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Evaluation of the nutrient contents of palm kernel cake fermented by microbial cocktails as a potential feedstuff for poultry

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ABSTRAK

Tujuan dari penelitian ini adalah untuk meningkatkan nilai gizi bungkil inti sawit dengan teknologi fermentasi menggunakan *Bacillus amyloliquefaciens, Trichoderma harzianum* dan mikroba koktail (kombinasi dari *Bacillus amyloliquefaciens* dan *Trichoderma harzianum*). Data dianalisis dengan menggunakan Rancangan Acak Lengkap pola Faktorial, dengan percobaan terdiri dari tiga jenis mikroba (*B. amyloliquefaciens, T. harzianum*, mikroba koktail) dan empat lama inkubasi (0, 3, 5, dan 7 hari). Parameter adalah protein kasar dan serat kasar untuk semua perlakuan, dan hasil analisis serat kasar terendah akan dilanjutkan dengan analisis NDF, ADF, lemak kasar, asam amino. Hasil penelitian menunjukkan bahwa ketiga mikroba tumbuh pada bungkil inti sawit pada inkubasi 3 hari dan tumbuh di dalam dan dipermukaan substrat pada inkubasi 7 hari. Koktail mikroba meningkatkan protein dan mengurangi serat kasar lebih baik daripada *B. amyloliquefacien* dan *T. harzianum* pada teknologi fermentasi bungkil inti sawit. Koktail mikroba dapat meningkatkan asam amino seperti metionin, arginin, dan asam glutamat, juga serat deterjen netral tetapi mengurangi ADF dan hemiselulosa setelah fermentasi. Disimpulkan, bungkil inti sawit yang difermentasi dengan koktail mikroba dapat mengurangi serat kasar dan meningkatkan protein kasar dengan masa inkubasi 7 hari .

Kata kunci: bungkil inti sawit, fermentasi, koktail mikroba, serat kasar, protein kasar

ABSTRACT

The aims of this research were to improve the nutritive value of palm kernel cake by fermentation technology using *Bacillus amyloliquefacien, Trichoderma harzianum* and cocktail microbes (combination of *Bacillus amyloliquefacien* and *Trichoderma harzianum*). Data were analyzed using Completely Randomized Design Factorial, with experiments consisting of types of microbes (*B. amyloliquefaciens*, *T. harzianum*, microbial cocktail) as treatment and incubation time (0, 3, 5, and 7 days). Parameters were crude protein and crude fiber for all treatments, and the lowest fiber analysis would continue with NDF, ADF, crude fat, and amino acids. Result showed that the three of microbes grew on palm kernel meal in third incubation and grew on and in the substrate at 7 days. microbial cocktails increased protein and reduced crude fiber better than B. amyloliquefacien and T. harzianum on palm kernel cake fermentation technology. Cocktail microbes enhanced amino acids such as

methionine, arginine, and glutamic acid, also neutral detergent fiber but reduced ADF and hemicellulose. It was concluded that the palm kernel cake fermented with microbial cocktails can reduced crude fiber and increase crude protein with an incubation period of 7 days.

Keywords:crude fiber, crude protein, fermentation, palm kernel cake, microbes cocktail

INTRODUCTION

The high price of feed ingredients spurred poultry nutritionists to look for alternative feed ingredients. One of them is palm kernel cake (PKC), because it has protein content of 13.98%, 8.61% fat, and crude fiber 15-18% (Puastuti *et al*., 2014; Sharmila *et al*., 2014) and also high production. Production of palm oil (CPO) in Indonesia around 23,096,541 tons in 2011, by 2015 increasing to 31,070,015 tons (SPI, 2017). Basic on CPO production that PKC production also increased from 2,078,689 tons in the year 2011 and increased to 31,070,015 tons by 2015 (Pasaribu, 2018). The nutrient composition of PKC, indicating it has the potential as a ruminant feed without any technology process because it has rumen microbes that can degrade fiber. While for monogastric or poultry such as chicken becomes a problem because of its high fiber content. To improve the nutritional value of PKC, the crude fiber content should be decreased and the protein content is increased. Fermentation technology using single or mixed microbes can improve the nutrient content of the agro-industry waste and is one of the efforts to improve the nutritional value of PKC (Pasaribu, 2018). Thus, fermented palm kernel cake can be used as poultry feed ingredients. Puastuti *et al*. (2014) reported that PKC fermentation with inoculant *Aspergillus niger* increased protein content from 13.98 to 25.78%. The palm oil sludge that fermented with *Lentinus edodes, Pleurotus ostreatus and Phanerochaete chrysosporium* in four days increased the protein from 14.04 to 21.86% (Nuraini *et al*., 2017). In addition to *A. niger* NRRL 337 or *L. edodes, P. ostreatus and P. chrysosporium*, the opportunity of using other microbes such as *B. amyloliquifaciens* bacteria and *T. harzianum* or the mixture of both which are called microbial cocktails is one effort to improve the nutritional value of PKC.

