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# Bio-processing Plantation by-products with White Oyster Mushroom (*Pleurotus ostreatus*) to Improve Fermentability and Digestibility Based on Substrate Type and Fermentation Time

**Yulia Riska Azzahra[1], Toto Toharmat[2*], and Iwan Prihantoro[2]**

[1]Feed Nutrition Study Program, Faculty of Animal Science, Bogor Agricultural University Postgraduate School, Bogor, 16689, Indonesia
[2]Department of Feed Nutrition Science, Faculty of Animal Science, IPB University, Bogor, 16689, Indonesia

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* Corresponding author:
Telp. +6811117505
E-mail: toharmat@apps.ipb.ac.id

**ABSTRACT**

Plantation by-products can be used as livestock feed with proper processing, optimizing the feed efficiency of palm kernel cake (PKC), empty oil palm bunch (EOPB), and acacia sawdust (AS). This study aimed to improve the usability of the byproducts as alternative feed through the *Pleurotus ostreatus* fermentation process. To this end, a 3×3 factorial, completely randomized design was applied. Factor A was the type of substrate, P1 = PKC, P2 = EOFB and P3 = AS. Factor B was the fermentation period, T1 = 0 d, T2 = 30 d and T3 = 60 d. The collected data were analyzed using analysis of variance (ANOVA), and the significantly different result was further tested using Duncan Test. The observed variables include Neutral Detergent Fiber (NDF), acid detergent fiber (ADF), hemicellulose, pH value, dry matter digestibility (DMD), organic matter digestibility (OMD), N-$NH_3$, and volatile fatty acids **(**VFA). The study result showed that *P.ostreatus* mushroom in various substrate types did not significantly affect the fiber fraction of NDF, ADF, and Hemicellulose. This study also found that the interaction of substrate type and fermentation duration significantly affects dry matter digestibility, organic matter digestibility, N-$NH_3$ production, and VFA values. This study concluded *P. ostreotus increased* the nutritional value and digestibility of the by-product from plantation processing. The best fermentation duration was 60 d PKC was found to have the highest digestibility value, and the best interaction was found in PKC with 60 d of fermentation.

Keywords: Acacia sawdust, Empty palm bunches, Fermentation, *In vitro*, Palm kernel cake, *Pleurotus ostreotus*

## Introduction

Feed in the farming industry constitutes 70% of the production cost and serves as the inhibiting factor of farming industry development in Indonesia. To address this issue, it is necessary to explore non-conventional, sustainable food sources available in a large number. Plantation products that are considered potential and expected to address the issue on feed are oil palm by-products, such as empty oil palm bunch, kernel cake, and wood processing by-products like sawdust. Palm oil plantation product continues to exhibit growing production recently. Indonesia has 11.92 million Ha area of palm oil palm plantation, producing 33.23 million tonnes (Kementan, 2016). The wood production reached 66,666 $m^3$, and assuming that the sawdust constitutes 65% of the production, the country's sawdust production is 9,999 $m^3$ (Ditjenbun, 2019).

Plantation processing product is a potential ruminant feed as the palm kernel cake contains 14.5-19.6% crude protein (CP), 13.0-20.0% crude fiber (CF), 9.60% eter extract (EE), 25.26% cellulose, 28.61% hemicellulose, 65.26% neutral detergent fiber (NDF), 36.65% acid detergent fiber (ADF), and 8-15% lignin (Rakhmani *et al.,* 2015). Empty oil palm bunch is reported to contain 48.8% CF, 3.2% EE, 3.7% CP, 81.8% NDF, and 61.6% ADF (Batubara *et al.,* 2003). Meanwhile, the sawdust contains 53.3% CF, 1.38% ash, 4.63% CP, and 0.32 EE (Ibrahim *et al.,* 2013). Using plantation by-products as animal feed, in general, have inhibiting factors. Thus, a biotechnological process is necessary before giving them to the animal. Among the biotechnological processes is the fermentation using oyster mushrooms *Pleurotus ostreotus*.

