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Comparisons of Nutrient Contents and Nutritional Values of Palm Kernel Cake Fermented by Using Different Fungi

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Abstract: An experiment has been conducted to compare the nutrient contents and nutritional values of palm kernel cake (PKC) from different fermentation processes. This experiment was designed in a Completely Randomized Design (CRD) with six treatments and five replicates. Treatments were A = unfermented PKC (control), B = fermented PKC with *Neurospora crassa*, C = fermented PKC with *Penicillium* sp., D = fermented PKC with *Trichoderma harzianum*, E = fermented PKC with *Aspergillus niger* and F = fermented PKC with *Aspergillus niger*+humic acid. Measured variables were nutrient contents (crude protein, crude fiber and ether extract) and nutritional values (nitrogen retention, crude fiber digestibility and metabolizable energy) of fermented and unfermented PKCs. Results of experiment indicated that the crude fiber content of fermented PKCs was very significantly lower ($p<0.01$) than that of control, but there was no difference ($p>0.05$) among the fermented PKCs. Fermentation with different fungi increased ($p<0.05$) the crude protein content of PKCs when it was compared with control. The crude protein content of *Aspergillus niger* and *Aspergillus niger*+humic acid fermented PKCs were significantly higher ($p<0.05$) than that of *Trichoderma harzianum*, *Neurospora crassa*, or *Penicillium* sp., fermented PKCs or control. The ether extract content of PKC was very significantly affected ($p<0.01$) by treatments. *Aspergillus niger*+humic acid, *Neurospora crassa* and *Aspergillus niger* fermented PKCs possessed the lower ether extract content ($p<0.05$) than that of *Penicillium* sp., or *Trichoderma harzianum* fermented PKCs, or control. The crude fiber digestibility of *Aspergillus niger* or *Aspergillus niger*+humic acid fermented PKCs was very significantly higher ($p<0.01$) than that of *Penicillium* sp., *Trichoderma harzianum*, *Neurospora crassa* fermented PKCs, or control. The nitrogen retention of *Aspergillus niger*+humic acid fermented PKC was significantly higher ($p<0.05$) than that of *Penicillium* sp., *Neurospora crassa* fermented PKCs, or control, but was not differed ($p>0.05$) from that of *Trichoderma harzianum* or *Aspergillus niger* fermented PKCs. The fermentation with different fungi improved ($p<0.05$) the metabolizable energy content of PKC. Thus, the improvement of the nutrient contents and nutritional values of *Aspergillus niger* fermented PKC was better than the other treatments.

Key words: Palm kernel cake, fermentation, *Aspergillus niger*, humic acid, *Penicillium* sp., *Trichoderma harzianum*, *Neurospora crassa*, nutrient content, nutritional value

INTRODUCTION

Indonesia is the biggest producer of crude palm oil in the world with the total production of 22.5 million tones per year. Seventy percent of the total production of crude palm oil was located in Sumatra Island (BPS, 2010). With the development of palm oil plantation, it will produce a huge amount of waste or by-product of palm oil industry in the form of Palm Kernel Cake (PKC) because 45-46% of the by-product of palm oil industry is PKC.

The nutrient content of PKC was as follows: crude protein 16,07%, crude fiber 21,30%, ether extract 8.23%, Ca 0.27%, P 0.94% and Cu 48.4 ppm (Mirnawati *et al.*, 2008), so that it can be utilized as an animal feed. Even though its crude protein content was rather high, its utilization in poultry diets was not optimal yet. According to Supriyadi (1997) the PKC could be included up to 10% in the duck ration and Rizal

(2000) found that PKC could be utilized up to 10% in the broiler diet to effectively replace 40% soybean meal.

The low in the utilization of PKC in poultry diets was due to the low in the nutritive value of this PKC. The low in nutritive value of PKC was related to the high in crude fiber content, low amino acids content and the high in Cu content (Nwokolo *et al.*, 1976; Ezhieshi and Olomu, 2004; Mirnawati *et al.*, 2008) and high in β -manan or mannose polymere (Dusterhof *et al.*, 1993; *et al.*, 2007). On the other hand, the ability of poultry to digest crude fiber is limited (Scott *et al.*, 1982).

