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Research Article
The Effect of Giving Probiotics and Palm Kernel Meal Subjected to Enzymatic Hydrolysis with Mannanase on Broiler Growth Performance

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Abstract

Background and Objective: Palm kernel meal is a byproduct of processing palm oil that can be used as animal feed. Palm kernel meal contains mannan in sufficiently high quantities that its use is limited to poultry. Enzymatic hydrolysis of palm kernel cake with the enzyme mannanase produces mannose and mannooligosaccharides (MOS), which can act as prebiotics. Prebiotics are used for the growth of probiotic microbes in the digestive tract of broilers. Probiotics are living microorganisms that are given in sufficient quantities and provide beneficial effects to the health of livestock while being able to increase livestock production. This study aimed to examine the effect of probiotics and palm kernel meal hydrolyzed with mannanase enzymes on broiler growth performance. **Materials and Methods:** This study used a completely randomized 3×6 factorial design with 3 replicates. The first factor was 3 doses of probiotics through the drinking water (0, 1 and 2%) and the second factor was 6 ration levels of palm kernel cake after hydrolysis with mannanase enzymes (0, 10, 15, 20, 25 and 30%). The variables observed included feed consumption, body weight gain, feed conversion ratio and mortality. **Results:** The supply of up to 30% palm kernel meal after enzymatic hydrolysis in broiler rations did not significantly influence ($p>0.05$) feed consumption, body weight gain and feed conversion ratio. Probiotic administration to the level of 2% through the drinking water also showed no significant effect ($p>0.05$) on feed consumption, body weight gain and feed conversion ratio but showed a significant effect ($p<0.05$) on mortality. The provision of 1 and 2% probiotics can reduce mortality compared to feed without probiotics. The interaction between the administration of palm kernel meal after enzyme hydrolysis and the administration of probiotics showed no significant effect ($p>0.05$) on all the observed parameters. **Conclusion:** Based on these results, it can be concluded that the administration of up to 30% palm kernel meal after enzyme hydrolysis has no negative effects in broiler rations. Probiotic administration to the level of 2% also had no negative effects on broiler growth performance and did not reduce survival. There was no interaction between the palm kernel meal and probiotics on broiler growth performance.

Key words: Enzymatic hydrolysis, growth performance, mannanase, palm kernel meal, probiotic

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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

Palm kernel meal (PKM) is a by-product of the process of separating palm kernel oil chemically (extraction) or physically (expeller) and contains nutrients that are useful as animal feed. PKM can be used in rations as a source of protein because the crude protein content can reach 14.5-19.6%[1]. The use of PKM in poultry rations is limited because of the high content of crude fiber (12-16%) and grittines[2] and the low content of the amino acids, methionine, tryptophan and cystine[3].

Palm kernel meal contains fiber that is high in non-starch polysaccharides (NSP) and β-mannan. Abdollahi *et al.*[4] reported that almost 96% of the total NSP in PKM was in the form of insoluble NSP. Saenphoom *et al.*[5] stated that content of β-mannan in PKM is 58%. The high content of nonstarch polysaccharides found in PKM increases intestinal viscosity, thereby reducing nutrient absorption[6].

Mannan absorption in poultry is very limited because there is no mannanase enzyme in the digestive tract of poultry. The addition of enzymes is one way to reduce the negative effects of mannan by increasing t he availability of nutrients in poultry rations. Hydrolysis of mannan polysaccharides with the help of enzymes reduce antinutrient factors and intestinal viscosity, release nutrients from cell walls and break down mannan into simple sugars, allowing nutrients and digestive enzymes to move more freely and increase nutrient absorption[7].

Hydrolysis of PKM with exogenous enzymes can reduce the components of cellulose, hemicellulose and lignin and can increase reducing sugars[5], while hydrolysis using mannanase enzymes can reduce cellulose, hemicellulose and crude fiber content in PKM[8-10]. The hydrolysis of PKM with mannanase enzyme from *Streptomyces cyaenus* produces manno-oligosaccharides (MOS) predicted as mannobiose, mannotriose, mannotetrosa, mannopentosa, mannohexose and mannoheptose[11]. Manno-oligosaccharides are nondigestible oligosaccharides and could be potentially applied as dietary fiber and prebiotics[12].

