Buletin Peternakan 42 (1): 37-44, February 2018

ISSTAP
Indonesian Society for Sustainable Tropical Animal Production

Bulletin of Animal Science

ISSN-0126-4400/E-ISSN-2407-876X Acredited: 36a/E/KPT/2016
http://buletinpeternakan.fapet.ugm.ac.id/

Doi: 10.21059/buletinpeternak.v42i1.27706

Utilization of Gamma Irradiated *Aspergillus niger* to Improve Oil Palm by-Product Digestibility

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Article history
Submitted: 24 August 2017
Accepted: 25 January 2018

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ABSTRACT

This study was conducted to determine the effect of fermentation using irradiated *A. Niger* on *in vitro* rumen fermentation characteristics of oil palm by-products. Completely randomized design with eight treatments and four replications was applied in this experiment. The treatments were kernel shell (CK), palm frond (PKS), oil palm empty bunches (TKKS), CK+PKS+TKKS (mix), fermented kernel shell (CKF), fermented palm frond (PKSF), fermented oil palm empty bunches (TKKSF) and fermented mix (mixF). The results showed that fermentation process reduced NDF content of PKS and TKKS by 7.42 and 7.09% respectively. Fermentation also decreased ADF content of TKKS by 7.35%. Maximum total gas production (a+b) of TKKS and mix sample decreased after fermentation process ($P<0.05$) by 52.92 and 35.60% respectively. Total VFA production increased on kernel shell and palm fronds samples after fermentation process ($P<0.05$) however CO_2:CH_4 ratio tended to be decrease. The conclusion of this study was the fermentation process by irradiated *A. niger* improved rumen fermentation characteristics of oil palm by-products, however more appropriate strategy is needed to reduce CH_4 emissions.

Key words: *Aspergillus niger*, Degradability, Fermentation, Gamma irradiation, Oil palm by-products

Introduction

Indonesia is a very massive growth country in palm oil plantations. In 2013, the area of large oil palm plantations (owned by the company) was 6,108,900 ha and increased to 6,404,400 ha in 2014. The area of smallholder palm oil plantations also increased from 4,356,090 ha in 2013 up to 4,551,850 in 2014 (BPS, 2016). Zahari *et al.* (2012) states that oil palm has a long economic value of about 20-25 years. This is a consideration that the oil palm by-products should be handled appropriately in order to provide a profitable added value. Proper by-products handling also supports environment-based management of intersectoral integration.

The oil palm by-products utilization as animal feed comes from two sources (direct by-products and by-products from oil palm processing industry). Oil palm empty bunches and palm fronds are plantation by-products, while kernel shell is one of industrial by-products (Zahari *et al*., 2012). Islam *et al.* (2000) explains that the oil palm by-products needs to be investigated for digestibility because it is a potential source of ruminant feed. The constraints of oil palm by-products utilization as ruminants feed are high fiber content and low digestibility (Antonius *et al*., 2015). Oil palm by-products including kernel shells also has a low palatability and characteristic of indigestible material (Chanjula *et al*., 2010). Oil palm empty bunches contain low quality fiber, high lignin content and resulting in low palatability performance (Akbar, 2007). Rahman *et al.* (2011) reported that the lignin content of palm fronds reaches 20% of dry biomass so that it is able to inhibit utilization as a fiber source. Various nutritional factors that inhibit the digestibility of feed in the rumen need to be processing first before being used as feed (Akbar, 2007).

