Fermented palm kernel meal by different fungi in broiler diets

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Abstract. The low quality of palm kernel meal (PKM) is due to the presence of high fibrous fraction. Fermentation of a low-quality feedstuff by using fungi could improve its quality. An experiment was done to determine the effect of fungi fermented palm kernel meal on the performance of broilers. Palm kernel meal was fermented by different fungi (Aspergillus niger, Trichoderma viride and Pleurotus ostreatus). The study used 200-day-old chicks and the chicks were randomly placed in brooder cages. The broilers were fed with starter and grower diets from days 1 to 21 and days 21 to 42 respectively. Feed and water were provided ad libitum. The treatments diets were R1: basal diet (0% PKM), R2: (10% PKM), R3: 10% Aspergillus niger fermented PKM, R4: 10% Pleurotus ostreatus fermented PKM, R5: 10% Trichoderma viride fermented PKM, R6: 20% PKM, R7: 20% Aspergillus niger fermented PKM, R8: 20% Pleurotus ostreatus fermented PKM, R9: 20% Trichoderma viride fermented PKM. A completely randomized design was used in this study. Data were analyzed by the analysis of variance. The inclusion of 20% PKM in the diet decreased body weight gain and feed intake. Supplementation of the diets with 20% fungi fermented PKM could maintain body weight gain to the same level of those birds fed the basal diets. The percentage of gizzard weight was higher in birds fed the basal diet than the other treatment birds. In conclusion, fermentation of PKM with Aspergillus niger, Trichoderma viride and Pleurotus ostreatus could improve its quality. The performance of broilers fed the 20% PKM could be maintained provided.

1. Introduction
Although, Indonesia has been the world’s largest palm oil producer, the use of its waste and by-products, particularly palm kernel meal, is still limited. There are two ways to optimize the use of palm kernel meal for poultry; either as a feed ingredient [1] or as a source of prebiotic [2]. Problems of using palm kernel meal in poultry diets have been well-reviewed by Sundu et al. [3]. Among the problems reported, high fibre content (cellulose and beta mannan) and contamination of nutshells (lignin) are the two biggest problems that need to be coped with. Early study of Sundu et al (2004) indicated that the supplementation of palm kernel meal with commercial enzymes did not significantly improve the growth rate of birds, compared to those of enzyme-unsupplemented birds [4]. It seems likely that the commercial enzymes used did not suitably match the nutrients profile of palm kernel meal, particularly the fibre fractions. The rationale behind this speculation is that most of the commercial enzymes available in the market were designed not specifically for palm kernel meal-based diet.
The use of fermentation technology has been widely used in the research level to improve the quality of various feedstuffs, such as copra meal [5], rice bran [6] and coconut dregs [7]. Due to the boom of the oil palm industry over the last three decades and thus increased palm kernel meal production, finding out a specific fungus that suitable for palm kernel meal becomes an important step to optimize the utilization of this by-product. Among the fungi available, filamentous fungi such as Aspergillus niger, Pleurotus ostreatus and Trichoderma spp are common fungi used for solid-state fermentation to enhance the feeding value of the substrate. These fungi were believed to have the capability to not only grow on the surface of the substrate particles but also penetrate through them [8].

The improvement of the quality of the feedstuffs due to fermentation was through the production of an enzyme [9], elimination of toxic compounds and enhancement of the aroma [10]. Accordingly, it is an urgent call to find out a fungus matching to the physical and nutritional properties of palm kernel meal. The finding might be beneficial to improve the quality of palm kernel meal that is produced in abundant quantity in Indonesia. This study was designed to determine the effect of the use of fermented palm kernel meal with different fungi through solid-state fermentation in broiler diets.

2. Materials and methods

2.1. Solid-state fermentation

Fermentation of palm kernel meal was conducted used a solid-state fermentation according to the procedure of Jacob and Prema [11]. Palm kernel meal was kindly provided by the local palm oil producer. The palm kernel meal was sieved to separate the nutshell from the kernel. The sieved palm kernel meal was finely ground to 1–2 mm particle size and used as a solid substrate for solid-state fermentation. The fine ground palm kernel meal was autoclaved for 20 min at 20 psi and then cooled to room temperature. The 20 kg substrates were then incubated with different fungi (40 g Aspergillus niger, 200 g Pleurotus ostreatus an 40 g Trichoderma viride). The substrates were mixed thoroughly and then moistened with distilled water. The Aspergillus, Pleurotus and Trichoderma-mixed substrates were aerobically incubated and kept the substrates for 6 days for fermentation. After fermentation was terminated, the substrates were then oven-dried at 50°C for 48 hours.

