J. Indonesian Trop. Anim. Agric.
39(3):167-174, September 2014
ISSN 2087-8273

EFFECT OF FERMENTED CACAO POD SUPPLEMENTATION ON SHEEP RUMEN MICROBIAL FERMENTATION

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Received April 25, 2014; Accepted July 28, 2014

ABSTRAK

Tujuan penelitian ini untuk meningkatkan nilai manfaat pod kakao sebagai bahan pakan domba yang pemberiannya mencapai 50% dari total pakan. Penelitian ini terdiri dua tahap. Tahap 1 adalah fermentasi pod kakao. Rancangan acak lengkap pola faktorial 3X3 digunakan pada tahap ini. Faktor I adalah dosis inokulum mikrobia 0%, 0,05% dan 0,1%. Faktor II adalah lama pemeraman 0, 3, dan 6 hari. Hasil penelitian menunjukkan perlakuan lama fermentasi enam hari dengan dosis inokulum mikrobia 0,05% dapat menurunkan NDF, ADF dan theobromin pod kakao. Dosis inokulum dan waktu fermentasi terbaik dari percobaan tahap 1 diaplikasikan pada penelitian tahap 2. Penelitian tahap 2 adalah percobaan fermentasi mikrobia rumen. Penelitian ini menggunakan rancangan bujur sangkar latin 3X3. Pada periode I domba diberi pakan perlakuan CF0 (*complete feed* tanpa fermentasi), periode II: CF1 (*complete feed* mengandung pod kakao fermentasi), dan periode III: CF2 (*complete feed* fermentasi berbasis pod kakao). Hasil penelitian menunjukkan nilai pH cairan rumen domba perlakuan CF0, CF1 dan CF2 masih dalam kisaran pH normal, dan tidak berpengaruh terhadap asam lemak volatil (VFA) dan amonia. Kesimpulan penelitian ini adalah pemberian *complete feed* yang mengandung pod kakao sampai 50% pada domba, baik difermentasi maupun tanpa fermentasi tidak mempengaruhi proses fermentasi mikrobia rumen.

Kata Kunci: pod kakao, fermentasi, complete feed, fermentasi mikrobia rumen, domba

ABSTRACT

The objective of this research was to improve beneficial value of cacao pod as sheep feed ingredients comprising up to 50% total feed. This research was conducted in two stages. Stage 1 was cacao pod fermentation. Completely randomized design with 3x3 factorial patterns was used in this stage, in which factor I was microbial inoculum dosage of 0%, 0.05% and 0.1% and factor II was incubation period of 0, 3 and 6 days. Result demonstrated that six-day fermentation with 0.05% microbial inoculum could lower cacao NDF, ADF and theobromine. The optimum inoculum dosage and fermentation time from stage 1 was applied to stage 2. Stage 2 was rumen microbial fermentation test. This research administrated 3x3 of latin square design. In period I sheep were fed with CF0 (non-fermented complete feed), in period II sheep were given CF 1 (complete feed containing fermented cacao pod) and in period III sheep were given CF2 (fermented complete feed based cacao pod). Result demonstrated that pH value of sheep microbial liquid in treatment of CF0, CF1 and CF2 was in normal pH range and did not affect volatile fatty acids (VFA) and ammonia. In conclusion, supplementing up to

50% of feed with complete feed containing fermented or non-fermented cacao pod did not affect the process of rumen microbial fermentation.

Keywords: cacao pod, fermentation, complete feed, rumen microbial fermentation, sheep

INTRODUCTION

Indonesia produces considerable amount of cacao. In 2012 cacao production reached 740,513 thousand tons with 3.8% average annual growth (Pusat Data dan Sistem Informasi Pertanian, 2013). Cacao pod is the biggest part of cacao fruit comprising 75% (Syamsu and Ishak, 2002). Cocoa pod is one of the major waste of agro-industry is the potential to be used as animal feed ingredients which are cheap. Nutrient content of the cocoa pod consists of 8% crude protein (CP), 40% crude fiber (CF), 50.8% total digestible nutrients (TDN), and its use by ruminants 30-40% (BPPT Sulawesi Selatan, 2008).

Utilizing cacao pod as feed is limited by anti-nutrition substance or alkaloid named theobromine (3,7–dimethylxantine). Theobromine in cacao pod can reduce feed consumption and overconsumption of theobromine may induce health disorder in sheep. Directly fed cacao pod to sheep will lower body weight because high lignocellulose causes low cacao pod digestibility. Cellulose and hemicellulose content in cacao pod are 35% and 11%, respectively (Alemawor *et al*., 2009). The relatively safe, applicable and environmental-friendly way to improve cacao pod quality is through biological treatment namely microbial inoculum fermentation.