Various strategies are done in overcoming these constraints so that oil palm by-products is able to be utilized as main feed or mixture in ruminant rations. In the previous study, Akbar (2007) used palm frond as a source of fiber, combined with protein by pass and defaunation treatment as sheep feed. Chanjula *et al.* (2010) utilize palm kernel cake (PKC) as a mixture in goat concentrate to accompany a *Paspalum plicatulum* based ration. Fariani *et al.* (2013) used

oil palm frond fermentation using white root fungi (WRF) which is then used in complete feed block (CFB) formulation for beef cattle. The strategy of increasing the digestibility of oil palm by-products is by fermentation treatment to facilitate the solubility of fiber fraction. Mulyana *et al*. (2015) using *Aspergilus niger* fungi that has been irradiated by 500 Gy dose as a fermentation agent to improve rice straw digestibility. Furthermore, it is explained that the gamma irradiation treatment in *A. niger* inoculum is able to increase cellulase enzyme activity and glucose substrate production. Potential *A. niger* that has been irradiated gamma can be utilized as a fermentation agent that able to improve the digestibility of oil palm by-products. Based on the description, the purpose of this study was to determine the effect of palm oil fermentation using *A. niger* which has been irradiated to rumen fermentation characteristics *in vitro*.

Materials and Methods

The research was conducted at Animal Nutrition Laboratory, Livestock Production Group, Agriculture Field, Center for Application of Isotope and Radiation, BATAN, from July to October 2016.

Sample preparation

The kernel shells (CK), palm frond (PK) and oil palm empty bunches (TKKS) were dried at 60°C for three days then milled to a size of 5 mm. Non fermented and fermented feed samples then milled at 2 mm for further analysis of nutrient content.

Fermentation of oil palm by-products

The fermentation of oil palm by-products using three main components (inoculum *A. niger*, nutrient solution and oil palm by-products substrate). Preparation of nutrient solution was done by dissolving 28.8 g of PDB; 1.2 g $(NH_4)2SO_4$; 0.6 g KH_2PO_4; 0.6 g of K_2HPO_4 and 0.24 g of $MgSO_4.7H_20$ in 1200 ml of distilled water and sterilized on autoclave for 15 minutes at 121°C. The inoculum used was *A. niger* which has been irradiated gamma using a ^{60}Co source irradiator at a dose of 500 Gy. A total of 200 ml of nutrient solution and 10 g of inoculum were dissolved into 200 ml of distilled water. The solution was mixed with 200 g of crude palm oil substrate which has been milled to a size of 5 mm then incubated into a 5 kg plastic bag and sealed with a cotton-clogged pipe. The substrate was incubated for 15 days.

***In vitro* incubation**

A total of 380 mg samples were inserted into a 100 ml glass syringe volume (Fortuna model, Germany). Rumen liquids were collected from buffalo fistula before feeding in the morning. The feed ration for the buffalo consists of a combination of forage and concentrates (50:50) which was needed to maintain a basic life and production. The stage of the incubation process of the sample, the glass syringe containing the sample was incubated at 39°C before the rumen buffered fluid filled into the syringe.

The rumen fluid was filtered using a four-layer cotton cloth. The incubation procedure uses Menke *et al*. (1979) which was modified by Blümmel *et al*. (1997). Incubation was carried out at 39°C for 48 hours. The sample was replicated as many as four replications. Observations of total gas production were performed at 0, 2, 4, 6, 8, 10, 12, 24 and 48 h incubation. Measurements of pH, ammonia (NH_3), and total volatile acids (VFA) and protozoa populations were performed on the sample and rumen-buffered fluid that has been incubated for 48 hours.

Experiment design and sample analysis

Complete randomized design (CRD) was used in this study. There were eight treatments combinations and four replications. The research treatment was: 1) Kernel shell (CK); 2) Palm frond (PKS); 3) Oil palm empty bunches (TKKS); 4) CK + PKS + TKKS (mix); 5) Fermented CK (CKF); 6) Fermented palm frond (PKSF); 7) Fermented TKKS (TKKSF) and 8) Mix fermentation (mixF).

Dry matter content (DM) and organic material (OM) (AOAC, 2010) were analyzed. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were also analyzed using Goering and Van Soest (1970) procedures. The rumen fluid used in the study collected from fistulated buffalo. Total gas production, methane gas concentration measurements, CO_2, and sample inoculum analysis were performed after 48 hours of incubation. Measurement of methane (CH_4) and CO_2 concentrations used MRU gas analyzer. Measurement of pH using Hanna instrument pH meter, NH_3 measurement using Conway (1950) micro diffusion method and total VFA measurement based on AOAC (2010) method.