For increasing the utilization of PKC in poultry diets, the processing through fermentation had been performed by using cellulolytic microbes such as: *Trichoderma harzianum*, *Aspergillus niger* and *Penicillium* sp. (Sabrina *et al.*, 2001; Nuraini and Yunara, 2001; Aziz *et al.*, 2003; Harnentis *et al.*, 2005; Mirnawati *et al.*, 2008), proteolytic such as: *Rhizopus* sp. (Sabrina *et al.*,

2001) and carotenogenic such as: *Neurospora* sp. (Sabrina *et al.*, 2001; Nuraini and Susilawati, 2006). Poultry response to the utilization of fermented PKC ranging from 15 to 25% (Sabrina *et al.*, 2001; Nuraini and Trisna, 2006; Nuraini *et al.*, 2007).

The nutrient content, nutritional value and poultry response to the utilization of fermented PKC are still varied, so that it is still necessary to further study the nutrient quality of processed PKC, so that it can be utilized in large amount in poultry diets and is available continuously in the market in the form of complete diets ready for feeding to poultry.

MATERIALS AND METHODS

This experiment was aimed to study the effect of fermentation by using different species of fungi on the nutrient contents and nutritional values of PKC and to find out the best fungi for fermenting the PKC through the utilization of *Neurospora crassa*, *Penicillium* sp., *Trichoderma harzianum* and *Aspergillus niger*. They were also compared with the unfermented PKC. A Completely Randomized Design (CRD) with 6 treatments and 5 replicates was employed in this experiment. The treatments were A = unfermented PKC (control), B = *Neurospora crassa* fermented PKC, C = *Penicillium* sp. fermented PKC, D = *Trichoderma harzianum* fermented PKC, E = *Aspergillus niger* fermented PKC and F = *Aspergillus niger*+humic acid fermented PKC.

The fermentation of PKC by using *Neurospora crassa* was performed with the inoculums dose of 9% and fermentation length of 5 days, *Penicillium* sp. with inoculums dose of 6% and fermentation length of 7 days, *Trichoderma harzianum* with the inoculums dose of 8% and fermentation length of 7 days, *Aspergillus niger* with the inoculums dose of 10% and fermentation length of 7 days and *Aspergillus niger*+100 ppm humic acid with the inoculums dose of 10% and fermentation length of 7 days. The total amount of substrate used for each treatment was 100 g, consisted of 80 g of PKC plus 20 g of rice bran.

Measured variables were nutrient contents (crude fiber, crude protein and ether extract) and nutritional values (crude fiber digestibility, nitrogen retention and metabolizable energy content) of fermented and unfermented PKCs. Crude fiber, crude protein and ether extract were determined according to proximate analysis procedures (AOAC, 1984). Crude fiber digestibility was measured according to Schneider and Flatt (1975). Nitrogen retention and metabolizable energy were performed according to McDonald (1981) and Sibbald (1975), respectively.

Data were analyzed by analysis of variance of CRD. Duncan Multiple Range Test (DMRT) according to Steel and Torrie (1980) was performed for testing the difference among treatments.

RESULTS

Effect of treatments on the crude fiber content of palm kernel cake: The means of crude fiber content of fermented and unfermented PKCs were illustrated in Table 1.

The result of the analysis of variance indicated that the crude fiber content of PKC was very significantly ($p < 0.01$) influenced by the treatments. The crude fiber of PKC fermented with *Neurospora crassa*, *T. harzianum*, *Penicillium* sp., *Aspergillus niger*, or *Aspergillus niger*+humic acid was not differed ($p > 0.05$). However, the crude fiber content of all fermented PKCs was very significantly lower than that of unfermented PKC.

Effect of treatments on crude protein content of palm kernel cake: The effect of treatments on the crude protein content of PKC was figure out in Table 2. The crude protein content of PKC was very significantly affected ($p < 0.01$) by treatments.

