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Digestibility and Methane Emission of Ration Based on Oil Palm By Products Supplemented with Probiotics and Banana Stem: An *In vitro* Study

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Abstract: The objective of the present study was to investigate feed digestibility and methane emissions of ration based on oil palm by-products on addition of probiotics, namely *Acetoanaerobium noterae* and *Saccharomyces cerevisiae* and banana stem *in vitro*. The substrate, i.e., oil palm by-products consisted of oil palm midrib, oil palm leaf, oil palm kernel cake and oil palm sludge in the ratio of 30, 30, 30 and 10%, respectively. The following treatments were tested: control (R0), R0+S. *cerevisiae* (R1), R0+A. *noterae* (R2), R0+S. *cerevisiae*+A. *noterae* (R3), R0+ banana stem (R4), R0+banana stem+S. *cerevisiae* (R5), R0+banana stem+A. *noterae* (R6) and R0+banana stem+S. *cerevisiae*+A. *noterae* (R7). The treatments were incubated *in vitro* with buffered-rumen fluid in four replicates (represented by three incubation units per replicate), conducted for 48 h at 39°C. Gas production and methane emission were measured at regular time point intervals. After the incubation, digestibility, Volatile Fatty Acids (VFA), ammonia and microbial counts were determined. Results showed that the highest dry matter digestibility was shown by R5 and the best reduction of methane emission was shown by R2 at 12 h of incubation. In conclusion, supplementation of probiotics did not affect the digestibility of ration based on oil palm by-products but A. *noterae* addition was potential to mitigate ruminal methane emission.

Key words: Acetogen, yeast, banana, methane, digestibility, rumen

INTRODUCTION

One of the causes of low ruminant productivity is due to insufficient nutrient intake. Provision of high-quality forages such as grasses and legumes is limited by land competition for various developmental reasons. Therefore, an opportunity to provide forage for livestock development is through utilization of agricultural by-products as animal feeds. Agricultural by-products, however, in general have a low quality which is characterized by high fiber and low protein contents. Such characteristics are found in oil palm by-products which highly available in Indonesia as the biggest producer country worldwide. Typical by-products from oil palm plantation are oil palm midrib, oil palm leaf, oil palm trunk, oil palm frond, empty fruit bunch, oil palm sludge and palm kernel cake. High fiber diet does not only lower feed utilization efficiency and livestock productivity, but also increases the emission of methane as a green-house gases. It has been reported that formation of methane in the rumen causes a loss of digestible energy for about 8-14% (Cottle *et al.*, 2011). Challenges of low productivity and negative impact of methane emissions of ruminant livestock should be answered with innovation technology that improves feed

management system. A promising approach is through optimization and manipulation of rumen microbial ecosystem, for instance, by using probiotics. A species of microbe that has been repeatedly used as probiotics and had been reported to increase feed digestibility, feed conversion and livestock productivity is yeast or *Saccharomyces cerevisiae* (Wina, 2000; Ando *et al.*, 2004). Apart from that, a class of microbes namely acetogen has been attempted to reduce ruminal methane emissions. Acetogen has the ability as methanogenesis inhibitor by using hydrogen to form acetate in the rumen (Lopez *et al.*, 1999; Fonty *et al.*, 2007). Thalib *et al.* (2008) isolated an acetogen species namely *Acetoanaerobium noterae* from the rumen of deer and such acetogen was shown to effectively inhibit methanogenesis under experimental environment. It would be interesting to observe the effect of a simultaneous addition of *S. cerevisiae* and *A. noterae* on methanogenesis and rumen fermentation. Further, supplementation of phytochemicals such as saponins and tannins were reported to decrease methane emissions in the rumen (Thalib *et al.*, 2004; Jayanegara *et al.*, 2013, 2014). Tropical plants generally contain high levels of plant secondary compounds

(Jayanegara *et al.*, 2011). A plant species that contain a considerable amount of tannins and saponins is the banana plant (*Musa paradisiaca*) (Wina, 2001).

