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THE EFFECT OF BIOPROCESS TECHNOLOGY IN OIL PALM TRUNK ON CHEMICAL COMPOSITION AND IN-VITRO FERMENTATION CHARACTERISTICS

AZHARY NOERSIDIQ1, YETTI MARLIDA2, MARDIATI ZAIN2, ANWAR KASIM3 AND DAN FAUZIA AGUSTIN2 1Graduate Students of Master Programs Leading to Superior Doctorate of Agricultural Science, Andalas University, West Sumatera, Indonesia 2 Department of Animal Nutrition and Feed Technology, Faculty of Animal Science, Andalas University, West Sumatera, Indonesia 3 Department of Agricultural Technology, Faculty of Agricultural Technology, Andalas University, West Sumatera, Indonesia

(Recieved 30 April, 2018; accepted 31 May, 2018) Key words: Bioprocess fchnology, Fermentation, In vitro, Oil palm trunk, Digestibility

Abstract- One effort to improve the quality of oil palm trunk nutrients as beef cattle feed can be done with fermentation technology,this technology is quite cheap and applicable for farmers. The purpose of the research was to evaluate of technology bioproces in oil palm trunk on chemical composition and in vitro fermentation characteristics. This research used Randomized Block Design (RBD) with 3 treatments and 4 replications. The treatment consisted of A1: oil palm trunk without treated; A2: fermented oil palm trunk using Phanerochaete chrysosporium; A3: fermented oil palm trunk using starbio. The results obtained showed that the treatment effect was higly significant differents ($P<0.01$) on In vitro digestibility dry matter (IVDM), In vitro digestibility organic matter (IVOM), In vitro digestibility crude protein (IVCP) and fiber fraction digestibility (NDF, ADF, cellulose and hemicellulose), whereas VFA, NH_3 and pH were not siqnificant different ($P>0.05$). This research we can conclude that the best treatment that produces the highest digestibility was treated A2 (fermented oil palm trunk using Phanerochaete chrysosporium) whereas IVDM : 61.56%, IVOM : 49.90%, IVCP :75.91%, andin vitro digestibility of ADF : 69.63%, NDF : 63.73%, Cellulose: 69.53%, Hemicellulose : 68.84% respectively, whereas rumen characteristic such as pH : 6.96, NH_3: 9.20 mM and VFA: 144.61 mM,

respectively. INTRODUCTION high starch and crude fiber that can be a source of energy for ruminants. Based on the analysis at The potential of oil palm plantation in Indonesia is ruminant laboratory (2016) , the oil palm trunk very high with the area of plantation ranging from . consisted of 44.43% crude fiber, 3.64% crude 10.754.801 ha in 2014 and is expected to increase to protein, 3.32% crude fat, 55,33% cellulose, 20.35% 11.672.861 ha in 2016 Statistical Plantation Book, hemiselulose, 15.41% lignin and 5.02% silica. 2014) while in West Sumatra, the area of oil palm However, the utilization of oil palm trunk as plantation is around 376.474 Ha and is expected to ruminant feed has limitation factor because of the increase until reaches 413.43 Ha in 2016 (Statistical lignin content was high in which lignin can bind Plantation Book, 2014) and around 40% of oil palm cellulose and hemicellulose and can block the plantation area in West Sumatra will be replanted. destruction of vascular and parenchymal cells The abundant quantity, the oil palm trunk can be Azemi et al., 1999), which is digestibility are very used as ruminants feed where until now the oil low. palm trunk from replantation have only been Technology bioprocess such as fermentation is burned or left decayed and made into boards. the right technology to break the bond between In terms of quality, the oil palm trunk contained lignin with cellulose and hemisellulose and can