The recently widespread additive fermentation for waste-based complete feed is microbial inoculum. Vast microbial diversity in Indonesia provides high probability of finding potential isolate to develop as inoculum in fermentation process. The objective of this research was to study microbial inoculum utilization in form of microbial composites such as cellulosic microbe, lactic acid and amylolytic to cope with theobromine anti-nutrition and cacao pod crude fiber and to examine the effect of administering fermented complete feed (cacao pod as raw material) on sheep rumen microbes.

MATERIALS AND METHODS

Cacao Pod Fermentation.

Cacao pod was obtained from plantation in Patuk, Gunungkidul Regency, Yogyakarta. Microbial inoculum of *Saus Burger Pakan* (SBP®) was produced by CV. Agro Indo Utama, Yogya. Molasses used was 2% of feed.

Eight hundreds g of sterilized ground cacao pod was thoroughly mixed with microbial inoculum (activated in solution molasses) according to treatment dosage, then it was added with sterilized aquadest to make up 40% water content. Dosage molasses was 2% of total cacao powder. The mixture were placed in mini silo (1 kg of sterile bottle), compressed to achieve the anaerobic conditions, measured, tightly sealed then was fermented.

Experimental design used was 3x3 Factorial completely randomized design. Factor I was inoculum microbial dosage, namely 0 mL/100g; 0.05% = 0.05 mL/100g and 0.1 = 0.1 mL/100g as feed. Factor II was fermentation period, that was 0, 3, and 6 days. Each treatment comprised three replicates. Data were tested by using analysis of variance, then it was followed by Duncan test for different result (Astuti, 2007). The observed parameters were fiber composition change (neutral detergent fiber/NDF, acid detergent fiber /ADF) and theobromine degradation. The optimum inoculum dosage and fermentation period at this stage was applied to cacao pod as raw material of complete feed and to observe the effect on rumen microbial fermentation.

Rumen Microbial Fermentation Test

Three rumen fistulated local male sheep (averaged about 33 kg) were used in the study. The ingredient of complete feed were elephant grass and cacao pod as fiber source. The other ingredients were rice bran, pollard, soybean meal and microbial inoculum (SBP®).

The experiment was conducted in 3x3 latin square design with three treatment feeds and three fermentation periods as replicates. In Period I sheep were given CF0 feed treatment or 50% non-fermented complete feed consisted of non-fermented cacao pod + 40% of concentrate + 10% of elepahant grass; in Period II sheep were given CF1 or fermented cacao pod-contained complete feed consisted of 50% of fermented cacao pod + 40% of concentrate + 10% of elephant grass, and in Period III sheep were given CF2 or cacao pod-based silage complete feed as a the fermentation

result of 50% of cacao pod + 40% of concentrate + 10% of elephant grass.

Feed was given for 10 days on each period, including adaptation period. Water was provided daily. The pH was determined after extracting rumen contents. The sample was filtered by using four-layer lint to measure rumen ammonia (NH_3) and volatile fatty acids (VFA) concentration. Each sample was placed in 25ml plastic bottle and stored in -5^oC freezer until NH_3 and VFA were analyzed. The obtained data were subject to analysis of variance and different result was followed by Duncan test (Astuti, 2007). The observed parameters comprised acidity (pH) (AOAC, 2005), carbohydrate metabolism: total VFA concentration (Doreau *et al.*, 1993), protein metabolism: N-NH_3 level (Chaney and Marbach, 1962).

RESULTS AND DISCUSSION

Conversion of Cacao Pod Fiber Composition during Fermentation

Cell wall components can be determined for instance by calculating NDF content (Jancik *et al*., 2008) in which the main components of NDF are cellulose, hemicellulose and lignin (Van Soest *et al*., 1991). The result demonstrated that treatment dosage and fermentation period significantly affected NDF content, and interaction was observed (Table 1). The lowest fiber content was in 0.5% microbial inoculum dosage with 6-day fermentation, shown by low NDF value of 54.9%. the highest NDF value or 71.2% was at 0% inoculum dosage with 0-day fermentation

It showed that cell wall restructuring microbial activity was still in effect after three days, while double dosage addition (0.1% at 40% KA) could decrease NDF because double dosage of microbial inoculum had failed to significantly increase the number of bacteria. Result of Wulandari *et al.* (2014) demonstrated that the total bacteria in fermented cacao pod with additional microbial inoculum was $7.6x10^4$ cfu/g became $5.8x10^4$ cfu/g for 0.05% and 0.1% dosage, respectively. Total bacteria consisted of cellulosic bacteria and lactic acid bacteria (LAB). Total bacteria needed for minimum anaerobic fermentation was $1.6x10^5$, consisted of $1.16x10^5$ cfu/g cellulosic bacteria (McDonald *et al*., 1991) and 1.0x105 cfu/g LAB (Kung, 2001). The average NDF content in cacao pod was more than 35% and therefore belongs to the first class feed (Hartadi *et al*., 2005).