Despite administration of probiotics or plant secondary compounds has been performed, however, the application is conducted separately; limited studies have attempted to observe their effects on methane emissions and rumen fermentation when being added simultaneously for assessing their possible interaction. Therefore, this study was aimed to investigate the effects of simultaneous addition of probiotics (*S. cerevisiae* and *A. noterae*) and phytogetic compounds (from banana stem) on methane emissions and rumen fermentation of ration based on oil palm by-products as performed *in vitro*.

MATERIALS AND METHODS

Sample preparation and chemical analysis: The substrate, i.e., oil palm by-products consisted of oil palm midrib, oil palm leaf, oil palm kernel cake and oil palm sludge in the ratio of 30, 30, 30 and 10%, respectively. Oil palm midrib and leaves were taken from at least three different trees, chopped, oven-dried at 50°C for 24 h and ground finely to pass 1 mm sieve. Ground samples from different trees were mixed homogeneously. Subsequently, they were mixed homogeneously with oil palm kernel cake and oil palm sludge. Samples were analyzed by the standard procedures of AOAC (1997) and Van Soest *et al.* (1991) for proximate composition and fiber fraction, respectively. Supplements given in this study were banana stem, *S. cerevisiae* and *A. noterae*. Banana stem used was from the Ambon variety banana (*M. paradisiaca*) that freshly harvested. The banana stem samples were taken from three different trees. Each tree was chopped, oven-dried at 50°C for 24 h, ground to pass 0.5 mm sieve and mixed homogeneously. Samples were analyzed for their phytogetic compounds and mineral contents. Tannins were determined according to Makkar (2003) and saponins were analyzed according to Hiai *et al.* (1976) by using a spectrophotometer (UV-Spectrophotometer U-1800-5930482, High-Technologies Corporation, Tokyo, Japan). *Saccharomyces cerevisiae* was grown in Potato Dextrose Broth (PDB) liquid medium, while *A. noterae* was grown in a liquid medium consisting of basal medium, vitamins and trace element solutions according to the procedures of Ogimoto and Imai (1980). *In vitro* incubation: *In vitro* incubation was performed by following the method of Theodorou and Brooks (1990). One gram of substrate and 100 mg of banana stem (1 mg/mL incubation medium), according to the experimental treatments, were added into each incubation tube. Incubation medium for each tube contained 90 mL of buffer solution (86 mL of basal solution and 4 mL of reducing agent) and 10 mL of rumen fluid. One liter of incubation medium was made of 860 mL basal solution (0.5 mL micro minerals, 200 ml bicarbonate buffer, 200 mL macro minerals, 2 mL

rezazurine 0.1% and 457.5 mL distilled water), 40 mL reducing agent and 100 mL rumen fluid. Rumen fluid was taken from three Etawa crossbred goats for each incubation run by stomach tube method before morning feeding. Goats were fed with grass (*ad libitum*), sugarcane leaf silage (300 g/goat/day) and a commercial concentrate (300 g/goat/day). After the collection, rumen fluid was taken to the laboratory, filtered through a nylon sieve and mixed with the buffer solution (100 mL rumen fluid+900 ml buffer solution). Incubation medium was saturated with CO₂ for 10 min to ensure the anaerobic condition, then put as much as 100 mL in each incubation tube (containing 1 g substrate and 100 mg banana stem, according to the treatments). Liquid preparation of *A. noterae* and *S. cerevisiae* was added 1 mL/tube (containing 10⁹ and 10⁶ cfu/mL, respectively) according to the treatments. The incubation tubes were closed immediately and put in a water bath incubator (39°C) and incubated for 48 h.

The treatments given in this study were probiotic supplementation (Factor A) and supplementation of banana stem (Factor B). The treatments were: control (R0), R0+*S. cerevisiae* (R1), R0+*A. noterae* (R2), R0+*S. cerevisiae*+*A. noterae* (R3), R0+banana stem (R4), R4+*S. cerevisiae* (R5), R4+*A. noterae* (R6) and R4+*S. cerevisiae*+*A. noterae* (R7).

