Physical, chemical and in vitro digestibility quality of oil palm midrib fermented by *Phanerochaete chrysosporium*

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**Abstract.** Oil palm midrib has potential to be used as ruminant feed, but fermented treatment is needed to increase their nutritional value. The study consisted of 2 phases of research. The first phase aims to find out the effect of oil palm midrib fermented by *Phanerochaete chrysosporium* on the physical and chemical qualities of feed, while the second phase to find out the effect on in vitro digestibility. The design of the first stage used the factorial completely randomized design. On the second phase the design used the completely randomized design. The first phase showed significant effect (P <0.05) on the physical and chemical quality. The best of incubation time was 15 days and the best dose was 10⁶ CFU/g. The second phase showed significant effect (P <0.05) on the digestibility of dry matter and organic matter. The best result of second phase is P3 treatment that used *Phanerochaete chrysosporium* + *Saccharomyces cerevisiae* in fermentation. It is concluded the use of *Phanerochaete chrysosporium* + *Saccharomyces cerevisiae* in fermentation can improve physical and chemical properties and increase the digestibility of oil palm midrib.

**1. Introduction**

The oil palm midrib have the potential to be used as alternative feeds. The high crude fibre content as well as the lignin content which is difficult for animals to digest becomes an obstacle because it causes a low level of digestibility and certainly will affect the growth of livestock. These constraints can be overcome by the application of animal feed processing technology, namely the fermentation method. The method of fermentation by utilizing microbes can reduce crude fibre, break down lignin, increase protein and nutrients in oil palm midrib.

*Phanerochaete chrysosporium* is a fungus that can degrade lignin and hydrolyse cellulose as well as hemicellulose. The method of fermentation using *Phanerochaete chrysosporium* is expected to improve nutrition in palm midrib so as to increase digestibility and affect optimal cattle growth such as increasing the body weight of cattle. Referring to the description, the authors are interested in researching more about processing fermented palm oil midrib with *Phanerochaete chrysosporium* physical, chemical and in vitro digestibility qualities.

**2. Materials and methods**

The study consisted of 2 phases of research. The first phase is the physical and chemical test of the fermentation of oil palm midrib with *Phanerochaete chrysosporium* and the second phase is the in vitro digestibility test of oil palm midrib.
2.1. Research phase I. Fermentation process of oil palm midrib with Phanerochaete chrysosporium on physical and chemical quality

2.1.1 Materials and research tools
Oil palm midrib that have been chopped and dried, Phanerochaete chrysosporium as fermenter, aquades, alcohol, autoclave, electric scales, desiccator, 105°C ovens and electric furnaces.

2.1.2 Methods
The research method used was Factorial Completely Randomized Design with 2 x 4 with 3 times repetitions. The treatment given consists of 2 factors. Factor A is the sample isolated with different doses which have 2 levels: P1 = fermented oil palm midrib with $10^4$ CFU/g Phanerochaete chrysosporium and P2 = fermented oil palm midrib with $10^6$ CFU/g Phanerochaete chrysosporium. Factor B is the incubation time with 4 levels: T0 = No incubation, T1 = Incubated for 5 days, T2 = Incubated for 10 days, T3 = Incubated for 15 days.

2.1.3 The oil palm midrib fermentation process
Taken 200 g of chopped oil palm midrib where the water content has been known before, then placed in a 20x25 cm container. The palm midrib was stirred evenly and an inoculum is added as much as 10% of the fermented material. After that added distilled water according to the water content needed for fermentation and covered with plastic, then stored at room temperature according to incubation time (0, 5, 10, 15 days). The fermentation product is then dried, then the sample is prepared for physical testing and chemical testing. The measurement of physical properties following the Khalil [3] method includes specific gravity, the density of the pile and the density of pile compaction. Meanwhile, chemical properties use proximate analysis [1].

2.1.4 The variables observed
The variables observed in the first phase study were physical tests (specific gravity, pile density and pile compacting density) and chemical tests (crude protein, crude fibre and crude fat).

2.2. Research phase II. In vitro test of fermented palm oil midrib with Phanerochaete chrysosporium
The second stage is carried out by taking the best results from the first stage then tested in vitro to see the digestibility value of dry matter and organic matter.

2.2.1 Materials and research tools
The ingredients consisted of oil palm midrib, MC Dougall solution, fresh rumen liquid, CO2 gas, 0.2% pepsin HCl solution, aquades, saturated HgCl2 solution, whatman no. 41 filter paper, Phanerochaete chrysosporium, Saccharomyces cerevisiae, Starbio. analytical scales, 50ml volume fermenter tubes, water bath shakers, centrifuges, vacuum pumps, dexticators, 105°C ovens, electric furnaces, pH meters.