Oyster mushroom from *Basidiomycetes* class is known to be edible and cultivated in various agricultural wastes. Sawdust fermentation using *P.ostreatus* is reported to degrade lignin and increase the dry and organic matter digestibility (Jafari *et al.,* 2007). A longer

fermentation period by *P.ostreatus* on sawdust is known to decrease *cellulose*, *hemicellulose*, and *lignin* (Hadrawi, 2014). Oyster mushroom can degrade lignin binding more extensively than other microorganisms (Sudrajat *et al.,* 2018). Lignocellulose degradation is expected to improve the feed quality sourced from agricultural and plantation by-products and improve the utility value of plantation products through *in vitro* fermentation.

## Materials and Methods

### Preparation media and isolate of fungi *Pleurotus ostreotus*

Sample of empty oil palm bunch (EOPB) was obtained from the palm oil processing plant PTPN VIII Kertajaya, Banten. The EOPB was dried to a moisture content of 10-15% and chopped ± 2-3 cm in size, palm kernel cake (PKC) and acacia sawdust (AS) are sieved using a special sieve. PKC, EOPB, and AS were sieved and inoculated on a laboratory scale (Herliyana *et al.,* 2008). PKC, rice bran, and corn flour (*Zea mays*) were obtained from a poultry shop. The AS was obtained from factory wood processing. The determination of chemical composition was based on proximate analysis. Prepared PKC, EOPB, and AS was as much as 74%. One of these samples was mixed with 15% rice bran, 10% corn flour, and 1% $CaCO_3$, then added water as much as to obtained a moisture content at 65-70%. The ingredients were mixed, put in a propylene bags, and sterilized at 100°C for 8 h. The bags were inoculated with ±15 g (3.75%) of seed and put in 22-28°C temperature and 60-80% relative humidity for 30 and 60 d of fermentation. Fermented media after 30 d and 60 d were composited and dried in an oven at 60°C for 2 d for Van Soest analysis and *in vitro* analysis (Van Soest, 1991).

### *In vitro* experiment

This study *in vitro* experiment was performed using the Tilley and Terry (1963) method. Rumen fluid as a source of inoculant was collected from ruminal fistulated Frisian Holstein (BW±510 kg) before morning feeding. The rumen liquor was filtered using two layers of cheesecloth. *In vitro* techniques were carried out using glass tubes with a capacity of 80-90 mL and filled with 0.5 g of sample, 40 mL of McDougall buffer solution, and 10 mL of fresh rumen fluid. The tube was shaken with $CO_2$ for 30 seconds and closed with a ventilated rubber cap. Tubes were put in a *water bath* at a temperature of 38°C to create the rumen conditions and incubated for 48 h. Samples were harvested for analysis of pH rumen, N-$NH_3$, volatile fatty acids (VFA), dry and organic matters digestibility. After 48 hours of incubation, the samples were harvested, and $HgCl_2$ two drops of saturated were added to stop the fermentation rate. Before dropping $HgCl_2$, the final pH was measured. The remainder was transferred to a polyethylene tube and then centrifuged at 3.000 rpm for 15 minutes. The supernatant was taken for the concentration analysis of N-$NH_3$ and VFA total. The N-$NH_3$ levels were determined using the Conway and O'Malley method (Conway and O'Malley, 1942). The VFA total levels were determined through steam distillation. In each tube, the sample was filtered using a vacuum machine and Whatman paper. The residue was used to analyse crude protein degradation, fiber fermentation, dry matter digestibility (DMD) and organic matter digestibility (OMD).

### Statistical analysis

The research method used a 3x3 factorial completely randomized design (CRD) with 3 replications. Factor A is the type of substrate, namely P1 = PKC, P2 = EOPB and P3 = AS. Factor B is the length of fermentation, consisting of T1 = 0 d, T2 = 30 d, and T3 = 60 d. Obtained from the observations were analyzed using ANOVA. If there is a significant difference, further testing was carried out using Duncan's test (Steel and Torrie, 1991). Data analysis was carried out using the SPSS version 25.

