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Research Article

Effects of the Combinations of Cassava Leaf Meal and Palm Kernel Cake Mixture Fermented by *Bacillus amyloliquefaciens* on the Alteration of their Dry Matter, Crude Protein, Crude Fiber and Crude Lipid Contents

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Abstract

Background: The utilization of Cassava Leaf Meal (CLM) and Palm Kernel Cake (PKC) in poultry diet is still limited due the high in crude fiber and other anti-nutrition compounds. The processing of these 2 feedstuffs, such as fermentation by using microbes did not perform much improvement in their utilization in poultry diets. Fermentation of feedstuffs by using fungi took a longer time than by using bacteria such as *Bacillus amyloliquefaciens*. Fermentation of the combination of CLM and PKC could elicit the complementary effect in supporting the growth of bacteria. **Objective:** Thus, an experiment had been conducted to determine the effects of the combinations of CLM and PKC mixture fermented by *Bacillus amyloliquefaciens* on the alteration of their dry matter, crude protein, crude fiber and crude lipid contents and the best combination of CLM and PKC for fermentation. **Materials and Methods:** This experiment was performed in a completely randomized design with 5 treatments and 4 replicates. The treatments were the combination of CLM and PKC as follows: 100:0% (A), 80:20% (B), 60:40% (C), 40:60% (D) and 20:80% (E). These combinations of CLM and PKC were fermented by using *Bacillus amyloliquefaciens* at the dose of 6% for 6 days. Measured variables were the reduction percentage in dry matter, crude fiber and crude lipid and the increasing percentage in crude protein. **Results:** Results of experiment indicated that the combinations of CLM and PKC mixture very significantly affected ($p<0.01$) the reduction percentage in dry matter and crude fiber and increasing percentage in crude protein, as well as significantly influenced ($p<0.05$) the reduction percentage in crude lipid. **Conclusion:** The lower reduction percentage in dry matter was in treatments A and B, the higher increasing percentage in crude protein was in treatments B and C, the higher reduction percentage in crude fiber was in treatments D and E and the higher reduction percentage in crude lipid was in treatments A, B and C. It is concluded that the best combination of CLM and PKC fermented by *Bacillus amyloliquefaciens* was 80:20% (treatment B).

Key words: Cassava leaf meal, palm kernel cake, *Bacillus amyloliquefaciens*, fermentation, nutrient alteration

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Indonesia is the 5th largest cassava (*Manihot esculenta* Crantz.) producer in the world after Nigeria, Somalia, Thailand and Brazil with the production upto 24.08 million t. This cassava is planted on 1.22 million ha land[1]. This cassava plantation produces a huge amount of Cassava Leaf Meal (CLM). According to Sudaryanto *et al.*[2], the production of fresh cassava leaf per hectare is ranging from 7-15 t. It means that the fresh CLM production in Indonesia reached 8.54-18.30 million t or approximately 2.0-4.0 million t of dried CLM.

The utilization of CLM in the poultry diet has been studied extensively for replacement part of soybean meal (SBM) because this CLM contains high Crude Protein (CP). According to Wyllie and Chamanga[3] the CP content of CLM is ranging from 17-28%, depends on age, position and/or combination with stalk. Its Crude Fiber (CF) content[3,4] is ranging from 11-21%. Ravindran and Blair[5] found that t he CLM also contains high HCN and is ranging from 400-600 ppm. Based on its nutrients and anti-nutrition contents, the CLM could be included in limited amount (5%) in broiler's diet[3]. Iheukwumere *et al.*[4], Ravindran and Blair[5] and Iheukwumere *et al.*[6] also found that 5% CLM inclusion in the diet did not affect growth rate, blood parameters, production performance, carcass production and the weight of digestive organs (gizzard and liver) of broilers. The utilization of CLM up to 10% in layer's diet could decline the egg yolk cholesterol content[7]. Wang *et al.*[8] reported that CLM could be included upto 10% in meat type duck diets.

To increase the utilization of CLM in poultry diets, one of several methods has been done, that is the biological processing by using microbes which is called fermentation. One of the microbes that is usually used, is fungi, such as *Aspergillus niger* and *Trichoderma viride.* Darma *et al.*[9] and Bakrie *et al.*[10] found that the fermentation with *Aspergillus niger* could augment the CP content and the digestibility of this CP of CLM, while it reduced the CF of CLM. This *Aspergillus niger* fermented CLM could be utilized upto 10% in broiler's diets[11]. Rizal *et al.*[12] also obtained that the fermentation of rutin isolated CLM with *Aspergillus niger* increased its CP content, while its CF and HCN contents decreased. This *Aspergillus niger* fermented rutin isolated CLM could be included upto 9% in the broiler diet[13]. The fermentation of CLM by using *Trichoderma viride* also decreased its CF, NDF and ADF, cellulose and hemicellulose contents[14], meanwhile its crude protein content increased[15].

