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FERMENTATION OF BLOOD MEAL ABSORBED BY OIL PALM FRONDS WITH *Bacillus amyloliquefaciens* AND *Lactobacillus plantarum*

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ABSTRACT: This study was aimed at improving the efficiency of blood meal (BM) use as feedstuff through the application of agricultural waste absorbance and fermentation technology. Blood was absorbed by oil palm fronds and fermented by using *Bacillus amyloliquefaciens* (BAF) and *Lactobacillus plantarum* (BLP) inoculants in 0, 60, and 120 hour incubation times. Quality was assessed by using Van Soest fiber analysis and *in vitro* digestibility trial on the best fermented product. Results showed that there was significant interaction effect (P<0.05) of inoculant type and fermentation times on the changes in fiber fraction of BM absorbed by oil palm fronds. Inoculant types were found to give significant effects (P<0.05) on ration digestibility rate and *in vitro* rumen condition characteristics. It was concluded that fermentation of BM absorbed with palm oil fronds with BLP in 120 hours resulted in BM with the best fiber fraction reduction, digestibility rate, and *in vitro* rumen condition characteristics.

Keywords: Agricultural waste, Feedstuff, Fiber fraction, Digestibility, Rumen condition.

INTRODUCTION

Agricultural wastes including oil palm fronds have been extensively used as animal feedstuff (Ooi et al., 2017). Blood meal, an animal farming waste, is also a potential source of protein and its availability for animal feed is plenty. It is high in protein (80-95%) and amino acid contents particularly lysine, arginine, methionine, cystine, and leucine but it is lacking in isoleucine and methionine (Odukwe and Njoku, 1987; Kerr et al., 2019). Fresh blood can be obtained from slaughtered cattle by 7-9% of body weight (BW) (Santoso, 1989) and contains 80% water while BM contains 16.5% water (Ramadhan et al., 2015). This indicates that fresh blood may contain 36.5% dry matter (DM). Meanwhile, Setyani and Soenarno (2020) found that cattle weighed 449 kg produced about 58.63 kg fresh blood (13.06% BW) which could be further be processed to produce about 11.73 kg BM (20%) (Ridla, 2014).

Time consuming drying process and low digestibility rate due to high iron content are the constraints found in the utilization of BM. Several processing methods including drying, absorption or mixing, and fermentation have been applied to overcome these constraints. Corn waste (Makinde and Sonaiya, 2011) and cassava peels as a substitute for 50% soybean cake meal (Onyimonyi and Ugwu, 2007) were used in BM processing by using a mixing method. Absorption or mixing method was found to be better than the drying method as the former could improve the utilization of BM up to 15% in poultry ration. This might be attributed to the findings that the absorption method accelerated drying process and improved nutrient quality. Mixing corn waste and BM shortened the drying process to less than 4 days, increased protein content, and reduced environmental pollution (Makinde and Sonaiya, 2007).

Common BM processing by using a heat drying method taking relatively longer time often results in protein denaturation. The use of absorbents is believed to accelerate BM drying process without destructing protein content. Oil palm frond, like other agricultural wastes, is potential to be used as an absorbent material as it contains high fiber (32.87-57.43%) and low protein (5.3%) (Imsya et al., 2013). Wide cell surface of fiber in oil palm frond makes water absorption from blood and blood drying process occur more extensively. In this study, the efficiency of BM production by using oil palm fronds as the absorbent was assessed. The resulted BM was fermented by using BAF and LBP inoculants and their effects on the improvement of nutrient contents ant the use of BM as animal feed were also assessed.

MATERIALS AND METHODS

Site of the study and ethical regulations

The study was conducted at Faculty of Agriculture, Sriwijaya University and supervised by the research committee of the Faculty of Agriculture, Sriwijaya Universisty in compliance with the Government Regulation (PP) Number 95 Year 2012 on Veterinary Public Health and Animal Welfare.

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Blood, oil palm fronds, and bacterial inoculant

Fresh cattle blood was obtained from a slaughterhouse in Palembang City, South Sumatera. Blood was placed in a container containing salt (8 g/liter) to avoid blood coagulation. Blood was immediately stirred and mixed with absorbent (chopped oil palm fronds). Oil palm fronds were obtained from oil palm plantation of Sriwijaya University, Palembang of South Sumatera. Bacterial culture was obtained from Biotechnology Laboratory of Faculty of Mathematics and Natural Sciences, Halu Oleo University, Kendari, Southeast Sulawesi (BAF FZB42) and Agrotech Laboratory, Yogyakarta (LBP Strain IS-10506).