## Results and Discussion

### Fiber fraction components of fermented media the *P. ostreotus*

A decreased NDF was noticed in each substrate treatment (Table 2). The most significant decrease was found in AS (3.34%), followed by PKC (2.04%) and EOPB (1.5%) in 60 d of fermentation. The significant ($p<0.05$) NDF decrease in AS during 60 d of fermentation occurs because AS exhibited the best mycelium growth and covers all fermentation media, resulting in increasing enzyme production. Mycelium growth is positively associated with enzyme production (Riswandi, 2014). The microbial

Table 1. Nutrient content of feed ingredient (% DM)

| Feed ingredients | MC | AC | FC | CP | CF | CHO |
|---|---|---|---|---|---|---|
| | | | (% DM) | | | |
| PKC | 7.1 | 4.21 | 10.33 | 16.34 | 15.36 | 53.42 |
| EOPB | 12.38 | 4.51 | 1.94 | 1.93 | 34.15 | 45.09 |
| AS | 15.82 | 0.39 | 0.63 | 1.94 | 60.76 | 20.46 |
| Bran | 17.37 | 10.48 | 3.12 | 9.86 | 8.79 | 50.38 |
| Corn Flour | 13.48 | 1.22 | 1.44 | 7.88 | 0.57 | 75.41 |

DM: Dry Mater, MC: Moisture Content, AC: Ash Content, FC: Fat Content, CP: Crude Protein, CF: Crude Fiber, CHO: Total Carbohydrate; PKC: palm kernel cake, EOPB: empty oil palm bunch, AS: acacia sawdust; Proximate analysis of feed ingredient based on laboratory analysis of Proyek Antar University (PAU).

Table 2. Fiber fraction content of mushroom fermentation media using *P. ostreotus* (%, DM)

| Parameter (% DM) | Type of Substrate (P) | Fermentation duration (d) | | |
|---|---|---|---|---|
| | | 0 | 30 | 60 |
| NDF | PKC | 64.36 | 62.78 | 62.32 |
| | EOPB | 78.21 | 77.53 | 76.71 |
| | AS | 79.70 | 77.07 | 76.36 |
| ADF | PKC | 35.93 | 34.32 | 33.79 |
| | EOPB | 63.60 | 61.68 | 60.28 |
| | AS | 63.68 | 61.29 | 60.45 |
| Hemicellulose | PKC | 30.57 | 28.00 | 26.85 |
| | EOPB | 16.53 | 14.23 | 16.43 |
| | AS | 16.02 | 14.23 | 16.43 |

NDF: neutral detergent fiber, ADF: acid detergent fiber, PKC: palm kernel cake, EOPB: empty oil palm bunches, AS: acacia sawdust.

fermentation process utilizes the cytoplasm to support the growth, followed by cell wall remodeling. Cytoplasm tends to be easier to use, and its cell wall remodeling is relatively slow as N substances are not easily soluble in NDF, which limits the enzyme activity in the cell wall remodeling process (Nurcahyani *et al.,* 2010). The decreased NDF content may affect the crude fat, showing that the lignocellulose enzyme produced by *P.ostreatus* can loosen up the lignin and hemicellulose complex bond.

