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Nutritional Qualities of Cocoa Pod Husk Treated with Bioconversion and or Provision of Nitrogen Sources in the Rumen

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ABSTRACT

The objective of this study was to investigate the effects of bioconversion using *Phanerochaete chrysosporium* and *Pleurotus ostreatus* and or inclusion of *Moringa oleifera* leaves and urea in the rumen on cocoa pod husk digestibility and fermentation in the rumen. There were 4 treatments tested: (1) 100% untreated cocoa pod husk (UCPH), (2) 55% UCPH + 43.7% *M. oleifera* + 1.30% urea (UCPHMU), (3) 100% bioconverted cocoa pod husk (BCPH), and (4) 55% BCPH + 44.5 *M. oleifera* + 0.5% urea (BCPHMU). Each of the treatments was replicated three times. Variables observed were dry matter and organic matter digestibilities and degradabilities, rumen VFA and ammonia concentrations, gas production, and calculated microbial biomass yields. Results indicated that the treatment increased dry matter (P<0.001) and organic matter (*P*<0.01) digestibility, with the highest for the BCPHMU and the lowest for the UCPH. The treatments also increased dry matter and organic matter degradability in the rumen (*P*<0.001), with the highest for the BCPHMU, followed by the UCPHMU, and then by the BCPH and the lowest was UCPH. The treatment affected rumen ammonia concentration (*P*=0.01), the highest value was found for the BCPHMU followed with UCPHMU and BCPH. Microbial biomass synthesis was affected (*P*<0.001) by the treatment and it was always higher when nitrogen was provided (UCPHMU and BCPHMU). Total VFA concentration or total gas production was higher for BCPHMU compared to other treatments. It can be concluded that nutritional quality of cocoa pod husk can be improved by either bioconversion with *P. chrysosporium* and *P. ostreatus* or inclusion of *M. oleifera* and urea in the rumen, but the best improvement can be obtained by the combination of bioconversion and provision of the nitrogen sources in the rumen.

Keywords: biological treatment, cocoa pod husk, digestibility, fermentation, white rot fungi

ABSTRAK

Tujuan penelitian ini adalah untuk mempelajari pengaruh biokonversi menggunakan *Phanerochaete chrysosporium* dan *Pleurotus ostreatus* dan atau penambahan *Moringa oleifera* (daun kelor) dan urea ke dalam rumen pada kecernaan dan degradasi kulit buah kakao di dalam rumen. Ada 4 perlakuan yang dicobakan: (1) 100% kulit buah kakao tanpa perlakuan (UCPH), (2) 55% UCPH + 43,7% *M. oleifera* + 1,30% urea (UCPHMU), (3) 100% kulit buah kakao biokonversi (BCPH), dan (4) 55% BCPH + 44,5 *M. oleifera* + 0,5% urea (BCPHMU). Tiap perlakuan diulang 3 kali. Peubah yang diamati adalah kecernaan dan degradasi bahan kering dan bahan organik pakan, konsentrasi VFA dan amonia rumen, produksi gas, dan produksi biomassa mikrob. Hasil penelitian menunjukkan bahwa perlakuan meningkatkan kecernaan bahan kering (P<0,001) dan bahan organik (P<0,01), kecernaan tertinggi diperoleh pada BCPHMU dan terendah pada UCPH. Perlakuan juga meningkatkan (P<0,001) keteruraian bahan kering dan bahan organik di dalam rumen, tertinggi pada BCPHMU, disusul oleh UCPHMU, BCPH, dan terendah pada UCPH. Perlakuan meningkatkan konsentrasi ammonia rumen (P=0,01), tertinggi pada BCPHMU diikuti oleh UCPHMU dan BCPH. Hasil biomasa mikroba juga dipengaruhi oleh perlakuan (P<0,001), dan hasil biomassa mikroba selalu lebih tinggi saat disediakan tambahan nitrogen di dalam rumen (UCPHMU dan BCPHMU). Konsentrasi total VFA atau produksi gas total pada BCPHMU lebih tinggi dibandingkan perlakuan lainnya. Disimpulkan bahwa kualitas nutrisi kulit buah kakao dapat diperbaiki, baik melalui biokonversi dengan *P. chrysosporium* dan *P. ostreatus,* maupun dengan penambahan *M. oleifera* dan urea ke dalam rumen, namun hasil terbaik diperoleh jika kedua perlakuan tersebut digabungkan.

