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The Use of Hydrolyzed Palm Kernel Cake After Addition by Buffalo Rumen Fluid Enzymes on Growth Performances and Relatively Organ Weight of Broilers

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ABSTRACT

This study aims to utilize a sufficient amount of palm kernel cake (PKC) and buffalo rumen fluid as a source of enzymes to improve the quality of PKC that can be used in broiler feed. The study was conducted in two stages. The first stage was to test the level of buffalo rumen fluid enzyme at various doses (0.0%, 0.75%, 1.5%, 2.25%, and 3.0% (v/w) in PKC incubation to measure dry matter (DM), organic matter (OM), crude fiber (CF), and dissolved glucose total (DGT) contents as hydrolyzed PKC. The second stage was to determine the usage level of hydrolyzed PKC in broiler feed. A total of 288 DOC MB202 strains were randomly allocated to one of five treatments with four replicated cages of 12 birds in a completely randomized design. Treatments were the various level of hydrolyzed PKC as following: 0%, 6%, 12%, 18%, 24%, and 30%. The variables were feed consumption, body weight gain (BWG), feed conversion ratio (FCR), final body weight (FBW), carcass, and digestive organs weight. The level of buffalo rumen fluid enzymes had a significant effect on DM, CF, and DGT content, while it was not significant on OM. The optimum level was 2.25% and it was used in the second stage. The use of hydrolyzed PKC up to 18% was not different (P>0.05) in BWG, FCR, and FBW compared to controls. The use of hydrolyzed PKC up to 24% decreased BWG, FCR, and FBW, but feed consumption did not differ (P>0.05) compared to control. Carcass and digestive organ weight were not affected (P>0.05) by the treatment. It can be concluded that the incubated buffalo rumen fluid enzymes at a level of 2.25% could improve the quality of PKC. The use of hydrolyzed PKC could be applied up to 18% without affecting the performance of broilers.

Keywords: Buffalo rumen fluid, Enzymes, Hydrolyzed, Incubation, Palm kernel cake

Introduction

There are quite abundant rumen contents of buffaloes that are considered wastes of slaughterhouses in Indonesia. Based on livestock statistical data, the number of buffaloes in 2019 reached 1,141,298 and increased to 1,177,254 heads in 2020. The slaughter of buffaloes in 2018 was 127,678 (Statistic Indonesia, 2021). If the amount of the buffalo rumen fluid is estimated to reach 30 L per head, then the total amount of buffalo rumen fluid based on the buffalo slaughter in 2018 in Indonesia could reach 3,830,340 L. If they are not handled properly, these wastes have the potential to pollute the environment. The results of previous studies found that some of the enzymes in the rumen fluid of buffaloes had been identificated with good activity and characteristics and have potential as a feed additive for poultry (Budiansyah *et al*., 2014; 2015). However, research on its use in broiler feed has not been widely carried out.

Buffaloes are a unique type of ruminants because of their prodigious ability to digest high fiber feed and utilize it for their growth very efficiently. Until now, no buffalo has ever been seen thin compared to cattle or other ruminants, even though they are only fed forage or field grass. This condition is because the enzymes that are produced by microbes in their rumen are particular, they have a solid ability to hydrolyze feed which has not yet been known and identified. The contents of the buffalo rumen from slaughterhouses have the potential to be a source of enzymes that can be used to replace some commercial enzymes in overcoming the problem of low-quality poultry feed.

Identification of the enzymes in buffalo rumen fluid and their characteristics has been carried out. The results showed that buffalo rumen fluid from the slaughterhouses contained the enzymes of xylanase, mannanase (Budiansyah *et al.,* 2014), cellulase, and amylase (Budiansyah *et al.*, 2015). Some of these enzymes had an enormous range of temperature pH to work, which are at a temperature of 39^{o}C to 80^{o}C and a pH of 4 to 8 (Budiansyah *et al*., 2014; 2015). An incubation of several feed ingredients as

constituents of broiler feed needs to be carried out to increase the quality of the feed ingredient such as palm kernel cake (PKC). The PKC is an alternative feed ingredient that is abundant in Indonesia. It is estimated that the PKC production in Indonesia in 2017 was almost 3.2 million tons (Pasaribu, 2018). Most researchers indicated that PKC was of low quality due to its high crude fiber content in the form of beta-mannan, low amino acids, and its quite coarse texture due to contamination of oil palm shells which could reach up to 15% (Sundu and Dingle, 2011). Of the total carbohydrates of palm kernel cake (PKC), 17% is mannan, 3% is arabinoxylan, 3% is glucuronoxylan, and 12% is cellulose (Sundu and Dingle, 2011). Crude fiber content reached 12.1% to 14.3% (Abd El Tawab *et al*., 2019).