*Corresponding author's email: yettimarlida@ansci.unand.ac.id degrade lignin through fermentation technology. Phanerochaete chrysosporium is an one of white rot fungus species which often being testing model in degradation of lignocelluloses component. It can be degraded in a selective way where it degrades lignin substrate which is brown colour and leave cellulose which white colour (Adaskaveg, et al., 1995; Blanchette et al, 1995). Phanerochaete chrysosporium has been reported to liberate lignin from plant tissue and the studies have shown that lignin is oxidized and degradated by a ligninolytic system composed of lignin peroxidase (LiP), manganese peroxidase (MnP) (Arora et al., 2002: Rothschild et al., 1999) , cellulase and hemicellulase (Wood et al., 1988) . Meanswhile Starbio is a commercial probiotic that has been widely used as inoculum for fermentation of rice straw and another feed fiber, its contents Cellulomonas Clostridium thermocellulosa as a degradated fatty acid, Agaricus and coprinus (lignin digesters), Klebssiella and Azozpirillum trasiliensis as protein digesters (Lembah Hijau Multifarm. 1999). It was assumed that probiotic microbes are able to penetrate the fibrolytic structure and cleave the binding of lignified carbohydtrate and in some extent, degrade cellulose and hemicellulose. The aim of the research was to evaluate technology bioproces in oil palm trunk on chemical composition and in vitro fermentation characteristics. MATERIALS AND METHODS Oil palm trunk fermentation Oil Palm Trunk (OPT) collected from field used as substrate, it was ground and sieved to 18-20 mesh by shredding machine. The shredded oil palm trunk was dried at 60 0C in an oven for 12 h. The dried oil palm trunk (OPT) was kept ready for the further use. Phanerochaete chrysosporium was maintained on Potato Dextrose Agar (PDA) slants at 4 0C, transferred to PDA plates at 37 0C for 7 days and subsequently grown on OPTs mixed with rice bran, the mineral solution from Brook et al (2012) was then added. The fermentation process was initiated by adding water to the OPTs until the water level reached 70%. Observations were after for 21 days. After 21 days, samples were taken for proximate analysis and fiber fraction determination. The same method was done using starbio, whereas 0.6% starbio was added to OPT and 0,3% urea then added by water to maintain the moisture of 60%. The proximate components were determined as described by AOAC (1995). The fiber fractions (NDF, ADF, hemicellulose, cellulose and lignin) were determined according to the method of Van Soest et al. (1982). In vitro digestibility assay In vitro digestibility was analyzed by Tilley and Terry (1963) . Fistula cow rumen fluid was diluted using McDougal Buffer (1:4) and dispensed into a 1 g substrate-prepared incubation tube, which was purged with CO2 to maintain an aerobic condition. The tubes were incubated in a water bath at 390C for 24 h. After fermentation, the erlenmeyer tube containing the sample was inserted into ice water to stop the fermentation. All samples were then centrifuged at 1.200 rpm for 15 min. The pH, NH3- N and total VFA of the supernatants were then recorded. The NH3-N concentration was measured using the micro-diffusion conway method and the total VFA concentration was measured using the steam distillation method. The previously incubated samples were vacuum filtered (Whatman No. 41) and dried at 60 0C in an oven. The dried samples were used to analyze the NDF, ADF and cellulose in the in vitro digestibility assay. Experimental design and statistical analysis The study

was carried out using a randomized block design (3× 4) with five replications. The treatment consisted

of A1: oil palm trunk without treated; A2: fermented oil palm trunk using Phanerochaete chrysosporium; A3: fermented oil palm trunk using starbio. Differences between treatment means were analyzed using Duncan's multiple range

