in this study followed that of Tilley and End Match Terry<sup>10</sup>. Rumen fluid was taken from a cow fistula at Bogor, LIPI. The End Match fermented tube was filled with 0.5 g of sample and 40 mL of McDougall solution was added. The tube was placed in a shaker bath at End Match 39EC and then filled with 10 mL of rumen fluid and shaken with CO<sub>2</sub> for 30 sec, the pH was checked (6.5-6.9) and End Match then the sample was covered with a ventilated rubber cap and fermented for 48 h. After 48 h, the rubber cap of the End Match fermented tube was removed and 2-3 drops of HgCl<sub>2</sub> were added to kill the microbes. The End Match fermented tube was centrifuged at 5,000 rpm for 15 min. The substrate 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 incubated for 48 h without a rubber cap. The remaining digested residue was

filtered using Whatman filter paper No. 41 (identified by its weight) with the help of a vacuum pump. The precipitate on the filter paper was placed into a porcelain dish, which was placed into an oven at 105EC for 24 h. After 24 h, the porcelain cup+filter paper+residue was removed, inserted into a desiccator and weighed to determine the dry matter content. Furthermore, the ingredients in the cup were placed in a kiln or in an electric furnace for 6 h at 450-600EC and then weighed to determine the amount of organic material. Residue originating from the fermentation without feed ingredients was used as a control. The proximate analysis of materials and residues followed the AOAC11 procedure.





Rumen protozoa population: Measurements were conducted using a counting chamber. Up to 0.5 mL of rumen solution was fixed with 0.5 mL of methyl green formalin saline (MFS) solution in tubes and thoroughly mixed. Up to 0.1 mL of this sample was placed in a counting chamber (hemocytometer) using a pipette and a coverslip was placed over the sample. The protozoa were quantified in the counter under a microscope at 40X magnification. According to the number of protozoa obtained by the above counting procedure, the number per 1 mL of rumen content was calculated using the following Eq: Protozoa population mL<sup>-1</sup> =  $\frac{n \times 1000}{d \times 0.1 \times 0.065 \times 6 \times 5}$  Where: n = Number of protozoa in the counting chamber d = Sample dilution

Rumen bacteria population Sample preparation: First, 1 mL samples of aseptic rumen fluid were pipetted into 9 mL of Butterfield's phosphate buffer diluent to obtain a 1:10 dilution. When homogenized, this sample was a solution at a 10G1 dilution. Analysis of total bacteria: Analysis of total bacteria used the Anoxomat system (MART Microbiology, B.V., Netherlands), for the cultivation of microorganisms in an oxygen-depleted or CO<sub>2</sub> enriched environment. For subsequent dilutions (10G2, 10G3, 10G4 and others, as required) of the sample, 1 mL of the previous solution (dilution of 10G1) was added to 9 mL of diluent using a sterile pipette. Then, the solutions were homogenized via vortexing. Next, 1 mL of any desired dilution was added to a sterile petri dish, this procedure was replicated twice. Then, 15-20 mL of NA medium (Nutrient Agar) was poured into a petri dish to test the total bacteria in the given sample. The media were homogenized by swirling the petri dish. Afterward, the samples were frozen. The petri dishes were inserted into an Anoxomat jar upside down to create anaerobic conditions according to the instructions for the Anoxomat system. Then, the petri dishes were incubated at 37EC for 48 h with the cup upside down. The number of colonies on a petri dish was counted, typically between 25 and 250 colonies. When the number of colonies was >250 colonies, the dish was considered TMTC (Too Many To Count). Colony calculations were performed.

Experimental design and statistical analysis: The study was conducted using a randomized block design (4×4) with 4 replications (Levels of FOPFs, tithonia and

elephant grass as the treatments). The differences between the treatment means were analyzed using Duncan's multiple range tests with confidence intervals of 5%  (p<0.05) and 1%  (p = 0.01). The treatments were  the following: C T1 = 20% FOPF+  80% (tithonia+elephant grass) C T2 = 40% FOPF+60% (tithonia+elephant grass) C T3 = 60% FOPF+40% (tithonia+elephant grass) C T4 = 80% FOPF+20% (tithonia+elephant grass)

