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In Vitro Digestibility of Ration Containing Different Level of Palm Oil Frond Fermented with *Phanerochaetae chrysosporium*

A. Imsya[a,*,#], E. B. Laconi[b,#], K. G. Wiryawan[b,#], & Y. Widyastuti[c]
[a]Major Program of Animal Nutrition and Feed Science, Graduate School, Bogor Agricultural University
[b]Department of Animal Science and Feed Technology, Faculty of Animal Science, Bogor Agricultural University
[#]Jln. Agatis, Kampus IPB Darmaga , Bogor 16680, Indonesia
[c]Research Centre for Biotechnology, Indonesian Institute of Sciences
Jln. Raya Bogor Km. 46 Cibinong 16911, Indonesia
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ABSTRACT

***Phanerochaete chrysosporium* was widely used to delignify agricultural waste product and improve biodegradation of the substrate as animal feed. The experiment was carried out to increase the use of palm oil fronds as a substitute material for napier grass through biodegradation process with *P. chysosporium*. A completely randomized design with four treatments and four replications was used. The treatments were ration containing 60% napier grass (R1), ration containing 40% napier grass and 20% fermented palm oil frond (R2), ration containing 20% napier grass and 40% fermented palm oil frond (R3), ration containing 60% fermented palm oil frond (R4). Fourty percent concentrate was included in all treatment rations. Parameters measured were *in vitro* digestibilities of dry matter, organic matter, crude fiber, NDF, ADF, NH_3, TVFA, and ruminal cellulolitic bacteria. Results showed that increasing level of fermented palm oil frond in the ration reduced (P<0.05) digestibilities of dry matter, organic matter, crude fiber, NDF, ADF, N-NH_3, TVFA concentration and number of ruminal cellulolytic bacteria. It is concluded that fermentation of palm oil frond—with *P. chysosporium* decrease lignin content by 47.79%, but increasing the fermented palm oil frond in the ration reduces nutrient digestibilities, N-NH_3 and TVFA concentrations and rumen cellulolytic bacteria counts. Fermented palm oil frond up to 40% could be used as a substitute for forages in ruminant rations.**

Key words: digestibility, fermentation, palm oil frond, Phanerochaete chrysosoporium

ABSTRAK

***Phanerochaete chrysosporium* secara luas digunakan untuk mendegradasi lignin dari produk limbah pertanian dan memperbaiki biodegradasi substrat sebagai pakan ternak. Penelitian ini bertujuan untuk meningkatkan kegunaan pelepah sawit yang difermentasi dengan kapang *P. crhysosporium* sebagai substitusi rumput gajah. Penelitian menggunakan rancangan acak lengkap dengan 4 perlakuan dan 4 ulangan. Perlakuan meliputi tingkat pemakaian pelepah sawit fermentasi, yaitu ransum 60% rumput gajah (R1), ransum 40 % rumput gajah + 20% pelepah sawit fermentasi (R2), ransum 20% rumput gajah + 40% pelepah sawit fermentasi (R3), dan ransum 60% pelepah sawit fermentasi (R4). Parameter yang diamati adalah kecernaan bahan kering, kecernaan bahan organik, kecernaan serat kasar, kecernaan NDF dan kecernaan ADF yang diuji *in vitro*. Hasil penelitian menunjukkan bahwa perlakuan memberikan pengaruh yang berbeda nyata (P<0,05) terhadap kecernaan bahan kering, bahan organik, serat kasar, NDF, ADF, konsentrasi N-NH_3, dan total VFA serta bakteri selulolitik. Kesimpulan penelitian ini adalah fermentasi pelepah sawit dengan *P. chysosporium* dapat menurunkan kandungan lignin pelepah sawit 47,79%, namun peningkatan pemakaiannya dalam ransum menurunkan kecernaan bahan kering, bahan organik, fraksi serat, kosentrasi TVFA dan N-NH_3 serta jumlah bakteri selulolitik. Pemakaian pelepah sawit fermentasi sampai level 40% dapat digunakan sebagai pengganti rumput gajah.**