2.2.2 Methods
The design used in this study was a completely randomized design (CRD) with 4 treatments and 3 replications. The treatments studied were: P0 = Unfermented oil palm midrib, P1 = Oil palm midrib fermented by Starbio, P2 = Oil palm midrib fermented by Phanerochaete chrysosporium, P3 = Oil palm midrib fermented by Phanerochaete chrysosporium + Saccharomyces cerevisiae.

2.2.3 Conducting research
Fermented oil palm midrib according to treatment P0, P1, P2 and P3. Fermentation lasts for 15 days according to the best treatment of the first stage.
2.2.4 Observed variables
The variables observed in the phase 2 study were dry matter digestibility coefficient (DMD) and organic matter digestibility coefficient (DMO).

3. Results and discussion

3.1. Research phase I. Fermentation process of oil palm midrib with Phanerochaete chrysosporium on physical and chemical quality
The result of statistical analysis by using ANAVA (Varian Analysis) showed significant result (P < 0.05) on the specific gravity, pile density and pile compacting density of fermented oil palm midrib.

Increasing specific gravity is due to changes in water content and particle structure of midribs that have been fermented by Phanerochaete chrysosporium. Phanerochaete chrysosporium degrades the structure of coarse oil palm midrib to be smoother and changes the content of water in the material. The specific gravity is affected by the chemical composition of feed [2]. The decrease in specific gravity is due to the space between the particles of the material already filled with distilled water in the measurement so that the specific gravity is low. If the particles get coarser, the particle size gets bigger and the density decreases so that the water more easily fills the space between the particles.

Table 1. Physical quality result of fermented oil palm midrib (Kg m⁻³)

| Parameters                      | Dosage (CFU/g) | Incubation time (days) | Average ± SD  |
|---------------------------------|----------------|------------------------|---------------|
|                                 |                | 0          | 5          | 10         | 15         |               |
| Specific gravity                | 10⁴            | 61.67      | 84.33      | 103.67     | 113        | 90.67 ± 22.72 |
|                                 | 10⁶            | 61.33      | 96.33      | 103        | 120        | 95.16 ± 24.66 |
| Average                         |                | 61.5c      | 90.33b     | 103.33ab   | 116.5a     |
| Pile density                    | 10⁴            | 78         | 100        | 133        | 133        | 111 ± 26.94  |
|                                 | 10⁶            | 78         | 122        | 133        | 133        | 116.5 ± 26.18 |
| Average                         |                | 78b        | 111b       | 133a       | 133a       |
| Pile compaction density         | 10⁴            | 86.67      | 143        | 214.67     | 211        | 163.83 ± 61.09 |
|                                 | 10⁶            | 88.67      | 161.67     | 185        | 211        | 161.58 ± 52.62 |
| Average                         |                | 87.67c     | 152.33b    | 199.83a    | 211a       |

Note: Different superscripts on the same line show significant differences (P <0.05)

The pile density of the oil palm midrib fermented by Phanerochaete chrysosporium with 10 and 15 days incubation showed the same and highest yield was 133 Kg m⁻³. The particle size becomes smaller because the fermentation process affects the density of the pile of material. The smaller air cavity between particles increases the pile density between particles. The value of pile density shows the porosity of the material, namely the amount of air cavity that exists between the particles of material [3].

Table 1 shows that the average pile compaction density of the palm midrib fermented by Phanerochaete chrysosporiumon 15 days incubation time was 211 Kg m⁻³. Finer material particles due to fermentation treatment cause cavities that can be filled with air or water to become smaller and stack density to be higher. Pile density is the ratio between the weight of the material to the volume of space it occupies after going through a compaction process such as shaking. [2] The density of pile compaction is influenced by the shape and size of the particle feed material. High pile compaction density means
that the material has high compacting capacity compared to other materials. The lower the compaction density of the pile produced, the lower the flow rate [4].

Table 2. Chemical quality result of fermented oil palm midrib (%)

| Parameters       | Dosage (CFU⁻) | Incubation time (days) | Average ± SD |
|------------------|---------------|------------------------|--------------|
|                  | 0             | 5                      | 10           | 15           |
| Crude Protein    |               |                        |              |              |
| 10⁴ CFU⁻         | 1.63e         | 2.08de                 | 6.76ab       | 6.33bc       | 4.2 ± 2.72 |
| 10⁶ CFU⁻         | 1.65e         | 2.57d                  | 5.83c        | 7.35a        | 4.35 ± 2.68 |
| Average          | 1.64          | 2.32                   | 6.29         | 6.84         |
| Crude Fibre      |               |                        |              |              |
| 10⁴ CFU⁻         | 53.95a        | 52.67a                 | 52.18a       | 43.78bc      | 50.64 ± 4.64 |
| 10⁶ CFU⁻         | 54.11a        | 52.73a                 | 46.65b       | 39.93c       | 48.33 ± 6.49 |
| Average          | 54.03         | 52.7                   | 49.37        | 41.85        |
| Crude Fat        |               |                        |              |              |
| 10⁴ CFU⁻         | 2.64          | 2.38                   | 2.08         | 1.64         | 2.18 ± 0.43 |
| 10⁶ CFU⁻         | 2.28          | 2.15                   | 1.57         | 1.47         | 1.87 ± 0.41 |
| Average          | 2.46a         | 2.26ab                 | 1.82bc       | 1.55c        |