Regarding to the aforementioned, to utilize PKC as a constituent of broiler feed for the starter and finisher periods, it is necessary to process PKC, one of the methods is to hydrolyze the PKC by using buffalo rumen fluid enzymes obtained from the slaughterhouses, especially the content of crude fiber and non-starch carbohydrates that cause low nutrient utilization. Therefore, this study is conducted to know the effect of hydrolyzed PKC after adding the buffalo rumen fluid enzyme on growth performances and relative organ weight of broilers. It is suspected that the use of buffalo rumen fluid enzymes in incubating palm kernel cake can improve the quality of palm kernel cake by reducing crude fiber content and increasing dissolved glucose total content as a result of hydrolysis of non-starch carbohydrates. In addition, the use of palm kernel cake which has been hydrolyzed with enzymes from buffalo rumen fluid in broiler feed can be increased in order to increase the use of local feed sources.

Materials and Methods

This study was carried out in two stages: The first stage was an invitro test to examine the optimum level of enzymes in the incubation of PKC. The research was conducted at the Laboratory of Animal Husbandry, Faculty of Animal Science, Jambi University, and at the Integrated Basic Laboratory, Jambi University. The tests were carried out on the hydrolysis ability of buffalo rumen fluid enzymes in the feed ingredients of palm kernel cake. The *in vitro* test procedure followed the method used by Budiansyah *et al.* (2011). A total of 25 g of PKC feed ingredients were weighed and placed in a closed container. Then, the buffalo rumen fluid enzymes were added to the feed sample with a dose of 0%; 0.75%;1.5%; 2.25%, and 3.0% (v/w)), and then they were stirred evenly. The volume of the enzyme was equated with the addition of the distilled water at the highest dose. Incubation was conducted for 24 hours at a temperature of 37.5^{o}C.

After the incubation was completed, measurements of the hydrolysis of the food substances by enzymes were carried out by measuring the hydrolysis of dry matter, organic matter, crude fiber, and non-starch carbohydrates. Hydrolysis measurements of dry matter, organic matter, and crude fiber by measuring the difference in the amount of those three matters before and after the feed incubation by enzymes were carried out by proximate analysis according to AOAC (2005). Hydrolysis measurements of non-starch carbohydrates by measuring the levels of dissolved glucose before and after the feed incubation by enzymes were conducted by measuring the levels of dissolved sugar (glucose) by the method described by Budiansyah *et al*. (2011).

A total of 1 g feed sample that had been incubated with rumen fluid enzymes was put into a test tube, then 5 mL of distilled water was added and stirred for approximately 1 minute. The tube was then centrifuged at 3,000 rpm for 15 minutes, then the resulted supernatant was used to measure the total dissolved glucose content of the feed following the method described by Budiansyah *et al*. (2011). The difference between the dissolved glucose total before and after hydrolyzing of carbohydrates was hydrolyzed carbohydrates during incubation with the enzyme level of added buffalo rumen fluid. The enzyme extraction of buffalo rumen fluid is depicted in Figure 1, while the research workflow in this study is presented in Figure 2.

The optimum result from the first stage was 2.25% and it used as a basic for the second stage. The second stage of the research was the application of palm kernel cake that was hyrolyzed with buffalo rumen fluid enzymes in broiler feed for starter and finisher periods. The research was conducted at the Experimental Cage for Poultry, Faculty of Animal Husbandry, Jambi University, and the laboratory analysis of feed ingredients was carried out at the Laboratory of the Faculty of Animal Husbandry, Jambi University.

A total of 288 one-day-old chicks (DOC) with broiler MB202 strain produced by Jafpa Comfeed Indonesia, Tbk was used in this study. The level of enzymes used in the incubation of palm kernel cake based on the *in vitro* testing results was 2.25%. The enzymes used were buffalo rumen fluid enzymes from the animal slaughterhouses of the Livestock Service of Jambi City. The feed was prepared with iso-calorie and iso-protein based on the needs of broilers in the starter period (0 - 3 weeks) and finisher period (4 - 6 weeks) according to the NRC (1994). The formulated feed consisted yellow maize, rice bran, soybean meal, fish meal, coconut meal, palm oil, mineral feed supplement, Top Mix NA, DL-methionine, and L-lysine. Representative of the formulated feed was analyzed for metabolizable energy (Kcal/kg), crude protein (CP), crude fiber (CF), calcium (Ca), and phosphor (P) according to established procedures described by AOAC (2005). The composition of formulated feed and the nutrient composition for each treatment showed in Table 1.