Kata kunci: kulit buah kakao, perlakuan biologis, jamur pelapuk putih, kecernaan, fermentasi

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INTRODUCTION

Indonesia is the third largest cocoa producing country in the world after Ivory Coast and Ghana (Witjaksono & Asmin, 2016). In 2016, the total area of cocoa plantation in Indonesia was estimated to be about 1.7 million hectares with a total cocoa production of 760,429 tonnes (DGEC, 2015). Assuming that cocoa pod husk constitutes about 75% of the whole cocoa fruit (Cruz *et al.,* 2013), there was about 530,322 tones of cocoa pod husk produced in Indonesia in 2016. As a by-product of cocoa plantation industry, the cocoa pod husk is currently not being utilized optimally by farmers. The common practice regarding the cocoa pod husk is to leave it dried and rotten or use it as a mulching material for the cocoa plant itself.

Cocoa pod husk is a potential biomass that can be used as an animal feed resource, especially for ruminants (Puastuti & Susana, 2014). However, the cocoa pod husk is known to have low nutritional properties such as high cellulose and hemicellulose contents (Suparjo *et al*., 2011; Daud *et al*., 2013). It also contains a considerable amount of lignin, *i.e* approximately 14.7% on dry matter basis (Daud *et al*., 2013), which is higher than that in rice straw. Inclusion of untreated cocoa pod husk in ruminant diets has been associated with low feed intakes and negative live weight gains (Saili *et al*., 2010).

Fermentative bioconversion of cocoa pod husk with white rot fungi seems to be a promising approach in attempt to increase its nutritional quality (Syahrir *et al.,* 2013; Laconi & Jayanegara, 2015; Yakin *et al*., 2016; Yakin *et al.,* 2017). Laconi & Jayanegara (2015) concluded that *Phanerochaete chrysosporium*, a species of white rot fungi capable of degrading lignin, was effective in improving the nutritive value of cocoa pod husk. In our previous study, we combined *P. chrysosporium* and *Pleurotus ostreatus* (another species of white rot fungi) and found that the two species acted synergistically in improving the nutritional properties of cocoa pod husk and its *in vitro* digestibility (Syahrir *et al.,* 2013).

Moringa oleifera is a multipurpose tree that grows well across Indonesia. *M. oleifera* leaves are known to contain high crude protein and several studies have indicated positive animal responses when it is included as a protein source or feed component in ruminant diets (Adegun *et al*., 2011; Mendieta-Araica *et al*., 2011; Asaolu *et al*., 2012; Babeker & Abdalbagi, 2015; Kholif *et al*., 2015; Sultana *et al*., 2015; Babiker *et al*., 2017; Syarifuddin *et al*., 2017). According to Soliva *et al*. (2005), crude protein in *M. oleifera* leaves is highly fermentable and is therefore expected to be used as an ammonia source for microbial protein synthesis rather than providing rumen by-pass proteins to the animals. *M. oleifera* leaves are also high in sulphur-containing amino acids (Makkar & Becker, 1997) and this may further benefit microbial protein synthesis in the rumen.

The objective of this study was to investigate the effects of bioconversion of cocoa pod husk with *P. chrysosporium* and *P. ostreatus* and or provision of *M. oleifera* and urea on its digestibility and fermentation in the rumen.

MATERIALS AND METHODS

Treatments

There were four dietary treatments tested consisting of untreated (UCPH) or bio-converted cocoa pod husk (BCPH) with or without *M. oleifera* leaves and urea that were mixed at different proportions as shown in Table 1.

Bioconversion of cocoa pod husk was done by first mixing dried and finely ground cocoa pod husk (10 kg) with rice bran (1.5 kg), CaCO3 (0.15 kg), urea (10 g), and water (70% of the total weight). The mixture was placed in sealed bags and autoclaved at 121°C and 2 atm for 1 h and allowed to cool at the room temperature for 24–36h. It was then inoculated with either *P. chrysosporium* (10^7cells/g) and or *P. ostreatus* (14.3×10^9cfu/mL) in a room temperature until the bags were completely covered with mycelium (after about 20 d of fermentation). Different dosages of the two white rot fungi were used and tested for the effects on the chemical composition of the cocoa pod husk and its *in vitro* digestibility (Syahrir *et al*., 2013). The best results were found for the cocoa pod husk that was treated with *P. chrysosporium* (8 g/kg) and *P. ostreatus* (30 g/kg), and this was selected to be used in the current study.