Data analysis

The gas kinetics variables were measured using the Ørskov and McDonald exponential models (1979) $p=a+b(1-e^{-ct})$. “a” and “b” are soluble fractions and insoluble fractions but may also be degraded respectively. While c is the constant fraction solubility rate per t time unit. Calculation of fractions a, b and c used the Neway fit curve software. The data were analyzed using SPSS 16.0 followed by Duncan's Multiple Range Test (DMRT) test. This test is used to determine differences between treatments (Steel and Torrie, 1980).

Result and Discussion

Organic matter and fiber contents

The content of DM, OM, NDF and ADF of oil palm by-products before and after fermentation was presented in Table 1. The fermentation process decreased ($P<0.05$) DM content by

Table 1. Organic matter and fiber contents of treatments

Treatment	Parameters value (%)			
	Dry matter	Organic matter	Neutral detergent fiber	Acid detergent fiber
CK	95.89^{a}	97.22^{a}	84.54^{c}	70.14^{c}
PKS	94.62^{d}	96.20^{ab}	94.88^{a}	74.39^{b}
TKKS	93.06^{e}	96.24^{ab}	94.67^{a}	72.33^{bc}
mix	91.99^{f}	94.51^{bc}	88.29^{b}	71.88^{bc}
CKF	94.73^{d}	97.38^{a}	90.83^{b}	79.15^{a}
PKSF	95.32^{b}	93.67^{c}	87.84^{bc}	72.23^{bc}
TKKSF	95.27^{bc}	95.20^{bc}	87.95^{bc}	67.01^{d}
mixF	95.03^{c}	95.69^{ab}	88.12^{b}	73.55^{b}
SEM	0.256	0.294	0.773	0.724

CK: kernel shell, PKS: palm frond, TKKS: oil palm empty fruit bunch, mix: CK+PKS+TKKS, CKF: fermented kernel shell, PKSF: fermented palm frond, TKKSF: fermented oil palm empty fruit bunch, mix: CKF+PKSF+TKKSF.
SEM (standard error mean).
different superscripts at the same row indicate significant differences (P<0.05).

1.21% in CK samples. Whereas, the fermentation process increased the DM content (P<0.05) in the PKS, TKKS and mix by 0.74; 2.38 and 3.30% respectively. Fermentation using irradiated *A. niger* was able to decrease (P<0.05) of OM content in PKS sample by 2.63%. The fermentation process has no significant effect (P>0.05) on the OM content in CK, TKKS and mix. The concentration of NDF fraction decreased (P<0.05) on post-fermentation of PKS and TKKS samples (7.42 and 7.09%). Fermentation also decreased the ADF fraction in TKKS by 7.35% (P<0.05), whereas in CK samples increased NDF and ADF fractions by 6.92 and 11.38% (P<0.05).

The effect of fermentation on DM content was different between CK sample with PK, PKS and TKKS. This was caused by differences in the characteristics of CK, PKS and TKKS samples containing higher water content than CK. Sufficient water content would support the fungi to grow more, so the fermentation process utilizing the water content increased the percentage of DM content. In a previous study, Wajizah *et al*. (2015) reported that *A. niger* absorbed water for its growth. The condition of the substrate was drier along with the length of the fermentation process. The difference in the effect of fermentation was also due to the high lignin content in the kernel shell. Okoroigwe *et al*. (2014) reported that the lignin content of the kernel shell was 53.85%. According to Saka *et al.* (2008), the lignin content of the kernel shell was 49.9% while the lower palm oil was 21.2%. The decrease in the percentage of OM in palm oil was occur because of the use of organic compounds contained in substrates that were not offset by the resulting organic product. The fermentation process was able to increase the OM content if there was sufficient source of dissolved carbohydrate (Wajizah *et al*., 2015). It further mentioned that rice flour and sago starch acts as a soluble carbohydrate added in the fermentation process.

