The Evaluation of Rumen Metabolism of Fries Holstein (Fh) Calves Fed Biofermented Cocoa Pods Using *Phanerochaete Chrysosporium*

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ABSTRACT

An *in vivo* experiment was conducted to evaluate cocoa pods to substitute forages for ruminant. The experiment was carried out using latin square design on 5 head of FH calves with 5 treatments and 5 replications. Ration was designed iso-protein (17%) and iso-TDN (65%) used cocoa pod as forages (35%) and other cocoa by-product were used as concentrate (65%). The treatment consisted of concentrate plus untreated cocoa pods (R1); urea ammonia treated of cocoa pods (R2); silage of cocoa pods (R3); bio fermented of cocoa pods using rumen content (R4) ; and bio fermented of cocoa pods using *P chrysosporium* (R5). Variables measured were pH, N-NH3, VFA, Microbial Protein, Alantoin, Non Glucogenic Ratio (NGR), Ration Utilization Efficiency (EPR) , Net Protein Utilizatin (NPU) . Data were analyzed using analysis of variance and Duncan multiple range test was further used to test the significant differences. Results showed that rumen metabolism variables such as pH, N-NH3, VFA, Microbial Protein on ration consisted of cocoa pods bio fermented by *P. Chrysosporium* were increased (P<0,01) compared to the others. There was positive correlation between microbial protein and alantoin. Microbial protein and alantoin excreted to urine indicated that there was an increase of rumen microbe population, while NGR value had positive correlation with methane production (CH4). Ration containing cocoa pods bio fermented by *P. Chrysosporium* showed the lowest NGR as indicator for optimum ration utilization efficiency for animal growth. It was concluded that cocoa pods bio fermented by *Phanerochaete chrysosporium* Burdsall ATCC 34541 is potential to be used as forages replacing elephant grass.

Key words: Cocoa Pod, Bio fermentation, Phanerochaete chrysosporium Fungi, Rumen Metabolism

INTRODUCTION

The shortage of agricultural land and low quality of the forages and roughages, encourage us to use waste such as cocoa plantations (Theobroma cacao L.) as energy source for ruminant. In Indonesia, Cocoa Plant Area is almost 1.5 millions Ha in 2008 and it produced 75% Cocoa Pod as by product. Utilization of cocoa pods as mulch around plants can be a host for growth of fungus *Phytophthora palmivora* known as Black Pod Diseases (Awuah and Frimpong, 2002) which can disrupt the development of cocoa plants. This fungus causes late blight, leaf blight and the cancer stem in cocoa plants. Nutrient quality of cocoa pods is equal to elephant grass, with 53,3% of TDN (Aregheore, 2002). Cocoa pods is potential as forage sources for ruminant, which have energy sources such as hemicelluloses and cellulose. Cacao pods contain approximately 6,28% protein; 39,9% crude fiber; 1,61% crude fat; 82,84% NDF, 78,74% ADF and 35,27% lignin (Laboratory of Feed Science and Technology IPB, 2005). Inhibitor factor in utilizing cocoa pods as feedstuff is high water content (85%) and lignin and also contains alkaloid *theobromine* (Tequia et al. 2004).

Utilization cocoa pods as energy sources were requires decomposition of lignin with polysaccharide bond becomes a simple product. Ration in high lignin can decrease consumption, ration digestibility and animal performances. Benefit values of cocoa pods as energy sources for animal could be improved by degradation of lignocelluloses bonds with biofermentation or ammonization (Taherzadeh, 1999). Lignin degradation could be done with bioprocesses by the ligninolytic fungi such as *Phanerochaete chrysosporium* (Amjed *et al.*, 1990) and rumen bacteria (Akin and Benner, 1988). P. *chrysosporium* fungi is one of the microorganisms White-rot fungi that can degrade lignocelluloses (Takano *et al.*, 2004; Coulibaly *et*

al., 2003). Lignin degradation by fungi involved ligninolytic enzyme activity such as lignin peroxidase (LiP), mangan peroxidase (MnP), and laccase (Takano *et al.*, 1987). Fermentation of cocoa pods by P.*chrysosporium* is able to decrease 18.36% lignin content (Laconi, 1999). Digestibility and nutrient metabolism process depends on the amount of rumen microbe and it's enzyme activities. Volatile fatty acid (VFA) is end product of carbohydrate hydrolysis namely acetatic acid, propionic acid, and butyric acid. Energy and protein balance on ration is needed by microbes to synthesize protein microbe. This research was carried out to observe rumen metabolism variables on FH calves fed biofermentation cocoa pod using *P. chrysosporium* such as Total and Partial VFA Concentration, N-NH3 concentration, microbial protein, and urine allantoin and also to evaluate nutrition quality of ration containing biofermentation cocoa pod.