Prebiotics are mixtures of indigestible oligosaccharides, consisting of three to ten carbohydrate monomers that may promote growth, colonization, or activity of the probiotic microorganisms in the gut[13]. A variety of molecules can be prebiotics but the majority are dietary fibers, such as oligosaccharides[14]. Studies have shown that prebiotics affect feed efficiency by improving the height and number of goblet cells in the jejunum of broiler chickens[15] and influence the gut immune system via stimulation of the specific bacteria metabolism[14]. Probiotics and prebiotics can increase body weight, carcass weight and mortality rates in broiler chickens[16,17]. Thus, probiotics and prebiotics have been suggested as alternatives to antibiotics in animal production[18,19]. Probiotic containing *Lactobacillus* species provide resistance to the host against disease causing agents, such as *E. coli*, *Salmonella*, *Campylobacter* and *Eimeriaa cervulina*[20].

Mannanase enzyme hydrolysis of PKM is expected to support greater levels of PKM in broiler rations and act as a prebiotic source for probiotic microbes. Therefore, it is necessary to conduct a study to measure the effect of probiotics and hydrolyzed PKM supplementation on the performance of broiler chickens.

MATERIALS AND METHODS

Production of mannanase crude enzyme extract: The enzyme used in this study came from the crude extract of the mannanase enzyme produced from *Bacillus cereus* V9 with enzyme activity of 29.5 UI mL^{-1} [21]. *Bacillus cereus* V9 bacteria were rejuvenated by growing in mannan liquid media with a composition of 0.5% locus bean gum (LBG), 0.05% yeast extract, 0.075% peptone and Mendels and Sternberg minerals[22]. Then, *Bacillus cereus* V9 was incubated in a shaker incubator for 88 h at room temperature with a rocking speed of 130 rpm[22]. Crude Mannanase extract was obtained by means of culture samples centrifuged at a speed of 12,000 rpm for 15 min at 4°C so that they were separated from the supernatant and pellet. The supernatant obtained was a crude extract of the mannanase enzyme and was then used to hydrolyze PKM.

Hydrolysis of PKM with mannanase enzyme from *Bacillus cereus* V9: The PKM used in this study came from PT Kurnia Tunggal Nugraha Jambi, Indonesia. PKM is first ground so that the particle size decreased without filtration. Enzyme filtrate was added to PKM with a ratio of 1 mL for each gram of PKM by spraying enzyme filtrate evenly on the PKM substrate and then incubated at room temperature for 5 h[9].

Research design: This study used a 3×6 factorial completely randomized design (CRD) with 3 replicates. The first factor was 3 doses of probiotic lactic acid consisting of *Lactobacillus fermentum, Lactobacillus acidophilus*, *Lactobacillus bulgaricus* and *Streptococcus thermophillus*[23,24] through the drinking water (0, 1 and 2%) and the second factor included 6 levels of hydrolyzed PKM (0, 10, 15, 20, 25 and 30%). The composition, nutrient (%) and energy (kcal kg^{-1}) content of the treatment ration are listed in Table 1. Drinking water

