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Improving Quality and Nutrient Content of Palm Kernel Meal with *Lactobacillus fermentum*

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ABSTRACT

Palm kernel meal (PKM) had the potential as an ingredient of broiler ration. PKM must be processed by fermentation with *Lactobacillus fermentum* to improve the nutritional quality. The aim of this study was to enhance quality and nutritional content of PKM through fermentation by integrating the composition of substrate and fermentation time. This research used a completely randomized design (CRD) with 3x3 factorial and repeated for 3 times. Factor A was substrate composition: A1 (100% PKM), A2 (90% PKM + 10% rice bran), and A3 (80%PKM +20% rice bran). Factor B was fermentation time: B1 (2 days), B2 (4 days), B3 (6 days). The observed variables were cellulase enzyme activity, mannannase enzyme activity, protease enzyme activity, crude protein (CP), crude fiber (CF), crude lipid (CL), nitrogen retention (NR), digestibility of crude fiber (DCF) and metabolic energy (ME) on Fermented PKM. The results showed that factor A and B had a very significant interaction ($P<0.01$) and those factors also exposed a very significant effect on cellulase activity, mannanase activity, protease activity, CP, CF, CL, NR, DCF and EM of fermented PKM. In conclusion the combination of substrate composition (80%PKM+20% rice bran) with fermentation time (4 day) showed optimal results as seen from aktivity selulase 17.63U/mL; mannanase 24.31U/mL; protease 10.34U/mL; CP 25.81%; CF 16.90%; CL 1.83%; NR 62.84%; DCF 54.37% dan ME 2743Kkal/kg.

Key words: Quality, Nutrient, Palm kernel meal, *Lactobacillus fermentum.*

INTRODUCTION

Indonesia occupies the top position as a major palm oil producing country in the world. According to Directorate General of Plantations, Ministry of Agriculture, in 2021 oil palm plantations sector will reach 15.081.021 hectares with CPO production of 49.7 million tons. CPO production has increased by around 6.8 million tons in the last 4 years. This increase in CPO production will certainly be followed by an rise palm oil processing by-products, one of which is palm kernel meal (PKM).

Based on the data above, PKM is an promise and feasible ingredient to be used as feed, especially for poultry. The nutritional quality of PKM as a poultry feed ingredient is quite good, such as CP 16.07%, CF 21.30%, CL 8.23%, Ca 0.27% and P 0.94% and Cu 48.4ppm (Mirnawati et al. 2018). From the data above, it turns out that PKM had high CP, but the utilization in the rations is still limited. Previous study using broiler chickens revealed the use of PKM up to 10% or replacing 40% of soybean meal in broiler rations (Rizal 2000). Improving the utilization of PKM in poultry rations can be made by processing the material. Processing is needed because without processing PKM has low quality (Olomu 2004; Sinurat 2003). The low quality of PKM is because 57.8% of the hemicellulose content of PKM consisted of β-mannan (Azman et al. 2016; Cerveró et al. 2010). To improve the efficiency of PKM, fermentation technology is needed in PKM which is expected to transform complex food substances into simpler ones through the work of microbes that secrete enzymes so the products are easier to be digested and utilized by poultry (Mirnawati et al. 2022a; Ciptaan et al. 2022a). Fermentation will usually produce smaller compounds that easier to digest than its orginal unfermented ingredient (Okonkwo and Igwilo 2022; Mirnawati et al. 2022b; Ciptaan et al. 2022b).

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Some factors that affect the fermentation were inoculum dosage and fermentation time (Sharma et al. 2020; Ajala et al. 2020). The right doses of inoculum will lead to a comfort growth of microbes to grow and develop rapidly, where the more doses of inoculum used, the more materials are remodeled and the time of fermentation affects in how much substances that are remodeled by microbes. Many factors need to be considered in the fermentation process including the inoculum doses, fermentation time, and substrate composition in order to provide optimum results (Mirnawati et al. 2022b; Ciptaan et al. 2022b).