ADF decrease was noticed in various substrates and fermentation duration. EOPB and AS exhibited the most significant decrease in 60 d of fermentation, respectively at 3.32% and 3.23%, compared to BIS at 2.14%. It is assumed that a longer fermentation period allows for more stable microbial activity, and the nutritional needs could be met. Thus microbial activities can be done optimally, an activity in which hemicellulose and cellulose are degraded by enzymes produced by *P. ostreatus* mycelium. Oyster mushroom secretes enzymes to degrade cellulose and hemicellulose (Nofrizal *et al.,* 2019). Various fermented substrates in this study were hemicellulose degradation into *Araban, galactan*, and *xylan* by *P.ostreatus* mycelium (Widiastuti *et al.,* 2008). Decreases of NDF and ADF in this study were lower than in Jafari *et al.* (2007) on rice straw fermented with *P. ostreatus* for 50-60 d of incubation. NDF and ADF decreased value in this study and in the previous study were accounted for by the types, structure, and texture of the substrate, in addition to the mushroom inoculum dose, physical pre-treatment prior to the fermentation process.

Hemicellulose content indicates the presence of microbial activity in utilizing carbohydrates available in the substrate. Microbes need carbohydrates to survive and grow (Fardiaz, 1992). Hemicellulose decrease occurs in the mycelium formation stage day $30^{th}$ of fermentation. The highest hemicellulose bond degradation occurs during the mycelium formation phase (Nicolini *et al.,* 1987). Hemicellulose is degraded by hemicellulase into a more simple polymer, even monosaccharides like glucose, fructose, mannose, galactose, and arabinose (Sari *et al.,* 2019). Hemicellulose is easily degraded into simple glucose and other product with higher digestibility than cellulose.

**pH value**

pH value defines the degree of fluid acidity of rumen receiving experimental feed after *in vitro* incubation. The pH value of *P.ostreatus* fermentation media based on types of substrate and length of *in vitro* fermentation are presented in Table 3. Types of substrate were found to significantly affect the pH value ($p<0.05$). EOPB (7.03) exhibited an insignificant difference from AS (7.04) and exhibited a higher value ($p<0.05$) than PKC (6.68). The low *in vitro* pH value of PKC is probably because the pH value of PKC substrate was lower (6.70) than EOPB (7.00) and AS (6.90).

The length of fermentation also significantly affects the pH value of *P.ostreatus* fermentation media. A longer fermentation period appears to lower the pH value because the fermentation process produces a soluble acid $CO_2$ ($H_2CO_3$). pH value decreases because the fermentation process produces organic acid from microbes, like malic acid, tartaric acid, lactic acid, butyric acid, and propionic acid (Putra and Amran, 2009). The interaction between types of substrate and length of fermentation ($p<0.05$) on pH value was noticed. The interaction shows that PKC with 60 d of fermentation was significantly ($p< 0.05$) lower (6.70) than other treatments, while experimental EOPB and AS were not significantly different in pH value. The pH value was normal, ranging from 6.70 to 7.03. This is one of the indicators of proper feed degradation because, in this pH value range, microbes producing crude fiber digesting engine can live optimally within rumen (Syahrir *et al.,* 2012). Rumen microbes

Table 3. The pH value of the mushroom fermentation product using *P. ostreotus* on different type of substrate and length of fermentation

| Type of substrate (P) | Fermentation duration (d) | | | Average |
|---|---|---|---|---|
| | 0 | 30 | 60 | |
| PKC | $6.97\pm0.06^{b}$ | $6.93\pm0.06^{b}$ | $6.70\pm0.00^{c}$ | $6.68\pm0.13^{b}$ |
| EOPB | $7.07\pm0.06^{ab}$ | $7.10\pm0,00^{a}$ | $6.93\pm0.12^{b}$ | $7.03\pm0.10^{a}$ |
| AS | $7.07\pm0.06^{ab}$ | $7.03\pm0.12^{ab}$ | $7.03\pm0.06^{ab}$ | $7.04\pm0.07^{a}$ |
| Average | $7.03\pm0.07^{a}$ | $7.02\pm0.10^{a}$ | $6.90\pm0.16^{b}$ | |

$^{a,b,c}$ Different superscripts in the same column/row show significant differences ($p<0.05$).
PKC = palm kernel cake; EOPB = empty oil palm bunch, AS = acaia Sawdut.