Microbial cocktail is a mixture of several microbes that are mixed into one in the fermentation process (Schwan, 1998; Moreira *et al*., 2017), in this study for PKC fermented is mixed are *B. amyloliquifaciens* and *T. harzianum*. The merging of these two microbes is based on the role of the enzyme Ekso-beta-glucanase from *B. amyloliquefaciens* which cuts the outer chains of polysaccharides and the endo-beta-glucanase enzyme of *T. harzianum* which intersects chains in polysaccharides (Wizna *et al.*, 2005; Kuge *et al.,* 2015).

It is expected that fermentation technology using microbial cocktails can decrease the levels of crude fiber and increase protein. With that fermentation technology that the by-product such as PKC can be used as alternative feed ingredients in poultry. The aim of this study was to improve the nutritive value of PKC with fermentation technology by using cocktail microbes (combination of *B. amyloliquefaciens* and *T. harzianum*).

MATERIALS AND METHODS

Palm kernel cake (PKC) was obtained from Bengkulu. Pure *B. amyloliquefaciens* cultures were obtained from Andalas University. The culture of *B. amyloliquefaciens* in this study was used at $18.7x10^{16}$ CFU/mL. The culture of *T. harzianum* obtained from IRIAP collection on $3.3x10^{2}$ CFU/mL.

Propagation of *B. amyloliquefaciens*

The ingredients used in the propagation of *B. amyloliquefaciens* were nutrient agar media and Paul Marjonoff (PM) liquid media. The propagation method of *B. amyloliquefaciens* was done according to Kompiang procedure (Personal Communication).

Fermentation Process

A total of 500 ml of *B. amyloliquefaciens* were mixed of PKC, then mixed until homogeneous and incubated for up to 7 days. Likewise for *T. harzianum* was did the same thing. As for microbial cocktail was carried out by mixing of PKC with of *B. amyloliquefaciens* and *T. harzianum*, then stirred until blended and incubated in the plastic tray for 7 days. Incubation was carried out at room temperature, with observations at days 0, 3, 5, and 7. The measured parameters were visual microbial growth during

fermentation, crude protein, and crude fiber. Analyzes of crude fat, ash, acid detergent fiber (ADF), neutral detergent fiber (NDF), essential amino acids (AAE) test were performed based on the lowest crude fiber results in 3, 5, and 7 days incubation.

Microbial Growth

Visualization of microbial growth is viewed descriptively with the value of + (mycelium growth does not exist yet); ++ (growth of mycelium is already visible (25%); +++ (mycelium growth is evenly distributed (50%); ++ ++ (mycelium growth uniformly on the surface and inside of the substrate (100%), during incubation (0, 3, 5, and 7 days).

Nutrition Analysis

Crude protein content was measured using the Kjeldahl method (AOAC, 2012). Determination of crude fiber, neutral detergent fiber (NDF), and acid detergent fiber (ADF was performed according to Van Soest procedure (1963). Determination of essential amino acids was carried out by using HPLC method.

Treatment and Experimental Design

The study was assigned in the completely randomized factorial design pattern with 3 kinds of microbial consisted of *B. amyloliquefaciens*, *T. harzianum*, and microbial cocktail. The incubation time consisted of 0, 3, 5 and 7 days. When the ANOVA test there was a significant difference then the analysis is continued with orthogonal comparison test (Steel and Torrie, 1995).

RESULTS AND DISCUSSION

Microbial Growth on Palm Kernel Cake Substrate

Fermentation from 0 to 2 days has not seen any grew of three kinds of microbes on PKC surface. On the third day, the grew of *B. amyloliquefaciens*, *T. harzianum*, and microbial cocktail has covered the substrate up to 25%(++ +). It grows well on the surface of PKC media, showing the color of white dots (mycelium). The fifth day, the grew of three kinds of microbes on PKC surface reached 50 %. The growth of mycelium is increasingly visible (++++), white color evenly. The seventh day, the grew of *B. amyloliquefaciens, T. harzianum*, and the microbial cocktail inside and on the surface of PKC reached 100% and the color of the PKC also changed. The growth of mycelium is increasingly visible (++++), white color uniformly within and on the surface of the PKC substrate.