Table 1: Composition, nutrient and energy of treatment rationing

	Palm kernel meal hydrolysis (PKMH)					
Feed materials	0%	10%	15%	20%	25%	30%
Corn yellow (%)	51.00	44.00	41.00	37.50	33.00	30.00
Bran (%)	4.00	3.50	3.00	2.50	3.00	3.00
Soybean meal (%)	26.00	23.50	23.00	21.00	21.00	18.00
PKMH[1] (%)	0.00	10.00	15.00	20.00	25.00	30.00
Fish flour (%)	15.00	15.00	15.00	15.00	15.00	15.00
Coconut oil (%)	3.00	3.00	3.00	3.00	3.00	3.00
$CaCO_3$ (%)	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin-mineral premix[2] (%)	0.25	0.25	0.25	0.25	0.25	0.25
DL-Methionine (%)	0.25	0.25	0.25	0.25	0.25	0.25
L-Lysin (%)	0.25	0.25	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00	100.00	100.00
Calculated nutrient						
Crude protein(%)	22.69	22.60	22.82	22.62	23.10	22.91
Crude fiber (%)	1.70	3.05	2.50	2.74	3.01	3.26
Crude fat (%)	5.66	6.45	5.34	5.62	5.91	6.12
Calcium (%)	1.41	1.43	1.43	1.43	1.45	1.46
Phosphorus (%)	0.71	0.72	0.72	0.72	0.73	0.73
Lysin (%)	1.83	1.79	1.76	1.71	1.71	1.68
Methionin (%)	0.96	0.95	0.95	0.94	0.95	0.94
Tryptophan (%)	0.39	0.41	0.42	0.42	0.44	0.45
ME (kal kg^{-1})[3]	3,086.33	3,084.25	3,090.40	3,090.90	3,065.60	3077.00

[1]Palm kernel meal Hydrolysis, [2]Content (per kg of ration); Vitamin A: 2,500 IU, Vitamin D: 3,500 IU, Vitamin E: 1.5 IU, Vitamin K3: 0.4 mg, Thiamine: 0.3 mg, Riboflavin: 1 mg, Pyridoxine: 1 mg, Cyanocobalamin: 2.4 µg, Vitamin C: 6 mg, Niacin: 7 mg, Calcium-d-pantothenate: 1 mg, Manganese: 20 mg, Iron: 5 mg, Iodine: 0.04 mg, Zinc: 20 mg, Cobalt: 0.04 mg, Copper: 0.6 mg, Antioxidant: 2 mg, Methionine: 7 mg and Lysine: 7 mg, [3]Metabolism energy

and rations were given ad libitum in 540-day-old chicks for 5 weeks of maintenance. The variables observed included ration consumption, body weight gain, feed conversion ratio and mortality.

Statistical analysis: All data were analyzed using 2-way analysis of variance (ANOVA) based on a 3×6 factorial design. If a significant main effect was found ($p<0.05$), then any differences were assessed using Duncan's Multiple Range Test (DMRT)[25].

RESULTS AND DISCUSSION

The effect of probiotics and the use of palm kernel meal hydrolyzed by mannanase enzyme on ration consumption, body weight gain and feed conversion ratio can be seen in Table 2.

Effect of diet on feed consumption: Based on the results of analysis of variance, the administration of probiotics and PKM hydrolyzed with mannanase enzymes and their interaction, had no significant effect ($p>0.05$) on the feed consumption of broiler chicken. In Table 2, it can be seen that the administration of hydrolyzed PKM to a level of 30% in the ration does not affect the consumption of feed. The results of this study indicate that the hydrolysis of PKM with mannanase enzymes can improve nutritional quality. Mairizal *et al.*[9] reported that hydrolysis of PKM using the mannanase enzyme from *Bacillus cerus* V9 with an enzyme activity of 29,500 UI kg^{-1} could reduce the crude fiber content from 16.78-9.68%. The hydrolysis of PKM with the mannanase enzyme from *Penicillium italicum* at a dose of 23,112 UI kg^{-1} decreased the crude fiber content by 64.02%[8] and the mannanase enzyme from *Aspergillus terreus* K1 at a dose of 9,000 UI kg^{-1} reduced the crude fiber content from 21. 99-13.40%[10].