Experimental Procedures

Feed dry matter and organic matter digestibilities were estimated with the two-step *in vitro* digestion technique of Tilley & Terry (1963) as modified by van Der Meer (1980). The *in vitro* gas production technique

Table 1. Proportions of untreated and bio-converted cocoa pod husk, M. oleifera leaves and urea used in the treatments

Treatments	Proportions (%)				Nutrients	
	UCPH	FCPH	*Moringa oleifera* leaves	Urea	DM (%)	CP (% DM)
UCPH	100	0	0	0	86.8	5.06
UCPHMU	55	0	43.7	1.3	86.5	16.1
BCPH	0	100	0	0	88.3	9.21
BCPHMU	0	55	44.5	0.5	88.9	16.1

Note: UCPH= untreated cocoa pod husk, UCPHMU= untreated cocoa pod husk + *M. oleifera* + Urea, BCPH= bio-converted cocoa pod husk, BCPHMU= bio-converted cocoa pod husk + *M. oleifera* + Urea.

of Menke *et al.* (1979) as modified by Makkar *et al.* (1995) was employed to study rumen fermentation. Both experiments were run in a randomized block design with time of rumen fluid collection serving as the block. Each of the dietary treatments was replicated five times.

The rumen fluids were obtained from a fistulated cow (BW ± 450 kg) maintained on a diet with a crude protein content of about 18%. The diet consisted of a concentrate feed (a mixture of cooked soybean peel and rice bran) given at 1% BW in the morning and *P. purpureum* that was given *ad libitum* after the concentrate. The fermentation lasted for 96 h, and gas production was recorded at 0, 2, 4, 8 16, 24, 36, 48, 72, and 96 h of incubation. At the end of the 96 h incubation, the fermentation was terminated by immersing the fermentation syringes in ice and the contents were then centrifuged (2.500 rpm; 15 min) to obtain the supernatants for NH_3 and VFA analyses. Residues from two syringes were taken and prepared for determinations of DM and OM contents, while the remaining syringes were prepared for determination of microbial biomass synthesis (Blummel *et al.*, 1997). For determination of microbial biomass, the syringe contents were transferred to a beaker glass and added with 100 mL of NDS solutions before it was refluxed for 60 min. The solution was then filtered with filter crucible (porosity 0.01 mm), washed with a least amount of hot water and followed with 75 mL of acetone and then dried in an oven (105°C; 4 h). Microbial biomass (mg) was calculated as the difference between apparent (substrate incubated–centrifuged residue) and true digestibility (substrate incubated–residu after NDS treatment).

Chemical Analysis

Feed dry matter content was determined by drying the feed samples to a constant weight at 60°C. The concentration of NH_3 in the fermentation supernatant was analysed with the Conway method (General Laboratory Procedure, 1966). One mL of each supernatant and 1 mL of saturated NaOH (40%) was placed oppositely on each side of the Conway disc. One mL of H_3BO_4 solution (4%) as indicator was also placed in the middle of the disc before the disc was covered air-tightly. The disc contents were then mixed by a gentle shaking before it was allowed to stand in a room temperature for 24h. The contents was then back titrated with 0.01N HCl until its color turned back to its original color before the titration. Ammonia concentration was calculated as:

$$NH_3\text{-N (mg/mL)} = (\text{mL HCl used} \times \text{N HCl}) \times NH_3 \text{ MW} \times 100^{-1} \text{ mg/mL}$$

The total VFA concentration was determined according to General Laboratory Procedure (1966) while that of individual VFA was done on a gas chromatographer (Shimadzu GC 9 AM equipped with a flame ionization detector and Shimadzu C-RGA integrator) according to Bachruddin (1996). For the individual VFA concentration, rumen fluid sample was first centrifuged (3,000 rpm; 10 min) and 2 µL of the supernatant was injected after the injection of a standard VFA solution.

Calculations

Gas production data were fitted to the exponential equation of $p = a + b (1 - e^{-ct})$ (Ørskov & McDonald, 1979), where p represents gas volume at time t, a the gas produced from the soluble fraction, b the gas produced from the insoluble but fermentable fraction, a+b the potential gas production, and c the rate constant of gas production during incubation (% h^{-1}).

Statistical Analysis

One way analysis of variance for the randomized experimental block design was performed to evaluate effects of the treatments on variables observed, and significant difference among individual variable means were identified with Duncan's Multiple Range Test (Steel & Torrie, 1991). Means differences were considered significant at $P<0.05$.