Microorganism have a life cycle that is divided into 4 growth phases, namely: 1. the lag phase (where the bacteria are metabolically active, not yet dividing), 2. exponential / log (bacteria exponentially developing), 3. stationary (the number of bacteria that divide is equal to the number of dead, is peak population growth, and death (a phase of decline in the number of living cells) (Pletnev *et al.,* 2015). In this study, when fermentation time in 0-2 days with *B. amyloliquefaciens, T. harzianum*, and microbial cocktails did not yet grow on the surface of the PKC. This indicates that the three types of bacteria were in the phase of lag (slow phase), where the activity of *B. amyloliquefaciens, T. harzianum*, and microbial cocktails was still in the metabolic process. Where microbes do not grew because cells undergo changes in chemical composition, increased size and intracellular substance, to prepare for bacterial cell division.

When fermentation time 3, 5, and 7 days, it was indicated that the grew of *B. amyloliquefaciens, T. harzianum*, and microbial cocktails that survived and died were the same, so the speed of grew was constant. This phase showed that *B. amyloliquefaciens, T. harzianum*, and microbial cocktails were growing in peak, namely the logarithmic/exponential phase. In this study showed that the growth peaks of *B. amyloliquefaciens, T. harzianum*, and microbial cocktails were shown in 7 days fermentation time. In this phase is the time for growing and microbial cell proliferation so that the number of cells increases, and is an important phase for the life of microorganisms in general, especially for *B. amyloliquefaciens, T. harzianum*, and microbial cocktails in this study. The growth is characterized by the presence of white color on the surface of the substrate on all three kinds of microbial treatments. This shows that nutrition, water, and the temperature required by *B. amyloliquefaciens, T. harzianum*, and microbial cocktails are fulfilled, so that grew increases. Microbes grow and multiply due to the availability of nutrients, water, and temperatures as required (Gotor-Vila *et al.,* 2017).

Crude Fiber Content of Palm Kernel Cake

The crude fiber content of PKC after fermentation is presented in Table 1. The result showed no significant difference between the

Table 1. The Crude fiber Content of Palm Kernel Cake After Fermentation with *B. amyloliquefaciens, T. Harzianum*, and Microbes cocktails (%)

Incubation Time (days)	Type of Microbes			Average
	Bacillus amyloliquefaciens	*Trichoderma harzianum*	Microbes cocktail	
0	14.27±0.33	14.04±0.86	13.98±0.00	14.10±0.46
3	13.70±0.08	13.82±0.04	13.42±0.50	13.65±0.29
5	13.13±0.18	13.25±0.41	12.79±0.41	13.06±0.34
7	12.81±0.40	12.85±0.43	11.64±0.72	12.43±0.71
Average	13.47±0.60	13.48±0.64	12.96±0.96	

three microbial treatments of crude fiber content. Fermentation for 7 days showed that crude fiber PKC that fermented with microbial cocktail had the lower (11.64%) than fermented with *T. harzianum* (12.85%) and *B. amyloliquefaciens* (12.81%). Rizal *et al.* (2013) reported that the fermentation of PKC with *T. harzianum* with dose 9% reduced crude fiber 26%, while on PKC that fermented by *T. harzianum* decreased crude fiber about 14%. The low of the decrease in crude fiber by *T. harzianum* in fermented PKC indicated that microbial mass development was slightly caused by low inoculant (about 6%) that resulting in low cellulase enzyme production, and therefore low degraded cellulose. The decrease in crude fiber in PKC were the result of endo-beta-glucanase enzyme activity in *T. harzianum* which intersects chains in polysaccharides (Castro *et al.,* 2010) and caused by cellulase enzyme activity of *B. amyloliquefaciens* (Moreira and Filho, 2008). Wizna *et al.* (2008) reported the same resulted, that fermentation of cassava by-product with inoculant *B. amyloliquefaciens* obtained a decrease of crude fiber content by 32%. The mechanism of crude fiber in PKC may decrease due to the hydrolysis process by endo-β-glucanase (CMC-ase). CMC-ase randomly breaks cellulose chains comprising glucose and cello-oligosaccharides (Bhavsar and Bhalerao, 2012), while the--β-glucanase produced by *B. amyloliquefaciens*, 1.4-β -D-glucan cellobiohydrolase, aviselase, and C1 invade the outside cellulose at the non-reduction end with cellobiase as the main structure. Then β-glucosidase, cellobiase hydrolyzes cellobiosa into glucose. The decrease in the crude fiber of PKC fermented by microbial cocktails was better than *B. amyloliquefaciens* and *T. harzianum*, indicated a positive association between *B. amyloliquefaciens* and *T. harzianum* in degrading cellulose.