Gas production and methane emission were measured at regular time point intervals using the method of Fievez *et al.* (2005). Gas was taken using a gas syringe (Sigma-Aldrich Z314382-1EA, Poulten and Graf GmbH, Wertheim, Germany) and it was flowed into an Erlenmeyer flask (1 l capacity) containing 6 N NaOH solution, connected to another syringe for estimating the volume of methane gas. The gas volume in the first syringe indicated the total gas volume. Carbon dioxide was trapped in NaOH solution, leaving out the methane gas into the second syringe.

Post fermentation analyses: Determination of dry matter digestibility was conducted after 48 h of incubation. The content of each *in vitro* tube was filtered using sinterglass and vacuum. The incubation residue was heated at 105°C for 24 h to calculate the dry matter digestibility (DMD) and organic matter digestibility (OMD). Supernatant was sampled for the analysis of VFA (volatile fatty acids), ammonia (NH₃) and the population of bacteria and protozoa.

Concentration of individual VFA was analyzed by Gas Liquid Chromatography (GLC Scion Bruker 436-GC, Bruker Daltonik GmbH, Bremen, Germany) using a column (BR-Wax fame, mmID 0.32, 0.25 mL df) and FID detector. An amount of 2 mL supernatant obtained from the *in vitro* incubation was added with 3 mg of sulfosalicylic acid dihydrate, centrifuged for 10 min at 12,000 rpm with a temperature of 7°C and then injected into the GLC column. Quantification of individual VFA was done by comparing it with the external standard

(known concentration of each VFA). Individual VFA unit was expressed in mmol/l and converted as a proportion to total VFA. Total VFA and total iso-VFA were obtained through the sum of each individual VFA.

Ammonia concentration was analyzed according to the method of Conway (1950). Briefly, boric acid (3%) was put into the center area of Conway apparatus as much as 3 mL and then added 1 drop of bromocresol green (BCG) and red methyl indicator solution. A total of 1 mL of NaOH and rumen fluid were put into each different area (either left or right) of the Conway apparatus. The apparatus was closed and then shaken gently to mix the NaOH and the rumen fluid. The sample was left for 24 hours and titrated with HCl until the boric acid color was changed to reddish yellow. The concentration of NH₃ was calculated by multiplying the HCl volume that was required for titration with HCl concentration and a dilution factor.

Protozoa population was counted by using counting chamber method and bacteria population was determined by the roll tube procedure (Ogimoto and Imai, 1980). A total of 4.5 ml of methyl green formal saline solution (MFS) was added into a test tube and added 0.5 mL of rumen fluid. Samples were vortexed, added into the counting chamber and the population was calculated directly by using a microscope. Protozoa population was the result of multiplying with the dilution factor. For total bacteria count determination, a total of 0.5 ml of rumen fluid was diluted 7 times and then grown on Rumen Fluid Glucose Cellobiose Agar (RGCA) medium and incubated for 21 days. Bacterial population was calculated manually by marking the colonies that formed on day 5, 14 and 21 of incubation. The calculation result was multiplied with the dilution factor.

Statistical analysis: The experiment employed a factorial randomized complete block design (4 x 2) with 4 groups (served as replicates) and each group was represented by two incubation tubes. Data were analyzed by SPSS statistical software version 16.0 by following the general linear model (GLM) procedure and the mean values among treatments were further tested with Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Nutrient composition and digestibility: The basal ration based on oil palm by-products, as expected, contained low level of protein and high level of fibre (as shown by crude fibre, NDF and ADF values; Table 1). The by-products used in the present study were unprocessed, either biologically or chemically, in order to observe the effects of adding *S. cerevisiae*, *A. noterae* and banana stem on *in vitro* rumen fermentation, digestibility and methane emission of a high fibre ration.

