In vitro nutrient digestibility, volatile fatty acids and gas production of fermented palm fronds combined with tithonia \((Tithonia \textit{diversifolia})\) and elephant grass \((\textit{Pennisetum Purpureum})\)

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Abstract. This study aims to determine the effect of the addition of phosphorus minerals to palm fronds fermented with \textit{Phanerochaete chrysosporium} mold on the digestibility of nutrient invtro and to evaluate the total gas production and concentration of volatile fatty acids in fermented palm fronds combined with elephant grass \((\textit{Pennisetum Purpureum})\) and Tithonia \((Tithonia \textit{diversifolia})\). This research was conducted with an experimental method through 2 experimental stages. Stage 1 consisted of 3 treatment doses of mineral P with 5 replications, namely: \(A = 1000\) ppm, \(B = 1500\) ppm and \(C = 2000\) ppm. Stage 2 consisted of 4 combined treatments of fermented palm fronds \((\text{FPF})\) with elephant grass \((\text{EG})\) and tithonia \((T\) with 4 replications, namely: \(A = 20\% \text{ FPF} + 80\% \text{ (EG} + T\), \(B = 40\% \text{ FPF} + 60\% \text{ (EG} + T), \(C = 60\% \text{ FPF} + 40\% \text{ (EG} + T)\) and \(D = 80\% \text{ FPF} + 20\% \text{ (EG} + T).\) The ratio of \(\text{EG} \text{ and} \text{ T} \) is 4: 1. The parameters measured in stage 1 were the digestibility of dry matter, organic matter, crude protein, and crude fiber, while in stage 2 were gas production and total and partial VFA concentrations. The results of the research in stage 1 showed that the treatment had a significant effect \((P < 0.01)\) on the digestibility of dry matter, organic matter, crude protein, and crude fiber, while in stage 2 were gas production and total and partial VFA concentrations. The results of the research in stage 1 showed that the treatment had a significant effect \((P < 0.01)\) on the digestibility of dry matter, organic matter, crude protein, and crude fiber. Gas production and total and partial VFA concentrations at stage 2 also showed significant differences \((P < 0.01)\) between treatments. This study concludes that the addition of a P dose of 2000 ppm \((C)\) in the fermentation process provides the highest nutrient digestibility value and the combination of 20\% FPF and 80\% \((\text{EG} + T)\) \((D)\) results in the highest gas production and VFA concentration.

Keywords: Gas production, \textit{inv tro}, Palm fronds, Phosphorus, \textit{Tithonia diversifolia}.

1. Introduction
The biggest obstacle experienced by ruminants when consuming feed derived from palm fronds is the lignin content. Lignin is a woody substance in plants that cannot be digested by rumen microbes. Enzymes from rumen microbes are difficult to utilize cellulose and hemicellulose in plants because of the very strong lignocellulose bond between lignin and cellulose and lignin with hemicellulose. Cellulose and hemicellulose are a source of energy for ruminants.

Fermentation of feed using \textit{Phanerochaete chrysosporium} mold is highly effective in reducing lignin levels so that the efficiency of utilization of nutrients will increase the digestibility value of food substances [1]. The work effectiveness of \textit{P. chrysosporium} depends on the availability of minerals in its growth substrate [2]. One of the macro minerals that have a massive role in the metabolism of living cells is mineral P [3].
The combination of various forage feeds is very good for complementing the deficiency status of certain nutrients in one type of feed ingredient. Fermented palm fronds combined with superior forages such as elephant grass and tithonia are expected to increase the productivity of ruminants. Tithonia contains amino acids, minerals, and high crude protein [4]. This study aimed to determine the addition of mineral P to the substrate of P. chrysosporium on digestibility in vitro and also to see the effect of the combination of fermented palm fronds with elephant grass and tithonia on gas production and Volatile fatty acids (VFA) concentration.

2. Materials and methods

2.1. Materials
Phanerochaete chrysosporium mold, palm frond, elephant grass, tithonia, distilled water, rice bran, mineral (P, Ca and Mn), rumen fluid, McDougall's solution, CO₂ gas, Whiteman filter paper no 41, NaOH solution, acetone, and in vitro paraphernalia.