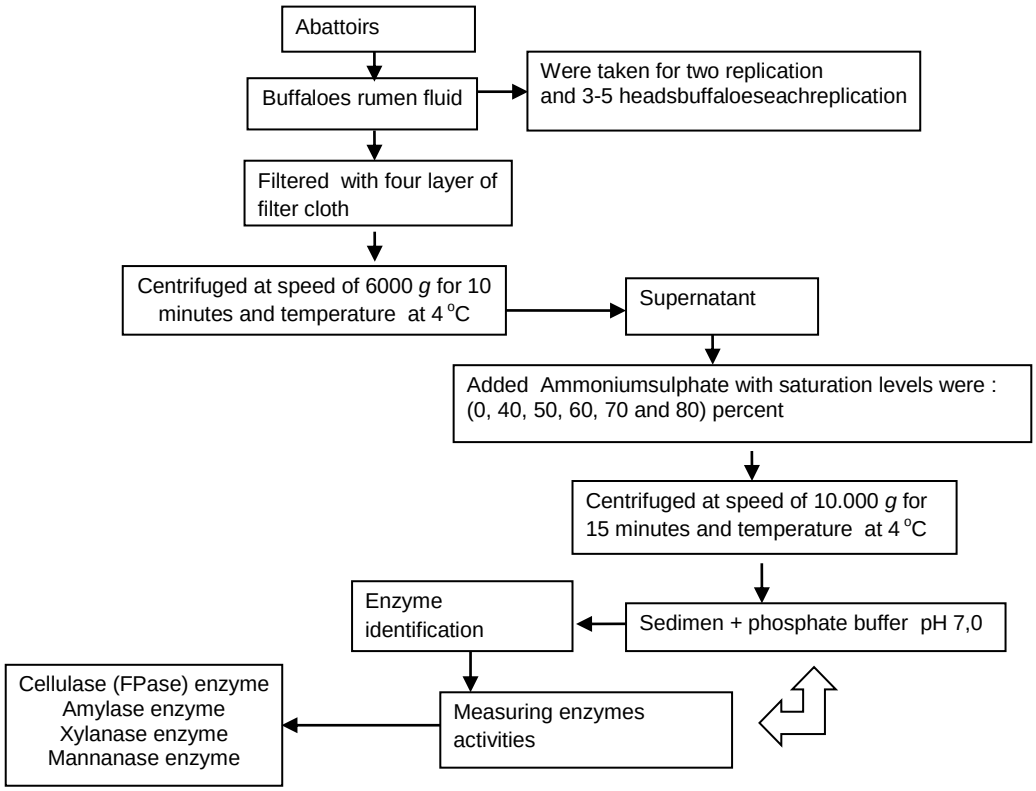

Figure 1. Workflow of enzyme extraction from the slaughterhouse in the research.

Each treatment was allocated as a completely randomized design (four replicates with 12 broilers per replication pen). Treatments were as follows: T0 = Feed without hydrolyzed PKC, T1 = feed with 6% hydrolyzed PKC, T2 = feed with 12% hydrolyzed PKC, T3 = feed with 18% hydrolyzed PKC, T4 = feed with 24% hydrolyzed PKC, and T5 = feed with 30% hydrolyzed PKC.

All broilers were placed in a cage of 1 × 1 meter for each unit treatment in a half open-housed. All broilers were allowed ad libitum access to feed and water from bell-shaped drinkers.

The variables observed were the broilers' performance (feed consumption, body weight gain, feed conversion, and final body weight), their slaughter weight and carcass weight (both absolute and relative), and their weight and length of digestive organs.

Data analysis

Analysis of variance (ANOVA) using a completely randomized design was carried out using Microsoft excel. Moreover, probability values were calculated using F testing according to Steel and Torrie (1980). The following mathematical model was used:

$$Y_{ij} = \mu + \alpha_i + \epsilon_{ij}$$

Where Y_{ij} was the parameters observed, μ was the overall mean, α_i the effect level of hydrolysis palm kernel cake, and ϵ_{ij} was the amount of error number. T0 = Feed without hydrolyzed PKC, T1 = feed with 6% hydrolyzed PKC, T2 = feed with 12% hydrolyzed PKC, T3 = feed with 18% hydrolyzed PKC, T4 = feed with 24% hydrolyzed PKC, and T5 = feed with 30% hydrolyzed PKC. The significant test continued using Tukey Test.

Results and Discussion

Table 2 presents the results of the study on the incubation effect of buffalo rumen fluid enzymes on palm kernel cake in dry matter, organic matter, crude fiber, and dissolved glucose total contents.

The result of the analysis showed that the treatment of the buffalo rumen fluid enzyme levels from the animal slaughterhouses in the incubation of palm kernel cake had a significant effect on dry matter, crude fiber, and dissolved glucose total contents, while the enzyme level treatment had no significant effect on organic matter.