The NDF and ADF fraction of the kernel shell become higher after the fermentation process. This can be caused by the resistance of high lignin content. The fermentation process would involve dissolved carbohydrates in the sample and decrease the percentage thereby increasing the percentage of fractions containing lignin (NDF and ADF). The fermentation process using irradiated *A. niger* resulted in different effects on the NDF fraction and the ADF of oil palm empty bunches. The fermentation process proved that it has been able to decrease the content of both crude fiber fractions. In palm frond, fermentation was also to decrease the ADF fraction. It proves that the existence of fiber-breaking mechanism by cellulase enzyme produced *by A. niger*. Puastuti *et al.* (2014) explains that the fermentation process was able to convert more complex fractions into simpler fractions in order to be utilized by microbes. Mahesh and Mohini (2013) reported that the use of fungi as a fermentation agent was to decrease the cell wall component (NDF) so as to improve the digestibility of feed.

Total gas production

Total gas production *in vitro* of oil palm by-products after incubation for 48 hours is presented in Table 2. Total gas production fluctuative greatly between treatments with incubation observation time from 2 to 48 hours. The difference was noticeable, occurring at the time of observation after 24 hours. At the time of incubation, TKKS and mix samples produced the highest total gas production (P<0.05) compared to the other six treatments. It was caused by the material characteristics of TKKS which were more easily fermented by rumen microbes. This affects the maximum gas production value (a + b), where TKKS produced the highest maximum of gas production (56.67 ml), and followed by mixed samples (37.81 ml). Different maximum gas production characteristics were shown between CK and PKS samples with TKKS and mix. In CK and PKS samples, the irradiated *A. niger* fermentation treatment had no significant effect on maximum *in vitro* total gas production. The fermentation treatment decreased (P<0.05) in TKKS and mix samples. The maximum total gas production was 52.92 and 35.60%, respectively. The “c” fraction (solubility rate fraction per t time unit) was not significantly different between

Table 2. Total gas production *in vitro* of treatments

Treatment	incubation time (hours)									
	2	4	6	8	10	12	24	48	a+b	c
CK	1.30^{c}	2.21^{c}	2.87^{c}	3.91^{cd}	4.43^{c}	5.34^{bc}	7.81^{d}	12.37^{d}	16.24^{d}	0.030
PKS	1.45^{c}	1.98^{c}	2.95^{c}	3.96^{cd}	4.36^{c}	5.28^{bc}	9.64^{c}	14.52^{cd}	29.81^{cd}	0.016
TKKS	1.61^{bc}	2.69^{bc}	3.35^{bc}	4.16^{bcd}	5.23^{b}	6.30^{ab}	17.84^{a}	32.06^{a}	56.27^{a}	0.015
mix	2.04^{bc}	3.39^{ab}	4.35^{ab}	5.43^{a}	6.52^{a}	7.06^{a}	13.45^{b}	21.32^{b}	37.81^{b}	0.019
CKF	1.19^{c}	2.11^{c}	2.64^{c}	3.29^{d}	3.56^{d}	4.35^{c}	5.93^{e}	10.15^{e}	18.13^{cd}	0.016
PKSF	1.83^{bc}	2.75^{bc}	3.67^{abc}	4.32^{bc}	5.11^{bc}	6.29^{ab}	9.69^{c}	14.54^{cd}	20.17^{cd}	0.026
TKKSF	2.36^{ab}	3.54^{ab}	4.33^{ab}	4.98^{ab}	5.77^{ab}	6.16^{ab}	9.31^{c}	14.94^{c}	26.49^{c}	0.017
mixF	3.02^{a}	3.81^{a}	4.73^{a}	5.52^{a}	6.30^{a}	6.57^{a}	9.46^{c}	14.31^{cd}	24.35^{cd}	0.018
SEM	0.114	0.127	0.147	0.139	0.172	0.165	0.561	1.064	2.053	0.001

CK: kernel shell, PKS: palm frond, TKKS: oil palm empty fruit bunch, mix: CK+PKS+TKKS, CKF: fermented kernel shell, PKSF: fermented palm frond, TKKSF: fermented oil palm empty fruit bunch, mix: CKF+PKSF+TKKSF.
a+b: maximum gas production, c: rate of fraction degradation.
SEM (standard error mean).
different superscripts at the same row indicate significant differences (P<0.05).

treatments. The fastest rate of solubility of the fraction was in the TKKS sample.