2.2. Research design
This study was carried out in two stages of experimental research consisting of: Stage 1 is in vitro test; Fermented palm fronds (FPF) using P. chrysosporium with the addition of P mineral. Stage 2 is In vitro test combination of the best level of FPF in stage 1 with a mixture of elephant grass and tithonia.

The method used in this study is an experimental method using a completely randomized design for stage 1 with 3 kinds of treatment levels of mineral P dose and 5 repetitions, where the treatments are as follows:
A = mineral P 1000 ppm
B = mineral P 1500 ppm
C = mineral P 2000 ppm

The method used in the second stage of the study was an experimental method using a Randomized Block Design with 4 types of combination treatment levels of FPF with different mixtures of elephant grass (EG) and tithonia (T) and 4 replications. The ratio of EG and T is 4:1 [5]. The data were analyzed using analysis of variance (ANOVA) according to [6], while Duncan's multiple distance test was used to determine the difference in the treatment mean at a confidence interval of 5% and 1%. The treatments in this experiment are:
Treatment A = FPF 20 % + (EG + T) 80 %
Treatment B = FPF 40 % + (EG + T) 60 %
Treatment C = FPF 60 % + (EG + T) 40 %
Treatment D = FPF 80 % + (EG + T) 20 %

2.3. Parameters measured
Stage 1: digestibility of dry matter, organic matter, crude protein, and crude fiber
Stage 2: total gas production, total VFA, and partial VFA

2.4. Research implementation
The milled palm fronds were then weighed and added with distilled water so that the water content reached 65%. Afterwards, mineral P was added according to the treatment and sterilized in an autoclave at 121°C for 25 minutes and then cooled to room temperature. After cooling, the palm fronds were inoculated with 10% of the substrate with P. chrysosporium, stirred evenly, transferred to a glass bottle, covered with rubber and gauze, and then incubated for 20 days. After the fermentation process is complete, the fermented products are weighed fresh and dried at 60°C for 24 hours and then analyzed for chemical composition [7, 8].

The best-fermented palm fronds in stage 1 were combined with elephant grass and tithonia according to the treatment. Elephant grass and tithonia were first dried and ground and analyzed for their chemical composition [7, 8]. The nutritional composition of FPF, Tithonia, and Elephant Grass is presented in
Table 1. The composition and nutritional content of the treatment rations are presented in Table 2. In vitro test was carried out based on the [9] method and measurement of total gas production using the [10] method.

Table 1. The nutritional composition of FPF, tithonia, and elephant grass.

| Chemical Compositions (%) | Raw Materials |
|---------------------------|---------------|
| Dry matter                | 72.01         |
| Organic matter            | 91.34         |
| Crude Protein             | 8.89          |
| Crude fiber               | 38.59         |
| NDF                       | 66.52         |
| ADF                       | 57.85         |
| Cellulose                 | 37.50         |
| Lignin                    | 18.35         |
| Titonia                   | 25.57         |
| Elephant grass            | 21.23         |
|                         | 84.01         |
|                         | 22.98         |
|                         | 18.17         |
|                         | 61.12         |
|                         | 40.15         |
|                         | 34.59         |
|                         | 4.57          |

Table 2. The composition and nutritional content of the treatment rations.

| Composition | Treatment rations |
|-------------|-------------------|
|             | A                | B      | C      | D      |
| FPF %       | 20               | 40     | 60     | 80     |
| Tithonia    | 16               | 12     | 8      | 4      |
| Elephant Grass % | 64     | 48     | 32     | 16     |
| Dry matter % | 93.58          | 92.33  | 90.88  | 89.73  |
| Organic matter % | 88.96    | 89.56  | 90.15  | 90.75  |
| Crude protein % | 12.42      | 11.54  | 10.65  | 9.77   |
| Crude Fibre % | 31.60         | 33.35  | 35.09  | 36.84  |
| NDF %       | 65.69          | 65.90  | 66.10  | 66.31  |
| ADF %       | 44.69          | 47.98  | 51.23  | 54.56  |
| Cellulose % | 34.91          | 35.56  | 36.20  | 35.85  |
| Lignin %    | 8.43           | 10.91  | 13.39  | 15.87  |

3. Results and discussion

3.1. In vitro digestibility

The data from the In vitro digestibility analysis of food substances due to the addition of mineral P combined with Ca and Mn in the palm fronds fermentation process using the P. chrysosporium are presented in Table 3. The analysis of variance showed that the addition of mineral P combined with Ca and Mn in the palm fronds fermentation process using P. chrysosporium had a very significant effect (P<0.01) on dry matter digestibility (DMD). Mineral P combined with Ca and Mn can play a role in supporting the growth and elongation of mold mycelia so that molds can produce optimum ligninolytic enzymes to degrade lignin that make rumen microbes digest food more easily.