test. RESULT AND DISCUSSION Loss of Nutrients After Incubated with Phanerochayte chrisosporium and Starbio Data on fermentation loss of OPT fermented by Phanerochayte chrisosporium (PC) and Starbio are presented in Table 1. Both treatments using PC and starbio caused a net loss of dry matter (DM), organic matter (OM), NDF, and ADF and concequantly in cellulose and hemicellulose of oil palm trunk (OPT) during fermentation. The chemicals composition the substrate was related to losses of nutritions caused of the fermentation with fungi and microorganism in strabio. In this study, fermentation with Phanerochayte chrysosporium decreased all the content of OPT nutrient which can be seen in Table 1. This happens because it has enzyme that degraded them like specific lignolitic enzyme and sellutic enzyme but it increased of Crude Protein (CP) content of OPT after 21 days of fermentation which was due to the PC supplied the increasing amount of protein from single cell protein be form the mycelium. Tripathi et al. (2008) found that Phanerochayte chrisosporium can degrade lignin in mustard straw and reduced lignin about 40% at 35 days incubation. Arora and Sharma (2009) added siqnificant losses in lignin and cellulose of wheat straw, which ranged from 22.1 to 30.5% and from 17.3 to 26.3%, respectively at an incubation of 30 days. Table 1. Loss of nutrients of OPT treated to technology bioprocess using Phanerochayte chrisosporium and starbio Variables (%) Technology bioprocess of OPT A1 A2 A3 Dry Matter Organic Matter Crude Protein Crude Fiber ADF NDF Cellulose Hemicellulose Lignin 49.54 33.41 87.56 74.60 3.64 5.37 44.43 31.34 75.75 57.24 96.10 75.20 55.33 46.12 20.35 17.96 15.41 8.71 37.01 78.91 6.36 37.68 63.54 81.95 49.17 18.40 10.54 Note : A1 : oil palm trunk (OPT) without treatment, A2 : fermented OPT using Phanerochaete chrysosporium (PC), A3 : fermented OPT using starbio Meanwhile, the fermentation with starbio gave the decreasing lower than fermentation with PC because the PC was the group of white rot fungi which it higher ability to degrade the component in high fiber or wood like OPT than bacteria in starbio as specially lignolitic enzyme. Starbio can be degrade the wood component because it has bacteria which can do that like proteolytic microbes 6 x 109 CFU/g of material, the usual type is formulated as: Nitrosomonas / Nitrobacter / Nitrospira / Nitrosococcus / Nitrosolobus, lignolytic microbes 6 x 109 CFU/g of material, commonly formulated types are: Clavaria dendroidea / Clitocybe alexandri / Hypoloma fasciculare. cellulolytic microbes 8 x 108 CFU/g of material, the usual colonies are formulated: Trichoderma polysporeum / Tricoderma viridae / Cellulomonas acidula / Bacillus cellulase disolven, lipolytic microbes 5 x 108 CFU/g of material, the usual type is formulated Spirillum liporerum (Multi farm, 1999) In Vitro

Fermentation Characteristics The analysis of the data for the fiber fraction digestibility (NDF, ADF, cellulose and

hemicellulose) due to the technology bioproces using Phanerochayte chrisosporium and starbio in the fermentation process of OPTs is presented in Table 2. Table 2. Digestibility of nutrient (Dry matter, Organic matter, Crude protein, NDF, ADF, cellulose and hemicellulose) due to the technology bioproces using Phanerochayte chrisosporium and starbio in the fermentation process of OPT In vitro digestibility Technology bioprocess of OPT (%) A1 A2 A3 Dry matter Organic matter Crude protein NDF ADF Cellulose Hemicellulose 39.63 c 61.56 a 30.17 c 49.50 a 52.17 c 75.91 b 33.30 c 69.63 a 35.21 c 69.73 a 36.76 c 69.53 a 43.93 c 68.84 a 54.14 b 42.70 b 78.26 ab 61.08 b 63.50 b 60.92 b 65.67 a Note : Means in the

same row with differenta,b letters are significant at P<0.05 NDF: Neutral detergent fiber, ADF: Acid detergent