Protein Content of Palm Kernel Cake

The protein content of PKC after fermentation is presented in Table 2. When PKC was incubated for 0, 3, 5, and 7 days the protein content was not significantly different among *B. amyloliquefaciens*, *T. harzianum,* and microbial cocktail treatment. Increased protein in PKC fermented by three microbial treatments was around 24-32%.

This showed that the activity of protein-breaking enzymes by *B. amyloliquifacien* and *T. harzianum* is no different. In this experiment, inorganic N elements were not added, so there would be no assimilation of proteins. An increased protein that occurs due to decreased levels of other elements and caused loss of dry matter during fermentation.

Rizal *et al.* (2013) reported fermentation in PKC using *A. niger*, *T. harzianum*, *Penicillium* sp, and *Neurospora crassa* increased from 0.5% to 21.9%. An increased protein was due to decreased levels of other elements and caused loss of dry matter during fermentation. *T. harzianum* has the ability to increase protein feed material and cellulose material. Fermentation of PKC with *T. harzianum* increased the crude protein from 23.30 to 26.21% (Rizal *et al.*, 2013), meanwhile increased protein in PKC fermented with *T. harzianum* in this research was 23.00% to 28.54%, higher than Rizal *et al*. (2013) research.

Increased protein in PKC with *B. amyloliquefaciens* was from 21.95 to 28.54%. Meanwhile, when *B. amyloliquefaciens* was combined with rumen content on sago pith

Table 2. The Protein Content of Palm Kernel Cake that Fermented with *B. amyloliquefaciens*, *T. harzianum*, and Microbes Cocktail with the Incubation Time of 0,3,5 and 7 Days

Incubation Time (day)	Protein (%) Fermented with		
	B. Amyloliquefaciens	*T. harzianum*	Microbes cocktail
0	21.95 ± 0.10	23.00 ± 0.42	21.66 ± 0.99
3	23.81 ± 0.24	24.17 ± 0.60	24.08 ± 0.40
5	25.49 ± 0.75	28.02 ± 0.34	26.92 ± 1.65
7	28.54 ± 0.30	28.54 ± 0.30	28.68 ± 1.36

fermentation at 9 days incubation, the protein increased 42% (Wizna *et al.,* 2008). Peptidoglycans of the bacterial wall also also has contributed to increasing the protein content of the pith of sago. Simce the fermentation in PKC is only done by one type of microbial namely *B. Amyloliquefaciens,* this leads to an increase in protein, although was not as great as on the sago pith fermentation. The actual increase in protein comes from microbes because in absolute terms protein is used by microbes for metabolic and growth needs that is relatively increased. Wizna *et al.* (2009) reported that the use of *B. amyloliquefaciens* on the fermentation of cassava by-product with the addition of minerals (urea, $MgSO_4$, ZA, KCl, NaH_2PO_4, $FeSO_4$) on the medium also increased the crude protein by 36%.

The result showed that fermentation with microbial cocktail increased crude protein from 21.66 to 28.68% at incubation 0 to 7 days. The increase in protein of PKC fermented with microbial cocktails is presented in Table 2. Increased protein on solid substrate comes from nucleic acids of bacteria which can contribute N because the bacterial cell wall contains peptidoglycan (glycoprotein). Thus the microbial cocktail can increase the protein content of PKC through the fermentation process. Also because the synergy of both of *B. amyloliquefaciens* and *T. harzianum* to degrade crude fiber and then the protein will released out of the cellular. The highest crude protein and the lowest crude fiber results among three microbial treatments were fermentation with microbial cocktail treatment.