Note: Different superscripts on the same line show significant differences (P < 0.05)

The fermentation of oil palm midrib with *Phanerochaete chrysosporium* showed significantly different results (P <0.05) on the increase in crude protein of palm midrib. There is a significant interaction between factors at a dose of 10⁶ CFU⁻ with a 15 days incubation period of 7.35%. The incubation time factor of 0 day dose 10⁶ CFU/g was not significantly different from the dose with 10⁴ CFU⁻ and 10⁴CFU⁻ dose combination with 5 days incubation duration. The highest crude protein value at 10⁴ CFU⁻ was found at 10 days of incubation but the value was not significantly different from 15 days of incubation. Fermentation of oil palm midrib with *Phanerochaete chrysosporium* showed the results of increased crude protein content. Molds develop both using crude fibre (carbohydrates) as a source of energy and releasing carbon dioxide. This causes protein fermentation products to increase. Crude protein levels increase with the incubation period. The longer the incubation time, the more substrate is broken down by fungi and more enzymes are produced so that it can increase the protein content. [5] The molds that have good growth and breeding will be able to change more components of the media into a mass of cells, so that proteins from the body of the mold itself will be formed and can increase the crude protein from the material. During the process of microbial fermentation will release an enzyme where the enzyme is a protein and the microbe itself is also a single cell protein source.

Table 2 shows a change in the crude fibre content of fermented palm midrib with *Phanerochaete chrysosporium*. The best fermentation average results is a decrease in crude fibre content with a 15 days incubation time. Decrease in crude protein levels in line with the incubation period of *Phanerochaete chrysosporium*. The longer the incubation period, the lower the crude fibre content in the palm midrib. The growth of fungal mycelium to obtain energy decomposes oil palm midrib fibres. *Phanerochaete chrysosporium* break down lignin in the fermentation process affecting the amount of crude fibre in the palm midrib. The longer the fermentation time, the more enzymes produced to break down lignin and break down the fibre.

The results of the analysis of the fermentation of oil palm midrib with *Phanerochaete chrysosporium* showed a significant difference (P <0.05) to the decrease in crude fat content of oil palm midrib and there was no significant interaction between factors. The longer the incubation time, the lower the gross fat content. This is due to changes in the chemical structure of the midrib due to the fermentation process by *Phanerochaete chrysosporium*. The process of silage fermentation, there is activity of bacteria that produce high enough fatty acids so that the fat content tends to increase. However, the gross fat content
that is too high in ruminant feed ingredients is also not very good because it can interfere with the feed fermentation process in the cattle rumen.

3.2. Correlation between physical test and chemical test

Table 3. The coefficient of determination between physical tests and chemical tests

| Parameters         | Specific Gravity | Pile Density | Pile Compaction Density |
|--------------------|------------------|--------------|-------------------------|
| Crude Protein      | 0.68             | 0.58         | 0.78                    |
| Crude Fibre        | -0.59            | -0.35        | -0.44                   |
| Crude Fat          | -0.34            | -0.13        | -0.32                   |

Increased levels of crude protein have an effect on increasing the physical properties of oil palm midrib. Table 3 shows the positive correlation value which means an increase in crude protein content by the fermentation of the *Phanerochaete chrysosporium* in line with the increase in specific gravity, pile density and pile compaction density. The increasing level of crude protein also increases the value of pile compaction density. Correlation value of 0.78 shows the close effect of protein content on the value of pile compaction density and oil palm midrib protein content.

The longer the fermentation of the material, the higher the protein content (Table 2) and the specific gravity (Table 1). *Phanerochaete chrysosporium* can degrade lignin more quickly and extensively than other microorganisms. The substrate for the growth of these microorganisms is cellulose and hemicellulose and lignin degradation occurs at the end of primary growth through secondary metabolism in nutrient deficiency conditions such as nitrogen, carbon or sulphur [6]. Degraded substrate into protein in the material changes the cell component and physical form of the material so as to improve the physical quality of the palm midrib.