The Tukey test results depicted that the incubation of buffalo rumen fluid enzymes at the levels of 2.25% and 3.0% significantly ($P<0.05$) increased the level of dry matter and decreased the crude fiber of this PKC, while the incubation of PKC at the level of 1.5% of buffalo rumen fluid enzymes significantly increased the dissolved glucose total content. This means that the incubation of buffalo rumen fluid enzymes on palm kernel cake was

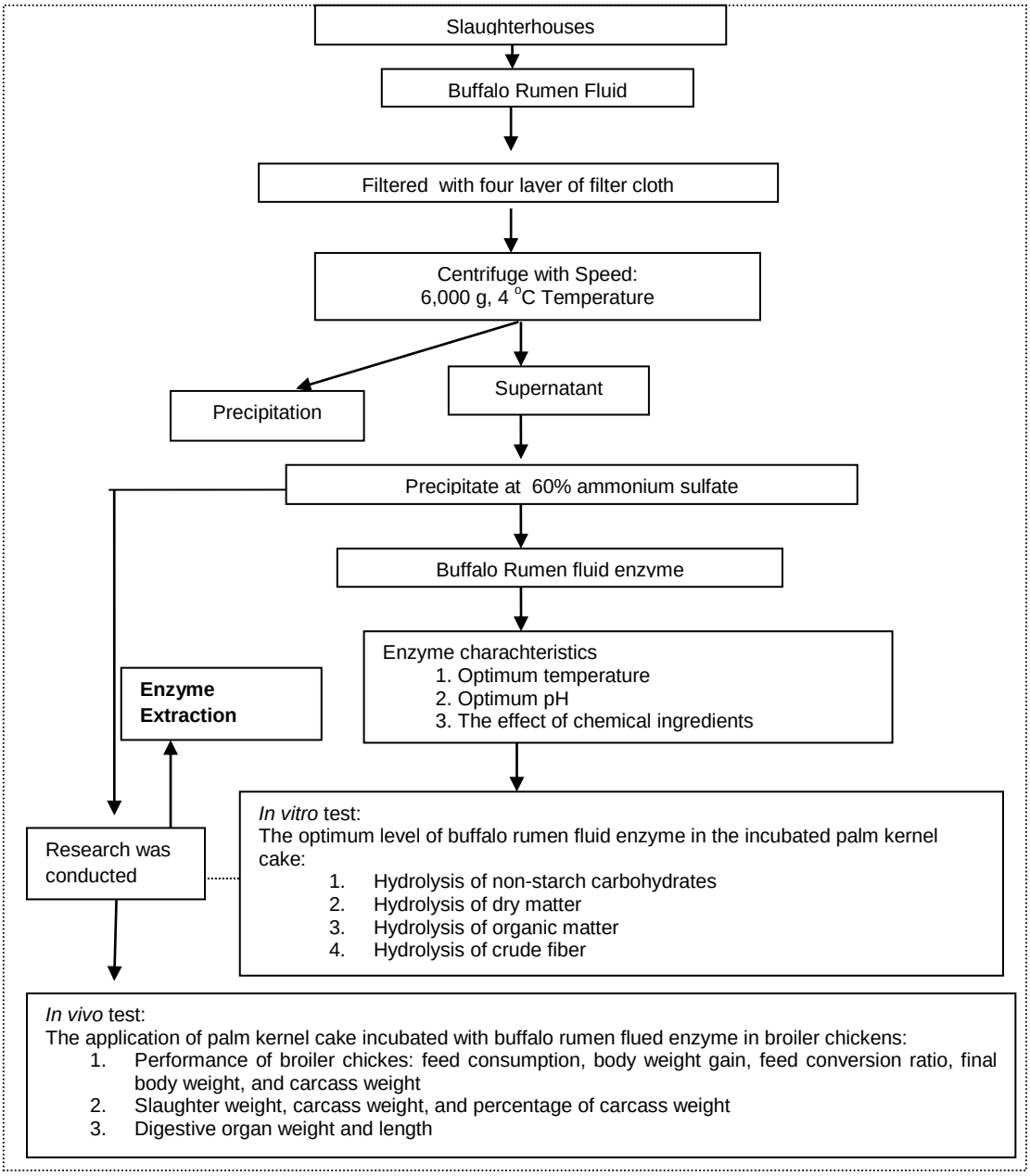

Figure 2. Research workflow conducted in the study.

able to hydrolyze feed by starting to give a good effect on the incubation of buffalo rumen fluid enzymes at a level of 1.5% as evidenced by the increase of dissolved glucose total content. However, a significant decrease in crude fiber was observed when incubation was carried out at the level of 2.25% of buffalo rumen fluid enzymes. It was identified that crude fiber was one of the factors that affected feed quality. High crude fiber tended to reduce the digestibility of food substances because undigested crude fiber would carry some of the food substances out with the feces. Kamran *et al.* (2002) reported that the incubation with the β-mannanase enzyme reduced the crude fiber content of fibrous feed ingredients at the level of 5.0% of the β-mannanase enzyme. This means that the use of enzyme from buffalo rumen fluid was less than the use of a β-mannanase enzyme to decrease the crude fiber content of feedstuffs.