Key words: kecernaan, fermentasi, pelepah sawit, Phanerochaete chrysosporium

*Corresponding author:
E-mail: aimsya@yahoo.com

INTRODUCTION

Oil palm fronds are an important co-product. However, the high neutral detergent fiber (NDF, 700 g/kg dry matter and lignin (205 g/kg contents are major constraints to OPF use as livestock feed (Zahari *et al.,* 2003; Wan Rosli *et al.,* 2004; Abdul Khalil *et al.,* 2006) this is consistent with low OPF digestibility in cattle (400 g/kg DM (Kawamoto *et al.,* 2001). Physical methods and chemical agents to disrupt the cell wall matrix in low quality forages and agriculture waste products have been investigated. Some methods are very effective in removing lignin and disrupting the cell wall matrix. Commercial application is plagued by disposal of chemical waste and hazards to persons applying treatments. One alternative is the use of biological agents like microbial for removal of lignin to increase digestibility of agriculture waste product as feedstuff for ruminants. The organisms predominantly responsible for lignocelluloses degradation are fungi, and the most rapid degraders in this group are basidiomycetes (Ten Have & Teunissen, 2001; Bennet *et al*. 2002; Rabinovich *et al.,* 2004)

Agriculture wastes like palm oil frond containing lignocellulose can be up grade by solid-state fermentation with white rot fungi. Moreover, lignin degradation is affected by the culture condition, among which aeration is highly important. Not even whiterot fungi are known to be capable of using lignin as a sole carbon and energy source, and it is generally believed that lignin break down is necessary to gain access to cellulose and hemicellulose. Although white-rot basidiomycetes have been shown to efficiently mineralize lignin, species differ gross morphological patterns of decay they cause *Phanerochaetae chrysosporium* strains simultaneously degrade cellulose, hemicellulose and lignin, whereas others such as Ceriporiopsis subvermispora tend to remove lignin in advance of cellulose and hemicelluloses (Sanchez, 2009).

The common pattern of attack on lignocellulose by white rot fungi is a simultaneous decay of polysaccharides and lignin, but preferential degradation of lignin may also occur. Much of the reported research has dealth with delignification of wood by white rot fungi, particularly *P. chrysosporium*. *P. chrysosporium* has been reported to liberate lignin from plant tissue, research has shown that lignin is oxidized and degraded by a ligninolytic system (Elisashvili *et al*., 2008; Rodrigues *et al.,* 2008) composed by lignin peroxidase (LiP), manganese peroxidase (MnP) and Laccase (Panagiotou *et al*., 2007). *P. chrysosporium* has recently been shown to produce an extracellular enzyme that catalyzes oxidative C_{α}- C_{β} cleavage in two important lignin substructures and thereby causes the partial depolymerization of natural lignin. Recent research reported about effect of temperature, pH, carbon and energy sources for *P. chrysosporium* activity, but little lignin degradation associated with decreased substrate digestibilty from waste agriculture product like palm oil frond has been reported.

The objective of this experiment was to study *in vitro* digestibility of ration containing different level of palm oil frond fermented by *P. chrysosporium.*

MATERIALS AND METHODS

This experiment was done in Dairy Nutrition Laboratory, Department of Animal Nutrition and Feed Technology, Faculty of Animal Science, Bogor Agricultural University.

Stater Preparation

P. chrysosporium (obtained from Biotechnolgy Laboratory, Bandung Institute of Technology, Bandung) was grown in potato dextrose broth (PDB). PDB was prepared following the modification method of MacFaddin (1985). *P. chrysosporium* (10^7 cfu/mL) was inoculated into 50 mL PDB medium and incubated for three days.

Fermentation

Palm oil frond was removed its skin, chopped, dried and milled with a size 5 mm. Fifteen grams of milled palm oil frond was autoclaved for 15 min at 121 ^oC in Erlenmeyer. Then, palm oil frond was inoculated with stater *P. chrysosporium* and incubated at 27 ^oC for 10 d.