The crude protein content of *Aspergillus niger* fermented PKC was not differed from *Aspergillus niger*+humic acid fermented PKC, but was higher ($p < 0.05$) than those of *Penicillium* sp., *Trichoderma harzianum* and *Neurospora crassa* fermented PKCs as well as unfermented PKC. The crude protein content of *Aspergillus niger* + humic acid fermented PKC was higher ($p < 0.05$) than those of *Trichoderma harzianum* and *Neurospora crassa* fermented PKCs as well as unfermented PKC, but was not different from *Penicillium* sp. fermented PKC. *Penicillium* sp. fermented PKC crude protein content was not differed from *Trichoderma harzianum* fermented PKC, but was higher than those of

Table 1: Crude fiber content of palm kernel cake as affected by treatments

Treatments	Crude fiber (%)
A (Control)	18.86 ^a
B (<i>Neurospora crassa</i>)	14.75 ^b
C (<i>Penicillium</i> sp.)	13.42 ^b
D (<i>Trichoderma Harzianum</i>)	14.04 ^b
E (<i>Aspergillus niger</i>)	14.34 ^b
F (<i>Aspergillus niger</i> + Humic Acid)	13.98 ^b
SEM*	0.68

^{a,b}Means with different superscript are very significantly different ($p < 0.01$). *Standard Error of the Mean

Table 2: Crude protein content of palm kernel cake as affected by treatments

Treatments	Crude protein (%)
A (Control)	23.30 ^a
B (<i>Neurospora crassa</i>)	24.49 ^{de}
C (<i>Penicillium</i> sp.)	26.34 ^{bc}
D (<i>Trichoderma harzianum</i>)	26.21 ^{cd}
E (<i>A. niger</i>)	28.41 ^a
F (<i>A. niger</i> + Humic Acid)	28.06 ^{ab}
SEM*	0.59

^{a,b,c,d,e}Means with different superscripts are significantly different ($p < 0.05$). *Standard Error of the Mean

Neurospora crassa fermented PKCs as well as unfermented PKC. *Trichoderma harzianum* fermented PKC was not differed from *Neurospora crassa* fermented PKC, but was higher than that on unfermented PKC. The crude protein content of *Neurospora crassa* fermented PKC was not different from unfermented PKC.

Effect of treatments on ether extract content of palm kernel cake: The means of the ether extract content of fermented and unfermented PKC can be seen in Table 3. The treatments affected the ether extract content of PKC very significantly ($p < 0.01$).

The ether extract content of unfermented PKC (control) was very significantly higher ($p < 0.01$) than those of fungi fermented PKCs. *Penicillium* sp. fermented PKC ether extract content was higher ($p < 0.05$) than that of *Trichoderma harzianum* fermented PKC and was very significantly higher ($p < 0.01$) than those of *Aspergillus niger*, *Neurospora crassa* and *Aspergillus niger*+humic acid fermented PKCs and unfermented PKC. The ether extract content of *Trichoderma harzianum* fermented PKC was not different ($p > 0.05$) from those *Aspergillus niger* and *Neurospora crassa* fermented PKCs, but was higher ($p < 0.05$) than that of *Aspergillus niger*+humic acid fermented PKC. There was no difference ($p > 0.05$) in the ether extract content of *Aspergillus niger*, *Neurospora crassa* and *Aspergillus niger*+humic acid fermented PKCs.

Effect of treatments on the crude fiber digestibility of palm kernel cake: The means of the crude fiber digestibility of fermented and unfermented PKC was seen in Table 4.

Results of the experiment indicated that treatments very significantly influenced the crude fiber digestibility of PKCs. This crude fiber digestibility of *Aspergillus niger* and *Aspergillus niger*-humic acid fermented PKCs was not differed ($p > 0.05$), but was significantly higher ($p < 0.01$) than those of *Neurospora crassa*, *Trichoderma harzianum* and *Penicillium* sp. fermented PKCs and unfermented PKC. The *Neurospora crassa* fermented PKC crude fiber digestibility was higher ($p < 0.05$) than those of *Trichoderma harzianum* and *Penicillium* sp., fermented PKCs and was very significantly higher ($p < 0.01$) than that of unfermented PKC. There was no difference in crude fiber digestibility between *Trichoderma harzianum* and *Penicillium* sp., fermented PKCs. However, both of them were differed ($p < 0.01$) from control.

Effect of treatments on the nitrogen retention of palm kernel cake: The effect of treatments on the nitrogen retention of fermented and unfermented PKC was depicted in Table 5. Treatments influenced the nitrogen retention of fermented and unfermented PKCs significantly ($p < 0.05$).