ADF main components are cellulose and lignin. Cellulosic bacteria in microbial inoculum added in fermented cacao pod could degrade cacao pod cell wall as shown in Table 2. The result of ADF analysis demonstrated that 0.05% of microbial inoculum could lower cacao pod ADF from 42.4 to 39.1% DM (dry matter) due to the cellulase enzyme produced by cellulosic bacteria developing during fermentation. According to Lin *et al*. (2012), cellulase enzyme is a complex enzyme that gradually cuts intermolecular at β-1,4-glycosidic bond, cutting cellulose chain into cellobiose units, then cutting cellobiose and oligosaccharide, producing glucose.

Theobromine Degradation during Cacao Pod Fermentation

Result showed that fermentation period

Table 1. NDF Content in Fermented Cacao Pod (%DM)

Fermentation Period (day)	Microbial Inoculum Dosage (%)			Mean	SEM
	0	0.05	0.1		
0	71.2^a	58.1^{bc}	60.1^{bc}	63.1^a	2.28
3	70.3^a	55.4^{bc}	60.7^{bc}	62.1^a	2.35
6	56.3^b	54.9^c	61.3^b	57.5^b	1.31
Mean	65.9^a	56.2^c	60.7^b		
SEM	2.59	1.24	0.65		

^{abc} Different superscript in the same column and line show significantly difference (P≤0.05).

Table 2. ADF Content in Fermented Cacao Pod (%DM)

Fermentation Period (day)	Microbial Inoculum Dosage (%)			Mean	SEM
	0	0.05	0.1		
0	43.3^{ab}	38.8^{bc}	40.0^{abc}	40.7	0.88
3	43.9^{a}	40.2^{abc}	38.6^{bc}	40.9	0.99
6	40.1^{abc}	38.2^{c}	43.7^{a}	40.7	1.22
Mean	42.4^{a}	39.1^{b}	40.8^{ab}	40.7	
SEM	1.01	0.82	0.93		

^{abc} Different superscript in the same column shows significantly difference (P≤0.05).

Table 3. Theobromin Content in Fermented Cacao Pod (ppm)

Fermentation Period (day)	Microbial Inoculum Dosage (%)			Mean	SEM
	0	0.05	0.1		
0	116.5	127.0	128.1	123.9^{a}	4.07
3	116.5	108.9	122.8	116.1^{a}	3.59
6	108.2	101.8	98.4	102.8^{b}	3.89
Mean	113.8	112.6	116.4		
SEM	4.55	5.70	5.94		

^{ab} Different superscript in the same column showssignificantly difference (P≤0.05).

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significantly affected theobromine content in fermented cacao pod as presented in Table 3. Six-day fermentation decreased 17.02% theobromine from 123.88 ppm at early fermentation to 102.79 ppm after six days. Bacteria needed carbohydrate resources or simple sugar probably resulted from cacao pod degradation to stimulate theobromine degradation. Accordingly, optimum theobromine degradation required proper period. Gokulakrishnan *et al*. (2006) reported that sucrose in media will stimulate the activity of caffeine and theobromine degrading enzyme from *Pseudomonas sp.* bacteria. Lactose enables disaccharide, comprising galactose and glucose, to degrade caffeine up to more than 90%.

Temperature raise during fermentation was likely to affect theobromine degradation causing theobromine content decrease. It was similar to theobromin degradation due to boiling or drying heat. Sukha (2003) stated that cacao pod is harmless feed, in which theobromine is removed by 1.5 of boiling or heating. From the high amount of theobromine degradation, it was observed that to lower amount of theobromine in fermented cacao pod containing 40% water was 6-days of fermentation.

Result of period I demonstrated that cellulosic bacteria from microbial inoculum growing in fermented cacao pod was capable of degrading fiber and lowering theobromine. The optimum and economical dosage of microbial inoculum was 0.05% with 6-days of fermentation, then applied to cacao pod as complete feed raw material in the next analysis stage.