In Vitro Digestibility of Palm Oil Frond in Ruminant Ration

In vitro digestibility trial was conducted based on modification of Tilley & Terry (1963) method. Cattle rumen liquor from RPH was filtered with four layers cheese cloth. One part of rumen liquor (10 mL) was mixed with 4 parts medium (40 mL) i.e. buffer solution, macro and micro mineral solution, resazurine and reduction solution (Goering & Van Soest, 2002). One gram of sample was added into the 100 ml incubation tube containing 50 mL mixed solution. Before incubation tube was closed, CO_2 gas was purged for 30 sec and incubated for 24, 48, and 72 h. When each incubation time finished, 2 drops of $HgCl_2$ was added into the cultures. The incubation tubes were then centrifuged at 4000 rpm for 10 min. Supernatant was used for determination cellulolytic bacterial, NH_3-N concentration and total VFA, then residue was added 50 mL pepsin-HCl 0.2% solution and incubation for 48 h. Residu filtered by whatman no 41, dried at 60 ^oC for 48 h. The dried sample was used for determination of *in vitro* digestibility of dry matter, organic matter, crude fiber, NDF, and ADF. Cellulolytic bacterial concentrations were estimated with the Hungate procedure (Ogimoto & Imai, 1981). NH_3-N concentration in rumen fluid was determined by microdiffusion technique Conway. Total VFA (TVFA) concentration in rumen fluid was determined by Markhams distillation

Experimental Design and Data Analysis

The experiment used completely randomized design with four treatments i.e: Ration 1 (R1) 60% napier grass, Ration 2 (R2) 40% napier grass 20% fermented palm oil fronds, Ration 3 (R3) 20% napier grass 40%

fermented palm oil fronds, Ration 4 (R4) 60% fermented palm oil fronds. Ration consists of 60% forage and 40% concentrate. The concentrate mixture was composed of corn (33.79%), rice bran (40%), soybean meal (1.89%), cassava waste meal (10.24%) and tofu waste meal (14.08%) (Table 1). Each treatment had four replications. Parameters observed were *in vitro* digestibility of dry matter, organic matter, crude fiber, NDF, and ADF. Data were analyzed by one-way analysis of variance (ANOVA) and the mean values were tested for difference using Duncan's New Multi Range Test (Steel & Torrie, 2002)

RESULTS AND DISCUSSION

Dry Matter and Organic Matter Digestibility

Digestibility of nutrient is one indicator to measure feed quality because it reflects the level of nutrients availability for livestock. The study showed that increasing level of fermented palm oil frond with *P. chrysosporium* in ruminant ration significantly reduced (P<0.05) digestibility of dry matter and organic matter. The average was 55.06%-71.27% for dry matter digestibility and 54.15%-69.66% for organic matter digestibility (Table 2). The dry matter digestibility of ration with 20% fermented palm oil frond had 6.73% lower than that of ration containing 60% napier grass. Meanwhile, ration containing 40% and 60% fermented palm oil frond had 18.15% and 22.74% lower than that of ration containing 60% napier grass respectively. Organic matter digestibility of ration with 20% fermented palm oil frond had 7.00% lower than of ration containing 60% napier grass, meanwhile ration containing 40% and 60% fermented palm oil frond had 17.18% and 22.26% lower than that of ration containing 60% napier grass respectively. The differences in dry matter and organic matter digestibility were caused by crude fiber contents in each treatment (Table 1). Cherdthong *et al.* (2010) found that the digestibility of DM and organic matter was negatively correlated with NDF and ADF contents in the diet. Davidson *et al.* (2003) reported that high of crude fiber contributed to decreased DM and OM digestibility. Griswold *et al.* (2003) reported that increasing trend in organic matter digestibility related with dry matter digestibility because the difference was only in the ash content. Ration containing 60% napier grass had the highest organic matter digestibility.

Dry matter and organic matter digestibility of ration containing fermented palm oil frond were higher compared to that ration containing palm oil frond without fermentation. Suryadi *et al.* (2009) reported that use of 42% palm oil frond without fermentation of in ration obtained dry matter digestibility 30.77% and organic matter digestibility 30.91%, meanwhile Syarief (2010) obtained dry matter digestibility of ration containing 60% non fermented palm oil frond was 39.28%, and organic matter digestibility was 44.35%. According to Islam *et al.* (2000), used of 60% fresh palm oil frond and 40% concentrate gave *in sacco* dry matter digestibility of 30.85%, meanwhile *in vivo* dry matter, organic matter and ADF digestibility were 52%, 56% and 26%, respectively.