Poultry do not have enzymes that break down fiber in the form of mannan in their digestive tract. PKM processing with fermentation technology is needed to raise PKM quality as a poultry feed ingredient by using cellulolytic and mannanolytic microbes (Meryandini et al. 2008; Purwadaria and Haryati 2003) which will diminish CF and mannan content of PKM and improve the quality of the PKM so that it can replace the use of soybean meal. Mirnawati et al (2019a) had conducted fermentation of PKM using *Bacillus subtilis* with 6 days of fermentaion duration which was able to give the greatest results in increasing the CP content of 24.65%, CF 17.35%, NR 68.47% and DCF of 53.25%. It was further explained that 6-days fermentation using *Bacillus subtilis* was able to provide mannanase activity of 24.27U/mL, cellulase of 17.13U/mL and protease activity of 10.27U/mL (Mirnawati et al. 2019b). Biological test results on broiler chickens showed that the PKM can be used until 25% in the rations (Mirnawati et al. 2020). Results from Seftiadi et al. (2020) showed that the addition of 80% PKM in the manufacture of *L. fermentum* as inoculum can increase the activity of cellulase (18.84U/mL), activity of mannanase (24.86U/mL) and activity of protease (10.45U/mL).

Based on these description, it is prominent to conduct a research to establish the effect of substrate composition on fermentation time with *L. fermentum* to enhance the quality and nutritional content of palm kernel meal (PKM).

MATERIALS AND METHODS

This study was conducted in August-November 2022. The material used were PKM obtained from Incasi Raya Padang company, NA agar media (Merck, Germany), distilled water, H_2SO_4 (Merck, Germany), acetone (Merck, Germany), alcohol, NaOH (Merck, Germany), selenium catalyst (Merck, Germany), laboratory materials for proximate analysis and broiler chickens with Cobb strain CP-707 as many as 30 birds aged 6 weeks weighing 1.5kg.

The equipment used in this study were ovens, autoclaves, incubators, analytical balances, plastic containers, porcelain dishes, metal cups, erlenmeyer, spray bottles, knives, ovens, furnaces, desiccators, incubators, beakers, grinding tools, filter paper, aluminum foil, tissue, syringe, ballistic bomb calorimeter and metabolic cage (equipped with a drinker).

This research used a completely randomized design (CRD) with 3x3 factorial and repeated 3 times for each treatment. The treatment consisted of 2 factors: factor A (substrate composition) which was a mixture of PKM and rice bran (RB) consisting A1 (100% PKM), A2 (90% PKM+10% RB) and A3 (80% PKM+20% RB). Factor B (length of fermentation) consists of B1 (2 d), B2 (4 d), and B3 (6 d). The parameters of this study were cellulase activity, mannanase, protease, CP, CF, CL, NR, DCF and ME of fermented Fermented PKM.

PKM Fermentation Processing

PKM and rice bran were used as substrates with a ratio of substrate composition, namely: (A1) PKM 100%, (A2) PKM 90% + rice bran 10%, (A3) PKM 80% + rice bran 20% and 70 mLL of distilled water was added to each treatments, then sterilized in the autoclave and cooled. The sterile substrate was then mixed with 7% inoculum and incubated for 2, 4 and 6 days, respectively. Then the bacteria were killed by placing them in an oven with a temperature of 80ºC for 2 hours until the heavy sample remained, aiming to stop bacterial activity during the fermentation process. After that the sample was milled and ready for analysis.

Parameter Measurement

Cellulase Activity (Jennifer and Thiruneelakandan 2015)

One mL of crude enzyme plus 1mL of extract (0.5mL of CMC+10mL of acetate buffer), incubated for 30min at 40˚C in a water bath shaker, 1mL is taken plus 1mL of Nelson AB, heated in boiled water for 20min, then 1mL of phospomolybdate was added plus 7mL of distilled water, then measured at the wavelength 575nm. To see the amount of cellulase activity, the formula used is:

$$\text{Cellulose activity (U/mL)} = \frac{\text{X } x \text{ P } x \text{ 1000}}{\text{T } x \text{ BM}}$$

Note: X = Conversion result of standard curve
P = Dilution
T = Time
BM = Glucose Molecular Weight

Mannanase Activity (Star 2010)