Table 1: Nutrient composition of basal ration based on oil palm by-products

Nutrient	Content (% dry matter)
Organic matter	93.2
Crude protein	9.2
Crude fibre	29.1
Ether extract	3.3
Nitrogen free extract	41.4
Neutral detergent fibre	81.3
Acid detergent fibre	67.6
Total digestible nutrient	50.5

Dry matter and organic matter digestibility were considered to be quite low, i.e., below 40% (Table 2). Such low digestibility was due to the considerably high fibre content in the diet based on oil palm by-products (Table 1). In addition, the digestibility values in this study represented only digestion process in the rumen only without considering the post-ruminal digestion. In general, supplementation of banana stem improved the digestibility of the diet, but less effect by the addition of probiotics (*S. cerevisiae* and *A. noterae*) on the digestibility. The highest DMD and OMD were demonstrated by the simultaneous supplementation of banana stem and *S. cerevisiae*. Such a response might be caused by the contribution of banana stem nutrient and *S. cerevisiae* that improve the growth and activity of rumen microbes. The chemical composition of *S. cerevisiae* is consisted of 50-52% crude protein, 30-37% carbohydrates, 4-5% fat and 7-8% minerals (Reed *et al.*, 1991) and therefore is considered as a source of vitamins, enzymes, cofactors and other nutrients for microbes and digestion in the rumen (Dawson, 1990). Mechanism of *S. cerevisiae* in improving fermentation activity in the rumen has been described by Yoon and Stern (1996), i.e., its respiratory activity can eliminate oxygen in the rumen so that the anaerobic condition can be maintained. This condition stimulates the utilization of ammonia and lactic acid, stabilizes rumen pH and increases population, growth and activity of rumen microbes and, as a result, fermentation process is optimized and digestibility is enhanced.

Regarding banana stem, it contains essential minerals that are needed by the rumen microbes, of which the most important ones are K, Fe, Zn and Ca (Wina, 2001). Mineral contents of banana stem used this study were consisted of K (1.62% DM), Mg (0.13% DM), Fe (150 ppm), Mn (99.5 ppm), Zn (5 ppm) and Cu (1.98 ppm). Thalib *et al.* (2002) reported that supplementation of Fe and Zn was shown to improve the feed digestibility up to 49% compared to the control. Further, digestibility increased because the phagocyte activity of protozoa was inhibited by phytochemicals present in the banana stem namely saponins. Saponins defaunate a certain population of protozoa by their interaction mechanisms with cell membranes of protozoa to rupture

Table 2: Total gas production at various incubation time intervals and digestibility of oil palm by-products supplemented with probiotics and banana stem (n = 4)

Probiotics	Banana stem	----- Gas production (mL) -----			DMD (%)	OMD (%)
		12 h	24 h	48 h		
Control	No	55.4	86.8 ^a	103.9 ^a	32.3 ^a	34.9 ^{ab}
SA	No	59.5	93.4 ^{ab}	113.7 ^{abc}	30.4 ^a	34.1 ^a
AN	No	59.6	92.4 ^{ab}	110.9 ^{ab}	33.3 ^{ab}	36.0 ^{abcd}
SA+AN	No	61.7	96.4 ^{ab}	114.2 ^{abc}	32.6 ^a	35.5 ^{abc}
Control	Yes	61.9	94.5 ^{ab}	114.8 ^{abc}	36.7 ^a	37.4 ^{bode}
SA	Yes	68.7	101.9 ^b	122.6 ^{bc}	38.9 ^a	39.6 ^a
AN	Yes	70.5	104.6 ^b	125.2 ^c	35.7 ^a	37.8 ^{ode}
SA+AN	Yes	64.1	102.3 ^b	124.0 ^{bc}	37.0 ^a	38.5 ^{de}
SEM		1.707	1.673	1.727	0.611	1.096
p-value						
Probiotics		0.464	0.144	0.099	0.990	0.720
Banana stem		0.014	0.004	0.001	<0.001	<0.001
Interaction		0.645	0.861	0.930	0.040	0.181