Even though, several attempts have been performed to improve the nutritive values of CLM but the results do not satisfy because the utilization of this CLM in poultry diet is still limited. It is still necessary to investigate other ways to improve the nutritive values of CLM and increase its utilization in poultry diets. One of many ways is to combine the CLM with other feedstuffs, viz., with Palm Kernel Cake (PKC) in order to elicit the complementary effect in nutrients for the microbial growth during fermentation process. Palm kernel cake is the by-product of oil extraction from the kernel of palm plantation through chemical and mechanical procedure[16].

According to BPS[1] the development of palm plantation increases every year in Indonesia with the growth rate of 13.89%. The area of plantation was 3,152,400 ha in 2001 and increased to 4,888,000 ha in 2009, while the crude palm oil production was 8,080,000 t in 2001 (lower than crude palm oil production in Malaysia) and increased to 16,600,000 t in 2007 (higher than in Malaysia) resulted in high palm kernel cake production.

Sabrina *et al.*[17] reported that PKC contained 16.80% CP, 8.05% EE, 21.97% CF, 0.97% Ca and 0.80% P. Meanwhile, Nuraini and Susilawati[18] found that nutrient and energy contents of PKC were as follows: 16.34% CP, 7.71% EE, 20.34% CF, 3.79% ash, 0.83% Ca, 0.86% P and 1750 kcal kg^{-1} ME. In another experiment by Mirnawati *et al.*[19] found that PKC contained 19.0% CP, 2.0% EE, 16.0% CF, 58.8% NFE, 4.2% ash, 0.34% Ca, 0.96% P and 48.04 ppm Cu. According to Rizal[20] PKC could be supplemented up to 10% (replace 40% SBM) in broiler's diet. Supriyadi[21] reported that PKC could also be included upto 10% in duck's diet.

For increasing the utilization of PKC in poultry diets, it should be processed for example by fermentation with fungi. Sabrina *et al.*[22] fermented PKC with three species of fungi (*Rhizopus* sp., *Trichoderma harzianum* and *Neurospora sitophila*) and found that fermentation by using *Trichoderma harzianum* was the best among the fungi. This *Trichoderma harzianum* fermented PKC could be included up to 15% in broiler's diet[22]. The fermentation of PKC by using *Neurospora crassa* augmented its CP and β-carotene contents from 16.34-22.09% and from 46.78-225.34 ppm, respectively. Udiati[23] and Desni[24] reported that there was an increase in CP and a decrease in CF of PKC fermented by *Bacillus amyloliquefaciens* at the dose of 6% and fermentation length of 6 days. It has been reported that *Bacillus amyloliquefaciens* produces several enzymes, such as α-amylase, α-acetolactase, decarboxylase, β-glucanase, hemicellulose, maltogenic amylase, protease and xylanase[25-30].

Based on the above information, there is an opportunity to perform the CLM and PKC fermentation for increasing their utilization in poultry diets. So far, there is no information available on the fermentation of the combined CLM and

PKC by using *Bacillus amyloliquefaciens*. The purposes of this study is to study the effects of combinations of CLM and PKC fermented by using *Bacillus amyloliquefaciens* and to investigate the appropriate combination of CLM and PKC for fermentation.

MATERIALS AND METHODS

Experimental design: This experiment was performed in a Completely Randomized Design (CRD) with 5 treatments (CLM and PKC combinations) and 4 replicates. Treatments were the combinations between CLM and PKC as follow: 100:0% (A), 80:20% (B), 60:40% (C), 40:60% (D) and 20:80% (E). These combinations of CLM and PKC were fermented by using *Bacillus amyloliquefaciens* at the dose of 6.0% and fermentation length of 6 days.

Measured variables: These measured variables were the percentage of the alteration (reducing or increasing) in DM, CP, CF and CL of *Bacillus amyloliquefaciens* fermented CLM and PKC mixture. The percentage of the alteration was obtained from the difference of each measured variable value between before and after fermentation that was divided by the value before fermentation and multiplied by 100% for all measured variables. The DM, CP, CF and CL contents of fermented CLM and PKC mixture were determined based on the proximate analysis procedure according to AOAC[31]. Crude protein analysis follows the procedure of Kjeldahl. Crude lipid were analyzed by using sohxlet apparatus.

Data analysis: All the data were analyzed by analysis of variance of CRD. The differences among treatment means were detected by DMRT according to Steel and Torrie[32].

RESULTS AND DISCUSSION

Reduction percentage in DM: The fermentation of CLM and PKC mixture by using *Bacillus amyloliquefaciens* significantly affected (p<0.05) reduction percentage in DM as it is figured out in Table 1. The reduction percentage in DM of combinations of A, B and D was not different (p>0.05). However, the combination of A was significantly differed (p<0.05) from the combinations of C and E and the combination of B was also differed significantly (p<0.05) from C and E. The combinations of C, D and E were not significantly different (p>0.05). The reduction percentage in DM of the combinations A and B was lower than the reduction percentage in DM of combinations C and E. According to Phuc *et al.*[33] and Phuc *et al.*[34] the DM digestibility of CLM was only 72.6 and 72.8%, respectively in growing pig, while Adesehinwa[35] reported that the DM digestibility in PKC was 73.77%. The low in the DM digestibility of CLM resulted in less reduction percentage in the DM of treatments A and B. The low in the reduction percentage in DM could also be the result of the synthesis of protein in treatments A, B and C was higher as it was showed in Table 1, the increasing in the CP in treatments B and C was also higher. The synthesis of protein contributed to the increase in DM content of fermented CLM and PKC in treatments A and B, so that the reduction percentage in DM in A and B was lower. The other possibility was that the degradability of CF in treatment A and B was also lower than the degradability of D and E.