Crude fiber of PKM contains high amounts of mannan[5]. Several studies have shown that enzymatic hydrolysis using β-mannanase significantly increases the content of mannose and mannan-oligosaccharides from PKM[11,26]. Mannose and mannan-oligosaccharides from enzymatic PKM hydrolysis can function as prebiotics for broilers. Prebiotics are a source of nutrition for good bacteria, such as *Lactobacillus* and *Bifidobacteria* and increase the populations of beneficial bacteria in the digestive tracts of broiler chickens. Nevertheless, the results of this study indicate that there is no relationship ($p>0.05$) between the administration of probiotics by giving PKM after hydrolysis with the mannanase enzyme with feed consumption of broiler chicken. This result is likely due to ineffective use of the prebiotics in hydrolyzed palm kernel meal by the probiotic lactic acid bacteria. Wang *et al.*[27] reported that mannan oligosaccharides (MOS)

Table 2: Average feed consumption, body weight gain, feed conversion ratio and mortality of broiler chickens fed probiotics and palm kernel meal from enzyme hydrolysis

Item/factors	Feed consumption (g/head/0-5 weeks)	Body weight gain (g/head/0-5 weeks)	Feed conversion ratio	Mortality (%)
PKM H (%)				
0	2,436.24	1,145.08	1.88	4.44
10	2,470.54	1,138.02	1.91	3.33
15	3,039.47	1,120.77	1.91	3.33
20	2,493.43	1,140.16	1.89	3.33
25	2,438.68	1,149.59	1.89	2.22
30	2,494.46	1,146.25	1.88	4.44
Probiotics (%)				
0	2,507.37	1,140.81	1.90	6.67[a]
1	2,771.61	1,145.25	1.90	2.22[b]
2	2,407.43	1,133.87	1.89	1.67[b]
PKM H-probiotics (%)				
0×0	2,533.51	1,152.98	1.89	6.67
10×0	2,513.56	1,138.38	1.90	6.67
15×0	2,496.38	1,102.05	1.92	6.67
20×0	2,514.22	1,154.04	1.90	6.67
25×0	2,534.20	1,159.07	1.91	3.33
30×0	2,452.32	1,138.31	1.90	10.00
0×1	2,399.24	1,146.91	1.86	3.33
10×1	2,490.64	1,139.66	1.92	3.33
15×1	2,478.13	1,154.33	1.93	0.00
20×1	2,543.00	1,140.42	1.89	0.00
25×1	2,429.55	1,146.64	1.89	3.33
30×1	2,489.12	1,143.55	1.89	3.33
0×2	2,375.97	1,135.34	1.89	3.33
10×2	2,407.43	1,136.01	1.91	0.00
15×2	2,343.90	1,105.93	1.89	3.33
20×2	2,423.08	1,126.01	1.89	3.33
25×2	2,352.29	1,143.05	1.88	0.00
30×2	2,541.93	1,156.87	1.87	0.00

[a,b]Different letters in the same column show a significant effect ($p<0.05$)

are not considered to be the direct substrate used for fermentation by microbes. However, MOS can inhibit growth of gram-negative pathogenic bacteria by offering competitive binding sites[28].

Probiotic administration of lactic acid bacteria (*Lactobacillus fermentum*, *Lactobacillus acidophilus*, *Lactobacillus bulgaricus* and *Streptococcus thermophilus*) to a level of 2% did not reduce feed consumption. Probiotic administration aims to balance the microbial population in the digestive tract of broilers. Probiotics are not important nutrients for livestock, so they will not affect the consumption of rations. According to Adams and Moss[29], feed additives, or "feed additive" or "nutricine", are ingredients that are mixed into feed that can affect the health and nutritional conditions of livestock, even though they are not nutrients. Similar results, namely, that the administration of probiotics does not affect the consumption of feed, were also reported by Khaksefi and Ghoorchi[30], Timmerman *et al.*[31], Jung *et al.*[32] and Mansoub[33].