Table 4. Dry matter digestibility of the mushroom fermentation product using *P. ostreotus* on different type of substrate and length of fermentation (%)

| Type of substrate (P) | Fermentation duration (d) | | | Average |
|---|---|---|---|---|
| | 0 | 30 | 60 | |
| PKC | 66.67±3.51$^{bc}$ | 72.50±4.97$^{b}$ | 88.80±6.44$^{a}$ | 75.99±10.88$^{a}$ |
| EOPB | 46.37±9.68$^{d}$ | 55.14±2.89$^{de}$ | 58.07±5.13$^{cd}$ | 53.19±7.74$^{b}$ |
| AS | 30.65±5.73$^{e}$ | 47.82±5.40$^{de}$ | 57.36±2.09$^{cde}$ | 45.28±12.92$^{c}$ |
| Average | 47.89±16.71$^{c}$ | 58.49±11.66$^{b}$ | 68.08±16.50$^{a}$ | |

$^{a,b,c,d,e}$ Different superscripts in the same column or row show significant differences ($p<0.05$).
PKC = palm kernel cake; EOPB = empty oil palm bunch, AS = acacia sawdust.

effectively degrade fibers if the pH value ranges between 6.5-7, and the fiber digesting activity slows down if the pH value is 6.2 (Usman, 2013).

**Dry matter digestibility**

Feed digestibility represents the amount of nutrition utilized by the animal. Analysis of Variance result showed that the interaction between types of substrate and length of fermentation period significantly affects ($p<0.05$) the dry matter digestibility (Tabel 4). The experimental PKC substrate with 60 d of fermentation showed higher dry matter digestibility and was significantly different from other interactions ($p<0.05$). The lowest dry matter digestibility was found in the experimental non-fermented AS. This result indicated that PKC substrate with 60 d of fermentation is the most optimal interaction to obtain the highest digestibility. PKC contains better nutritional content and relatively low crude fiber, allowing rumen microbes to digest the substrate dry matter. PKC contain 14.5-19.6 CP, 17.18% CF, 25.26% cellulose, 28.61% hemicellulose, 65.26 NDF, 36.65 ADF, and 9-15% lignin (Rakmani *et al.,* 2015). Empty oil palm bunch is reported to contain 48.8% CF, 3.2% EE, 3.2% CP, 81.8% NDF, and 61.6% ADF (Batubara *et al.,* 2003). Meanwhile, sawdust contains 53.3% CF, 1.38% ash, 4.63% CP, and 0.32 EE (Ibrahim *et al.,* 2013). Low AS digestibility may be accounted for by its high crude fiber, as higher crude fiber results in lower digestibility. Digestibility is closely associated with chemical composition, especially crude fiber (Yanuarianto *et al.,* 2015). A fermentation period of 60 d increases the possibility for microbes to grow and perform the fermentation, causing mycelium to spread over the substrate particles and produce a high number of the extracellular enzyme while degrading the lingo-hemicellulose component into more simple bonds, making it more soluble and digestible (Hambakodu and Ina, 2019). Substrate perfectly covered by mycelium causes high enzyme concentration and cell wall to further degraded (Chang and Miles, 1989).

**Organic matter digestibility**

Analysis of Variance result showed that the interaction between types of substrate and length of fermentation period significantly affects ($p<0.05$) the organic matter digestibility (Tabel 5). The experimental PKC substrate with 60 d of fermentation showed higher organic matter digestibility and was significantly different from other interactions ($p<0.05$). The lowest dry matter digestibility was found in the experimental non-fermented AS. This shows that PKC substrate with 60 d of fermentation is the most optimum interaction to produce the best organic matter digestibility. The increase in organic matter digestibility is in line with the increase in dry matter digestibility because most dry matter components comprise organic matter. In other words, factors affecting dry matter digestibility will likely affect organic matter digestibility (Utomo, 2017). High organic matter digestibility of PKC with 60 d of fermentation was accounted for by the substrate's more complex nutrition and better solubility compared to other substrates.