fiber The digestibility of dry matter, organic matter and crude protein can be seen in Table 2. It showed that technology bioprocess can increase the digestibility twice, this is due to work done by enzymes produced Phanerochayte chrysosporium and starbio in hydrolyzing organic compounds from OPT, thereby providing convenience to rumen microbes attack. The technology bioprocess higly siqnificantly (P<0.01) affects to the dry matter, organic matter and crude

protein digestibility. The higher digestibility of dry matter, organic matter and crude protein in the treatment A2 compared to A3, because during the fermentation of all components of organic matter undergoes changes due to the work of lignolytic enzymes produced by Phanerochayte chrysosporium (PC) and starbio (Table 1). The highest dry matter, organic matter and crude protein digestibility associated with the optimal degaradation of lignin by PC (43.5%), give opportunity more nutrients availability and produce the highest dry matter digestibility. The same result also found by Febrina et al. (2015) reported that low lignin content can increase dry matter digestibility of OPF that fermented by PC added mineral Ca, P and Mn on the fermentation process. Zhao et al., (2015) reported that Phanerochayte chrysosporium and Lentinula edodes degradated about 45% of lignin and enhanced in vitro digestibility of dry matter (IVDM). Cell content includes carbohydrates, organic acids, lipids, proteins, nitrogenous substances and most of inorganic constituents.

Digestibility of organic matter includes digestible cell content and digestible cell wall content. While cell content is

digestible from almost 100%, the level of cell wall degradation is different. Digestibility of organic matter has a negative correlation with NDF, ADF and hemicelluloses. A significant negative correlation was found between digestible

organic matter and NDF (%) in organic matter (Èerešňáková et al., 1996). The digestibility of protein with fermentation by starbio gave the higher result compared with PC because it has microbe which specific degrade protein enzyme like Nitrosomonas / Nitrobacter / Nitrospira / Nitrosococcus / Nitrosolobus higher than proteolytic enzyme which has by PC. The technology bioprocess such as fermentation by Phanerochayte chrisosporium and starbio of OPT showed that higher fiber fraction digestibility compared digestibility of OPT without treated, whereas among the technology bioprocess, fermented by Phanerochayte chrysosporium was higher compared fermented by starbio. The technology bioprocess higly siqnificantly ($P<0.01$) affects the NDF, ADF, cellulose and hemicellulose digestibility of OPT. The NDF, ADF, cellulose and hemicellulose digestibility was lowest 61.08% , 63.50%, 60,92% and 65.87%, respectivelly in the treatment A3 (fermentation with starbio). Low NDF, ADF, cellulose and hemicellulose digestibility because in the inoculum starbio there are many microbial mixtures that not only play a role in degrading ligin but also can degrade cellulose, protein and fat. Microbial contents of the starbio was proteolytic microbes 6 x 109 CFU/g of material, the usual type is formulated as: Nitrosomonas / Nitrobacter / Nitrospira / Nitrosococcus / Nitrosolobus, lignolytic microbes 6 x 109 CFU/gram of material, commonly formulated types are: Clavaria dendroidea / Clitocybe alexandri / Hypoloma fasciculare. cellulolytic microbes 8 x 108 CFU/g of material, the usual colonies are formulated: Trichoderma polysporeum / Tricoderma viridae / Cellulomonas acidula / Bacillus cellulase disolven, lipolytic microbes 5 x 10 8CFU/g of material, the usual type is formulated Spirillum liporerum (Mussato and Teixeira . 2010) The low digestibility of the fiber fraction (NDF, ADF, cellulose and hemicellulose) in OPT, because the limiting factor of the OPT is lignin, while the microbes break down the lignin contained in starbio are microbes that do not have a high ability to break lignin compared of the Phanerochayte chrysosporium. Phanerochaete chrysosporium is the model white rot fungus because of its specialized ability to degrade the abundant aromatic polymer lignin, while leaving the white cellulose nearly untouched. It releases extracellular enzymes to break-up the complex three-dimensional structure of lignin into components that can be utilized by its metabolism. It could decompose lignin in the substrate, penetrating the cellulose and hemicellulose attached to the lignin matrix. Release of the lignocellulosic bonds and lignohemicellulose result in cellulose and hemicellulose that can be used by fungi to grow and develop so that the process of