Experimental design

This study was designed to assess the effects of bacterial inoculant (BAF and LBP) and incubation times (0, 60, and 120 hours) on the quality of BM produced by an absorbance method using oil palm fronds. Six replicates were allocated to each treatment.

Inoculant preparation

Inoculant was prepared by using 100 g rice bran as a medium. Rice bran was sterilized in an autoclave at 121 °C, 1 atm pressure for 15 minutes before it was left cool at room temperature (24°C). A streak of pure BAF culture was diluted in 100 ml distilled water. Ten-millilitre of this mixture was taken and diluted in 90 ml distilled water. This procedure was repeated 6 times until a mixture containing 10^6 cpu of BAF was obtained. No dilution procedure was conducted for LBP culture as it was obtained in the form of diluted one. Diluted culture of both bacteria was each poured into sterilized rice bran medium which was then incubated for 24 hours before it was ready to use in the fermentation process.

Blood meal processing by using absorbent

Blood meal was made by using a method of Makinde and Soniya (2010) with some modifications. Oil palm fronds were chopped into fiber before it was sundried for 24 hours. Chopped oil palm fronds were mixed with fresh blood in 1:1 ratio weight/weight (w/w). This blood-absorbent mixture was dried for 3-4 hours before it was mixed with more blood in 5:4 ratio (w/w). This final mixture was subsequently fermented.

Fermentation process

Aerobic fermentation was conducted in tightly closed plastic containers. Microbial inoculant as much as 3% (w/w) was added to the blood-absorbent mixture. The mixture was further incubated at 40 °C for 0, 60, and 120 hours before they were dried at 60 °C for 24 hours. Dried fermented materials were analysed for their nutrient contents. Fiber fractions were determined by using Van Soest analysis.

In vitro test

In vitro test was conducted to fermented BM-oil palm frond absorbent (BMOPFA) mixtures which were incubated for 120 hours. Three treatments rations including 60% king grass (KG) + 40% BM (P0, control), 60% KG + 40% BMOPFA fermented with BLP (P1), and 60% KG + 40% BMOPFA fermented with BAF (P2) were used.

Digestibility determination

Digestibility was determined by using the in vitro technique of Tilley and Terry (1963). Rumen fluid was obtained from the rumen of a fistulated cow and filtered by using 4-layer cheese clothes. One part of rumen fluid (10 ml) was mixed with 4 parts of media solution (40 ml) consisting of buffer solution, macro and micro mineral solution, resazurin, and reduction solution (Goering and Van Soest, 1970). This mixed solution (50 ml) was added into 1 g of sample placed in a 100-ml incubation tube. CO_2 gas was flown into the tube for 30 seconds before the tube was covered. The filled tubes were incubated for 24, 48, and 72 hours. At the end of each incubation time, 2 drops of $HgCl_2$ were added into each tube. Tubes containing samples and incubation media were centrifuged at 4000 for 10 minutes. Supernatant was taken out from the tubes and analysed for volatile fatty acids and N-NH_3 concentration. Meanwhile, the residue was added with 50 ml pepsin-HCl 0.20% and incubated for 48 hours. When the incubation finished, the solution was filtered by using Whatman No. 41 filter paper and dried at 60 °C for another 48 hours before it was analyzed for its nutrient contents.

Statistical analysis

Data were subjected to an analysis of variance and a Duncan test. Differences were considered to be statistically significant at $P<0.05$.

RESULTS

Changes in fiber fractions of fermented BMOPFA

Types of inoculant and fermentation times gave significant interaction effects ($P<0.05$) on changes in fiber fractions including neutral detergent fiber (NDF), acid detergent fiber (ADF), hemicellulose, cellulose, and lignin of fermented BMOPFA (Table 1). Neutral detergent fiber and ADF contents of fermented BMOPFA significantly decreased ($P<0.05$) with

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the interaction of between inoculant type of BAF and BLP and incubation times. Bacterial incubation within 120 hours reduced NDF contents by 1.05 percentage points (BAF)) and 1.60 percentage points (BLP). Significant reduction in ADF content by 2.50 percentage points was found in BMOPFA inoculated with BAF for 120 hours but not in BMOPFA inoculated with BLP. Significant interaction effects of inoculant types and incubation times on hemicellulose, cellulose, and lignin contents of BMOPFA were also revealed. There were fluctuated changes of hemicellulose contents in BMOPFA fermented with both bacteria. Hemicellulose contents were found to increase with the use of BAF and decrease with the use of BLP. However, these changes in hemicellulose contents were not statistically different.