RESULTS

Nutrient contents of cocoa pod husk and nitrogen supplements are presented in Table 2. *In vitro* dry matter and organic matter digestibilities of treatment feeds in the rumen fluid are shown in Table 3 while those of rumen fermentation parameters and gas productions are presented in Tables 4 and 5, respectively. Results indicated that dry matter and organic matter digestibilities among treatments differed one another, being the highest was found in the bioconverted cocoa pod husk added with *M. oleifera* and urea (BCPHMU) and the lowest was found in the untreated cocoa pod husk (UCPH). Digestibilities of dry matter (48.0±1.03%) and organic matter (48.0±1.97%) for the untreated cocoa pod husk provided with nitrogen sources (UCPHMU) were higher (P<0.01) than the corresponding values for the bioconverted cocoa pod husk (BCPH) which was 46.0±1.09% and 45.0±1.65%, respectively (Table 3).

Total VFA concentration, individual VFA proportion (Table 4), and total gas production (Table 5) were not affected by the treatments, except for the BCPHMU that were higher (P<0.05) than the other treatments. However, NH_3 concentration for the BCPHMU was higher than the others, while the NH_3 concentrations for the UCPHMU and the BCPH remained similar. Results

Table 2. Chemical compositions of feed ingredients (%)

Nutrients	UCPH	BCPH	*M. oleifera* leaves	Urea
Dry matter	86.60	88.30	89.60	99.50
Crude protein	5.84	10.10	24.50	288.00
Crude fiber	44.40	38.40	18.30	
Ether extracts	2.75	2.56	4.40	
Organic matter	76.10	76.30	81.40	
N-free extract	23.51	25.24	34.20	

Note: UCPH= untreated cocoa pod husk; FCPH= bio-converted cocoa pod husk.

Table 3. Dry matter and organic matter digestibilities of feed treatments determined with two-steps *in vitro* incubation

Treatments	*In vitro* digestibility (%)	
	Dry matter	Organic matter
UCPH	40.5 ± 1.08[a]	38.6 ± 1.15[a]
UCPHMU	48.0 ± 1.03[c]	48.0 ± 1.97[b]
BCPH	46.0 ± 1.09[b]	45.0 ± 1.65[b]
BCPHMU	49.9 ± 0.68[d]	50.2 ± 2.65[d]
SEM	0.06	0.18
p-value	0.000	0.001

Note: UCPH= untreated cocoa pod husk, UCPHMU= untreated cocoa pod husk + *M. oleifera* + Urea, BCPH= bio-converted cocoa pod husk, BCPHMU= bio-converted cocoa pod husk + *M. oleifera* + Urea. Means in the same column with diferent superscripts differ significantly (P<0.05).

for dry matter and organic matter degradabilities followed a similar trend with those for digestibilities in which the BCPHMU exhibited the highest values, followed by the UCPHMU, and then by the BCPH and the lowest one was UCPH. Microbial biomass synthesis was higher when nitrogen sources were provided in the rumen.

DISCUSSION

This study indicated that bioconversion of cocoa pod husk with a combination of *P. chrysosporium* and *P.ostreatus* had desirable effects on the nutritional values of the cocoa pod husk for ruminants. Significant higher dry matter and organic matter digestibilities were found in the bio-converted than in the untreated cocoa pod husk (BCPH *vs* UCPH; Table 3). There was about 14% increase in dry matter digestibility and 17% in organic matter digestibility due to the biological treatment of the cocoa pod husk. The increased feed digestibility found in the current study was consistent with the results reported previously (Syahrir *et al.,* 2013) and this was probably related to the reduction in lignin contents of the cocoa pod husk after the bioconversion that had provided more sites for microbial fermentation in the rumen. Provision of nitrogen sources in the form of *M. oleifea* leaves and urea in the rumen also improved the dry matter and organic matter digestibilities. The improvement in digestibility due to the provision of nitrogen sources was higher (for dry matter) and similar (for organic matter) compared to that for the bioconversion treatment. Dry matter or organic matter digestibility was further improved by the combination of nitrogen source and bioconversion compared to each of these treatments *per se*.

Similar increases due to the bioconversion treatment and or the provision of nitrogen were also found

Table 4. Fermentation variables of feed treatments after 96 h of incubation

Variables	Treatments				SEM	p-value
	UCPH	UCPHMU	BCPH	BCPHMU		
Total VFA concentration (mM)	60.50[a]	62.70[a]	62.00[a]	63.40[b]	0.12	0.04
Acetate, C2 (%)	75.40	74.00	74.40	74.00	0.08	0.07
Propionate, C3 (%)	15.40	15.90	16.30	15.90	0.05	0.06
Butyrate, C4 (%)	9.55	10.10	9.37	10.10	0.05	0.41
C2:C3 ratio	5.09	4.67	4.58	4.64	0.04	0.45
NH_3 concentration (mM)	8.65[a]	9.29[b]	9.11[b]	10.30[c]	0.11	0.01
DM degradability (%)	38.70[a]	49.00[c]	45.70[b]	51.40[d]	0.12	0
OM degradability (%)	37.00[a]	48.10[c]	42.30[b]	50.30[d]	0.09	0
Microbial biomass synthesis (mg)	82.20[a]	85.10[bc]	83.10[ab]	86.00[c]	0.03	0

Note: Means in the same row with diferent superscripts differ significantly (P<0.05).

