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

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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 CO₂:CH₄ 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 CH₄ 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 Aspergillus 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 (NH₄)₂SO₄; 0.6 g KH₂PO₄; 0.6 g of K₂HPO₄ and 0.24 g of MgSO₄.₇H₂O 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 ⁶⁰Co 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 BlümmeLPet 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 (NH₃), 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 (PK); 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, CO₂, and sample inoculum analysis were performed after 48 hours of incubation. Measurement of methane (CH₄) and CO₂ concentrations used MRU gas analyzer. Measurement of pH using Hanna instrument pH meter, NH₃ 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
The condition of the substrate was drier also due to the high lignin content in the kernel shell. According to Saka et al. (2008), the lignin content of the kernel shell was 53.85%. The difference in the effect of fermentation was noticeable, occurring at the time of observation from 2 to 48 hours. The difference was 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 fluctuated 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>
<thead>
<tr>
<th>Treatment</th>
<th>Dry matter (%)</th>
<th>Organic matter (%)</th>
<th>Neutral detergent fiber (%)</th>
<th>Acid detergent fiber (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK</td>
<td>95.89</td>
<td>97.22</td>
<td>84.54</td>
<td>70.14</td>
</tr>
<tr>
<td>PKS</td>
<td>94.62</td>
<td>96.20</td>
<td>94.88</td>
<td>74.39</td>
</tr>
<tr>
<td>TKKS</td>
<td>93.06</td>
<td>96.24</td>
<td>94.67</td>
<td>72.33</td>
</tr>
<tr>
<td>mix</td>
<td>91.99</td>
<td>94.51</td>
<td>88.29</td>
<td>71.88</td>
</tr>
<tr>
<td>CKF</td>
<td>94.73</td>
<td>97.38</td>
<td>90.83</td>
<td>79.15</td>
</tr>
<tr>
<td>PKSF</td>
<td>95.32</td>
<td>93.67</td>
<td>87.84</td>
<td>72.23</td>
</tr>
<tr>
<td>TKKSF</td>
<td>95.27</td>
<td>95.20</td>
<td>87.95</td>
<td>67.01</td>
</tr>
<tr>
<td>mixF</td>
<td>95.03</td>
<td>95.69</td>
<td>88.12</td>
<td>73.55</td>
</tr>
</tbody>
</table>

SEM (standard error mean)
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 (CO₂ and CH₄) and came from indirect gas production through a buffering mechanism of VFA in the form of CO₂ and CH₄ gas production because the carbohydrates on the substrate. Increasing the amount of waste energy in the form of gas that indicated the low efficiency of feed utilization. CH₄ gas production indicated the amount of waste energy in the form of gas that indicated the low efficiency of feed utilization. CH₄ gas was a fermentation product that was not utilized and contributed to environmental pollution. Mahesh and Mohini (2013) explain that enteric CH₄ 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 CO₂: CH₄ gas in oil palm empty bunches and substrate mix became lower after the fermentation process using A. niger. This is different from the calculation of CO₂ concentration ratio: CH₄, the TKKS and mix samples decreased respectively by 49.69 and 53.63% after the fermentation treatment. 

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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
<th>12</th>
<th>24</th>
<th>48</th>
<th>a+b</th>
<th>c</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK</td>
<td>1.30</td>
<td>2.21</td>
<td>2.87</td>
<td>3.91</td>
<td>4.43</td>
<td>5.34</td>
<td>7.81</td>
<td>12.37</td>
<td>16.24</td>
<td>0.030</td>
</tr>
<tr>
<td>PKS</td>
<td>1.49</td>
<td>1.92</td>
<td>2.95</td>
<td>3.96</td>
<td>4.36</td>
<td>5.28</td>
<td>9.64</td>
<td>14.52</td>
<td>29.81</td>
<td>0.016</td>
</tr>
<tr>
<td>TKKS</td>
<td>1.61</td>
<td>2.69</td>
<td>3.35</td>
<td>4.16</td>
<td>5.23</td>
<td>6.30</td>
<td>17.84</td>
<td>32.06</td>
<td>56.27</td>
<td>0.015</td>
</tr>
<tr>
<td>mix</td>
<td>2.04</td>
<td>3.39</td>
<td>4.35</td>
<td>5.43</td>
<td>6.52</td>
<td>7.06</td>
<td>13.45</td>
<td>21.32</td>
<td>37.81</td>
<td>0.019</td>
</tr>
<tr>
<td>CKF</td>
<td>1.19</td>
<td>2.11</td>
<td>2.64</td>
<td>3.29</td>
<td>3.56</td>
<td>4.35</td>
<td>5.93</td>
<td>10.15</td>
<td>18.13</td>
<td>0.016</td>
</tr>
<tr>
<td>PKSF</td>
<td>1.83</td>
<td>2.75</td>
<td>3.67</td>
<td>4.32</td>
<td>5.11</td>
<td>6.29</td>
<td>9.69</td>
<td>14.54</td>
<td>20.17</td>
<td>0.026</td>
</tr>
<tr>
<td>TTKSF</td>
<td>2.36</td>
<td>3.54</td>
<td>4.33</td>
<td>4.98</td>
<td>5.77</td>
<td>6.16</td>
<td>9.31</td>
<td>14.94</td>
<td>26.49</td>
<td>0.017</td>
</tr>
<tr>
<td>mixF</td>
<td>3.02</td>
<td>3.81</td>
<td>4.73</td>
<td>5.52</td>
<td>6.30</td>
<td>6.57</td>
<td>9.46</td>
<td>14.31</td>
<td>24.35</td>
<td>0.018</td>
</tr>
</tbody>
</table>