MATERIALS AND METHODS

Experimental Rations and Animals

Ration was designed iso-protein (17%) and iso-TDN (65%) used cocoa pod as forages (35%) and cocoa seed shell, cocoa powder and palm kernel meal were used as concentrate (65%). The treatments consisted of concentrate plus untreated cocoa pod (R1); 1.5% urea ammonia treated of cocoa pod (R2); silage of cocoa pod (R3); biofermented of cocoa using 3.5% rumen liquor (R4) and cocoa pod bio fermented by *P chrysosporium.* Five rations were used cocoa pod as a sources of forages (35%) and other cocoa by-product were used as concentrate fed on *in vivo* research of 5x5 latin square design five head of FH calves. Ration in pellet form and fed twice each day and *ad lib* drinking water.

Experimental Procedures

The experimental design was Latin square design on 5 head of FH calves (95-100 kg body weight) with 5 rations as treatment and 5 time period as replication. Each treatment had 20 days of preliminary and 10 days for data collecting. The variables measured were rumen metabolism variables such as pH, N-NH3 (Micro diffusion Conway Technique), VFA-Total (steam distillation technique), VFA-Partial (Gas Chromatography Technique) , microbial protein synthesis (SPM) by rate of incorporation ^{32}P tracer counting (Swandyastuti, 1986), urine allantoin (Larson, 1954), methane production by Non Glucogenic Ratio (NGR) approach and calves average daily gain (kg/day). While ration quality such as Biological Value (BV), ration utilization efficiency (EPR) and Net Protein Utilization (NPU) were calculated. Data were analyzed using analysis of variance and Duncan multiple range test was further used to test the significant differences (Steel and Torrie, 1980).

Partial VFA concentration was analized using gas chromatography techniques. Rumen liquor taken by stomach tube was filtered and 5 ml of this liquor was added 1 ml protein coagulant (metaphosphoric acid), centrifuged 10 000 rpm for 15 minutes on temperature 40^{0}C. Amount 1µ supernatant was injected into the gas chromatograph. The calculation of the partial VFA concentration rumen liquor using equation:

VFA-Partial (mM) = (Sample Area /Standard Area) x Fp x Standard Concentration

Analysis of allantoin urine (Larson, 1954), using phosphortungstic acid to deproteination. Phosphortungstic acid solution (1.5 g/5ml aquadest) was added 5 ml urine sample, centrifuged at temperature of 40^{0}C for 90 minutes until clear. Pb-acetate was added 5 ml and centrifuged, added again 5 ml of H_2SO_4 5%, centrifuged until homogenize. Amount of 2 ml homogenized sample was inserted into the Follin-Wu tube 100 ml volume, neutralized with 100 ml 5% NaOH pH 7.0. Folin ammoniacal copper added 2 ml and water bath heating for 10 minutes, cooled, add 2 ml molibdic acid and 2.4 --dinitro phenil hydrazine (2,4-DNPH), conducted reading by Spectrophotometer with 520 nm wave length. Allantoin standard solution created for the 1 mg compared with the standard. Calculation allantoin urine levels using equation:

Allantoin (mg/100 ml) = {(allantoin standard/alantoin sample) x 1 x 100/5}

RESULTS AND DISCUSSION

Cocoa pod contains lignocelluloses composed of celluloses and hemicelluloses are bound by lignin. Lignin contains potential energy, but very hard to revamped by rumen microbes, especially the aromatic ring solution. Improving the nutritional value of cocoa pod through the application of technology 1.5% urea

ammonization and bio fermentation with fungus *Phanerochaete chrysosporium* Burdsall ATCC 34,541 were significantly (P <0.01) decrease NDF, ADF and lignin and increasing crude protein and Beta-N (P < 0.05) (Laconi, 1999). Lowest lignin content of the cocoa pod bio fermentation *Phanerochaete chrysosporium* ATCC Burdsall was 31.66%. Cocoa pod biofermentation *P. chrysosporium* can break and soften the fiber cell walls of cocoa pod effectively, so that micro fibril ribbons can be easily digested by rumen microbes. Digestibility of high fiber rations needs cooperation among rumen microbes; higher fibrolytic activity of rumen fungi which can penetrate cell wall fiber rations and create access for rumen bacteria. Increasing nutrient digestibility gave implication that rumen microbial population are not disrupted, this showed that rations made from cocoa waste and palm kernel waste can provide good environment for rumen microbial growth in the rumen. Delignification can reduce lignin and increase the surface area of cell wall of high-fiber rations and easier penetration process.