fermentation in the rumen can better function. It has been proven that high fiber fraction digestibility (NDF, ADF, cellulose and hemicellulose) in this treatment. Feng et al. (2011) added the biodegradation of lignin is a key process for lignocellulosic waste composting, in which the increased use of carbon increases the activity of micro-organisms that cause

the enzyme activity of ligninolytics, thus increasing the degradation of lignin. Actually, lower the digestibility of fiber fraction of OPT assosiated with the composition of nutrients after treated with technology bioprocess that can be see in Table 1, whereas the limiting factor such as lignin of OPT reduce about 43.5% with fermentation using Phanerochayte chrysosporium and 31.6% with starbio. Some fungi (the white-rot fungi) degrade lignin faster than they degrade polysaccharides Feng et al., 2011: Hadar et al., 1992; Reid and Deschamps, 1990). Lignin is a very complex molecule constructed of phenylpropane units linked in a large three-dimensional structure.Three phenyl propionic alcohols exist

as monomers of lignin: p- coumaryl alcohol, coniferyl alcohol and sinapyl alcohol. Lignin is closely bound to cellulose and hemicellulose and its function is to provide rigidity and cohesion to the material cell wall, to confer water impermeability

to xylem vessels, and to form a physico –chemical barrier against microbial attack (Valmasade et al., 1990). Due to its molecular configuration, lignins are extremely resistant to chemical and enzymatic degradation (Valmasade et al., 1990). Biological treatments, based on the use of brown-, white- and soft-rot fungi have been commonly used to degrade the lignin, being considered a cheap and effective method of delignification (Fengel et al,. 1989). In terms of rumen characteristics, the treatment gave a significantly different effect ($P<0,05$) to pH, NH_3 and VFA concentration (Table 3). After further testing using DMRT it was shown that the pH, NO_3 in A2 treatment was significantly different ($P<0.05$) with other treatments and among treatments were mutually exclusive as well. However, the pH of rumen fluid was not significantly different ($P>0.05$) between treatments. The highest values of pH, NH_3, and VFA were obtained from A2 treatment using Phanerochaete chrysosporium of 6.96, 9.20 mM, 144.61 mM respectively. This is because the role of Phanerochaete chrysosporium which degrades maximally is characterized by the growth of mycelium mold to produce more enzymes for the perfect lignin degradation process in palm oil palm and can break the lignocellulose and lignohemiselulose bond so that rumen microbes can utilize Cellulose and Hemicellulose to be converted to VFA. Meanwhile, the pattern of fermentation in the rumen can be influenced by several factors such as microbial species, absorption and fermentability of the carbohydrate source feed (Palmqvist et al., 2000). Table 3. The characteristics rumen fluid due to the technology bioprocess using Phanerochayte chrysosporium and starbio in the fermentation process of OPTs Variables (%) Technology bioprocess of OPT A1 A2 A3 pH NH3 (mM) VFA (mM) 6.89 a 6.46 c 116.88c 6.96 a 9.20 a 144.61a 6.94 a 8.43 ab 132.87b

Means in the same row with differenta,b letters are significant at $P<0.05$ VFA: Volatyl Fatty Acid, NH_3: Ammonia The average pH value generated in this study ranged from 6.89 to 6.94 and is still in normal rational pH. while the ideal pH range for cellulose digestion is between 6.4 - 6.8 (Hindratiningrum et al., 2011). The same results also reported by Astuti et al (2015) examining the characteristics of fermented oil palm waste with local microorganisms of livestock wastes yielding pH rumen of 6.85 - 6.88. The process of growth and microbial metabolism is not disturbed in normal pH rumen conditions, so that microbial activity runs normally and the process of digestion of feed ingredients will be optimal. According to Sung et al (2007) said that if the pH of rumen is below 6.0 it can reduce fiber digestibility. The mean concentration of NH_3 produced in this study were A1 (6.46 mM), A2 (9.20 mM), and A3 (8.43 mM) and the result is still within the normal range. The same results also reported by McDonald et al. (2002) who reported that the optimum concentration of NH_3 in the rumen is 6-21 mM. This increased percentage of NH_3 values is caused by OPT containing high starch and fiber which are food and easily degradated into energy to form NH_3. Orskov (1992) reported that in ruminant livestock some of the proteins