Table 3: Ether Extract Content of Palm Kernel Cake as Affected by Treatments

Treatments	Ether extract (%)
A (Control)	9.18 ^a
B (<i>Neurospora crassa</i>)	2.78 ^{c,d}
C (<i>Penicillium</i> sp.)	4.39 ^b
D (<i>Trichoderma harzianum</i>)	3.35 ^c
E (<i>A. niger</i>)	3.00 ^{c,d}
F (<i>A. niger</i> + Humic Acid)	2.34 ^d
SEM*	0.25

^{a,b,c,d}Means with different superscripts are significantly different ($p < 0.05$). *Standard Error of the Mean

Table 4: Crude fiber digestibility of palm kernel cake as affected by treatments

Treatments	Crude fiber digestibility (%)
A (Control)	12.02 ^d
B (<i>Neurospora crassa</i>)	27.89 ^b
C (<i>Trichoderma harzianum</i>)	23.48 ^c
D (<i>Penicillium</i> sp.)	22.40 ^c
E (<i>Aspergillus niger</i>)	38.71 ^a
F (<i>Aspergillus niger</i> + Humic Acid)	40.86 ^a
SEM*	4.23

^{a,b,c,d}Means with different superscripts are very significantly different ($p < 0.01$). Standard Error of the Mean

Table 5: Nitrogen retention of palm kernel cake as affected by treatments

Treatments	Nitrogen retention (%)
A (Control)	18.67 ^c
B (<i>Neurospora crassa</i>)	28.44 ^c
C (<i>Penicillium</i> sp.)	34.48 ^{b,c}
D (<i>Trichoderma harzianum</i>)	56.40 ^{ab}
E (<i>A. niger</i>)	42.31 ^{abc}
F (<i>A. niger</i> + Humic Acid)	63.73 ^a
SEM*	8.26

^{a,b,c}Means with different superscripts are significantly different ($p < 0.05$). *Standard Error of the Mean

Table 6: Metabolizable energy of palm kernel cake as affected by treatments

Treatments	Metabolizable Energy (Kcal/Kg)
A (Control)	2252 ^b
B (<i>Neurospora crassa</i>)	2368 ^a
C (<i>Trichoderma harzianum</i>)	2371 ^a
D (<i>Penicillium</i> sp.)	2404 ^a
E (<i>Aspergillus niger</i>)	2411 ^a
F (<i>Aspergillus niger</i> +Humic Acid)	2424 ^a
SEM*	40

^{a,b}Means with different superscripts are significantly different ($p < 0.05$). *Standard Error of the Mean

The nitrogen retention of the *Aspergillus niger*+humic acid, *Trichoderma harzianum* and *Aspergillus niger* fermented PKCs was significantly higher ($p < 0.05$) than those of *Penicillium* sp. and *Neurospora crassa* fermented PKCs and control, but it was not different among them. The *Penicillium* sp., fermented PKC nitrogen retention was not different ($p < 0.05$) from *Neurospora crassa* fermented PKC and unfermented

PKC. The nitrogen retention of *Neurospora crassa* fermented PKC was also statistically the same as the unfermented PKC.

Effect of treatments on the metabolizable energy of palm kernel cake: The means of the metabolizable energy content of fermented PKC by using different species of fungi and unfermented PKC are depicted in Table 6.

Based on the result of analysis of variance, the fermentation by using different kind of microbes significantly affected ($p < 0.05$) the metabolizable energy content of PKC. The metabolizable energy content of *Neurospora crassa*, *T. harzianum*, *Penicillium* sp., *Aspergillus niger*, *Aspergillus niger*+asam humat fermented PKCs was higher ($p < 0.05$) than the unfermented PKC.

DISCUSSION

Effect of treatments on the crude fiber content of palm kernel cake: The low in crude fiber of PKC after fermented with *Neurospora crassa*, *T. harzianum*, *Penicillium* sp. and *Aspergillus niger*, was due to the cellulolytic characteristics of these fungi which possess high cellulase activity (Mirnawati *et al.*, 2011), so that they can degrade cellulose into glucose which in turn at the end of fermentation process the declining in crude fiber occurs. The result of the reduction of crude fiber was in accordance with the results of experiments by Sabrina *et al.* (2002) and Nuraini and Yunara (2001).

Effect of treatments on crude protein content of palm kernel cake: When it was compared with the unfermented PKC (control), there was an increase in crude protein content of fermented PKCs (treatments B, C, D, E and F). It was clearly found that there was the growth of fungi in each fermented PKC. The growing fungi contribute to the increase in crude protein content of PKC, so that there was an augmenting in crude protein of fermented PKC. Crueger and Crueger (1989) reported that fungi contained high crude protein (40-60%). According to Carlile and Watkinson (1995), the increase in crude protein content of substrate after fermentation was associated with the process of protein enrichment which was similar to the formation of a single cell protein and in this process it was not separated between protein from fungi and substrate.