Rumen Metabolism and Allantoin Urine

The increasing of rations fermentation can be done by providing a source of carbohydrate and nitrogen balance and sustainable in the rumen. The influence of cocoa pod processing treatment on rumen metabolism variables such as pH, N-NH3 concentration, total VFA concentration, synthesis of protein microbe, allantoin urin and gas non glucogenic ratio (NGR) are given in Table 1.

Result showed that rumen metabolism variables (NH3, VFA, Microbial protein) on ration consisted of bio-fermented cocoa pods by P. *chrysosporium* were increased (P <0.01), but rumen liquor pH in the normal range of 6.06 to 6.38, where cellulolytic microbes can live in the rumen (Jean-Blain, 1991). Dynamics concentration of ammonia and total VFA in rumen liquor illustrates effectiveness of the fermentation process. Concentration of ammonia ranged from 4.18–6.30 mM was lower than that recommended by Mc Donald *et al.* (2002). This reflects the fermentation process work better or protein in the ration difficult to be degraded in the rumen. Total VFA concentrations between treatments was significant different (P <0.01), ranged from 85.50-114.74 mM. This value is still within the range of VFA concentrations that support the optimum conditions of 60-120mM (Waldron *et al.*, 2002). Microbial protein synthesis (SPM) describes the contribution of microbial protein to the animal host. Rations with cocoa pod bio fermented by *P.chrysosporium* had the highest yield of microbial protein synthesis (SPM) values (520.44 g /d/ head) and urine allantoin 5.10 g/head. Allantoin is intermediate metabolite from rumen bacterial digestion in the small intestine. There was positive correlation between microbial protein and allantoin. Increase of microbial protein and allantoin excreted to urine as indicator that there was an increase of rumen microbial population. Blood sugar as the main energy source of organ function. The range of blood glucose concentration was 68.00-74.40 mg/100ml. It was still in normal category fulfilled energy sources required for normal function of animal organs. Concentration of total VFA reflects the balance of production rate and it's usage in the rumen. Partial Volatile Fatty Acid (P-VFA) concentration is influenced by the composition of the feed in the ration (Table 2).

Table 1. Rumen metabolism variables on various rations

Parameters	Treatments					P Values
	R-I Control	R-II Ammoniation	R-III Silage	RIV Silage of Rumen Content	R-V P. *Chrysosporium*	
Rumen Metabolism						
pH Rumen	6.06	6.26	6.21	6.15	6.38	NS
N-NH_3 (mM)	4.69^{b}	6.30^{b}	4.18^{b}	4.84^{b}	5.90^{a}	0.01
T- VFA (mM)	85.50^{b}	120.62^{a}	90.23^{b}	102.77^{b}	114.74^{a}	0.01
Protein Microbe (g/h) (SPM)	253.23^{b}	298.90^{b}	317.5^{b}	330.54^{b}	520.44^{a}	0.01
Allantoin (g/h)	3.32^{bc}	3.98^{b}	3.69^{bc}	2.85^{c}	5.10^{a}	0.01
Non Glucogenic Rasio (NGR)	3.23^{b}	3.15^{b}	3.69^{ab}	4.44^{a}	2.86^{b}	0.05
Blood Glucose (mg/100 ml)	68.80^{c}	91.80^{a}	67.80^{c}	68.00^{c}	78.40^{b}	0.05

Note: Different superscript in the same row indicates significantly different (P <0.05) and (P <0.01).
RI = 65% concentrate +35% Cocoa Pod; R-II = 65% concentrate +35% Cocoa Pod Urea Ammonization ; R-III = 65% concentrate +35% Silage Cocoa Pod; R-IV = 65% concentrate +35% Cocoa pod bio fermentation Rumen liquor; and RV = 65% concentrate +35% Cocoa Pod bio fermentation *P chrysosporium* Fungi.