Fiber Digestibility

The increasing level of fermented palm oil frond with *P. chrysosporium* in ruminant ration significantly reduced (P<0.05) digestibility of NDF, ADF, and crude fiber. The average was 35.99%-55.90% for NDF digestibility, 41.02%-58.58% for ADF digestibility, and 32.87%-57.43% for crude fiber digestibility. The increased level of fermented palm oil frond in ration decreased NDF, ADF, and crude fiber digestibility. NDF, ADF, and crude fiber digestibility of ration containing 60% napier grass (R1) were higher than those rations containing 20% fermented palm oil frond (R2), 40% fermented palm oil frond (R3) and 60% fermented palm oil frond (R4), meanwhile the ration containing 60% fermented palm oil frond had the lowest NDF, ADF and crude fiber digestibility (Table 2). This indicated that although biodegradation of palm oil frond with *P. crhysosporium* capable to decrease lignin contents (Table 3) but the nutritive value was still not equivalent to napier grass.

Tabel 1. Chemical composition of ration treatments

Nutrient (%)	Ration			
	R1	R2	R3	R4
Dry matter	89.56	89.91	89.56	88.92
Organic matter	70.66	69.16	69.61	71.57
Crude protein	10.90	11.56	12.22	12.87
Crude fiber	14.23	15.19	16.16	17.12
NDF	26.86	25.76	24.65	23.55
ADF	21.08	20.28	19.48	18.67
Cellulose	20.23	17.06	13.89	10.72
Hemicelullose	5.78	5.47	5.17	4.87
Lignin	6.85	7.22	7.58	7.95

Note: R1= 60% napier grass; R2= 40% napier grass, 20% fermented palm oil fronds; R3= 20% napier grass, 40% fermented palm oil fronds; R4= 60% fermented palm oil fronds.

Table 2. Digestibility of dry matter, organic matter, and fiber fraction of fermented palm oil frond different level in ration

Digestibility (%)	Ration			
	R1	R2	R3	R4
Dry matter	71.27±1.53[a]	66.47±0.51[b]	58.34±2.15[c]	55.06±2.02[d]
Organic matter	69.66±1.24[a]	64.78±0.95[b]	57.69±1.48[c]	54.15±2.01[d]
NDF	55.90±0.48[a]	48.78±0.43[b]	41.24±1.37[b]	35.99±2.09[c]
ADF	58.58±0.36[a]	52.15±0.35[b]	44.52±0.71[c]	41.02±1.19[c]
Crude fiber	57.43±5.60[a]	48.98±6.75[b]	38.58±8.51[c]	32.87±5.28[c]

Note: Means in the same row with different superscripts differ significantly (P<0.05). R1= 60% napier grass; R2= 40% napier grass, 20% fermented palm oil fronds; R3= 20% napier grass, 40% fermented palm oil fronds; R4= 60% fermented palm oil fronds.

Biodegradation of palm oil frond with 10^7 cfu/mL *P. crhysosporium* and incubated for 10 d showed the best yield for lignin degradation. The lignin content of fermented palm oil frond was 13.27% while palm oil frond without biodegration has lignin content 25.42% (Table 3). The result also showed that lignin content of fermented palm oil frond was higher than lignin of napier grass (11.42%). Lignin in the ration could decrease digestibility as reported by Lynd *et al.* (2002) that ration with lignocelulolytic contents generally have high structural carbohydrate by some level of crystalline and make stick to lignin so digested of ration is difficult. Digestion of ration with fiber content could be increased through that can disperse lignin and carbohydrater string (Annison *et al.,* 2002). Rumen microbes can digest the nutrient in the ration easier as the content of lignin decreasing. Van Soest (2002) mentioned that lignin in the plant cell is important factor in limiting the digestibility of the nutrient. A positive relation between total lignin loss and *in vitro* DM degradability has been reported in a few studies, particularly for sugarcane bagasse colonized with *L. edodes* and *C. subvermispora* for 16 wk (Okano *et al.,* 2006). Similar positive relationships between lignin loss and *in vitro* DM degradability have been reported for other substrates and fungi, such as sugarcane bagasse colonized with *P. eryngii* (Okano *et al.,* 2007) The negative relationship that seems to exist between lignin concentration and forage digestibility has been recorgnized for many years, because lignin as a component of the cell wall, the effect of lignin on forage digestibility is assumed to be a direct influence on wall digestibility rather than on digestibility of total forage organic matter (Van Soest, 2002). Nutrient digestibility was influenced with physical of fibrous component in the rations (Toharmat *et al.* 2006).