One mL of crude enzyme was reacted with 1mL of mannan substrate (0.5 mannan plus 10mL of phosphate buffer), after that being incubated for 30min in a shaker water bath at 60˚C, 1mL was taken, and 1mL of Nelson AB was added and then cooled at room temperature, 1mL phosphomolybdate and 1mL of distilled water were added, it was then read at a wavelength of 575nm. To see the amount of mannannase activity, the formula is used:

$$\text{Mannannase activity (U/mL)} = \frac{\text{X } x \text{ P } x \text{ 1000}}{\text{T } x \text{ BM}}$$

Note: X = Conversion result of standard curve
P = Dilution
T = Time
BM = Glucose Molecular Weight

Protease Activity (Cupp and Enyard 2008)

Casein solution 2.5mL of 1% was added with 1.5ml of phosphate buffer (0.1M pH 7). Incubation was done in a water bath (37˚C, 10min), and 1mL of enzyme extract was added and then being incubated in water bath (50˚C, 10min). Enzyme activity in the blank solution was stopped by adding 5mL of 20% TCA, and then its being mixed with a vortex and chilled in the refrigerator for 30min to agglomerate the protein. Centrifuged (5000rpm,

15min at 4ºC) filter and the supernatant was removed. Supernatant about 2mL was added with 5mL of 0.5N NaOH and 0.5mL of Folin-Ciocalteau reagent and leave for 10min. The absorbance then was read at a wavelength of 650nm. The amount of protease activity was counted by formula:

$$\text{Protease Activity (U/mL)} = \frac{Y\,x\,a}{b} \text{ x } \frac{1}{t}$$

Note: Y = Sample absorbance
a = Value a of the regression curve Y = a + bx
b = Value b of the regression curve Y = a + bx
t = time of incubation

CP, CF and CL were measured using proximate analysis, while NR, DCP and ME were measured using the Sibbald method (1976). This experiment used 30 broiler chickens aged 6 weeks. Before given the treatments, chickens were unfed for 36h to dodge the effect of previous feed. The treatment started with feeding chicken about 2grams/head of fermented products, then the chickens were located in a metabolic cage that was provided with a drinking container and feces storage. Feces was collected hourly for 30h and each hour was sprayed with 0.3N H_2SO_4 to dodge evaporation of nitrogen. The feces were dried in an oven at 50˚C and then being grounded to a fine powder and then analyzed for NR, CFD and ME of the feces.

Statistical Analysis

All data were tested by statistical analysis according to Steel and Torrie (2002), any difference between treatments was tested by Duncans multiple range test (DMRT) (P<0.01).

RESULTS AND DISCUSSION

Cellulase Activity

The result obtained represented a very significant interaction (P<0.01) between the factors (A &B) on the activity of the cellulase enzyme. The interaction between factor B and factor A indicated that cellulose activity of A3B1 was very significant (P<0.01) higher than A2B1 and A1B1 where the two treatments were related. The A3B2 treatment was also significantly (P<0.01) higher than the A2B2 and A1B2 treatments. The A3B3 treatment was very significant (P<0.01) higher than the A2B3 and A1B3. The graph of cellulase enzyme activity for each treatment is shown in Fig. 1.

From Fig. 1, it can be seen that cellulose activity increased from day 2 to day 6 along with the addition of rice bran. The inclusion of rice bran to the composition of the fermentation substrate gave a higher cellulase enzyme activity than without its addiction. This is because the addition of rice bran causes more available nutrients to be used for the growth of *L. fermentum*. Wizna et al. (2014) stated that rice bran was as an energy source for microorganisms to grow and develop, the high growth of microbes will lead to the greatest cellulase enzyme produced (Mirnawati et al. 2019; Mirnawati et al. 2022b).

The alleviate in cellulase enzyme activity that occurred on day 6 was due to *L. fermentum* entering a stationary phase where *L. fermentum* was not productive to produce enzymes metabolically. This agrees Moriki et al. (2019) and Fadahunsi et al. (2020) who claimed that there was a degrade of cellulose activity by *L. fermentum* along with the longer fermentation. High cellulase activity resulted in A3B2 treatment, namely 17.63U/mL. The result was better than result obtained by Mirnawati et al. (2019) who found that PKM fermentation with *Bacillus subtilis* had cellulose activity of 17.13U/mL. However, the inclusion of 300ppm humic acid conduce the enhance of cellulase activity of fermented PKM with *B. subtilis* (Mirnawati et al. 2022b).