Table 2. Total and Partial Volatile Fatty Acid (P-VFA) concentration at various ration

Parameters	Treatments					P Values
	R-1 Control	R-II Ammonia tion	R-III Silage	RIV Silage of Rumen Content	R-V P. *Chrysosporium*	
T- VFA (mM)	85.50[b]	120.62[a]	90.23[b]	102.77[b]	114.74[a]	0.01
VFA Partial (mM)						
Acetate (C2)	63.31[b]	73.86[a]	69.71[a]	69.51[b]	72.50[b]	0.05
Propionate (C3)	22.51[b]	26.59[a]	22.98[b]	19.69[b]	**29.34[a]**	0.01
Butyrate (C4)	5.10	5.25	5.74	6.09	5.05	NS
Ratio C2/C3	2.84[b]	2.80[b]	3.24[ab]	3.88[a]	2.54[b]	0.05

Table 3. Nitrogen retention and ration quality of various treatments

Parameters	Treatments					P Values
	R-I Control	R-II Ammoniation	R-III Silage	R-IV Silage of Rumen Content	R-V P. *Chrysosporium*	
Nitrogen Retention (g/kg BB $^{0.75}$/h)	1.06[b]	1.45[a]	1.12[b]	1.16[b]	**1.60[a]**	0.01
Ration Quality						
Ration utilization efficiency (EPR)	0.17[b]	0.31[a]	0.20[b]	0.15[b]	**0.29[a]**	0.01
Biological Values ,BV (%)*	97.03	96.62	96.59	96.90	96.11	NS
Utilization Protein Net, NPU (%)**	40.58[b]	50.14[ab]	41.38[b]	42.99[ab]	**53.03[a]**	0.05
Average Daily Gain (kg/h)	0.76[b]	1.56[a]	0.94[b]	0.75[b]	1.46[a]	0.01

Ration Quality

Propionic acid concentration increased in rations containing cocoa pod bio fermented by *P. chrysosporium*, whereas the C2/C3 ratio was not significantly different with the control (P >0.05). Ration fermentation system in the rumen that leads to the synthesis of propionate which use many H2 gas will influence available free H2 gas and reduce formation of methane (CH4) gas. Reduction non glucogenic ratio as an indicator decrease production of methane gas (CH4). NGR values have positive correlation with production of methane gas (CH4). Non Glucogenic Ratio (NGR) of ration with cocoa pod bio-fermented by *P. chrysosporium* was the lowest (2.26) but it is still in the range of 2.25-3.00. It was an indicator of optimum utilization efficiency of ration on growing period of FH calves. Ration with cocoa pod biofermented by *P. chrysosporium* can increase microbial protein synthesis as a contribution to the host protein and propionic acid synthesis. Propionic acid is a precursor formation of muscle meat. NGR value was the lowest (2.86) obtained in the range 2.25-3.00 as for growth and fattening cattle. Ration quality of various treatments are presented in Table 3.

The indicator of rations protein quality is reflected by biological value (BV). Application of processing cocoa pods as a forage sources did not significant affect biological value, but significantly (P<0.05) increased net protein utilization (NPU) and ration utilization efficiency (EPR). Ration contained the cocoa pod bio-fermented by *P. chrysosporium* had the highest value of NPU (53.30%) and EPR (0.29). In management of livestock production, EPR value as the basis of the decision making, greater value of the EPR would be advantageous because it can reduce feed costs as the biggest cost component in production of livestock.

CONCLUSION

Cocoa pod biofermented by *Phanerochaete chrysosporium* Burdsall ATCC 34541 is potential used as forage sources replacing elephant grass and other by product of cocoa and palm kernel oil as concentrate for fed calves in growing period.

Another reason, method of enzyme production in this study may be suitable for *Aspergillus niger* rather than for *Trichoderma spp.* Further experiment is needed to prove this rationale.

Since the pattern of weight gain of broilers fed different enzyme treatments did not correlate with their intake, this may indicate that the factor which is mostly responsible for weight gain is feed digestibility. Sundu *et al.* (2006) found that dry matter, protein and lipid digestibilities were improved due to enzyme supplementation in copra meal based diets. In this current study, broilers fed diet supplemented with coprase produced by Aspergillus niger ate less feed but produce higher body weight. Therefore, their feed conversion ratio was much lower than those feed without any coprase suppmentation (1.58 vs 2.04).

CONCLUSIONS

Addition of 30% copra meal in the diet deteriorate feed quality and thus impairs the performance of birds. Enzyme "coprase" produced by *Aspergillus niger* made a large improvement in body weight gain of birds and reduced feed conversion ratio.

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