Cellulose contents were found to be significantly reduced ($P<0.05$) as the results of interaction effects of inoculant types and incubation times. Compared to that of unfermented BMOPFA, cellulose content of BMOPFA incubated with bacteria in 120 hour was found to be lower by 3.29 percentage points (BAF) and 5.40 percentage points (BLP). Meanwhile, BMOPFA fermentation within 120 hours by using BAF and BLP was shown to lower lignin content by 0.92 and 1.66, respectively.

Table 1 - Changes in fiber fractions of fermented BMOPFA

Inoculant	Incubation times (hours)	Fiber fractions (%)				
		NDF	ADF	Cellulose	Hemicellulose	Lignin
No inoculant	0	39.11^{c}	27.07^{c}	17.55^{d}	12.04^{ab}	6.69^{c}
B. amyloliquefaciens	60	38.17^{b}	26.87^{bc}	14.32^{b}	11.30^{a}	6.55^{c}
	120	38.16^{b}	26.79^{bc}	14.26^{b}	11.37^{a}	5.77^{b}
L. plantarum	60	38.60^{bc}	26.01^{b}	15.85^{c}	12.59^{b}	5.79^{b}
	120	36.51^{a}	24.57^{a}	12.15^{a}	11.94^{ab}	5.03^{a}

Different superscripts in the same column indicate significant differences ($P< 0.05$), NDF: Neutral detergent fiber, ADF: Acid detergent fiber

Digestibility rates and in-vitro rumen condition characteristics of fermented BMOPFA

It was revealed that inoculant types significantly affected ($P<0.05$) DM and organic matter (OM) digestibility rates of BMOPFA (Figure 1). The highest DM (78.10%) and OM (73.56%) digestibility rates were found in BMOPFA fermented with BLP followed by those in BMOPFA fermented with BAF (66.11 and 60.98%), and those in unfermented BMOPFA (52.59 and 48.54%). Compared to those in unfermented BMOPFA, DM and OM digestibility rates of fermented BMOPFA increased by 48.51 and 51.55% (BLP) and 25.56 and 25.63% (BAF), respectively.

Fermentation also gave significant effects ($P<0.05$) on crude protein (CP) digestibility and rumen N-NH_3 concentration of BMOPFA (Figure 2). BMOPFA fermented with BLP and BAF was found to have increased CP digestibility and rumen N-NH_3 concentration. CP digestibility rates of inoculated BMOPFA were 67.33% (BLP) and 56.08% (BAF). These figures were higher than that (48.72%) of inoculated BMOPFA. These findings indicated that fermentation of BMOPFA with BLP and BAF improved CP digestibility by about 38.42 and 15.11%, respectively. Similar findings were revealed in rumen N-NH_3 concentration, which was higher in inoculated BMOPFA, namely 32.67% (BLP) and 28.67% (BAF), than that in inoculated BMOPFA (25.00%). These were increases of 30.68 and 14.68% of rumen N-NH_3 concentration resulted from BMOPFA fermentation.