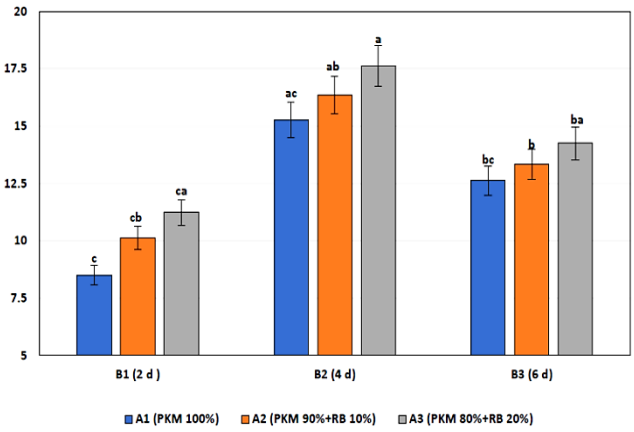

Fig. 1: Cellulase enzyme activity (U/mL) of fermented palm kernel meal.

Mannanase Activity

The result represented a very significant effect (P<0.01) between the factors on the mannanase enzyme activity. The A3B1 treatment was very significant (P<0.01) higher than A2B1 and A1B1. The A3B2 treatment was also very significant (P<0.01) higher than A2B2 and A1B2. A3B3 treatment was very significant (P<0.01) higher than A2B3 and A1B3. The average activity of the mannanase enzyme for each treatment can be seen in Fig. 2.

Based on the Fig. 2, it can be concluded that the more the inclusion of rice bran substrate of fermentation, the higher the mannanase activity in the 2-, 4- and 6-days

treatments. Rice bran has high porosity because it forms pores on the substrate which can facilitate bacterial growth in the fermentation medium (Murni et al. 2008). The more microbial growth, the more enzymes produced so that the enzyme activity increases (Mirnawati et al. 2022b; Ciptaan et al. 2022b). Along with the longer fermentation, the activity of the mannanase enzyme decreased on the 6th day. The decrease in mannanase enzyme activity that occurs because *Lactobacillus fermentum* enters a stationary phase where *Lactobacillus fermentum* is not productive to produce metabolically enzyme.

Protease Activity

The result represented a very significant effect (P<0.01) between the factors on protease activity. The interaction between factor B and factor A showed that

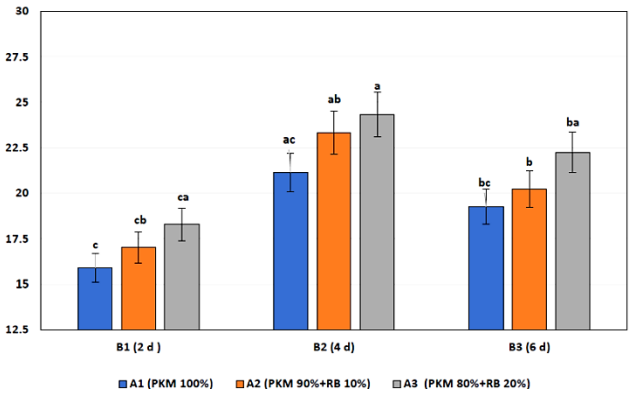

Fig. 2: Mannanase activity (U/mL) of fermented palm kernel meal.

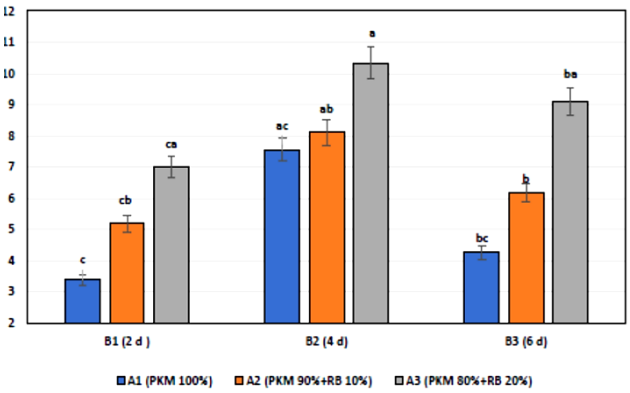

Fig. 3: Protease enzyme activity (U/mL) of fermented palm kernel meal.