A longer fermentation period will likely result in more mycelium. Hence, more enzymes are diffused into the substrate and increase the nutritional content of the fermented substrate product. One of the enzymes produced by the microbe is phenol oxidase, which contains peroxidase and lactase, and aryl-alcohol oxidase which can degrade lignocellulose (Andini *et al.,* 2015). The increased nutrition with *P. ostreatus* was accounted for by the additional nutrition from *P.ostreatus* mycelium during incubation. The complex compounds, lignocellulose and lingo-hemicellulose, are degraded into simpler compounds. The chemical activity of the enzyme produced by the mushroom decomposes and helps rumen microbes to digest. The amount of digested nutrients in the rumen will increase the organic matter digestibility (Santi *et al.,* 2012).

**N-$NH_3$ production**

N-$NH_3$ refers to the amount of protein fermented in the rumen. Table 6 displays the N-$NH_3$ values of *P.ostreatus* fermentation media in terms of substrate and fermentation period. The interaction between substrate types and fermentation length significantly affected the N-$NH_3$ value ($p<0.05$). PKC substrate with 60 d of fermentation exhibited the highest N-$NH_3$ value (14.30 mM) among other experimental substrates. The crude protein content of PKC substrate is higher, 14.5-19.6% (Rakhmani *et al.,* 2004). EOPB has 3.7% CP content (Batubara *et al.,* 2003), while AS has 4.63% CP (Ibrahim *et al.,* 2013). Longer fermentation duration causes the number of microbes to increase, resulting in higher crude protein levels. A higher number of microbes will increase protease production. Protease will degrade the substrate's protein into amino acid, causing soluble nitrogen and protein

Table 5. Organic matter digestibility of the fermented product of fungus *P. ostreotus* on different type of substrate and the length of fermentation

| Type of substrate (P) | Fermentation duration (d) | | | Average |
|---|---|---|---|---|
| | 0 | 30 | 60 | |
| PKC | 71.44±2.64$^{bc}$ | 76.34±4.15$^{b}$ | 91.30±7.52$^{a}$ | 79.69±10.02$^{a}$ |
| EOPB | 49.50±7.36$^{d}$ | 64.80±7.55$^{c}$ | 66.01±4.81$^{c}$ | 60.10±9.85$^{b}$ |
| AS | 34.74±544$^{e}$ | 52.05±4.05$^{d}$ | 64.41±2.86$^{c}$ | 50.39±13.42$^{c}$ |
| Average | 51.89±16.69$^{c}$ | 64.40±11.55$^{b}$ | 73.90±13.89$^{a}$ | |

$^{a,b,c,d,e}$ Different superscripts in the same column/row show significant differences (p<0.05).
PKC = palm kernel cake; EOPB = empty oil palm fruit bunch; AS = acacia sawdust.

Table 6. Production of N-$NH_3$ (mM) in the fermented product of the fungus *P. ostreotus* on different type of substrate and the duration of fermentation

| Type of substrate (P) | Fermentation duration (d) | | | Average |
|---|---|---|---|---|
| | 0 | 30 | 60 | |
| PKC | 7.79±1.17$^{b}$ | 8.04±1.57$^{b}$ | 14.30±1.44$^{a}$ | 10.04±3.42$^{a}$ |
| EOPB | 6.54±0.17$^{b}$ | 7.00±0.28$^{b}$ | 7.22±0.67$^{b}$ | 6.92±0.48$^{b}$ |
| AS | 6.68±0.56$^{b}$ | 6.91±0.20$^{b}$ | 7.27±0.77$^{b}$ | 6.95±0.55$^{b}$ |
| Average | 7.00±0.88$^{b}$ | 7.32±0.97$^{b}$ | 9.60±3.64$^{a}$ | |

$^{a,b}$ Different superscripts in the same column/row show significant differences (p<0.05).
PKC = palm kernel cake; AS = acacia sawdust, EOPB = empty oil palm bunch.