Total gas production increased with increasing hours of observation. It represented a fermentation product by a microbe that turned into a gas form. The total gas produced came from direct substrate fermentation (CO_2 and CH_4) and came from indirect gas production through a buffering mechanism of VFA in the form of CO_2 released from the bicarbonate buffer during the fermentation process (Getachew *et al.,* 1998; Jayanegara *et al.,* 2009). The dynamics of total gas production was influenced by differences in the fiber profile of the treatment substrate (Table 1). The data in Table 1 shows that there was a decrease in NDF percentage due to fermentation by A*. niger*. The decrease in the NDF fraction was in line with the decrease of maximum gas production on PKS, TKKS and mix substrate. Wahyono (2015) reported that the maximum gas production of incubation *in vitro* feed substrates was affected by fiber content and solubility rate. The maximum decrease in total gas production in TKKS samples appeared to be very massive (Table 2). This may be due to a decrease in the ADF fraction related to the fermentation treatment using *A. niger* (Table 1). In the fraction of the ADF was obtained component of cellulose which was the source of carbon element (C) in the fermentation process.

The efficiency of feed material utilization was not a result from the calculation of total gas production, therefore the production of gas in the form of CO_2 and CH_4 need to be measured so that the effectiveness of feed material utilization was able to be determined. The results of this study are in accordance with the Jahromi *et al.* (2010) who reported that although the fermentation process can decrease the content of ligno-cellulase in the feed substrate, it also has the effect of decreasing total gas production. Furthermore, it is explained that *A. niger* inoculum has antagonistic effect on rumen microbe so it can affect feed degradation process, and needs to be investigated further. The same results are also found in the study of Kaur *et al.* (2010) that reported if total gas production *in vitro* of rice straw decreased after fermentation process.

CH_4 and CO_2 gas production

The higher total gas production produced the higher CH_4 and CO_2 production. Therefore, the measurement of gas production based on the type of gas produced which determined from CH_4 and CO_2 gas concentration. The results of concentration of CH_4, CO_2 and CO_2: CH_4 ratio were showed in Figure 1.

CH4 concentrations of CKF and PKSF samples were 27.27 and 4.46% respectively lower than CK and PKS (P<0.05). Different things are shown on TKKSF samples which result in higher CH4 concentrations of 34.72% compared to TKKS samples (P<0.05). The fermentation process using *A. niger* had no significant effect on mixed samples. Fermentation using *A. niger* decreased CO_2 concentration in CK, TKKS and mix samples (P<0.05). From the calculation of CO_2 concentration ratio: CH_4, the TKKS and mix samples decreased respectively by 49.69 and 25.63% after the fermentation treatment.

The effect of fermentation using irradiated *A. niger* differed greatly against the concentration of CH_4 and CO_2 on each substrate. This was influenced by differences in the structure of carbohydrates on the substrate. Increasing production of CH_4 and CO_2 gas because the production of excess CH_4 gas that accompanied the release of CO_2 also decreased feed efficiency. Baker (1999) reported that CH_4 gas production indicated the amount of waste energy in the form of gas that indicated the low efficiency of feed utilization. CH_4 gas was a fermentation product that was not utilized and contributed to environmental pollution. Mahesh and Mohini (2013) explain that enteric CH_4 production came from microbial fermentation to the structural carbohydrate sources contained in the feeds of cellulose and hemicellulose.