Lignin seems to exert its negative effect on cell-wall polysaccharaide digestibility by shielding the polysaccharides from enzymatic hydrolysis (Jung & Deetz, 2003). This is apparently a steric hindrances such that the polysaccharides cannot align themselves properly with their substrate for hydrolysis to occur (Tripathi *et al*. 2008). In this experiment based on *in vitro* experiment, the value of dry matter, organic matter, crude fiber, NDF, ADF digestibility was negatively correlated to lignin content it seems in determination koefisien of correlation value. Determination koefisien (R) for correlation value of lignin content with dry matter digestibility= -0.97098 (y= -15.52x+177.3; R^2= 0.942), organic matter digestibility= -0.97351 (y= -14.26x+166.9; R^2= 0.947), NDF digestibility= - 0,99831 (y= -11.70x + 158.30; R^2= 0.996), ADF digestibility= -0.97417 (y= -7.077x + 130.10; R^2= 0.949) and crude fiber digestibility= -0.9032 (y= -19,38x+213.00; R^2= 0.986).

Fiber digestibility of ration with 20% fermented palm oil frond had 6.9% lower than ration with 60% napier grass. Meanwhile, ration with 40% and 60% fermented palm oil frond had fiber digestibility of 14.45% and 20.89%, respectively. Based on the result above, 60% of fiber in fermented palm oil frond could be digested by rumen microbes. This is the result of laccase and ligninase enzymes activity from *P. crhysosoporium* which capable of decreasing lignin content in palm oil frond up to 47.79%.

Crude fiber digestibility of fermented palm oil frond in the ration was higher than the ration with palm oil frond without fermentation. This result support the experiment conducted by Syarief (2010) that NDF and ADF digestibility of ration with 42% palm oil frond without fermentation had 62.66% and 54.83%, respectively.

Tabel 3. Chemical compotition of fermented and unfermented palm oil frond

Nutrient (%)	Palm oil frond	Fermented palm oil frond
Dry matter	88.14	86.27
Crude protein	5.28	12.13
Crude fiber	39.85	28.54
NDF	65.59	39.29
ADF	52.72	31.14
Cellulose	27.79	17.87
Hemicelullose	12.87	8.12
Lignin	25.42	13.27

Concentrations of $N\text{-}NH_3$, TVFA, and Cellulolytic Bacteria

The concentrations of $N\text{-}NH_3$ and TVFA and the number of cellulolytic bacteria were found to be decreasing with the increasing use of fermented palm oil frond in the rations. It was found that the average $N\text{-}NH_3$ concentration was about 6.5-8.71 mM, the average TVFA concentration was about 150.06-163.47 mM, and the number of cellulolytic bacteria was about 6.02-8.27 log cfu/ml. The figures of these three parameters were found to be higher in ration containing 60% napier grass (R1) than those in rations containing 20%, 40%, and 60% fermented palm oil frond (R2, R3, and R4). Ration containing 60% fermented palm oil frond was found to have the lowest $N\text{-}NH_3$ and TVFA concentrations and the number of cellulolytic bacteria (Table 4).

The decreasing $N\text{-}NH_3$ concentration with an increased use of fermented palm oil frond was in line with

Tabel 4. Celulolytic bacteria, $N\text{-}NH_3$ consentration, and VFA total at different level fermented palm oil frond in ration

Ration	Celulolitic bacteria (log cfu/mL)	$N\text{-}NH_3$ (mM)	Total VFA (mM)
R1	8.27±0.58[a]	8.71±0.72[a]	163.47±0.65[a]
R2	7.42±0.44[ab]	7.72±0.31[b]	159.82±2.53[b]
R3	7.15±0.29[b]	7.20±0.47[bc]	153.42±2.93[c]
R4	6.02±0.94[c]	6.50±0.72[c]	150.06±2.10[d]

Note: Means in the same column with different superscripts differ significantly ($P<0.05$). R1= 60% napier grass; R2= 40% napier grass, 20% fermented palm oil fronds; R3= 20% napier grass, 40% fermented palm oil fronds; R4= 60% fermented palm oil fronds.