Table 7. Concentration of volatile fatty acid (mM) through *in vitro* study from fermentation products by *P.ostreotus* on different types of substrate and length of fermentation

| Type of substrate (P) | Fermentaton duration (d) | | | Average |
|---|---|---|---|---|
| | 0 | 30 | 60 | |
| PKC | 53.42±0.13$^{f}$ | 125.00±0.09$^{c}$ | 150.39±12.06$^{a}$ | 109.60±43.97$^{b}$ |
| EOPB | 76.38±0.30$^{e}$ | 95.47±6.28$^{d}$ | 130.55±5.74$^{bc}$ | 100.80±24.17$^{c}$ |
| AS | 101.47±3.19$^{d}$ | 123.16±0.41$^{c}$ | 136.97±2.88$^{b}$ | 120.53±15.65$^{a}$ |
| Average | 77.09±43.97$^{c}$ | 114.54±15.65$^{b}$ | 139.30±24.17$^{a}$ | |

$^{a,b,c,d,e,f}$ Different superscripts in the same column/row show significant differences (p<0.05).
PKC = palm kernel cake; AS = acacia sawdust, EOPB =empty oil palm bunch.

values to increase (Hastuti *et al.,* 2011). Crude protein content will likely determine the N-$NH_3$ value. Higher protein degradation in rumen will increase the N-$NH_3$ concentration (Riswandi, 2014). The N-$NH_3$ product is used by rumen microbes to synthesize its body. The increase in the microbial population is helpful for animals as it improves the digestibility of rumen.

The average score of rumen fluid ranges from 6.54-14.3 mM. Fiber substrate as the growth media of *P.ostreatus* exhibited a good performance as it produces adequate N-$NH_3$ concentration and meets the animal needs. Based on the calculation result, the optimum N-$NH_3$ value in rumen ranges between 6–21 mM (McDonald *et al.,* 2002).

**Volatile fatty acids**

The VFA is the final product of carbohydrate fermentation and the main source of energy from rumen. The fermented carbohydrate VFA in rumen also produces $CO_2$ and $CH_4$ (McDonald *et al.,* 2002). Table 7 presents the effect of the treatment on VFA production.

The interaction between substrate types and fermentation duration was found to significantly affect the VFA concentration (p<0.05). PKC substrate with 60 d of fermentation exhibited the highest VFA value (150.39 mM). This is because PKC substrate contains higher total carbohydrate (CHO) 53.42% than EOPB 45.09% and AS 20.46% (Table 1). CHO has more easily soluble compound than CF, making it degraded more easily (Muslimah *et al.,* 2020). Microbes produced by the mushroom loosen up the hemicellulose and cellulose bonds from lignin, the source of non-structural carbohydrate, and convert them to simple glucose in the form of organic acid (*acetate, lactate, propionate*, and *butyrate*) as a source of energy. Ammoniac concentration in rumen also determines the efficiency of microbial protein synthesis, which eventually affect the result of organic matter fermentation VFA, the main source of energy for animals (Utomo, 2017). The amount of formed VFA is highly influenced by the digestibility and the quality of additional feed.

An increased VFA production occurs along with increased fermentation duration because longer fermentation increases opportunities for microbes to grow and perform fermentation, resulting in more microbes to decompose fibers. This process allows feed to be more fermentable and digestible by rumen microbes. Each substrate has a high VFA value to meet the standard 80-160 mM (McDonald *et al.,* 2002). VFA is absorbed into the circulatory system through gluconeogenesis and turned into blood glucose. The blood glucose partially supplies the energy needed by the ruminants (Lenhinger, 1992).

## Conclusions

The use of *P. ostreotus increased* the nutritional value and digestibility of the by-product from plantation processing. Fermentation of 60 d showed the best fermentation time to increase the nutritional value and digestibility of feed. The type of PKC substrate showed the best digestibility value among other substrates.

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