The height in crude protein content of PKC *Aspergillus niger*, *Aspergillus niger*+humic acid, *Penicillium* sp. and *Trichoderma harzianum* fermented PKCs was also related to the enzymes (cellulase, protease, etc.) produced by these fungi as the protein which could contribute to the height in crude protein content of the PKC as compared with *Neurospora crassa* fermented PKC and control. According to Hidayat (2007) fermentation is the activity of microbes in food or feed to

produce high quality products through increasing the nutrient content and nutritional value of the products.

Effect of treatments on ether extract content of palm kernel cake: There was a reduction of ether extract content when the PKC was fermented by using fungi. The dramatic decrease in ether extract content of PKC occurred at the fermentation by using *Aspergillus niger*, *Neurospora crassa* and *Aspergillus niger*+humic acid. According to Falony *et al.* (2006) fungi was known as a microbe which produce high lipase and *Aspergillus niger* was one of fungi that produce high amount of lipase. *Penicillium* sp. was also a high lipase producer (Pimentel *et al.*, 1997; Lima *et al.*, 2003). Ulker *et al.* (2010) found that *Trichoderma harzianum* was also a fungi which produce lipase. *Neurospora crassa* was also well known as a lipase producer (Kundu *et al.*, 1987). Thus, the lipase produced by each fungi could hydrolyze lipid content in fermented PKC, so that the ether extract/lipid content of fermented PKC declined.

Effect of treatments on the crude fiber digestibility of palm kernel cake: The crude fiber digestibility among *Neurospora crassa*, *A. niger* and *A. niger*+humic acid fermented PKC was relatively high. This was due to the height in cellulolytic activity of these species of fungi. The higher the cellulolytic activity of the fungi, the more cellulose could be degraded into glucose, so that at the end of fermentation the amount of crude fiber decreased. This finding was in accordance with the results of experiments by Nuraini and Susilawati (2006) and Mirnawati *et al.* (2010) who reported that there was a decline in crude fiber content of PKC fermented by using *Neurospora crassa* and *Aspergillus niger*.

Effect of treatments on the nitrogen retention of palm kernel cake: The height in nitrogen retention of *Aspergillus niger*+humic acid fermented PKC was related to the height in crude protein of this fermentation product (28.06%), so that the amount of protein consumed increased. The high amount of protein consumed resulted in the high in retained nitrogen in the body which could be utilized by animals. According to Farrell (1974) the nitrogen retention was the retained feed protein in the animal body. The nitrogen retention was influenced by several factors such as crude protein content of feed, amino acid content and protein digestibility. Lloyd *et al.* (1978) said that nitrogen retention was one of methods for determining the protein quality of feed or diet.

Effect of treatments on the metabolizable energy of palm kernel cake: The metabolizable energy (ME) of fermented PKC by using *Neurospora crassa*, *Trichoderma harzianum*, *Penicillium* sp., *Aspergillus niger* and *Aspergillus niger* + humic acid was higher

than that of unfermented PKC (control). The increase in this ME was resulted from the fermentation process which lead to the degradation of crude fiber into the simple carbohydrates. These simple carbohydrates are readily digested and absorbed by animals. Fardiaz (1989) reported that the biosynthesis of macromolecules such as protein, lipid, polysaccharide and nucleic acid was originally produced from variety of monomer units such as amino acids, fatty acids, simple carbohydrates, etc.

The result of this experiment was also in accordance with the result of experiment by Desserheine (1998) who reported that there was an increase in ME of PKC which was fermented by using *Aspergillus niger* from 1844 to 2103 kcal/kg. The increase in the ME content of *Aspergillus niger* fermented PKC was due to the increase in the crude fiber digestibility of this fermented PKC which produced simple sugars as the source of energy by animals.

Conclusion: The fermentation of palm kernel cake by using *Aspergillus niger* was the best when it was compared with the fermentation by using *Neurospora crassa*, *Trichoderma harzianum*, *Penicillium* sp. or *Aspergillus niger*+humic acid, or without fermentation (control).

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