Rumen Microbial Fermentation

Acidity (pH) of rumen liquid

Result of pH measurement is presented in Table 4. Composite result of rumen liquid extraction for 24 hours showed that the three

Table 4. pH Value and Total VFA Concentration, NH_3 (Amonia), Sheep Rumen Liquid Fed on Ration with 50% Cacao Pod

24 Hour Composite	Treatment		
	CF0	CF1	CF2
pH	$6.4^{ab} \pm 0.03$	$6.3^{b} \pm 0.06$	$6.5^{a} \pm 0.06$
VFA^{ns}	128.3 ± 78.31	127.4 ± 6.07	129.7 ± 29.90
NH_3 /amonia^{ns}	6.4 ± 3.92	6.4 ± 0.30	6.5 ± 1.49

^{abc} Different superscript in the same column and line show significantly difference (P≤0.05).
^{ns} non-significant

treatments (CF0, CF1 and CF2) demonstrated significantly different in pH. The CF1 had the lowest pH value because the administered cacao pod was priorly fermented, therefore more cellulose degradation occurred as shown by the declining NDF and ADF content in cacao pod (Table 1 and Table 2) and being more fermentable in rumen into organic acids like VFA and lactic acid. Cellulase enzyme of microbial rumen would degrade cellulose into glucose. LAB would fermented glucose into lactic acid and affected pH decline (Wulandari *et al.*, 2014).

The average pH value of 24 hour rumen liquid composite in CF0, CF1 and CF2 was 6.4 ± 0.03; 6.3 ± 0.06 and 6.5 ± 0.06, respectively, or within normal range for sheep pH value. Jasmin *et al.* (2011) stated that normal sheep rumen pH ranges from 6.4 to 6.8, therefore, pH less than 5.5 or more than 7 is considered as abnormal pH. It proved that complete feed containing 50% DM fermented or non-fermented cacao pod supplementation can be administered to sheep without interrupting rumen fermentation.

Observation result on rumen liquid pH kinetics is presented in Figure 1 showing that rumen pH decrease after feed was given to all treatments. The pH decrease in CF1 was significant particularly within 2 to 3 hours post feeding (10 and 11 a.m.), that was 6.0 ± 0.08 and 5.8 ± 0.26, respectively. Rumen liquid was acid because during 1-3 hour post feeding the CF1 containing the fermented cacao pod was digested more quickly and produced organic acids like VFA. The pH less than 5.5 would be acidotic without clinical symptoms (Jasmine *et al*., 2011). The pH kinetics of rumen liquid in CF0 and CF2 was in normal bacterial range (6-7); accordingly,

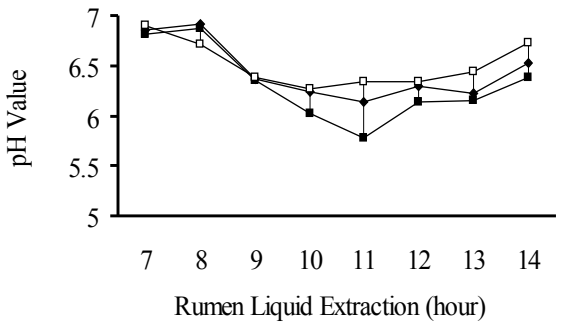

Figure 1. The pH Kinetics of Sheep Rumen Liquid Fed on Ration with 50% Cacao Pod. The symbols represent CF0= non-fermented complete feed (♦), CF1= complete feed containing fermented cacao pod (■), and CF2= fermented complete feed (□).

it did not interrupt rumen fermentation process. Conducive rumen environment would support the maximum microbial growth (Uhi *et al*., 2006).

Carbohydrate Metabolism: Total VFA

Effect of non-fermented (CF0) or fermented (CF1 and CF2) cacao pod-based complete feed on rumen liquid VFA is presented on Table 4. Result demonstrated that utilizing of cacao pod based-complete feed in CF0, CF1 and CF2 showed non significantly different result towards VFA concentration of sheep rumen liquid. The average VFA concentration (Mmol) in all treatments from rumen liquid composite extracting for 24 hours were 128.3 ± 78.31 (CF0), 127.4 ± 6.07 (CF1) and 129.7 ± 29.90 (CF2), showing optimal VFA

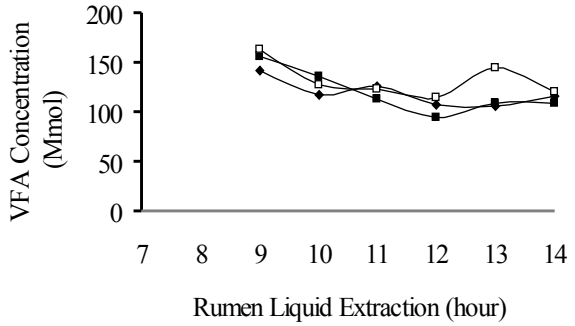

Figure 2. VFA Kinetics Concentration of Sheep Rumen Liquid Fed on Ration with 50% Cacao Pod. The symbols represent CF0= non-fermented complete feed (♦), CF1= complete feed containing fermented cacao pod (■), and CF2= fermented complete feed (□).