Palm Kernel Cake by Microbial Cocktail

The chemical composition of PKC before and after fermented with microbial cocktail showed that protein content and acid detergent (ADF) fiber increased. While crude fiber and neutral detergent fiber (NDF), hemicellulose, crude fat, and ash decreased (Table 3). The total amino acid composition of PKC before and after fermentation is presented in Table 8. The data showed that total amino acid composition was decreased (2.13%) in PKC after fermentation. But the glutamic acid, arginine, and methionine showed improvement, respectively 2.62 to 3.08%; 1.88 to 2.41% and 0.71 to 0.73%.

Increased protein on PKC fermented by three kind of microbial treatments around 24-32%. In while previous research showed that protein increased in PKC fermentation from 14.19 to 36.43% (Sinurat *et al.*, 1996, Supriyati *et al.*, 1998). The difference of protein content between them because the fermentation the previous research was attributed to the addition of minerals (urea, $MgSO_4$, ZA, KCl, NaH_2PO_4, $FeSO_4$) in previous studies, whereas in this experiment did not add some minerals at the media of fermentation. These minerals will support more optimal microbial growth because nutrients needed by microbes are available on PKC media. Wizna *et al.* (2008) reported that *B. amyloliquefaciens* also increased the crude protein around 36% in fermented onggok.

Analyzes of fat, acid detergent fiber (ADF), neutral detergent fiber (NDF), and amino acid analyzes were performed only on the highest protein and the lowest crude fiber results obtained on fermentation with microbial cocktail treatment. The chemical composition of PKC after fermented with microbial cocktail showed protein content and ADF increased, while crude fiber NDF, hemicellulose, crude fat, and ash decreased (Table 3). During the PKC fermentation process, NDF decreased indicating due to the breaking of lignocellulose and hemicellulose bonds so that cellulose and lignin can be detached from the bond by cellulase and lignocellulase enzymes.

Table 3. The Chemical Composition of of Palm Kernel Cake Before and After Fermentation with Microbial Cocktail

Parameters	Chemical Composition	
	Before Fermentation	After Fermentation
Crude Protein (%)	21.66	28.68
Crude Fiber (%)	13.98	11.64
NDF (g/100 g)	62.99	56.39
ADF (g/100 g)	42.21	45.95
Hemicellulose (g/100 g)	20.78	10.44
Ash (g/100 g)	6.81	4.34

NDF (neutral detergent fiber); ADF (acid detergent fiber)

Table 4. Essential Amino Acid Composition of Palm Kernel Cake Before and After Fermentation by a Microbial Cocktail

Amino Acids	Before Fermentation	After Fermentation
	---------- (% w/w) ----------	
Aspartic acid	0.54	1.33
Glutamic acid	2.62	3.08
Serin	0.74	0.73
Histidine	0.54	0.52
Glycine	0.82	0.73
Threonin	0.7	0.59
Arginine	1.88	2.41
Alanin	0.85	0.66
Tyrosine	0.58	0.52
Methionine	0.71	0.73
Valin	0.91	0.87
Phenylalanine	1.15	1.01
I-leusine	0.67	0.57
Leusin	1.05	0.96
Lysine	1.24	0.92
Total of Amino Acids	15.99	15.65

% w/w = percentage of weight per weight

While the breakdown of ADF was slow, it is indicated that degradation has not occurred in 7 days incubation.

The decrease in fat content after fermentation from 12.23 to 11.46% was due to the use of fat contained in PKC by microbial cocktails for growth. The same research happened to the coconut cake and PKC that fat content was decreased when fermentation with *A. niger* NRRL 337 (Supriyati *et al.,* 1998).

The total amino acid composition (TAA) in PKC before and after fermentation is summarized Table 4. During the fermentation process, oxidation takes place and various reactions on the PKC substrate are assimilated and dissimilated. Microbial cocktails used sugars that are already hydrolyzed by enzymes. The growing microbial population then produced beneficial substances such as amino acids. Increased amino acid, i.e glutamate, arginine, and methionine after fermentation indicated that the three amino acids are synthesized by microbial cocktails. Meanwhile, other types of amino acids did not increase even decrease. Amino acids that did not increase indicated nutrients needed in PKC are not available, so the microbial cocktails do not synthesize them.

In general, the availability of amino acids such as methionine in poultry feed ingredients is very small even in certain materials not available. Increased content of methionine is an added value to the PKC fermented microbial inoculum cocktail.

CONCLUSION

The nutritional content of palm kernel cake increased after fermentation using a microbial cocktail so that it could be used as poultry feed ingredients

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