Increasing percentage in CP: The increasing percentage in CP was very significantly influenced (p<0.01) by *Bacillus amyloliquefaciens* fermented CLM and PKC combinations as showed in Table 1. The increasing percentage in CP of combination of E was not significantly different (p>0.05) from D but was significantly different (p<0.05) from combinations of A, B and C. The combination of D was significantly different (p<0.05) from A, B and C. The combination of A was differed significantly (p<0.05) from B and C. However, the combination of B and C did not significantly differ (p>0.05). The higher increasing percentage in CP was in treatments B and C. It seems that there was a complementary effect of protein and other nutrients in CLM and PKC, so that the synthesis of

Table 1: Effects of *Bacillus amyloliquefaciens* fermented CLM and PKC combinations on the reduction percentage in their DM, CF and CL and increasing percentage in their CP contents

	Measured variables			
Treatments (CLM:PKC)	Reduction percentage in DM	Increasing percentage in CP	Reduction percentage in CF	Reduction percentage in CL
A (100:0%)	4.0^{a}	8.17^{b}	22.49^{a}	17.68^{b}
B (80:20%)	6.0^{a}	9.99^{c}	27.72^{a}	16.55^{b}
C (60:40%)	9.0^{b}	9.55^{c}	29.41^{a}	16.24^{b}
D (40:60%)	7.0^{ab}	6.91^{a}	40.87^{b}	5.31^{ab}
E (20:80%)	9.0^{b}	5.70^{a}	36.85^{b}	3.43^{a}
SE*	0.02	0.26	1.30	2.30

a-c Means with different superscript at the same column are significantly differed (p<0.05), *Standard error of the mean protein was higher in treatments B and C than the other treatments. Ravindran *et al*.[36] reported that the protein of CLM could be utilized efficiently by the pig.

Reduction percentage in CF: *Bacillus amyloliquefaciens* fermented CLM and PKC combinations very significantly affected (p<0.01) the reduction percentage in CF as depicted in Table 1. The reduction percentage in CF of the combination of A was not differed (p>0.05) from the reduction percentage in CF of the combinations of B and C but was differed (p<0.05) from the combinations of D and E. The combinations of B and C were significantly different (p<0.05) from combinations D and E. However, the reduction percentage in CF of the combination D was not different (p>0.05) from the combination E. The reduction percentage in CF in treatments D and E was higher than those in treatments A, B and C. It seems that the degradability of CF was higher in PKC than that in CLM. Phuc *et al*.[33] found that the CF digestibility of CLM was only below 50% in growing pis, while the CF digestibility in PKC was around 70%[35]. This result was also in accordance with the results of experiments by Wizna *et al*.[37,38] who found that the fermentation by using *Bacillus amyloliquefaciens* decrease the crude fiber content in tapioca by-product and rice bran, respectively.

Reduction percentage in CL: The reduction percentage in CL was significantly influenced (p<0.05) by the *Bacillus amyloliquefaciens* fermented CLM and PKC combinations. The reduction percentage in CL of combination E was not significantly different from combination D but it was significantly differed (p<0.05) from combinations of A, B and C. The reduction percentage in CL of the combination D was not significantly different (p>0.05) from the reduction percentage in CL of combinations A, B and C. The reduction percentage in CL in treatment E was the lowest when it was compared with the other treatments, except the treatment D. It was most likely that the lipid content of CLM could be degraded easily when it was compared with lipid content of PKC. In contrary the lipid digestibility in CLM was very low[39]. Based on the results of this experiment it is seen that the treatment B is better than the other treatments, because the reduction percentage in DM, the increasing percentage in CP and the reduction percentage in CL were found mostly in treatment B.

CONCLUSION

The combination of *Bacillus amyloliquefaciens* fermented CLM and PKC influenced the alteration of dry matter and nutrient contents. The best combination of CLM and PKC fermented by using *Bacillus amyloliquefaciens* was 80:20% (treatment B).

SIGNIFICANCE STATEMENTS

This study is about the attempt to include the cassava leaf meal and palm kernel cake mixture fermented with bacteria *Bacillus amyloliquefaciens* in the diet of laying-hens. Cassava leaf meal and palm kernel cake are the waste/by-product of harvesting cassava and processing of palm oil. These two wastes have been used in the diet of poultry, but their utilization is still limited due to the low in their nutritive values. By fermenting the combination of these two feedstuffs, there is an improvement in their nutritive values.

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