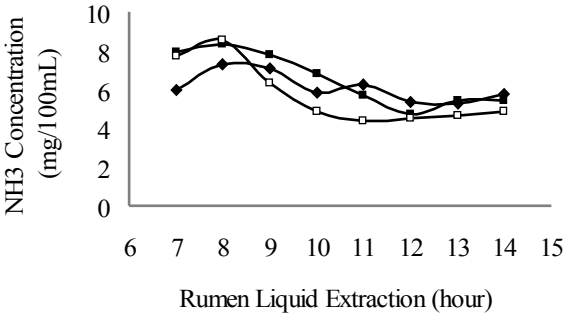

Figure 3. Amonia (NH_3) Kinetics Concentration of Sheep Rumen Liquid Fed on Ration with 50% Cacao Pod. The symbols represent CF0= non-fermented complete feed (♦), CF1= complete feed containing fermented cacao pod (■), and CF2= fermented complete feed (□).

for microbial rumen activity. Sufficient VFA concentration for rumen microbial growth is 80 - 180Mmol (Fathul and Wajizah, 2009).

Kinetic of VFA concentration is presented in Figure 2. It showed a fast increase after feeding due to fast fermentation rate as a result of high soluble carbohydrate in feed either in form of additional molasses in complete feed (in CF0, CF1 and CF2) or product of fermented anaerobe restructuring with microbial inoculum addition (CF1 and CF2). However, CF2 (fermented complete feed with additional microbial inoculum) tended to produce the highest VFA. It showed that CF2 had the highest fiber digestibility due to silage process with additional microbial inoculum containing cellulosic bacteria. The final result of carbohydrate fermentation among which was VFA (McDonald *et al*., 2002).

VFA rumen liquid decreased in kinetics concentration was due to cell-wall absorption. Saqifah *et al*. (2010) reported that a part of VFA was produced in rumen diminished from ruminal wall absorption.

Protein Metabolism: NH_3

Ammonia in rumen liquid was the sign of protein degradation entering the rumen and protein synthesis process by rumen microbes, half of which would be degraded by protolithic enzyme of rumen microbes. Concentration of rumen ammonia extracted periodically within 24 hours and rumen liquid composite are presented in Table 4.

Result showed no significant difference between CF0, CF1 and CF2, in which ammonia concentration from composite were 6.4 ± 3.92; 6.4 ± 0.30 and 6.5 ± 1.49 mg/100mL, respectively. It proved that ammonia concentration of rumen liquid in the treatments of either fermented or non-fermented cacao pod-based complete feed was still within normal range for rumen microbial growth. According to Satter and Slyter (1973), rumen microbial growth requires 4 - 8mg/100mL ammonia concentration. Ammonia concentration reflected protein fermentability level in rumen (Fathul and Wajizah, 2009).

Figure 3 shows kinetics change in ammonia concentration (NH_3), in which the highest ammonia occurred one hour post-feeding in CF0, CF1 and CF2, that were 7.3 ± 3.52; 8.4 ± 1.88; and 8.5 ± 3.60 mg/100mL, respectively. This was due to protein feed degradation by protease rumen microbes where protein feed was degraded into amino acid and ammonia, then ammonia product would be reused by rumen microbes for its growth. According to Arora (1995), utilizing ammonia by microbial rumen was ever increasing up to 8.5 mg/100 mL.

Result of research stage II showed that cacao pod supplementation (as complete feed) up to 50% feed either fermented or non-fermented was utilizable for sheep without disrupting rumen fermentation process. VFA and ammonia concentration were in normal range for rumen microbial growth.

CONCLUSION

Saus Burger Pakan (SBP) microbial inoculum was viable fermenter to improve digestibility and reduction of cacao pod theobromine with optimum dosage of 0.05% within six day incubation with 40% water content. Feeding complete feed containing 50% cacao pod for sheep did not affect the rumen microbes' fermentation process. VFA and ammonia concentration was within normal range for microbial growth.

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