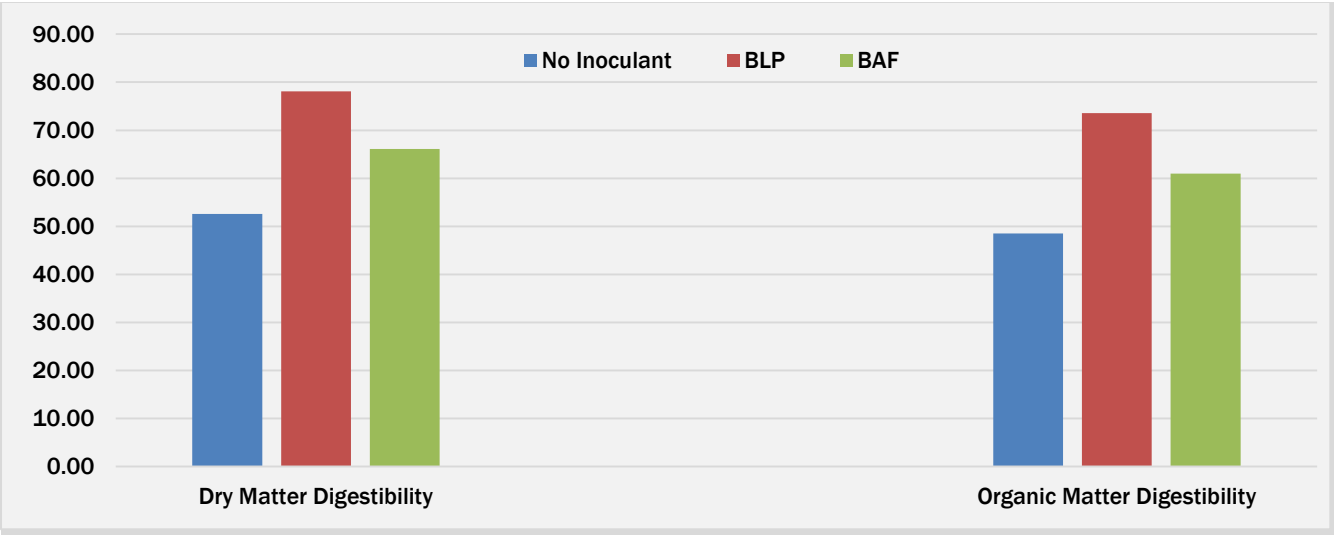

Figure 1 - Dry matter and organic matter digestibility rates of fermented BMOPFA (%). BLP= *Lactobacillus plantarum*; BAF= *Bacillus amyloliquefaciens*

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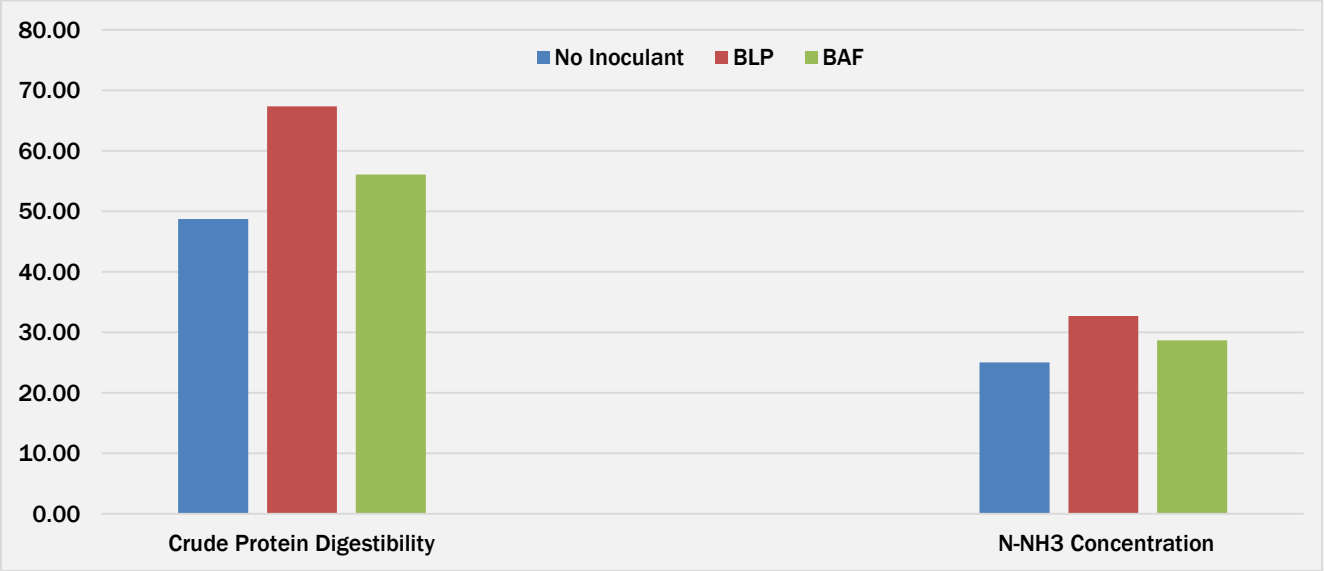

Figure 2 - Crude protein digestibility rates and rumen N-NH_3 concentration of fermented BMOPFA (%). BLP= *Lactobacillus plantarum*; BAF= *Bacillus amyloliquefaciens*