In this study, the increase in dissolved glucose total content was markedly after the incubation of buffalo rumen fluid enzymes at a level of 1.5%, and the highest dissolved glucose total content was obtained at the level of 3.0% of the incubation of buffalo rumen fluid enzymes. In previous studies, the highest dissolved glucose level was obtained by the incubation of cattle rumen fluid enzymes at a level of 2.0% (Budiansyah *et al*., 2011). Mishra *et al*. (2013) and Baurhoo *et al*. (2007) reported that an enzyme incubation significantly increased glucose total content in fibrous feed ingredients.

Table 1. Composition of feed ingredients and nutrients content in the treatments

Feed ingredient	Treatment (%)					
	T0	T1	T2	T3	T4	T5
Yellow corn	49	49	44	42	39	35
Wheat bran	9.5	5.5	4.5	2.5	0.5	0.5
Soybeans	25	25	26	23	23	19
Fish flour	12	10	9	10	9	11
Palm kernel cake	0	6	12	18	24	30
Vegetable oil	2.5	2.5	2.5	2.5	2.5	2.5
Mineral feed supplement[1]	0.75	0.75	0.75	0.75	0.75	0.75
Top mix[2]	0.75	0.75	0.75	0.75	0.75	0.75
DL-methionine	0.25	0.25	0.25	0.25	0.25	0.25
L-Lysine	0.25	0.25	0.25	0.25	0.25	0.25
Total	100	100	100	100	100	100
Calculated nutrient contents						
Crude protein (%)	23.08	23.03	23.03	23.06	23.06	23.09
Crude fat (%)	11.06	6.93	6.8	6.9	6.78	699
Crude fiber (%)	5.56	5.06	5.28	5.09	5.02	5.36
Calcium (Ca; %)	0.74	0.68	0.63	0.68	0.66	0.67
Phosphorus (P; %)	0.61	0.58	0.56	0.59	0.59	0.6
Metabolic energy (Kcal/kg)[3]	3229	3245	3228	3201	3235	3237

[1]The *"Mineral feed supplement" contained* the following per 1 kg: calcium (Ca), 32.5%; phosphorous (P), 1.0%; iron (Fe), 6 g; manganese (Mn), 4 g; iodine (I), 0.075 g; copper, (Cu) 0.3 g; zinc (Zn), 3.75 g; vitamin B_{12}, 0.5 mg; and vitamin D_3, 50,000 IU.
[2]The *"Top Mix"* premix contained the following per 10 kg: vitamin A, 12,000,000 IU; vitamin D_3, 2,000,000 IU; vitamin E, 8,000 IU; vitamin K, 2,000 mg; vitamin B_1, 2,000 mg; vitamin B_2, 5,000 mg; vitamin B_6, 500 mg; vitamin B_{12}, 12,000 g; vitamin C, 25,000 mg; calcium-D-pantothenate, 6,000 mg; niacin, 40,000 mg; choline chloride, 10,000 mg; methionine, 30,000 mg; lysine, 30,000 mg; manganese, 120,000 mg; iron, 20,000 mg; iodine, 200 mg; zinc, 100,000 mg; cobalt, 200 mg; copper, 4,000 mg; zinc bacitracin, 21,000 mg; and excipient q.s., 10,000 mg.
[3]Metabolizable energy was calculated by determining (combustion) gross energy of the entire diet multiplied by a ME to GE-conversion factor (0.725).
T0 = Feed without hydrolyzed PKC, T1 = feed with 6% hydrolyzed PKC, T2 = feed with 12% hydrolyzed PKC, T3 = feed with 18% hydrolyzed PKC, T4 = feed with 24% hydrolyzed PKC, and T5 = feed with 30% hydrolyzed PKC.