Different superscripts in the same column are significantly different at p<0.05

AN: *Acetonaerobium noterae*

DMD: Dry matter digestibility

OMD: Organic matter digestibility

SA: *Saccharomyces cerevisiae*

SEM: Standard error of the mean

Table 3: Methane production (ml) and methane concentration (% total gas) *in vitro* of oil palm by-products supplemented with probiotics and banana stem at various incubation time intervals (n = 4)

Probiotics	Banana stem	----- CH ₄ production (mL) -----			----- CH ₄ concentration (% total gas) -----		
		12 h	24 h	48 h	12 h	24 h	48 h
Control	No	15.1 ^{ab}	22.2 ^{bc}	28.2 ^b	27.9 ^b	26.0	27.5
SA	No	14.8 ^{ab}	22.5 ^{bc}	29.7 ^{bc}	25.2 ^{ab}	24.4	26.5
AN	No	13.2 ^a	20.5 ^a	26.9 ^a	23.2 ^a	22.7	24.6
SA+AN	No	14.0 ^{ab}	21.3 ^{ab}	27.6 ^{ab}	23.6 ^{ab}	22.6	24.6
Control	Yes	15.7 ^{bc}	23.2 ^{bc}	29.8 ^{bc}	26.4 ^{ab}	25.0	26.2
SA	Yes	17.8 ^c	25.8 ^c	33.0 ^c	26.7 ^{ab}	25.7	27.1
AN	Yes	15.7 ^{bc}	23.9 ^{cd}	31.1 ^{cd}	24.1 ^{ab}	23.7	25.5
SA+AN	Yes	15.1 ^{ab}	23.9 ^{cd}	31.1 ^{cd}	24.0 ^{ab}	23.8	25.4
SEM		0.378	0.359	0.388	0.609	0.470	0.412
p-value							
Probiotics		0.065	0.085	0.029	0.046	0.088	0.102
Banana stem		0.001	<0.001	<0.001	0.890	0.501	0.839
Interaction		0.227	0.346	0.551	0.740	0.696	0.670

Different superscripts in the same column are significantly different at p<0.05

AN: *Acetonaerobium noterae*

SA: *Saccharomyces cerevisiae*

SEM: Standard error of the mean

the cells (Wina, 2012). The reduction of population and activity of protozoa provides greater opportunities for *S. cerevisiae* to optimize anaerobic condition in the rumen as well as the microbial activity in digesting feed.

Total gas production and methane emissions: Supplementation of banana stem significantly increased total gas production at 12, 24 and 48 h of incubation (p<0.05; Table 2). No significant effects of probiotics addition as well as the interaction between probiotics and banana stem were observed. Total gas production showed similar trend with the dry matter digestibility in which the better result was the combination between supplementation of banana stem with *S. cerevisiae* and *A. noterae*. Gas production was a direct result of substrate fermentation (CO₂ and CH₄) and the result of VFA buffering mechanism (CO₂) indirectly (Getachew *et al.*, 1998). In general, the longer of incubation process occurred, the more gas is produced. The rate of gas production decreased as the length of incubation increase since the amount of available substrate for digestion is depleted.

The increase of total gas production is usually followed by the increase of CH₄ production (in mL) because CH₄ is a component within the gas. Therefore, the best parameter representing CH₄ emission was the concentration of CH₄ in the total gas production (in percent of total gas production; Table 3). Supplementation of *A. noterae* at 12 h of incubation decreased CH₄ concentration by 17% compared to the control (p<0.05). The acetogens inhibited the formation of CH₄ by utilizing hydrogen in the rumen to form acetate. Asetogenic microbes (including *A. noterae*) utilize CO₂ and H₂ for their growth and produce acetic acid as a metabolic product (Lopez *et al.*, 1999). Asetogenic microbes are belong to hydrogenotrophic microbes that can reduce carbon dioxide to form acetate, reduce sulfate to form hydrogen sulfide and reduce fumarate to form succinate (Morvan *et al.*, 1996).