The digestibility of food substances from feed ingredients greatly determines the quality of the feed ingredients. The higher the digestibility of a feed ingredient means that the feed ingredients are of good quality for livestock consumption and utilized for their body's metabolic processes. Table 3 shows that the highest DMD was found in treatment C (43.41%), followed by B (39.60%) and the lowest was in treatment A (38.00%). The low DMD in treatment A indicated that the dose of P 1000 ppm was not
optimal to support the growth and elongation of the mycelia of molds as a result of the mold not being able to produce optimal ligninolytic enzymes to degrade lignin. In essence, there was still a lot of cellulose and hemicellulose bound to lignin [11] stated that lignin is a fraction that is difficult to degrade. The binding of cellulose and hemicellulose to lignin causes rumen microbes to not optimally degrade lignin so that DMD in this treatment is still low.

Table 3. In vitro digestibility of fermented palm fronds.

| Digestibility (%) | Treatments |
|-------------------|------------|
|                   | A  | B  | C  |
| Dry matter        | 38.00<sup>a</sup> | 39.60<sup>b</sup> | 43.41<sup>c</sup> |
| Organic matter    | 36.65<sup>a</sup> | 38.60<sup>b</sup> | 40.86<sup>c</sup> |
| Crude protein     | 72.57<sup>a</sup> | 78.84<sup>b</sup> | 79.78<sup>c</sup> |
| Crude fiber       | 43.36<sup>a</sup> | 50.02<sup>b</sup> | 54.89<sup>b</sup> |

Note: different superscripts (a,b,c) on the same line show a very significant difference (P<0.01).

The addition of mineral P to 2000 ppm in treatment C showed the highest DMD value. Increasing the dose of P to 2000 ppm was able to increase the activity of the ligninase enzyme because is needed in reactions related to the release of energy to form ATP and is an enzyme activator. The higher the activity of the ligninase enzyme, the more lignin is degraded and the more food substances that can be digested by rumen microbes resulting in the highest DMD. The higher the DMD, the higher the quality of the feed [12].

The DMD of the results of this study ranged from 38.00 to 43.41%. This value is higher than that of [1] obtaining DMD of palm fronds from the fermentation of the <i>P. chrysosporium</i> with the addition of minerals Ca and Mn of 34.04% - 41.91%. It is lower than [13] which was 61.14% fermented on a solid substrate by <i>Pleurotus ostreatus</i> and [14] on the fermentation of cocoa pod skin by the <i>P. chrysosporium</i> with the addition of minerals Ca and Mn of 40.14% - 59.61%. This difference in value is caused by the type of media and microorganisms used in the fermentation [15] stated that different types of microorganisms have different abilities from one type of material to another.

The results showed that the addition of mineral P in the palm fronds fermentation process using <i>P. chrysosporium</i> had a significant effect (P<0.05) on the digestibility of organic matter. This shows that the addition of P mineral in the palm frond fermentation process affects the digestibility of organic matter. [16] stated that the addition of organic macro minerals in the ration resulted in higher organic matter digestibility (OMD).

The lowest OMD was 36.65% in treatment A and the highest was 40.86% in treatment C. The OMD pattern was in line with the DMD pattern because most of the dry matter consisted of organic matter and the difference was the ash content. Increasing the dose of mineral P in this study was able to increase DMD which was also followed by an increase in OMD. [17] reported an increase in DMD along with an increase in Zn minerals in the ammonium corn cob ration and also followed by an increase in OMD. The combination of minerals in treatment C was able to degrade lignin more due to optimal nutrients in the form of minerals provided so that molds grew and produced optimal ligninase enzyme activity as well. The release of lignocellulosic and lignohemicellulose bonds due to the action of enzymes produced by the <i>P. chrysosporium</i> will facilitate rumen microbes to digest cellulose and hemicellulose which are carbohydrates. Carbohydrates are the most influential component among the components of organic matter in determining the digestibility of organic matter because carbohydrates as energy producers (VFA) are the largest component in feed [18].