Table 2. Effect of enzyme levels in the incubation of palm kernel cake on dry matter, organic matter, crude fiber, and dissolved glucose total contents

Variables	Buffalo rumen fluid enzyme level				
	0.0%	0.75%	1.5%	2.25%	3.0%
Dry matter (%)	90.5732^{a}	90.2262^{a}	90.9035^{ad}	91.2758^{bcd}	91.9043^{bc}
Organic matter (%)	95.7182	96.2919	95.7765	95.7356	95.5291
Crude fiber (%)	17.6289^{a}	15.7905^{a}	14.5312^{ac}	10.4431^{b}	12.1863^{bc}
Dissolved glucose total (%)	2.1275^{a}	2.8125^{ac}	3.1500^{bc}	3.3550^{bc}	3.3700^{bc}

^{a,b,c,d} Different lowercase superscripts in the same line show significant differences at a level (P<0.05).

A significant decrease in crude fiber content (P<0.05) was obtained after the incubation of buffalo rumen fluid enzymes at a level of 2.25%. This was because the buffalo rumen fluid enzymes contained carbohydrase enzymes, some of which were crude fiber digesters consisting of cellulase, amylase, xylanase, and mannanase. The activity of these enzymes after their precipitation with ammonium sulfate was 13.6073±7.9986 µmol/mL/hour for cellulase, 4.1751±0.0927 µmol/mL/minute for amylase (Budiansyah *et al*., 2015), 1.8864±0.5226 µmol/mL/minute for mannanase and 0.6595±0.0525 µmol/mL/minute for xylanase (Budiansyah *et al.*, 2014). The activity of carbohydrate enzymes between cattle and buffalo rumen fluid was almost the same according to Budiansyah *et al.* (2010; 2014; 2015).

The treatment effect of using the hydrolyzed palm kernel cake with buffalo rumen fluid enzymes (hydrolyzed PKC) in feed on broilers' performance is presented in Table 3.

The result of the study using hydrolyzed PKC showed that the treatment of the various levels of hydrolyzed PKC in broiler feed based on the local feed had a significant effect (P<0.05) on feed consumption, body weight gain, and feed conversion ratio. The treatment showed that the use of hydrolyzed PKC had a significant effect (P<0.05) on feed consumption. The treatment displayed that the use of hydrolyzed PKC had a significant effect (P<0.05) on feed consumption. Feed consumption was similar when the use of hydrolyzed PKC up to a level of 24% compared to the control treatment, but the use of hydrolyzed PKC of more than 24% in the feed significantly reduced feed consumption. This means that the use of hydrolyzed PKC at a higher level in the feed (30% in the T5 treatment) tends to be less palatable. Rehman *et al.* (2016) reported that the use of fibrous feeding ingredients with the addition of enzymes to a level of 12.5% did not affect the feed consumption, but in this study, the use of the hydrolyzed PKC at a level of 30% significantly reduced feed consumption. Sundu and Dingle (2011) suggested that the use of oil palm cake in the poultry feed was limited by three factors: first, palm kernel cake was physically "gritty" (rocky/contains grit) and is not palatable; second, it contained ingredients/substances such as mannan or galactomannan, and xylan or arabinoxylan, which could reduce nutrient absorption; and third, the use of PKC in ruminants was economically more profitable than when used for poultry feed. Furthermore, Sundu and Dingle (2011) stated that of the total carbohydrates of palm kernel cake, 78% were mannan, 3% were

Table 3. Treatment effect of the hydrolyzed palm kernel cake with buffalo rumen fluid enzymes on broiler's performance

Treatment	Feed consumption (g/head/day)	Body weight gain (g/head/day)	Feed conversion ratio
T0	54.1375±0.9984[a]	31.0475±1.9053[a]	1.7500±0.1101[c]
T1	52.7725±1.0692[ab]	29.0400±2.0629[a]	1.8225±0.1102[bc]
T2	53.9150±0.1436[a]	27.2725±1.6344[ab]	1.9825±0.1233[bc]
T3	53.0850±1.9834[ab]	26.7850±1.0832[ab]	1.9825±0.0665[bc]
T4	51.9300±0.8213[ab]	22.3325±3.0149[c]	2.3525±0.3036[a]
T5	51.3500±1.0056[b]	23.6725±1.8596[bc]	2.1775±0.1198[ab]

[a,b,c] The same lowercase superscripts in the same column show no significant difference at the level (P>0.05).
T0 = Feed without hydrolyzed PKC, T1 = feed with 6% hydrolyzed PKC, T2 = feed with 12% hydrolyzed PKC, T3 = feed with 18% hydrolyzed PKC, T4 = feed with 24% hydrolyzed PKC, and T5 = feed with 30% hydrolyzed PKC.

arabinoxylan, 3% were glucoronoxylan, and 12% were cellulose.

The result of the variance analysis showed that the treatment of using the hydrolyzed PKC in the feed had a significant effect (P<0.05) on the body weight gain of the broilers. The results of the Tukey test showed that the use of hydrolyzed PKC up to a level of 18% (T3) resulted in body weight that was not significantly different (P>0.05) compared to the control treatment (T0).