DISCUSSION

In a fermentation process, incubation time and type of inoculant affect nutritive values of fermentation substrate (Wang et al., 2019; Suprayogi et al., 2022). This can be seen from the stages of the fermentation process. In the initial stage, no or very slow growth of inoculant is observed as it is acclimatizing to pH, nutrients, and temperature in a new medium. In an initial (lag) phase, bacteria are adapting to new environment and do not reproduce or undergo cell division (Rolfe et al., 2012). In this lag phase, cell growth but not cell proliferation may take place. As incubation time progresses, inoculants enter exponential phase when and stationary phases. In the exponential phase, bacteria grow very fast while in the stationary phase bacterial growth takes place in the same rate as the bacterial death (Urnemi et al., 2012). Faster growth of inoculant in stationary phase results in production of enzymes to degrade substrates. Changes in dry matter degradation occurs because of the growth of fungi, substrate decomposition, and changes in water content as a result of evaporation, substrate hydrolysis, or metabolic water production (Gervais 2008). Imsya et al. (2013) found that degradation of lignocellulose components in oil palm fronds by fungi increased nutrient availability and improved inoculant growth. Decreased DM and OM contents was caused degradation of OM by fungi which produced more water. This finding was in line with what Dinis et al. (2009) found that cycles of nutrient availability continued to occur during the fermentation process. This led to fluctuations in DM and OM contents as the degradation and utilization of nutrients as an energy source by inoculants progressed. Decreased DM and OM contents of BMOPFA as the substrate used in this study might be attributed to this notion.

Inoculant types also affect nutrient changes in substrate as each inoculant has an ability to produce different kinds of enzymes having different activities. BAFs produce significant amount of amylase making them excellent degraders of simple carbohydrates. Having this characteristic, BAFs are considered as a biocatalyst in starch hydrolysis. Meanwhile, BLPs are cellulase producers (Turker and Ozcan, 2015).

Decreased NDF and ADF contents of BMOPFA in this study might be caused by cell wall degrading activities of enzymes produced by BAF and BLP. Fluctuating decreases in substrate NDF and ADF contents were observed in a fermentation process (Nelson and Suparjo, 2011). Decreases in NDF and ADF contents are the reflections of accumulated decreases in fiber components (cellulose, hemicellulose, and lignin). Cell walls are composed of NDF components including cellulose, hemicellulose, and lignin and ADF components including cellulose and lignin (Van Soest, 2002).

Significant changes in fiber fraction contents, particularly cellulose and lignin of BMOPFA in this study were caused by activities of BAF and BLP inoculants. BAFs and BLPs were found as bacteria which could produce extracellular enzymes including cellulases and hemicellulases (Wizna et al., 2007). In another study by Ramadhan et al. (2016), it was found that the absorption treatments of BM by using coconut pulp and palm kernel cake decreased fiber contents by 6.78-14.72%. Cellulase is a mixture of hydrolytic enzymes which hydrolyse β-1,4-glycocide bonds found in cellulose (Dashtban et al., 2010). Based on their specific activities, these main hydrolytic enzymes include endoglucanase or endo-1-4-β-glucanase (EC 3.2.1.4), cellobiohydrolase (EC 3.2.1.91), and β-glucosidase (EC 3.2.1.21) (Rabinovich et al., 2004; MekonnenTeto, 2021). Cellulose degradation is initiated by endoglucanase which hydrolyzes the amorphous part of cellulose to result in end groups of cellulose. These cellulose end groups are further hydrolyzed by cellobiohydrolase to form cellobiose (Rabinovich et al., 2002; Shrotri, 2017). Monomers and dimers are removed from the end of glucan chains by cellobiohydrolase and these dimers are then hydrolyzed by β-glucosidase to produce glucose (Rabinovich et al., 2002).