the possible low protein digestibility as reflected from the decreasing organic matter digestibility. It was found that the reducing organic matter digestibilities in R4, R3, and R2 (22.26%; 17.18%; and 7%, respectively) compared to that in R1 resulted in reducing N-NH_3 concentrations (3.09, 2.11, and 1.38 mg N/dl, respectively). This might be explained by the notion that in ruminants, protein entering the rumen will be degraded to ammonia by proteolytic enzymes produced by rumen microbes. Ammonia production depends on feed protein solubility, feed protein content, feed retention in rumen, and rumen pH (Orskov, 1992).

N-NH_3 concentration found in this study was considered adequate for optimum growth. Cherdthong *et al.* (2011) N-NH_3 concentration of rumen is 15.7 mg N/dl, meanwhile Tripathi *et al.* (2008) found N-NH_3 concentration 20.34 mg N/dl in rumen of diet containing mustard (*Brassica compestris*), and 10-15 mg N/dl rumen fluid (Alcaide *et al*. 2003) were required for optimum growth. Nagadi *et al.* (2000) found that NDF fermentation rate increased as N-NH_3 concentration increased. An N-NH_3 concentration of 8-15mg N/dl was found to be optimum for NDF degradation and intake (Dermann, 2009) and 6.4 mg N/dl rumen fluid was found by Islam *et al*. (2000) in a ration containing 60% palm oil frond and 40% concentrate.

In this study, the increasing level of fermented palm oil frond with *P. chrysosporium* in ruminant ration significantly reduced ($P<0.05$) number of cellulolytic bacteria. Reducing number of cellulolytic bacteria was influenced by decreasing of N-NH_3 concentration. This was attributed to the fact that the resulted ammonia was the main source of nitrogen for rumen microbes. A deficiency of N-NH_3 concentration resulted in a reduction in rumen microbial population. Decreasing of N-NH_3 concentration could be reflection about sintetic rumen bacteria (Karabulut *et al*., 2007; Kongmun *et al.*, 2010; Cutrignelli *et al.,* 2010). Most ammonia (82%) is used by rumen microbes for multiplication, especially in their cell synthesis. The ammonia level needed for rumen microbe growth is about 5.9-9.4 mg N/dl (Jalc *et al*. 2009). *In-vitro* N-NH_3 measurement can be used to estimate protein degradation and utilization by rumen microbes. A strong correlation appeared between the digestible organic matter intake and the microbial protein synthesis (Pathak, 2008).

Volatile fatty acids (VFA) are the end products of carbohydrate metabolism by rumen microbes. TVFA concentration of 150.06-163.47mM in this study was found to be adequate for microbial growth as it was higher than the amount required (80-160 mM) for optimal rumen microbial growth (Sutardi, 1980).

The decreasing VFA concentration as the amount of palm frond use increased was found to be correlated with the decreaing fiber digestibility. Lower fiber digetibility resulted in lower VFA concentration. NDF digestibilities were found to be lower by 16.68%, 9.88%, and 6.44% (in R4, R3, and R2, respectively) than that in R1. These reducing NDF digestibility figures were correspondingly accompanied by decreasing TVFA concentrations of 13.41, 10.05, and 3.65 mM in R4, R3, and R2, respectively. High lignification level in feed was known to limit rumen microorganisms in fermenting cellulose and hemicellulose to produce energy in the forms of VFAs (Tripathi *et al*., 2008). The improvement of access of cellulose and hemicellulose enzymes should be initiated by the breakdown of lignocellulose bonds of the cell wall. The degradation process in the rumen requires protein availability to ensure the optimum growth of microbes (Alcaide *et al*., 2003). TVFA concentration in this study was found to be higher than that (22,13-91,95mM) resulted from fermented cocoa pods by *P. chrysosporium* (Nelson *et al*., 2011)

CONCLUSION

Fermentation of palm oil frond with *P. crhysosporium* decreases lignin content by 47.79%, but it can not improve nutrient digestibilities, N-NH_3 and TVFA concentrations, and rumen cellulolytic bacterial counts. Fermented palm oil frond can be used as a substitute for napier grass up to 40% in ruminant rations.

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