A3B1 was very significant (P<0.01) higher than A2B1 and A1B1. Treatment A3B2 also had a very significant (P<0.01) higher protease activity than A2B2 and A1B2. A3B3 treatment was very significant (P<0.01) higher than A2B3 and A1B3. The high and low of each treatment on the protease activity can be seen in Fig. 3.

Fig. 3 revealed that the high addition of rice bran in the A2, A3 treatments of B1, B2 and B3 had effected in the higher of protease activity. The increase in protease activity was due to bacteria being able to grow and develop properly. The high growth of bacteria is related to the addition of bran, this is because the bran serves as an energy source for microbial growth.

Musaalbakri et al. (2005) stated that the more microbes grow and develop on the substrate, the higher the protease activity of fermented PKM. Further added by Musaalbakri et al. (2005) and Mirnawati et al. (2022b) microbes activity during fermentation can enhance the protease activity of the fermented material. Mannanase activity decreased both on day 4 and on day 6 of fermentation. This agrees with Mirnawati et al. (2022b) who describe that fermentation time is one of the determining factors in the material fermentation process where the longer the time of fermentation will cause more substrates to be overhauled by microbial enzymes so that enzyme activity will increase. However, fermentation time that is too long will cause the nutrients in the substrate to decrease so that the microbial population will decrease so that the enzyme activity will also decrease (Ciptaan et al. 2022b).

Crude Protein (CP)

The resulted showed that both factor A and B highly affected (P<0.01) CP content of PKM. The interaction revealed that treatment A3B1 was very significant (P<0.01) higher than A2B1 and A1B1 while A3B2 was very significant (P<0.01) higher than A2B2 and A1B2. The A3B3 treatment was also very significant (P<0.01) higher than the A2B3 and A1B3. The graph of the average CP content of Fermented PKM can be seen in Fig. 4.

Based on the Fig. 4, it showed that the more the addition of bran, the higher the crude protein of PKM in the 2-, 4- and 6-day fermentation treatments. While the length of fermentation, there was an increase in CP on the 4th days then on the 6th day there was a decrease. The improve in CP was caused by the increase in bacterial growth where the more bacterial growth, the more cell mass of bacteria, resulting in an increase of protein. This result agrees with Iyayi et al. (2004) which stated that the improve of CP is thought to be due to the inclusion of protein contributed by microbial growth that produce single cell protein (SCP) or cell biomass which contain about 40-65% protein. This was in agree with Ciptaan et al. (2022b) who revealed that CP after fermentation was increased due to the contribution of microbes activity.

The increase in CP in A3B2 treatment was also caused by the high protease activity, where the higher enzyme activity will lead to a higher enzyme production, while the enzyme itself is a protein. This is as what stated by Mirnawati et al. (2022b) that microbes will produce enzymes which are proteins in fermentation and the microbes itself were single cell proteins so that an increase in CP was found after fermentation. The CP produced in the A3B2 treatment was 25.81%. This was higher than PKM fermentation with *Bacillus subtilis* that produced 24.65% CP (Mirnawati et al. 2019a) and PKM fermentation with *Bacillus amyloliquefaciens* that had 13.13% CP (Pasaribu et al. 2019).

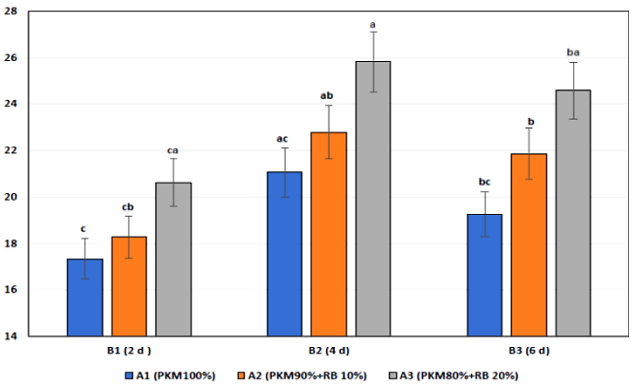

Fig. 4: Crude protein contents (%) of fermented palm kernel meal.