Apart from *A. noterae*, all other supplemented treatments numerically decreased CH₄ concentration at 12, 24 and 48 h of incubation in comparison to the control treatment, including *S. cerevisiae* and banana stem. The decrease of CH₄ is due to three principal

Table 4: Volatile fatty acid (VFA) profile of oil palm by-products supplemented with probiotics and banana stem (n = 4)

Probiotics	Banana stem	Total VFA (mM)	(% total VFA)					
			C2	C3	C4	IsoC4	C5	IsoC5
Control	No	100.0	52.2	17.9 ^a	15.1 ^{ab}	2.66 ^d	7.16 ^c	4.97 ^b
SA	No	109.5	52.1	17.9 ^a	17.6 ^{bc}	2.07 ^{bcd}	4.98 ^{bc}	5.27 ^b
AN	No	96.8	53.8	21.6 ^b	13.2 ^a	2.21 ^{cd}	5.26 ^{bc}	3.94 ^{ab}
SA+AN	No	121.4	52.0	20.9 ^b	18.2 ^{bc}	1.57 ^{ab}	3.23 ^{ab}	4.15 ^{ab}
Control	Yes	100.0	49.5	20.0 ^b	19.1 ^b	1.97 ^{bc}	5.02 ^{bc}	4.36 ^{ab}
SA	Yes	88.1	53.4	16.8 ^a	19.9 ^b	1.48 ^{ab}	3.58 ^{ab}	4.90 ^b
AN	Yes	107.9	50.4	17.2 ^a	23.4 ^d	1.80 ^{abc}	2.85 ^{ab}	4.34 ^{ab}
SA+AN	Yes	104.8	55.6	20.9 ^b	17.1 ^{bc}	1.32 ^a	2.02 ^a	3.16 ^a
SEM		4.833	0.982	0.349	0.747	0.105	0.400	0.185
p-value								
Probiotics		0.251	0.271	<0.001	0.520	0.001	0.001	0.011
Banana stem		0.290	0.771	0.055	<0.001	0.001	0.003	0.204
Interaction		0.194	0.071	<0.001	<0.001	0.721	0.850	0.431

Different superscripts in the same column are significantly different at p<0.05. AN: *Acetanaerobium noterae*
 C2, acetate C3, propionate C4, butyrate C5, valerate
 IsoC4, iso butyrate IsoC5, iso valerate SA, *Saccharomyces cerevisiae* SEM, standard error of the mean

Table 5: pH, N-NH₃ concentration and bacteria (log cfu/ml) and protozoa population (log cell/ml) of oil palm by-products supplemented with probiotics and banana stem (n = 4)

Probiotics	Banana stem	pH	N-NH ₃ (mM)	Bacteria (log cfu/mL)	Protozoa (log cell/mL)
Control	No	6.87	22.7	10.40 ^b	7.20 ^c
SA	No	6.78	22.4	10.35 ^b	7.21 ^c
AN	No	6.88	20.4	10.36 ^b	6.77 ^b
SA+AN	No	6.82	19.7	10.36 ^b	6.87 ^b
Control	Yes	6.89	20.9	10.15 ^a	7.10 ^c
SA	Yes	6.82	19.4	10.28 ^b	6.82 ^b
AN	Yes	6.96	19.4	10.34 ^b	6.52 ^a
SA+AN	Yes	6.77	16.5	10.37 ^b	6.83 ^b
SEM		0.024	0.675	0.017	0.039
p-value					
Probiotics		0.071	0.204	0.150	<0.001
Banana stem		0.578	0.080	0.006	<0.001
Interaction		0.621	0.905	0.012	0.041

Different superscripts in the same column are significantly different at p<0.05
 AN: *Acetanaerobium noterae* SA: *Saccharomyces cerevisiae* SEM: Standard error of the mean