The relationship between crude fibre content and weight of palm midrib (Table 3) shows a negative value of -0.59, which means that the crude fibre content has a strong influence on the physical properties of palm midrib. Negative value means that the level of crude fibre decreases, the weight value increases. The crude fibre of palm oil midrib which has decreased due to hydrolysis by *Phanerochaete chrysosporium* and physically changes the material so that the value of pile density also changes to high. The fermentation of palm midrib by *Phanerochaete chrysosporium* can break down cellulose, hemicellulose and lignin into simple forms so that the feed ingredients are easily digested by the rumen microbes. The process will also include microbial body cells and enzymes that contain proteins and other metabolites, thus producing better quality feed products, especially protein and crude fibre.

The relationship between crude fat content and specific weight of palm oil midrib shows a number of -0.34 (Table 3) which means that the crude fat content in the palm midrib does not have a close effect on the specific gravity. Table 3 shows the negative correlation between crude fat and the physical quality of oil palm midrib. Decreased fat levels are in contrast to increasing physical quality values. The fermentation process reduces crude fat content (Table 3) is not in accordance with the research [7] which showed the crude fat content of fermented palm midrib increased by a percentage of 30.68%.

3.3. Research phase II. In vitro test of palm oil midrib with *Phanerochaete chrysosporium*

The in vitro method is carried out in the laboratory and is used to simulate the work of the rumen effectively and easily because it allows to adjust the fermentation conditions as needed. The in vitro method makes it easy to get the value of the digestibility of various feed ingredients.

Food substance digestibility is defined as the amount of food that is not excreted in faeces or assuming that the food substance is digested by animals, if expressed in percentages it is called digestive coefficient [8].
3.3.1 Digestibility coefficient of dry matter
The highest average dry matter digestibility coefficient was P3 treatment (Table 4), which was *Phanerochaete chrysosporium* + *Saccharomyces cerevisiae* fermented palm midrib by 50.68%. The lowest average is P0 which is a fermented palm midrib that is equal 33.47%.

| Treatment | Repitation | Average ± SD (%) |
|-----------|------------|------------------|
| P0        | 33.28      | 33.66            | 33.48 | 33.47±0.190<sup>d</sup> |
| P1        | 40.46      | 40.93            | 40.99 | 40.79±0.290<sup>c</sup> |
| P2        | 45.42      | 45.97            | 46.37 | 45.92±0.476<sup>b</sup> |
| P3        | 50.72      | 50.46            | 50.87 | 50.68±0.207<sup>a</sup> |

Note: Different superscripts on the same line show significant differences (P <0.05)

The fermentation of palm midrib by *Phanerochaete chrysosporium* can break down cellulose, hemicellulose and lignin into simple forms so that the feeds are easily digested by the rumen microbes. The process will also include microbial body cells and enzymes that contain proteins and other metabolites, thus producing better quality feed products, especially protein and crude fibre. The presence of microbial activity in the digestive tract greatly affects digestibility [10]. Its high digestibility reflects the magnitude of certain nutritional donations in livestock, while feed which has low digestibility indicates that the feed is less able to supply nutrients to be absorbed in meeting basic life needs and production goals [11].

3.3.2 Digestibility coefficient of organic matter

| Treatment | Repitation | Average ± SD (%) |
|-----------|------------|------------------|
| P0        | 50.34      | 51.38            | 50.82 | 50.85±0.520<sup>d</sup> |
| P1        | 62.06      | 62.10            | 62.71 | 62.29±0.364<sup>c</sup> |
| P2        | 66.11      | 66.13            | 66.46 | 66.23±0.196<sup>b</sup> |
| P3        | 70.34      | 70.83            | 70.94 | 70.70±0.319<sup>a</sup> |

Note: Different superscripts on the same line show significant differences (P <0.05)

The results of the analysis of various fermentation with various types of microbes showed a significantly different effect (P <0.05) on the digestibility level of organic oil palm midrib. The ability to digest feed is determined by several factors such as the type of livestock, the chemical composition of feed and feed storage.

Organic matter is part of dry matter, so that increasing consumption of dry matter will increase the consumption of organic matter. Increased digestibility of organic matter in line with the increase in dry matter digestibility, because most of the dry matter components consist of organic matter so that the factors that influence the high and low digestibility of dry matter will also affect the high and low digestibility of dry matter.

4. Conclusions
Fermented palm midrib using *Phanerochaete chrysosporium* can increase crude protein content, decrease in crude fibre and crude fat content. The crude fibre content has a close relationship to the physical properties of oil palm midrib. The use of *Phanerochaete chrysosporium* in oil palm midrib fermentation compared with unfermented has increased 36.51% and 30.24% dry and organic
digestibility. The best treatment was oil palm midrib fermented with *Phanerochaete chrysosporium* plus *Saccharomyces cerevisiae* with an increase in the value of digestibility of dry matter and organic matter by 50.21% and 39.04%.

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