Crude Fiber (CF)

The resulted represented that both factor A and B highly affected (P<0.01) the CF content of fermented PKM. The interaction between factor B and factor A indicated that treatment A1B1 was very significant (P<0.01) higher than treatment A2B1 and A3B1. Treatment A1B2 was very significant (P<0.01) higher

than A2B2 and A3B2. The A1B3 treatment was also very significant (P<0.01) higher compared to A2B3 and A3B3. The graph of CF content for each treatment was shown in Fig. 5.

Data in Fig. 5 showed that the more addition of bran, the lower CF content of fermented PKM, both in the 2-, 4- and 6-day fermentation treatments. While the length of fermentation there was a decrease in crude fiber on day 4 and day 6. Fermentation can reduce fiber contents in agricultural by-products (Abid et al. 2019; Aboragah et al. 2020). The alleviation of CF in PKM after fermentation on day 6 was due to the large number of bacteria that grew which then degraded crude fiber of PKM. The improvement in bacterial growth increases the activity of the cellulase enzyme to alter cellulose from PKM into glucose so that the CF content would decrease after fermentation. This was in agree with Mirnawati et al. (2012) who stated that cellulase enzyme have ability to alter cellulose into glucose to produce energy so that CF of the material after fermentation will decrease. The results were lower than Mirnawati et al. (2019a) who found that fermentation of PKM using *Bacillus subtilis* was able to reduce CF by 17.35% and cellulase enzyme activity by 17.13U/mL.

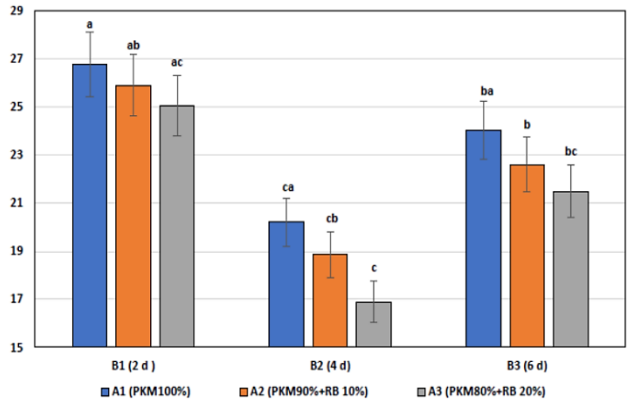

Fig. 5: Crude fiber contents (%) of fermented palm kernel meal.

Crude Lipid (CL)

The results revealed a very significant interaction (P<0.01) between factor A and B on CL of Fermented PKM. The interaction between factor B and factor A showed that treatment A1B1 was highly significant (P<0.01) higher than A2B1 and A3B1. The A1B2 treatment was very significant (P<0.01) higher than A2B2 and A3B2. A1B3 treatment was very significant higher (P<0.01) than A2B3 and A3B3. The average CF content of fermented PKM for each treatment can be seen in Fig. 6.

Based on the Fig. 6, it showed that the more the addition of rice bran, the lower CF found in the 2-, 4- and 6-days treatment. In the long fermentation treatment, there was an alleviate in the CL content of PKM on day 4 and on day 6. The decrease in CL was due to the number of bacteria that grew during fermentation, this could be seen in the number of colonies that grew where the more bacteria grew, the more lipase enzymes produced to alter fat into fatty acids and glycerol. Furthermore, fatty acids can be utilized by microbes as a source of energy for their growth, resulting in a alleviate of CF content after fermentation. This result agrees with Cuevas-Rodriguez et al. (2004) who reported that the alleviate in fat during fermentation is due to oxidation and the use of fatty acids by bacteria as an energy source.

The enhance of CF in the B3 treatment was caused by the longtime of fermentation that resulted in the decrease of the availability of nutrients in the substrate caused by microbial growth. At the end of the fermentation there was an improve in the CF content of PKM. This was in agree with Agustina et al. (2015) which stated that the availability of nutrients in the fermentation media will decrease with increasing fermentation time which can cause bacteria to die so that the CF of the material will increase.