**RESULTS AND DISCUSSION**

**Nutritional content of FOPFs:** The nutritional contents of OPFs before and after fermentation are presented in Table 3. Phanerochaete chrysosporium is a white mold that is currently used as a model for many scientists to degrade existing lignin in waste-based feedstocks. Table 3 shows a 40.08% decrease in lignin (from 30.86-18.35%), which was due to the supplementation with mineral (Ca, P and Mn) during fermentation to increase the enzyme activity of ligninase produced by Phanerochaete chrysosporium. Gupte et al.<sup>13</sup> stated that P. chrysosporium reduced the lignin content by 20.5% on the 10th day of incubation and showed maximum enzyme activity in degrading lignin. Supplementation of Ca and Mn minerals to palm fronds fermented by P. chrysosporium can reduce lignin by 25.77% and increase in vitro digestibility<sup>4</sup>. Fermentation of olive pomace by P. chrysosporium decreased lignin by 63.9% after 20 days of fermentation and by 46.44% after a fermentation time of 10 days<sup>14</sup>. These decreases are likely related to the availability of nutrients resulting from the reshuffling of lignocellulose components by the mold for its own growth. The change in lignin content in the substrate occurs because of the reshuffling of the lignin structure into simpler components<sup>15</sup>. Fermentation using P. chrysosporium  that was supplemented with Ca, P and Mn minerals  also increased the  protein content  of the feed and reduced the content of crude fiber. The amount of substrate protein increased by 144.23% and the amount of crude fiber decreased by 28.72%. The increased percentage of the crude protein content of fermented palm bark is caused by mold growth contributing to the increase in protein. Due to the growth of mold, the more mycelia are there, the more nitrogen is supplied to the body of the mold, which is the factor responsible for the increase of protein observed in the FOPFs. Nelson and Suparjo<sup>15</sup>, suggest that an increase in protein content occurs because of the bioconversion of sugar into a mycelium protein or a single cell protein. The decrease in crude fiber content

10 9 9.36a 9.2b 9.17b 9.05c Cells mLG1 (log 10) 8 7 6 5 4 4.61 4.7 3 3.41 2 2.23 1 Protozoa 0 Bacteria T1 T2 T3 T4 Treatments

Fig. 1: Rumen microbial populations of various combinations of FOPF levels with tithonia and elephant grass a-dMeans in the same color with different letters are highly significantly different (p<0.01)

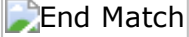
Table 3: Nutritional content of OPFs before and after fermentation

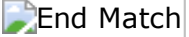
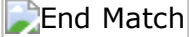
Raw material	OPF	FOPF	Dry matter (%)	Organic matter (%)	Ash (%)	Extract ether (%)	Crude protein (%)	Crude fiber (%)	NDF (%)	ADF (%)	Cellulose (%)	Hemicellulose (%)	Lignin (%)
	83.96	72.01	83.96	72.01	5.87	8.66	3.64	49.80	89.98	73.21	41.35	16.78	8.67
	94.13	91.34	83.96	72.01	5.87	8.66	8.89	38.59	66.52	57.85	37.50	8.67	8.67

30.63 18.35 NDF: Neutral detergent fiber, ADF: Acid detergent fiber due to enzyme activity by the mold can also increase the percentage of crude protein content. Good protein quality and low levels of crude fiber result in increased digestibility of the feed. Population of rumen microbes: Protozoa and bacteria are the most abundant in cattle rumens. In degrading feed ingredients through the production of enzymes, bacteria are very useful, whereas at high densities, protozoa decrease the rumen bacterial population. Rumen protozoan and bacterial populations of the [fermented oil palm fronds](#)

 combined [with tithonia and elephant grass](#) 

are presented in Fig. 1. Figure 1 shows total protozoan population values that range from 2.23-4.70 cells mLG1 (Log 10). These values are lower than the range of protozoa obtained by Jamarun et al.<sup>16</sup> [5.312-6.295 cells mLG1 (Log 10)] from 100% palm fronds fermented using *Phanerochaete chrysosporium* fungus [supplemented with Ca, P](#)

[and Mn minerals.](#) The  values are also lower than the range described by Febrina et al.<sup>17</sup> from a 100% substrate of fermented palm fronds by *Phanerochaete chrysosporium* with the

[addition of](#)  minerals [\(P, S and Mg\)](#),  which was 5.851-5.864 cells mLG1 (Log 10). These differences are because in this study, tithonia plants were used that contained anti-nutritional tannins and saponins. Tannins and saponins in tithonia are anti-nutrients that can function as defaunation agents<sup>18</sup>. Defaunation is the reduction and elimination of rumen protozoa. With a decrease in protozoan population values, the numbers of bacteria increase because protozoa prey on bacteria. Excessive protozoa kill the bacteria, reducing the population of bacteria in the rumen. The reduction in the bacterial population reduces feed degradation, resulting in low digestibility of the feed. Different levels of FOPFs combined with tithonia and elephant grass did not significantly affect ( $p > 0.05$ ) the protozoan populations. However, as shown in Fig. 1, an increasing trend in the protozoan population was observed with reduced doses of tithonia in the treatments. T1 has the lowest protozoan population, which was due to the highest dose of tithonia that was equal to 16% of the feed ingredients. T4 had the highest protozoan population because the dose of tithonia was only 4% of the feed ingredients. The population of rumen bacteria was inversely proportional to the protozoan population. Figure 1 shows the range of rumen bacteria at 9.05-9.36 cells mLG1 (Log 10), which is within the normal limits [9-12 cells mLG1 (Log 10)] reported by Stewart<sup>19</sup>. As levels of fermented palm bark increased and those of tithonia decreased, the rumen bacterial population decreased significantly ( $p < 0.01$ ). T1 had the highest population of bacteria, whereas the lowest population was in T4. The development and growth of bacteria are influenced by the ease or difficulty of degradation of feed ingredients. The T1 treatment contained lower lignin levels than those of the other treatments (Table 1). Low lignin levels allow more bacteria to degrade feed and feed degradation also produces a source of food for bacteria to grow and multiply. In addition to the influence of low lignin levels, the increasing population of bacteria was also due to the effects of tithonia plants which is containing tannin and saponin anti-nutrients as defaunation agents. Therefore, with reductions in