mechanisms, i.e., (1) direct inhibition of methanogens population, (2) decrease of H₂ concentration in the rumen and (3) the provision of alternative electron acceptors of H₂ to divert the methane formation reaction (McAllister and Newbold, 2008). Phytochemical compounds of banana stem apparently inhibit the growth of methanogenic microbes directly and protozoa as the host of some methanogen population. Banana stem also contains a number of minerals that have ability to bind H₂ such as Fe and sulfate; these substances have a higher affinity for hydrogen than the CO₂ (Thalib, 2004). With regard to *S. cerevisiae*, the yeast can stimulate the growth and activity of asetogetic microbes in the rumen to form acetic acid (Chaucheryras *et al.*, 1995). Limited effects of *A. noterae*, *S. cerevisiae* and banana stem in mitigating ruminal methane emissions *in vitro* were apparently related to the small amounts of addition. One milliliter of probiotics per 100 mL of incubation medium and 1 mg of banana stem per mL of incubation medium in such low nutritious oil palm by-products have not been able to reduce methane emissions significantly. Increasing amounts of supplementation and the balance among the three components are an interesting topic for further investigation.

Rumen fermentation and microbial population: The end products of fermentative digestion in the rumen were volatile fatty acids (VFA), ammonia (NH₃) and gas, consisted of CH₄, CO₂, as well as other gases in small amounts (Campbell *et al.*, 2003). McDonald *et al.* (2002) explained that VFA is the end product of carbohydrate digestion that consisted of acetate, propionate and butyrate with average molar ratio of 65, 21 and 14%, respectively. In addition, VFA also contains valerate and branched-chain fatty acids such as isobutyrate and isovalerate (Damron, 2006). The VFA content of each treatment was relatively normal, i.e., about 88.1-121.4 mM (Table 4). Van Soest (1982) suggested that the ideal concentration of total VFA in the rumen is about 80-160 mM. In this study, all treatments did not result in a significant effect on total VFA and acetate proportion, but the treatments had significant effects (p<0.05) on the proportions of propionate, butyrate, iso-butyrate, valerate and iso-valerate. Addition of *A. noterae*, *A. Noterae*+*S. cerevisiae*, banana stem and banana stem+*A. noterae*+*S. cerevisiae* increased the propionate proportion compared to the unsupplemented control (p<0.05). Individual VFA profiles have an effect on methane emissions and feed energy efficiency.

Formation of propionate in the rumen required hydrogen (H_2), while the formation of acetate and butyrate produced H_2 (Martin *et al.*, 2008). Hydrogen in the rumen is utilized by methanogens to reduce CO_2 during formation of CH_4 . On the other hand, ammonia, free amino acids and oligopeptides are used by rumen microbes to form microbial protein. The optimum concentration of NH_3 in the rumen is around 85 to 300 mg/L, equivalent to 6-21 mM (McDonald *et al.*, 2002), in order to sufficiently support microbial protein synthesis. Ammonia concentration in the present study was similar among treatments (Table 5). Rumen pH was also no difference and it was within the comfortable range for rumen microbes to proliferate.

Populations of bacteria and protozoa are presented in Table 5. Supplementation of banana stem only decreased the bacterial population from 10.40 log cfu/mL (control) to 10.15 log cfu/mL ($p < 0.05$). The decrease of bacterial population was occurred because of the activity of phytochemical compounds present in the banana stem, particularly tannins. Bacterial cells have a large affinity to tannins and may cause cell rupture (Min *et al.*, 2003). Moreover, tannins bind cell membrane of rumen bacteria, inhibit their growth and the activity of their enzymes (Smith *et al.*, 2005). Protozoa population was affected by both supplementation of probiotics and banana stem by reducing their population ($p < 0.05$). *S. cerevisiae* and banana stem supplementation singly did not decrease protozoa population, but their combination decreased the protozoa significantly compared to control ($p < 0.05$). It seems that saponins present in banana stem causes defaunation effect on protozoa by the interaction mechanism with sterol of protozoa cell membrane and the subsequent cell lysis (Hart *et al.*, 2008; Wina, 2012).

Conclusion: Supplementation of probiotics did not significantly affect the digestibility of ration based on oil palm by-products. When the probiotics were combined with banana stem, the digestibility was improved. *Acetoanaerobium noterae* addition was potential to mitigate ruminal methane emission, but it did not show any interaction effects with additions of *Saccharomyces cerevisiae* and banana stem.

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