The treatment also gave a significantly different effect (P<0.01) on crude protein digestibility. This significant difference in crude protein digestibility is the same as DMD and OMD. This is because crude protein is part of organic matter and organic matter is part of dry matter. The increase in crude protein digestibility in treatment C was influenced by the increase in the crude protein content of the fermentation substrate. The increase in crude protein content was caused by an increase in the mass of mold cells. The increase in protein content in in line with the growth of fungi because the mushroom
body consists of nitrogen-containing elements. According to [19] and [20], fungal cell walls contain 6.3% protein, while cell membranes in hyphal fungi contain 25-45% protein and 25-30% carbohydrates. In addition, the enzymes produced by fungi are also proteins. Secretion of extracellular enzymes by *P. chrysosporium* also plays a role in increasing the protein content of the fermented substrate biomass [21].

Treatment C resulted in higher crude fiber digestibility compared to treatments A and B. This indicated that the palm midrib fermentation process was able to loosen the lignocellulosic and lignohemicellulose bonds thereby increasing crude fiber digestibility. [17] stated that the chemical composition that has the most influence on digestibility is the content of crude fiber which is the building block of cell walls that are difficult to digest. The combination of mineral doses in treatment C was the most optimal to help enzymes work in degrading lignin.

### 3.2. VFA production

The average total VFA from the combination of fermented palm fronds with elephant grass and tithonia is presented in Table 4.

| Treatments | VFA (mM) |
|------------|----------|
| A          | 126.40   |
| B          | 111.25   |
| C          | 91.10    |
| D          | 82.83    |
| SE         | 1.54     |

Note: different superscripts in the same column show very significant differences (P <0.01).

The high Volatile fatty acids in treatment A was due to the degradation of cell wall components (NDF and ADF) into VFA (simple molecules) which was greater than the B, C and D treatments. The higher the level of fermentability of a feed ingredient, the greater the VFA produced other than those from protein. VFA is not only derived from carbohydrates but also protein. The production of VFA can be used as a benchmark for the level of feed digestibility where the higher the digestibility level of the feed, the greater the VFA produced [22]. In this research, the total mean value of VFA obtained was sufficient for the needs of rumen microbes for maximum development and growth. [23] stated that the total rumen VFA concentration was 60-120 mM. [24] stated that the optimum total VFA conditions for rumen bacteria to carry out their activities were 80-160 mM. When viewed from the proportion of partial VFA (Figure 1), the highest proportion ratio in treatment A was 86: 21: 13 for acetate, propionate, and butyrate, respectively. [25] stated that the proportion of a good partial VFA ratio in the rumen was 63: 21: 16, while [26] stated that the molar proportion of acetic acid in the rumen of various good feed formulations was in the range of 53-72, the molar proportion of propionate 15-30 and the molar proportion of butyrate 7-21. The tendency of a higher molar proportion of acetate in this study indicates the potential for higher energy production for livestock due to the higher ATP production in the substrate [27].
3.3. Gas production

The highest gas production after 48 hours of incubation was produced by treatment A, namely 50.13 ml/200 mg dry matter, the lowest gas production was produced by treatment D, namely 35.05 ml/200 mg dry matter (Figure 2). The low gas production in treatment D was caused by the high lignin content. Lignin is reported to inhibit rumen microbial activity in degrading feed. This hurts gas production. There was a very significant difference (P>0.01) in gas production along with differences in the lignin content of the treatment. The low gas production in treatment D was also due to the low protein content of the D diet so that the production of microbial biomass was slow. The CO₂ and CH₄ gases produced are the results of microbial activity in the rumen fluid digesting the food substances contained in the feed [28].

![Partial VFA from FPF combination with Elephant Grass and Titonia](image1)

![GAS PRODUCTION FROM FPF COMBINATION with Titonia and Elephant Grass](image2)
4. Conclusion
1. The addition of 2000 ppm mineral Phosphorus (P) in the palm fronds fermentation process using the P. chrysosporium was able to increase the digestibility of food substances.
2. The combination of 20% fermented palm fronds with an 80% mixture of tithonia and elephant grass resulted in the highest concentration of VFA and gas production.

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