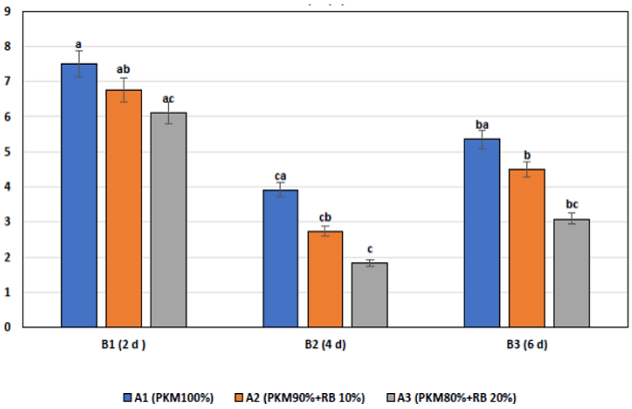

Fig. 6: Crude lipid contents (%) of fermented palm kernel meal.

Nitrogen Retention (NR)

The results revealed a very significant interaction (P<0.01) between factor A and B on NR. The interaction between factor B and factor A revealed that treatment A3B1 was very significant (P<0.01) higher than A2B1 and A1B1. The A3B2 treatment was very significant (P<0.01) higher than the A2B2 and A1B2. The A3B3 treatment was also very significant (P<0.01) higher than A2B3 and A1B3 treatments. The correlation between fermentation time and substrate composition on NR can be seen in Fig. 7.

Based on Fig. 7, it revealed that the more addition of rice bran the higher the nitrogen retention in both the 2-, 4- and 6-day fermentation treatments, while the 2-, 4- and 6-days of fermentation increased NR on the 4th days and then on the 6th days. The data above showed that high NR in the A3B2 was due to high protease activity in the A3B2. High protease activity lead to a high enzyme's ability to alter protein into amino acids so that it enhanced the product quality. This was in agree with Mahfud et al. (2004) that microbial enzymes can alter protein sructures into amino acids so it would increase nitrogen retention. In addition, the high nitrogen retention in the A3B2 Fermented PKM was also caused by the amino acid content of the fermented products which was better than the PKM before fermentation (Mirnawati et al. 2022a). Wahju (1997) which stated that the balance of amino acids greatly determines material quality, seen from the high value of NR. The highest NR (62.84%) was found in the A3B2 treatment. This was lower than result found by Mirnawati et al. (2019a), that fermentation of PKM using *Bacillus subtilis* had NR of 68.47%.

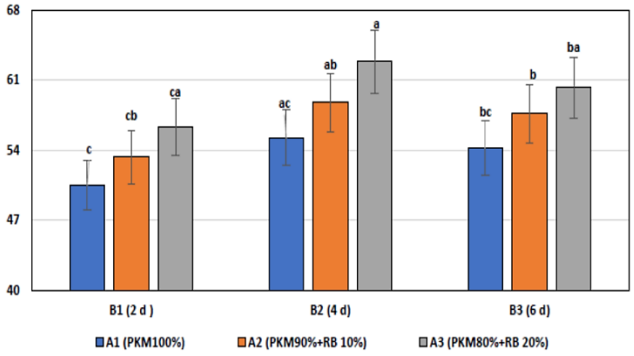

Fig. 7: Nitrogen Retention (%) of fermented palm kernel meal.

Digestibility of Crude Fiber (DCF)

The results revealed a very significant interaction (P<0.01) between factor A and B on DCF of PKM. The interaction between factor A and factor B showed that treatment A3B1 was very significant (P<0.01) higher than A2B1 and A1B1 while A3B2 was very significant (P<0.01) higher than A2B2 and A1B2. The A3B3 treatment was very significant (P<0.01) higher than the A2B3 and A1B3. The graph of high and low DCF of fermented PKM for each treatment can be seen in Fig. 8.

Based on the Fig. 8, it can be seen that the more addition of rice bran, in the A2 and A3 treatments, the higher the digestibility of CF in both the 2, 4 and 6 days fermentation treatments. Meanwhile, during the fermentation period, the digestibility of CF increased on the 4th days and then on the 6th days there was a decrease. The high DCF in the A3B2 treatment was caused by the low CF content in the A3B2 treatment. The lower CF content of feed will lead to an increase of DCF. This was in agree with Mirnawati et al. (2017), that DCF rely on the CF of feed, the higher CF would make a lower DCF found due to the limit of poultry ability to digest CF. DCF relies on the content of CF in feed and the amount of CF consumed. The high CF in the feed has a negative effect on nutrients digestion and absorption in poultry (Mirnawati et al. 2022b).