The use of hydrolyzed PKC at a higher level, namely at the level of 24% (T4) and 30% (T5), showed a lower body weight gain than the control treatment. The same result was also shown by the treatment effect on the feed conversion ratio. The use of hydrolyzed PKC up to a level of 18% did not depict a significant difference (P>0.05) compared to the control treatment without hydrolyzed PKC (T0), but a higher use of hydrolyzed PKC resulted in significant higher feed conversion (P<0.05) compare to the control treatment. This indicated that the use of hydrolyzed PKC could only be carried out to a level of 18% because a higher use would result in lower broiler performance. Adedokun *et al.* (2015) reported that the use of fibrous feed ingredients fermented with *Trichoderma reesei fungus* in monogastric did not cause differences in body weight gain and feed efficiency. Meanwhile, Almeida *et al*. (2013) reported that the use of high fiber feed ingredients fermented with *Aspergillus oryzae* in monogastric was not significantly different from the control treatment without high fiber feed ingredients. As stated above, fibrous feed ingredients are feed ingredients that have low quality. In addition, palm kernel cake had low nutritional value due to the high cellulose and non-starch carbohydrates, such as mannan compounds which reached 78%. The PKC is also heavily contaminated with oil palm shells which led to PKC having a gritty (sandy) texture. This situation could cause gastrointestinal damage of broilers. Although the hydrolyzed PKC could help reduce fiber content, it was suspected that the hydrolyzed PKC would not help much because of the shell contamination on PKC could cause gastrointestinal damage. Therefore, the use of PKC at a high level tended to be non-palatable and caused a decrease in the performance of broilers.

The safety limit on the use of PKC ingredients in monogastric including for poultry feed has been reported by several previous researchers to vary from 5% (Sinurat *et al.*, 2000) until 10% (Son *et al*., 2012; Mok *et al.*, 2013; Almaguer *et al*., 2014). This is due to differences in the process of producing PKC and its nutritional content. The results of the study using palm kernel cake in this research were much higher than that reported by Sinurat *et al.* (2000). The PKC is known as a feed ingredient that is not favored by livestock because it is dry and rough like sand and contains high fiber (Chong *et al*., 2008). This is also a limiting factor in the use of PKC in poultry feed, especially broilers.

The treatment effect of the hydrolyzed PKC with the buffalo rumen fluid enzymes on the final body and carcass weight of broilers is presented in Table 4.

Based on analysis regarding the effect of the various treatments on final body weight and carcass weight (percentage of carcass weight), it was shown that the treatment using hydrolyzed PKC in the feed had a significant effect on final body weight, but no significant effect on carcass weight.

The results of the Tukey test on final body weight showed that the treatment using hydrolyzed PKC up to 18% did not show a significant difference (P>0.05) compared to the control treatment, but a higher usage of it significantly decreased (P<0.05) the final body weight. This result indicated that the hydrolyzed PKC could only be used up to 18%. This result was the same as the treatment effect on body weight gain that had been stated above. The

Table 4. Treatment effect of the hydrolyzed palm kernel cake with buffalo rumen fluid enzymes on final body weight and carcass weight of broilers

Treatment	Final body weight (g/head)	Carcass weight (%)
T0	1343.25±79.50[a]	69.99±6.91[a]
T1	1258.59±86.47[a]	71.18±0.63[a]
T2	1183.56±68.76[ab]	71.42±4.42[a]
T3	1163.77±44.82[abc]	67.52±2.68[a]
T4	975.33±127.31[c]	69.97±2.08[a]
T5	1032.36±78.31[bc]	70.00±2.03[a]

[a,b,c] The same lowercase superscripts in the same column show no significant difference at the level (P>0.05).
T0 = Feed without hydrolyzed PKC, T1 = feed with 6% hydrolyzed PKC, T2 = feed with 12% hydrolyzed PKC, T3 = feed with 18% hydrolyzed PKC, T4 = feed with 24% hydrolyzed PKC, and T5 = feed with 30% hydrolyzed PKC.

results of the Tukey test on (the percentage of) carcass weight showed that the treatment using hydrolyzed PKC was not significantly different from the treatments. This result indicated that the carcass weight depended on the slaughter weight (in this case the final body weight). If the slaughter weight was high, the carcass weight would also be high, and vice versa. If the slaughter weight was low, the carcass weight would also be low. This result was different from the study by Adedokun *et al.* (2015) that the use of fibrous feed ingredients fermented with *Trichoderma reesei* fungus could reduce (the percentage of) carcass weight. In this study, the percentage of carcass weight was maintained. The range of the relative carcass weight in this study was between 67.52% to 71.42%. It was assumed that various processes of PKC would produce different quality outputs of PKC so that the relative carcass weight being produced was also different.