2.2. Location and animals used in the study

The study was conducted in the animal house at the Universitas Tadulako, Palu, Indonesia. A total of 200-day-old unsexed Cobb chicks were purchased from the local hatchery. The birds were placed in brooder pens for one week. After 7 days, 108 birds were selected to be used as experimental animals and kept them for another 5 weeks. Each cage was equipped with a feeder and drinker and those were placed inside the cage. The cage and drinker were cleaned routinely and the surroundings were also regularly cleaned.

2.3. Feed and feeding

The birds were fed with starter diets from days 1 to 21 and grower diets from days 21 to 42. Diets were formulated to meet the nutrient requirements of starter broilers (see table 1), using the UFFF computer program version 1.11 [12]. The ingredients used in this study were purchased locally and mixed them all by using a horizontal feed mixer. The nine diets imposed in this study are described in table 2. Feeds were offered ad libitum twice a day at 08.00 a.m. and 04.30 p.m. and water was available at all times. Feed mixing was done every two weeks to maintain the freshness and quality of the mixed diet.
Table 1. Ingredient and nutrient composition of the experimental diets (%).

| Dietary components       | Starter diet (%) | Grower diet (%) |
|--------------------------|------------------|-----------------|
|                          | control          | PKM10 | PKM20 | control | PKM10 | PKM20 |
| Palm Kernel Meal         | 0                | 10.00 | 20.0  | 0       | 10.00 | 20.0  |
| Maize                    | 53.60            | 47.50 | 38.00 | 56.90   | 48.00 | 40.30 |
| Soybean                  | 25.00            | 26.00 | 25.00 | 24.00   | 25.00 | 26.00 |
| Fish Meal                | 13.00            | 13.00 | 13.00 | 10.00   | 10.50 | 9.10  |
| Rice bran                | 6.00             | 2.15  | 1.10  | 6.65    | 4.75  | 1.20  |
| Vegetable oil            | 0                | 0     | 1.50  | 0       | 0     | 1.50  |
| Dicalcium Phosphate      | 1.75             | 0.80  | 0.80  | 1.50    | 1.20  | 1.20  |
| Premix                   | 0.20             | 0.20  | 0.20  | 0.30    | 0.20  | 0.20  |
| DL-Methionine            | 0.20             | 0.20  | 0.15  | 0.25    | 0.20  | 0.20  |
| L-Lysine                 | 0.05             | 0.05  | 0.05  | 0.10    | 0.05  | 0.10  |
| Salt                     | 0.20             | 0.20  | 0.20  | 0.10    | 0.10  | 0.20  |

Calculated composition;

|                          | ME (kcal/kg) | Protein | Methionine | Lysine | Calcium | Available phosphorus |
|--------------------------|--------------|---------|------------|--------|---------|---------------------|
| Starter                  | 3130         | 22.20   | 0.65       | 1.35   | 1.27    | 0.86                |
| Grower                   | 3128         | 22.02   | 0.65       | 1.37   | 1.12    | 0.75                |
|                          | 3107         | 22.06   | 0.60       | 1.36   | 1.18    | 0.81                |
|                          | 3139         | 20.60   | 0.65       | 1.24   | 1.03    | 0.72                |
|                          | 3121         | 20.63   | 0.61       | 1.26   | 1.06    | 0.74                |
|                          | 3119         | 20.61   | 0.59       | 1.25   | 1.04    | 0.76                |

ME: Metabolizable energy; kcal: Kilo calori

Table 2. Details of experimental treatments.

| Diets | Details                                      |
|-------|----------------------------------------------|
| T-1   | Control                                      |
| T-2   | 10% palm kernel meal (PKM10) without fermentation |
| T-3   | 10% Aspergillus niger-fermented palm kernel meal (PKM10) |
| T-4   | 10% Pleorotus ostreatus-fermented palm kernel meal (PKM10) |
| T-5   | 10% Trichoderma viride-fermented palm kernel meal (PKM10) |
| T-6   | 20% palm kernel meal (PKM20) without fermentation |
| T-7   | 20% Aspergillus niger-fermented palm kernel meal (PKM20) |
| T-8   | 20% Pleorotus ostreatus-fermented palm kernel meal (PKM20) |
| T-9   | 20% Trichoderma viride palm kernel meal (PKM20) |

2.4. Gizzard, intestine and carcass measurement

On day 43 after excreta collection, two birds with body weight closest to the mean bodyweight were taken from each cage and fasted overnight. On day 44, the birds were individually weighed and slaughtered. After plucking and eviscerating by removing the head, the shank and the internal organs, the eviscerated carcasses, breast meat and legs (thighs and drumsticks) were individually recorded as a percentage of live body weight [13]. The empty weight of the gizzard was also individually weighed and expressed as a percentage of live body weight and the length of the small intestine was measured from the gizzard to ileo-caecal junction and expressed in cm.