Effect of diet on body weight gain: Including hydrolyzed PKM in rations showed no significant effect ($p>0.05$) on body weight increase in broiler chickens. Broiler chickens provided rations containing 30% hydrolyzed PKM had an average body weight of 1,146.25 g, which was not different from the average body weight of chickens with rations that did not contain hydrolyzed palm kernel cake (1,145.08 g). The research ration was formulated to be isoprotein and isoenergic, so it did not affect body weight gain. Previous research also reported that ioscaloric and isoprotein rations containing PKM did not have a negative effect on broiler chickens growth[34]. Supplementation of exogenous enzymes into broiler diets containing PKM to improve growth performance has been reported by Yahya *et al.*[35], Iyayi and Davies[36] and Sekoni *et al.*[37]. The results of this study prove that the hydrolysis of PKM with mannanase enzyme can reduce anti-nutritional factors in the form of fibers, especially from nonstarch polysaccharides so as not to affect broiler performance. High NSP intake can increase digesta viscosity in the digestion of broilers, thus inhibiting enzyme contact with digesta, which results in low digestibility of starch, fat and protein[38-41].

Probiotic administration to the level of 2% through the drinking water (P2) did not show a significant effect ($p<0.05$)

on the weight gain of broiler bodies. Probiotics can only play a role in improving the health status of livestock but have not been shown to play a role in increasing the digestibility of nutrients. The results of this study are in line with those reported by Awad *et al.*[42] that the addition of probiotics to broilers did not show a significant effect on body weight compared to the control group. Similarly, Appelt *et al.*[43] found that the addition of prebiotics did not significantly influence the weight gain of broiler chickens

Effect of diet on feed conversion ratio: Providing rations with hydrolyzed PKM had no significant effect ($p>0.05$) on feed conversion ratio. Broiler chickens who were given rations containing as much as 30% enzyme-hydrolyzed PKM had the same feed conversion ratio as the control ration (0% PKM Hydrolysis). This result is because the research ration was formulated to be isoprotein and isoenergic (Table 1) so that it did not affect feed consumption or body weight gain. High-quality feed that is palatable and balance protein and energy will result in low feed conversion ratio[44]. Increased nutrient digestibility of PKM enzyme-hydrolyzed as a result of reduced crude fiber content likely causes a decrease in viscosity of the digesta so as not to affect feed conversion ratio. Sundu and Dingle[2] stated that the addition of enzymes in rations containing PKM can reduce digesta viscosity by 27% when compared with no enzyme administration.

The provision of probiotics to a level of 2% did not to reduce feed conversion ratio, with or without rations containing enzyme-hydrolyzed PKM. Giving probiotics only increases the health status of livestock by balancing the microflora in the digestive tract but has not been shown to increase absorption of food substances. Mountzouris *et al.*[45] stated that probiotics can change mucin movements and microbial populations in the chicken's small intestine, so that their presence can improve intestinal function and health, improve the composition of the caecal microflora and increase absorption of nutrients. The results of this study are in line with those reported by Mutus *et al.*[46] and Vargas-Rodriguez *et al.*[47] who stated that the administration of probiotics did not increase feed consumption, body weight gain or feed conversion ratio in broilers.

Mortality: Probiotics showed a significant effect ($p<0.05$) on mortality over 35 days of maintenance, whereas the supply of hydrolyzed PKM and its interaction with the administration of probiotics showed no significant effect ($p>0.05$) on mortality. The mortality rate during the study based on treatment is presented in Table 2. The highest mortality rate was found in the treatment without probiotics, which was 6.67%, while probiotic administration at 1% (2.2%) and at 2% (1.67%) were not significantly different ($p>0.05$) in terms of mortality. The mortality observed in this study is in line with Awad *et al.*[42] who stated that the broiler mortality rate was lower for the group supplemented with probiotics when compared to the mortality rate for the group without probiotics. Jin *et al.*[48] stated that the addition of probiotics in broiler feed can increase endurance so that mortality is low.

The results of this study indicate that palm kernel cake can be optimized in broiler rations after being pretreated with enzymatic hydrolysis. Probiotic administration of lactic acid bacteria can only reduce mortality but cannot increase the weight gain of broiler bodies

CONCLUSION

PKM after enzyme hydrolysis can be added to a level of 30% in broiler rations. Probiotic administration to the level of 2% did not have a negative effect on broiler growth performance and reduced mortality. There was no interaction between the provision of enzyme-hydrolyzed palm kernel meal and the provision of probiotics to improve broiler growth performance.

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