The low DCF in the 6-days fermentation caused by the long fermentation time where the longer of fermentation will lead to the less nutrients in the substrate so that microbial growth decreases which causes the improve of CF which in turn will lessen the digestibility of crude fiber. High DCF was produced in the A3B2 treatment, which was 54.37%. The result was higher than the result from Mirnawati et al. (2019) who revealed that PKM fermentation with *Bacillus subtilis* produced DCF of 53.25%.

Metabolizable Energy (ME)

The results revealed a very significant interaction (P<0.01) between factor A and B on the energy content of PKM metabolism after fermentation. More details about the energy metabolism of fermented PKM can be seen in Fig. 9.

Based on Fig. 9, it can be seen that the more addition of bran in both the A2 and A3 treatments, the higher the ME of PKM in the 2-, 4- and 6-days treatments. Meanwhile, during the fermentation period, there was an increase in metabolic energy on day 4 which then decreased on day 6 by microbes as an energy source. The composition of 80% PKM substrate + 20% rice bran with 4 days fermentation is the best composition for microbial growth that can increase metabolic energy after fermentation of PKM. In agrees with Widjastuti et al. (2007) which stated that during fermentation, there will be changes in complex molecules into simpler and easily digestible molecules. In addition, there was an increase in the glucose content, which is a product of hydrolysis of cellulose from Fermented PKM cellulase during fermentation, where then glucose will be counted as metabolic energy. In accordance to Gerharzt (1990) that cellulase is a complex enzyme that works gradually or simultaneously to break down cellulose into glucose units.

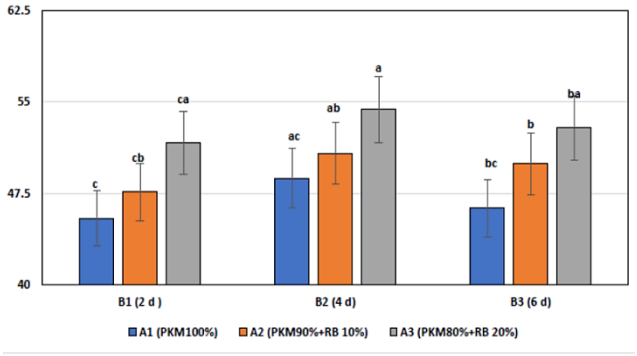

Fig. 8: Digestibility of crude fiber (%) of fermented palm kernel meal.

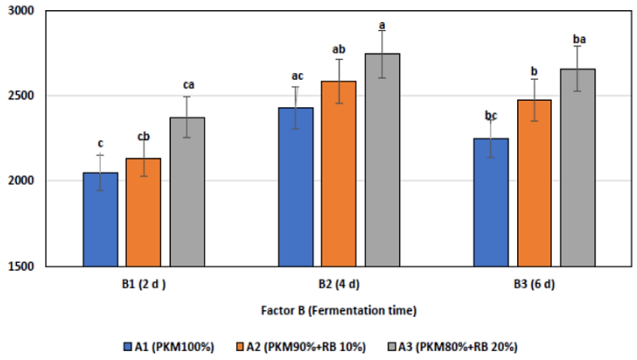

Fig. 9: Metabolizable energy (Kkal/kg) of fermented palm kernel meal.

Conclusion

This study demonstrated that the composition of the substrate consisting of 80% PKM + 20% rice bran with 4 days fermentation is the best treatment. This can be seen from the cellulase activity of 17.63U/mL; mannanase activity of 24.31U/mL; protease activity of 10.34U/mL; 25.81%; crude fiber 16.90%; crude fat 1.83%; nitrogen retention 62.84%; digestibility of crude fiber 54.37% and metabolic energy 2743Kcal/kg.

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Author's Contribution

Mirnawati was in charge to supervise the experiment and writing the original script. Gita Ciptaan, Imana Martaguri and Ferawati conducted the experiment and analyzed the data. Anifah Srifani finalize the manuscript.

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