that enter into the rumen will experience a reshuffle / degradation into ammonia by proteolytic enzymes

produced by rumen microbes. The production of ammonia depends on the solubility of the dietary protein,

the amount of protein ration, the duration of the feed being in the rumen and the rumen pH. High levels of NH_3 produced in A2 treatment are due to the high fiber digestibility of A2 treatment that is influenced by the role of Phanerochaete chrysosporium in degrading lignin where the higher the fiber digestibility, the higher the NH_3 content is produced. This is in accordance with the opinion of Nagadi et al. (2000) who found that the rate of NDF fermentation increases with the increase of N-NH_3 concentration, this is in line with the results of the research conducted, where NDF digestibility decreases with decreasing N-NH_3 concentration. The mean concentration of VFA produced in the study were A1 (116,88 mM), A2 (144,61 mM), and A3 (132,87 mM). This study ii higher than Astuti et al. (2015) founded VFA concentrations of 54.46-72.26 mM. While the VFA levels required to support optimal rumen microbial growth of 70-150 mM

(Sung et al., 2007) .The increase is due to the high starch in oil palm pith that are easily degraded in the rumen to produce higher energy. The content of starch in oil palm trunk according to Azemi et al (1999) amounted to 84.49%. In addition, VFA levels are determined by the digestibility of crude fiber where the higher the digestibility the higher the VFA content is produced. In this case, A2 treatment using Phanerochaete chrysosporium mold gave the highest fiber fraction digestion to produce high VFA level which was 144.61 mM. This is supported by a statement by Liu et al. (2002) who suggested that there is a strong correlation between the digestibility of organic matter and dry matter to the total production of VFA. High levels of In-vitro digestibility of organic matter and coarse fiber will increase the total production of rumen VFA (Liu et al., 2002). The high level of lignification in the feed ingredients used limits rumen microorganisms in fermenting cellulose and Hemicellulose to produce energy as a volatile fatty acid. The high levels of VFA produced are supported by research using this fungi (Nagadi et al., 2005), where the VFA content produced from fermented oil palm trunk is 150.06 mM. CONCLUSION Bioprocess technology significantly increased in all nutrient digestibility and rumen liquid characteristics compared to Control. The highest values of digestibility and concentration in A2 treatment were followed by A3 and A1 treatment. From this research, it can be concluded that fermentation technology using 7% Phanerochaete chrysosporium is best used as oil palm trunk processing technique that produces the highest digestibility was treated A2 (fermented oil palm trunk using Phanerochaete chrysosporium) whereas IVDM : 61.56%, IVOM : 49.90%, IVCP :75.91%, andin vitro digestibility of ADF : 69.63%, NDF : 63.73%, Cellulose: 69.53%, Hemicellulose : 68.84% respectively, whereas rumen characteristic such as pH : 6.96, NH3: 9.20 mM and VFA: 144.61 mM, respectively. ACKNOWLEDGMENTS This study was funded by the Ministry of Research, Technology and Higher Education of The Republic of Indonesia through PMDSU No: 1387/E4./2015. We are very grateful to their support. REFERENCES A.O.A.C. 1995. Official Methods of Analysis of the Association of Official Analytical Chemists. Washington: AOAC. Adaskaveg, J.E., Gilbertson, R.L. and Dunlap, M.R. 1995. 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