Table 5. Total gas production and gas production variables after 96 h of incubation

Variables	Treatments				SEM	p-value
	UCPH	UCPHMU	BCPH	BCPHMU		
Gas production (mL/500 mg DM)	69.3[a]	69.9[a]	70.4[a]	73.2[b]	0.08	0
a	-4.33	-3.89	-4.55	-4.05	nd	nd
b	76.1	75.6	77.6	79.8	0.19	0.07
c	0.04	0.04	0.04	0.04	0	0.12

Note: Means in the same row with diferent superscripts differ significantly (P<0.05). nd= not determind; a= the gas produced from the soluble fraction; b= the gas produced from the insoluble but fermentable fraction; and c= the rate constant of gas production during incubation (% h^{-1}).

for dry matter and organic matter degradabilities of cocoa pod husk in the rumen (Table 4). Provision of *M. oleifera* and urea in the rumen increased rumen dry matter and organic matter degradabilities by 27% and 30%, respectively, for the untreated cocoa pod husk; while the respective corresponding values for the bio-converted cocoa pod husk were 13% and 19%. As for feed digestibility mentioned above, *M. oleifera* combined with urea exhibited higher effects on dry matter and organic matter degradabilities of cocoa pod husk in the rumen compared to the bioconversion treatment. The higher effect on rumen *in-vitro* dry matter digestibility of cocoa pod husk when added with urea than when treated with *Phanerochaete chrysosporium* was also found by Laconi & Jayanegara (2015). Results of this study and those of Laconi & Jayanegara (2015) show that provision of additional nitrogen sources to the rumen is probably more preferable approach to increase the cocoa pod husk digestibility than biologically treating it with white rot fungi.

The rumen fluid ammonia concentrations found in this study (8.65–10.3 mM) were well above the optimum concentrations of 3.5–5.7 mM for microbial protein synthesis in the rumen suggested by Satter & Slyter (1974). However, other studies have found optimum microbial protein synthesis at higher rumen ammonia concentrations (Hume *et al*., 1970; Allen & Miller, 1976). The ammonia concentrations were also similar or above the suggested rumen ammonia concentrations of *ca.* 8.8 mM for optimum rumen degradability of fibrous diets (Preston & Leng, 1987). Based on the observed rumen ammonia concentrations in this study, rumen microbial growth and fermentation may have been proceeded normally.

While both provision of nitrogen sources in the rumen and bioconversion in this study increased the rumen ammonia concentrations significantly, higher microbial biomass production was only shown when *M. oleifera* and urea were added to either untreated or bio-converted cocoa pod husk (Table 4). It was probably that other forms of nitrogen than ammonia (amino acids and small peptide) were also available and beneficial for the growth and synthesis of rumen microbes when *M. oleifera* was present. *M. oleifera* leaves are known to contain high levels of sulphur-containing amino acids (Makkar & Becker, 1997), and the S requirement of rumen microbes for the synthesis of its own S-containing amino acids is apparent (McSweeney & Denman, 2007; Akinlade & Ososanya, 2016).

Provision of additional nitrogen sources in the rumen or treating the cocoa pod husk biologically with *P. chrysosporium* and *P. ostreatus* had no effect on total or individual VFA concentrations and gas productions and parameters in the rumen (Table 4 and 5). The facts that the higher feed degradability was not followed with higher fermentation products might have indicated that the degraded feed organic matter was probably used for microbial biomass synthesis rather than being fermented. The presence of sulphur-containing amino acids in *M. oleifera* and higher ammonia concentrations may have promoted a more efficient microbial growth in the rumen.

CONCLUSION

It can be concluded that nutritional quality of cocoa pod husk can be improved by either bioconversion with *P. chrysosporium* and *P. ostreatus* or inclusion of *M. oleifera* and urea in the rumen, but the best improvement can be obtained by the combination of bioconversion and provision of the nitrogen sources in the rumen.

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