SEM (standard error mean).

### CH₄ and CO₂ gas production

The higher total gas production produced the higher CH₄ and CO₂ production. Therefore, the measurement of gas production based on the type of gas production which determined from CH₄ and CO₂ gas concentration. The results of concentration of CH₄, CO₂ and CO₂: CH₄ 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 TTKKS 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 CO₂ concentration in CK, TTKS and mix samples (P<0.05). From the calculation of CO₂ concentration ratio: CH₄, the TTKS 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 CH₄ and CO₂ on each substrate. This was influenced by differences in the structure of carbohydrates on the substrate. Increasing production of CH₄ and CO₂ gas because the production of excess CH₄ gas that accompanied the release of CO₂ also decreased feed efficiency. Baker (1999) reported that CH₄ gas production indicated the amount of waste energy in the form of gas that indicated the low efficiency of feed utilization. CH₄ gas was a fermentation product that was not utilized and contributed to environmental pollution. Mahesh and Mohini (2013) explain that enteric CH₄ 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 CO₂: CH₄ gas in oil palm empty bunches and substrate mix became lower after the fermentation process using A. niger. This is
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 CH₄ 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 CH₄ production. Acetate and butyrate produced by carbohydrate fermentation feed substrate will provide H⁺ ions and methanogenic bacteria will utilize the H₂ ion as a CH₄ forming component (Wahyono et al., 2015). The mechanism of propionic acid formation will otherwise antagonistic with CH₄ gas formation (Li et al., 2014).

**Characteristics of rumen fermentation**

*In vitro* rumen fermentation results including pH, NH₃ 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 NH₃ 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

<table>
<thead>
<tr>
<th>Variable</th>
<th>CK</th>
<th>PKS</th>
<th>TKKS</th>
<th>mix</th>
<th>CKF</th>
<th>PKSF</th>
<th>TKKSF</th>
<th>mixF</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.16a</td>
<td>7.16a</td>
<td>7.03b</td>
<td>7.14a</td>
<td>7.16a</td>
<td>7.14a</td>
<td>7.11a</td>
<td>7.17a</td>
<td>0.009</td>
</tr>
<tr>
<td>NH₃ (mg/100 ml)</td>
<td>4.44bc</td>
<td>3.78b</td>
<td>4.26ab</td>
<td>4.08bc</td>
<td>4.80bc</td>
<td>4.80bc</td>
<td>4.80bc</td>
<td>5.40bc</td>
<td>5.10ab</td>
</tr>
<tr>
<td>VFA total (mM)</td>
<td>89.10a</td>
<td>79.20b</td>
<td>96.53b</td>
<td>102.09b</td>
<td>133.65a</td>
<td>133.03a</td>
<td>97.76b</td>
<td>96.53b</td>
<td>4.244</td>
</tr>
</tbody>
</table>


Different superscripts at the same column indicate significant differences (P<0.05).

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 NH₃ concentration of the eight treatments also corresponds to the normal range of NH₃ produced in a closed culture. Wanapat et al. (2013) reported that optimal NH₃ 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 CH₄ gas (Figure 1). The process of producing acetate and butyrate produces H₂ ions used in CH₄ 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 CO₂: CH₄ 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 CO₂: CH₄ ratio.

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