The results of this study indicate that the ratio of CO_2: CH_4 gas in oil palm empty bunches and substrate mix became lower after the fermentation process using *A. niger.* This is

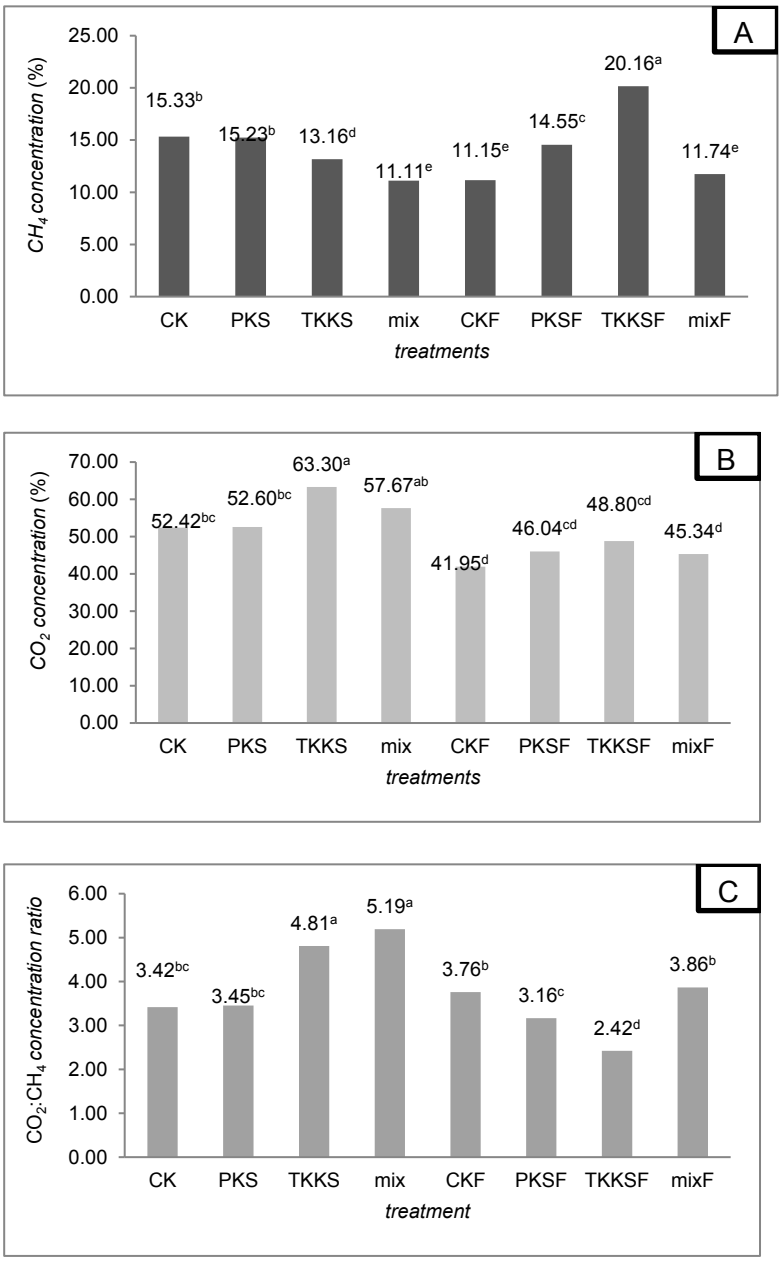

Figure 1. CH_4 concentration (A), CO_2 concentration (B) and CO_2:CH_4 concentration ratio (C) of gas production *in vitro* analyze of treatment sample after 48 hours of incubation.
CK: kernel shell, PKS: palm frond, TKKS: oil palm empty bunches, mix: CK+PKS+TKKS, CKF: fermented kernel shell, PKSF: fermented palm frond, TKKSF: fermented oil palm empty bunches, mix: CKF+PKSF+TKKSF. Different superscripts in similar chart bars indicates significant differences ($P<0.05$).

different from the Mahesh (2012) study which states that the fermentation treatment using fungi is able to improve the quality of feed substrates that impact on the decline in CH_4 production. This difference of results was thought to be caused by differences in fermented yields of partial VFA (Acetate, propionate, butyrate) each of which has a significant effect on CH_4 production. Acetate and butyrate produced by carbohydrate fermentation feed substrate will provide H+ ions and methanogenic bacteria will utilize the H_2 ion as a CH_4 forming component (Wahyono *et al*., 2015). The mechanism of propionic acid formation will otherwise antagonistic with CH_4 gas formation (Li *et al*., 2014).