2.5. Statistical analysis

A completely randomized design was adopted in this experiment with 9 treatment diets and four replicate cages. Data were analyzed by analysis of variance using the Minitab 14 statistical program
The significance of the differences between pairs of treatment means within any overall treatment effects, found significant by analysis of variance, was tested by the Tukey Test [15].

3. Results and discussion

3.1. Results
Data on the effect of the treatments on live weight gain, feed intake, feed conversion ratio (FCR), carcass percentage, the length of the small intestine and the weight of gizzard are shown in the table 3 and table 4. Treatments produced a significant effect (P<0.05) on body weight gain, feed intake and weight of gizzard while the effect of treatments on FCR, length of intestine and carcass percentage was not significantly different. The birds fed the 20% unfermented palm kernel meal was lighter than those of birds fed the control soy-bean based diets and 20% the *Aspergillus niger*-fermented palm kernel meal diets. The broilers fed the 20% unfermented palm kernel diet consumed less than all the other treatments birds. The gizzard of broilers fed the control diet was smaller than the birds fed the unfermented palm kernel diets, 20% the *Aspergillus niger*-fermented palm kernel and *Trichoderma viride*-fermented palm kernel diet. Data on mortality was not reported due to only 2 out of 108 birds died. All the cases of death that took place during the first week were not correlated to any specific treatment.

Table 3. The effect of diets on body weight gain, feed intake and feed conversion ratio.

| Diets | Parameters       | Body weight gain | Feed intake | Feed conversion ratio |
|-------|------------------|------------------|-------------|-----------------------|
| R-1   |                  | 2099             | 3560        | 1.70                  |
| R-2   | ab               | 2021             | 3596        | 1.79                  |
| R-3   | a                | 2085             | 3583        | 1.73                  |
| R-4   | ab               | 2016             | 3558        | 1.77                  |
| R-5   | ab               | 2011             | 3581        | 1.78                  |
| R-6   | ab               | 1787             | 3365        | 1.89                  |
| R-7   | a                | 2092             | 3574        | 1.71                  |
| R-8   | ab               | 2059             | 3585        | 1.75                  |
| R-9   | ab               | 2021             | 3594        | 1.78                  |

Values with the different superscript within a column were significantly different (P<0.05)

Table 4. The effect of diets on carcass, gizzard weight and small intestine length of broilers

| Treatments | Parameters       | Carcass (%) | Breast meat (%) | Leg (%) | Gizzard (%) | Length of SI (cm) |
|------------|------------------|-------------|-----------------|---------|-------------|-------------------|
| T-1        |                  | 69.2        | 24.7            | 22.1    | 1.25        | 205.0             |
| T-2        |                  | 67.3        | 22.5            | 22.8    | 1.85        | 229.3             |
| T-3        |                  | 68.8        | 24.4            | 22.2    | 1.64        | 212.0             |
| T-4        |                  | 67.7        | 23.5            | 22.3    | 1.61        | 223.0             |
| T-5        |                  | 68.6        | 24.7            | 21.6    | 1.72        | 212.8             |
| T-6        |                  | 68.8        | 22.5            | 22.4    | 2.03        | 223.5             |
| T-7        |                  | 68.3        | 24.5            | 22.3    | 1.84        | 215.8             |
| T-8        |                  | 68.8        | 24.6            | 22.0    | 1.74        | 216.3             |
| T-9        |                  | 68.6        | 23.9            | 22.2    | 1.92        | 220.3             |

SI: Small intestine; Values with the different superscript within a column were significantly different (P<0.05)
3.2. Discussions
Studies on the addition of palm kernel meal for broilers have been well-reviewed by Sundu et al [3]. The results found by the previous studies of Onwudike [16], Panigrahi and Powell [17] and Sundu et al. [4] were inconsistent. In this present study, the inclusion of 10% palm kernel meal diets did not deteriorate feed quality as the growth of the birds was not significantly different from those of control-fed birds. It seems that up to the level of 10% palm kernel meal in the diet, the effect of its inclusion has not been negatively affected. However, the birds fed the 20% palm kernel meal diet had lower body weight gain and feed intake than those of birds fed the control diet. The tolerable level of 10% palm kernel meal found in this study was inconsistent with the previous study of Sundu et al (2005), who found that the use of palm kernel meal up to 30% did not impair body weight gain of broilers [18]. This is possible because of the problems of diets used and the location of the study, rather than the level of inclusion of palm kernel meal per se. Sundu et al (2005) used diets with 23% protein and metabolizable energy of 3200 kcal/kg [18]. In this current study, the experimental starter diet was 22% protein and 3100 kcal/kg for metabolizable energy. The location of the study might also play a role in affecting the growth of birds. Our previous study in 2015 was done in Australia with having a lower temperature than the current location in Central Sulawesi, Indonesia.