protozoa, the number of bacteria increased. Digestibility of dry matter, organic matter and crude fiber in vitro: Increased levels of FOPFs and decreased levels of tithonia and elephant grass had a highly significant effect on digestibility ( $p < 0.01$ ), with decreases in dry matter, organic matter and crude fiber digestibility in vitro as shown in Fig. 2. The average digestibility ranged from 38.01-58.17% for the digestibility of dry matter, 38.35-58.23% for the digestibility of organic matter and 33.81-57.35% for the digestibility of crude fiber. T4 33.81d 38.35d CFD 38.01d OMD DMD Treatments T3 36.2c 43.85cc 43.19 T2 40.53b 47.78b 47.2b T1 57.35a a 5588..2137a 0 10 20 30 40 50 60 70 Percentage Fig. 2: In vitro digestibility of dry matter, organic matter and crude fiber of various combinations of FOPF levels with tithonia and elephant grass a-dMeans in the same color with different letters are highly significantly different ( $p < 0.01$ ) Figure 2 shows a trend of decreased digestibility of dry matter, organic matter and crude fiber from treatment T1 to T4. The decrease in dry matter digestibility, organic matter and crude fiber was caused by the differences in crude fiber content and lignin content of each treatment. Imsya et al.20 stated that the digestibility of dry matter and organic matter decreases because of high levels of crude fiber content in the ration. T4 showed the highest crude fiber and lignin contents (36.84% crude fiber and 15.87% lignin) and the lowest dry matter, organic matter and crude fiber digestibility. These results suggested that although OPFs were fermented [using \*P. chrysosporium\* supplemented with Ca, Mn and P minerals](#), which decreased [the lignin content](#) the nutritional value produced by the fermentation process was not able to achieve the nutritional value with [tithonia and elephant grass](#) (Table 1). Fermentation of OPFs with [P. chrysosporium supplemented with Ca, P and Mn minerals](#) for 20 days showed a good lignin degradation rate. The content of lignin of OPFs without fermentation was 30.63%, whereas lignin content of fermented OPFs was 18.35% (Table 3). This result showed that although the lignin content could be reduced by the fermentation process, the lignin content remained higher than the lignin contents in tithonia and elephant grass, which were 4.57 and 6.29%, respectively (Table 1). Thus, at the high levels of FOPFs used in the treatment rations, the levels of digestibility remained low. The high digestibility of T1 suggested that this combination of forages was more easily degraded by rumen microbes than the other treatments. Low lignin content is very helpful for rumen microbial breakdown of cellulose and hemicellulose feed. The contribution of tithonia to this treatment, in addition to donating proteins, was a reduction in the protozoan population (Fig. 1) such that the number of bacteria significantly increased. The pattern of digestibility of organic materials follows that of dry matter digestibility, i.e., the low digestibility of the dry matter causes the low digestibility of organic materials and vice versa. Thus, an increase in the digestibility of dry substrate materials is followed by an increase in the digestibility of organic substrate materials<sup>21,22</sup>. Jamarun et al.<sup>9</sup> also observed the same trend combining tithonia with elephant grass. The values of DMD, OMD and CFD in this study are higher than those in the treatment of 100% in



vitro FOPFs used by Jamarun et al.21, Mariani 23, Rahayu et al.24 and Febrina et al.4,17. This is because this treatment combines several feed ingredients such that the composition of nutrient substances donated, particularly the mineral content and amino acids, was more complete. Minerals and more complex amino acids optimize the metabolic activities in the rumen. Rumen microbes also optimally degrade feed ingredients, resulting in increased dry matter, organic matter and crude fiber feed digestibility. In contrast,

Febrina25 and Febrina et al. 26 reported that fermented OPFs by Phanerochaete chrysosporium supplemented with Ca and Mn minerals matched the digestibility and consumption of elephant grass rations. The difference in these results was likely due to the influence of the addition of concentrate to the ration. CONCLUSION The combination of 20% FOPF and 80% (tithonia+elephant grass) (T1) yielded the best in vitro nutrient digestibility values and microbial populations. SIGNIFICANCE STATEMENT The effect of FOPFs combined with tithonia and elephant grass on microbial populations and in vitro nutrient digestibility was evaluated in this study. The results indicated that the combination of 20% FOPF with 80% (tithonia and elephant grass) was highly effective in rations derived from plantation waste products, such as oil palm fronds. This study help researchers to realize that the combination of FOPFs with tithonia and elephant grass is important to support growth and microbial activity and therefore to improve the digestibility of nutrients. Thus, this research could lead to a new theory on the importance of combination feed materials in rations derived from plantation waste products. ACKNOWLEDGMENTS This research was funded by the Andalas University Professor Grant 2017 and was part of the Ph.D. dissertation by Roni Pazla in Animal Nutrition, Faculty of Animal Science, Andalas University.

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