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Similar kinds of enzymes in greater amount are involved in hemicellulose degradation as hemicellulose is more heterogenous than cellulose (Malherbe and Cloete, 2002). Degradation of hemicellulose results in monomeric sugars and acetic acid. The main carbohydrate contained in hemicellulose is xylan and degradation of it requires collaborative work of various hydrolytic enzymes (MekonnenTeto, 2021). Hemicellulases are divided based on their activities into endo-1,4-β-xylanase (EC 3.2.1.8) producing oligosaccharides from xylan degradation and xylan 1,4-β- xylosidase (EC 3.2.1.37) producing xylose from xylan oligosaccharides. Furthermore, supplementary enzymes including xylan esterases, ferulic and p-coumaric esterases, α-1-arabinofuranosidases, and α-4-O-methyl glucuronosidases which synergically work to hydrolyze xylans and mannans (Perez et al., 2002; Juturu et al., 2013; Houfany et al., 2020). Degradation of O-acetyl-4-O-methylglucuronxylan, the main form of hemicellulose, requires four hemicellulolytic enzymes including endo-1,4-b-xylanase (endoxylanase), acetyl esterase, a-glucuronidase and b-xylosidase. Meanwhile, degradation of O-acetylgalactoglucomannan is initiated with the breakdown of it by endomannases. This is followed by the removal of acetyl groups and galactose residues by acetylglucomannan esterases and a-galactosidases, respectively. In the last stage, endomannases-generated oligomers b-1,4 bonds are broken down by b-mannosidase and b-glycosidase (Perez et al., 2002).

Similarly, lignin degradation also occurs in fermentation processes involving microbial organisms including fungi and bacteria (Janusz et al., 2017). Lignin is the hardest part of cell wall components designed to protect carbohydrates from bacterial degradation. Therefore, in nature, lignin is more effectively degraded by white-rot fungi (Zabel and Morrell, 2020). In ruminant animals, however, rumen bacteria are also found as lignin degraders (Kuhad et al., 2013). There are two important groups of enzymes involved in lignin degradation namely lignin-modifying enzymes (LME) and lignin-degrading auxiliary (LDA) enzymes. Lignin-modifying enzymes including lignin peroxidase, manganese peroxidase, versatile peroxidase, and laccase can work on their own or cooperatively with others. Meanwhile, LDA enzymes cannot work by their own but are required to complete the degradation process (da Silva Coelho-Moreira et al., 2013; Janusz et al., 2017) into simpler components (Nelson and Suparjo, 2011). Changes in lignin degradation in this study were found to be small compared to those of cellulose and hemicellulose. This might be attributed to the fact that the fermentation process in this study were conducted by using bacterial inoculants (BAF and BLP) which were less effective in degrading lignin component than fungi (Zabel and Morrell, 2020).

Increases in BM and OM digestibility of BMOPFA in this study occurred because of changes in nutrient contents resulted from absorbent and fermentation treatments. Fermentation process resulted in decreased fiber fraction contents (Table 1) which, in turn, made it easier for rumen microbes to degrade other nutrients contributing to higher DM and OM digestibility. It is well accepted that DM and OM digestibility is negatively correlated with fiber fraction contents (Cherdthon et al., 2010). Lower fiber content increased DM and OM digestibility and DM digestibility was affected by OM digestibility as OM is part of DM in feeds (Davidson et al., 2003; Griswold et al., 2003; Imsya et al., 2013).

Likewise, increased protein content because of absorbent and fermentation treatments was found to enhance protein digestibility of BMOPFA in this study. Increased activities of proteinase enzymes were observed in BM treated with fermented agricultural wastes (Ramadhan et al., 2016). Fermentation by inoculants degraded protein into simpler peptides and improved protein digestibility. In vitro CP was also found to increase in BM as protein solubility increased (Paz et al., 2013).

Increased CP and OM digestibility was indicated by increased rumen N-NH_3 concentration (Imsya et al., 2013). Rumen N-NH_3 concentration resulted from BMOPFA in this study was adequate to meet the requirement of ruminant animals (10-15.7 mg N/dl) for their optimal growth (Alcaide et al., 2003; Cherdthong et al., 2011).

CONCLUSION

It was concluded that types of inoculants and lengths of incubation time interactively affected changes in concentration and digestibility of fiber fractions of BMOPFA. Fermentation with BLP in 120-hour incubation time produced the best BMOPFA containing 36.51% NDF, 24.57% ADF, 12.15% cellulose, 11.94% hemicellulose, and 5.53% lignin. This BMOPFA had 78.10% DM digestibility rate, 73.56% OM digestibility rate, 67.33% CP digestibility rate, and 32.67 mM rumen N-NH_3 concentration. In-vivo feeding studies on the use of BMOPFA fermented with LBP in 120-hour incubation time and its effects on the performance of ruminant animals are suggested.

DECLARATIONS

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Authors' contribution
All authors contributed equally to this research work. All authors read and approved the final manuscript

Conflict of interests
The authors declare no conflict of interests.

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