Although the use of 20% palm kernel meal diets decreased body weight gain of broilers, compared to the control birds, using 20% Aspergillus niger-fermented palm kernel meal could increase body weight gain of birds to the same level of body weight gain of birds fed the control diet. All the birds fed the 20% fungi-fermented palm kernel meal had the same body weight as found in the control birds. This might indicate that the positive effect of fermentation of palm kernel meal becomes evident when the diets were supplemented with 20% palm kernel meal. The effect of supplementation of fermented palm kernel meal below 20% in the diet on body weight gain of birds was statistically undetected. The improvement of body weight gain of birds fed the 20% fermented palm kernel meal may be due to the fact that birds fed those diets (T-7, T-8 and T-9) increased feed intake by 13–17%. It is hard to rationalize the improved feed intake due to fermentation as this only becomes detected in 20% palm kernel meal diets, not in 10% palm kernel meal diets. It can be speculated here that the palm kernel meal used in this study was of low quality and fermentation could play a significant role in hydrolyzing components of the diet through the action of enzyme produced by the fungi. Accordingly, the more palm kernel meal used, the more the quality of the diet adversely affected.

Interestingly, despite the fact that birds fed 20% the fungi-fermented palm kernel meal diet consumed more diets than the birds fed the 20% the unfermented palm kernel meal diet, a significant increase in body weight gain was only found in the 20% Aspergillus niger–fermented palm kernel meal diet, compared to the birds fed 20% the unfermented palm kernel meal diet. Among the fungi used in this current study, Aspergillus niger appears to be slightly more effective in improving the quality of palm kernel meal by breaking down mannan as the major fibrous fraction in palm kernel meal, than Pleurotus ostreatus and Trichoderma viride. This is possibly because this filamentous fungus (Aspergillus niger) had an ability to penetrate their filaments inside the palm kernel meal particle and thus could hydrolyze fibre fractions of palm kernel meal into smaller particle size. Studies on the digestibility either in-vitro or in-vivo, are needed to clarify this rationale. The study of coconut by-product by Bahri et al (2019) indicated that the fermentation of coconut by-product using Aspergillus niger could produce mannanase enzyme [19]. The finding of Bahri et al (2019) might add up to the reason why Aspergillus niger was effective in improving the quality of palm kernel meal [19].

It is well recognized that the gizzard weight of broilers was affected by the type of diets consumed. A higher concentration of fibre in the diet may lead to larger sized gizzard due to possibly an increased frequency of gizzard contraction for finely grinding fibrous feed and thus increased development of the muscle of the gizzard [20]. It is for this reason that the gizzard weight of birds fed the unfermented palm kernel meal-based diets had larger gizzard size than those of birds fed control com-soy based diet.
There is a tendency that the size of the gizzard increased linearly over the increased level of palm kernel meal in the diet. Fermentation may decrease the workload of the gizzard and thus may decrease the relative weight of the gizzard. Although the addition of a 10% unfermented palm kernel meal produced a larger gizzard than the gizzard of the control birds, fermentation could minimize the work of gizzard as the gizzard size of birds fed the fungi-fermented palm kernel meal was not different from the gizzard size of birds fed the control diet. However, when palm kernel meal was 20% included in the diet, the only birds had the same gizzard size as the control birds were the birds fed 20% of the Pleurotus ostreatus-fermented palm kernel meal diet.

It is unexpected that the higher body weight gain of broilers fed the control soybean-based diets and Aspergillus niger-fermented palm kernel meal diet had the same carcass percentage. This might indicate that those birds with high body weight gain possessed a high percentage of non-carcass organs as well. The effect of fermented palm kernel meal on carcass traits of broilers has been studied by Alshelmani et al (2017), who found the same results as this present study [21]. The same pattern was also found on the length of the small intestine, in which all the birds from different treatment diets had similar length of the small intestine.

4. Conclusions

Birds fed 20% unfermented palm kernel meal in the diet were smaller than those birds fed the control and Aspergillus niger-fermented palm kernel meal either 10 or 20% in the diets. Feed intake of birds fed the 20% unfermented palm kernel meal consumed less than all the other treatments. The gizzard percentage of birds fed the control diet was smaller than those of birds fed the unfermented palm kernel meal. Feed conversion ratio, carcasses percentage and the length of the small intestine were not affected by experimental diets.

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