Characteristics of rumen fermentation

In vitro rumen fermentation results including pH, NH_3 and VFA are presented in Table 3. The lowest pH measurement results were TKKS samples ($P<0.05$), whereas differences were not seen in comparison among other treatments. The fermentation treatment using *A. niger* did not affect NH_3 concentration. Total of VFA production in fermented kernel and palm kernel shells was higher ($P<0.05$) 50 and 67.97%,

Table 3. Characteristics of rumen fermentation results *in vitro* on the treatment sample

Varieables	(treatment)								SEM
	CK	PKS	TKKS	mix	CKF	PKSF	TKKSF	mixF	
pH	7.16[a]	7.16[a]	7.03[b]	7.14[a]	7.16[a]	7.14[a]	7.11[a]	7.17[a]	0.009
NH_3 (mg/100 ml)	4.44[ab]	3.78[b]	4.26 [ab]	4.08 [ab]	4.80 [ab]	4.80 [ab]	5.40[a]	5.10 [ab]	0.164
VFA total (mM)	89.10[b]	79.20[b]	96.53[b]	102.09[b]	133.65[a]	133.03[a]	97.76[b]	96.53[b]	4.244

CK: kernel shell, PKS: palm frond, TKKS: oil palm empty fruit bunch, mix: CK+PKS+TKKS, CKF: fermented kernel shell, PKSF: fermented palm frond, TKKSF: fermented oil palm empty fruit bunch, mix: CKF+PKSF+TKKSF, NH_3 : Ammonia, VFA: volatile fatty acids, SEM: standard error mean.
Different superscripts at the same coloumn indicate significant differences ($P<0.05$).

respectively, than before fermentation. The fermentation treatment did not affect the total VFA production of the sample of empty bunches of palm oil and mixture.

The pH value of rumen fermentation tended to be the same between treatments except TKKS treatment. Nevertheless, the pH value of the eight treatments was still within the normal range so as not to interfere with the performance of rumen microbes especially cellulolytic bacteria. Martinez *et al*. (2010) reported that the rumen pH that continues to decrease, it will negatively affect population and activity of cellulolytic bacteria that assist the rumen fermentation process. Cardozo *et al*. (2000) identified that optimum and suboptimal pH values in the rumen were 6.4 and 5.5, respectively. The NH_3 concentration of the eight treatments also corresponds to the normal range of NH_3 produced in a closed culture. Wanapat *et al*. (2013) reported that optimal NH_3 concentrations in *in vitro* cultures of closed systems were about 5 mg / 100 ml.

Characteristics of total VFA production of kernel shell substrate and palm oil frond increased after fermentation using *A. niger*. This is according to Mahesh and Mohini (2013) study which explains that the fermentation process can increase total VFA production. VFA production in oil palm by-products tended to be more toward acetate and butyrate. This can be proved by a considerable increase in CH_4 gas (Figure 1). The process of producing acetate and butyrate produces H_2 ions used in CH_4 gas formation. Total VFA production of the eight treatments is still within the normal range, where the total ideal VFA production ranges from 80-160 mM (Van Soest, 1982).

Conclusion

Fermentation used irradiated *A. niger* affected the fiber profile in each of the oil palm by-products. In the kernel shell, the fermentation increased the NDF and ADF fractions while in the empty palm oil bunch, the NDF and ADF fractions decreased after fermentation. The fermentation treatment resulted in the lowest total *in vitro* production of gas in the sample of oil palm empty bunches and a mixture of the three oil palm by products. Fermentation was able to increase total VFA production in kernel shell and oil palm frond samples. The fermentation process using irradiated *A. niger* tends to decrease CO_2: CH_4 ratio. This affected the decreased efficiency of energy consumption contained in the feed so that further research was needed. The best treatment was mixed treatment (CK + PKS + TKKS) because it produced the best CO_2: CH_4 ratio.

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