The treatment effect of the use of hydrolyzed PKC on digestive organs (liver weight, weight of proventriculus, ventriculus weight, small intestine weight and length) of broilers is presented in Table 5.

The result of the variance analysis on the average liver weight (per 100 g of live weight) presented in Table 5 showed that the effect of using of hydrolyzed PKC in the feed on the weight of chicken broilers' liver did not lead to a significant effect (P>0.05). This result was similar to the study by Haroen *et al.* (2019) that the use of buffalo rumen fluid enzymes in broiler's feed did not significantly affect the chicken broiler's liver weight. Liver weight in this study was still in normal condition. Abbasi *et al.* (2015) reported that the relative liver weight of 35-day-old chicken broilers ranged from 2.91% to 3.14% of body weight. The function of the liver is as an organ that neutralizes anti-nutritional substances that are toxic to the body (Bhanja *et al*., 2004; Bhattacharyya *et al.*, 2007). Thus, if the feed ingredients in the feed did not contain anti-nutritional compounds or toxins, the liver function would not be too over to neutralize toxins that went inside the body, so the resulting liver weight was the same as the control.

The results of the variance analysis of the use of hydrolyzed PKC in the feed on the proventriculus weight (per 100 g of live broilers) showed no significant effect (P>0.05). This result was the same as a study by Haroen *et al.* (2019) on the use of buffalo rumen fluid enzymes in broiler's feed. The proventriculus is a glandular stomach that functions to secrete pepsin and HCl so that when feed enters the ventriculus, it can be moistened, digested, mashed, and crushed. Then, when it leaves the ventriculus and enters the small intestine, it is in the form of a paste. The same weight of proventriculus shows that pepsin and HCl secretion is not affected by PKC treatment in the feed. The proventriculus work system between treatments in secreting pepsin and HCl was relatively the same (Sklan and Noy, 2000).

The results of the variance analysis of the use of hydrolyzed PKC on the ventriculus weight of broilers (per 100 g of live weight) showed a significant effect (P<0.05). Tukey's test results showed that the ventriculus weights in treatment T0 (control), T1, and T2 were not significantly different, but T0 and T1 were significantly lower (P<0.05) than the ventricular weights T3, T4, and T5. Meanwhile, the ventriculus weights among the treatments of T3, T4, and T5 and the treatments of T2, T4, and T5 were not significantly different (P>0.05). These results indicated that the ventriculus weight was not affected when it was up to 12% of the use of hydrolyzed PKC. However, a higher the use of hydrolyzed PKC (more than 12%), the more increased the ventriculus weight would be. This was different from what was stated by Haroen *et al.* (2019) stated that the use of buffalo rumen fluid enzymes in broiler feed on the ventriculus weight also had no significant effect. It was assumed that hydrolyzed PKC still contained a lot of hard shells or shells that could not be crushed, so it affected the work of the ventriculus (Sundu and Dingle, 2011), in addition to the high crude fiber content in PKC of 17.63% (Pasaribu, 2018). Sundu and Dingle (2011) reported that the weakness of PKC as feed is the presence of a gritty (rocky) PKC shell which could reach 15%, while Pasaribu (2018) reported that PKC shell reaches 9.21 to 22.8%.

The results of the variance analysis of the use of hydrolyzed PKC on the small intestine weight (per 100 g of body weight), and the small intestine length (cm per 100 g of body weight) of broilers showed that the weight of the small intestine did not different (P>0.05), but the treatment had a significant effect on the length of the small intestine (P<0.05).

Table 5. Treatment effect of the hydrolyzed palm kernel cake with buffalo rumen fluid enzymes on broilers' digestive organs (liver weight, proventriculus weight, ventriculus weight, small intestine's weight and length)

Treatment	Liver weight (g/100 g)	Proventricular weight (g/100 g)	Ventriculus weight (g/100 g)	Intestine weight (g/100 g)	Intestine length (cm/100 g)
T0	1.91±0.18	0.57±0.17	1.65±0.23^{a}	4.06±0.70	13.37±1.56^{ac}
T1	2.05±0.33	0.59±0.03	1.74±0.13^{a}	4.56±0.27	14.50±0.97^{a}
T2	2.16±0.11	0.62±0.13	1.86±0.19^{ac}	4.58±0.32	15.72±0.96^{b}
T3	2.15±0.15	0.69±0.07	2.13±0.04^{b}	4.46±0.55	15.52±1.16^{ab}
T4	2.19±0.25	0.76±0.26	2.05±0.12^{bc}	4.69±0.42	17.58±1.71^{b}
T5	2.15±0.12	0.62±0.03	2.04±0.17^{bc}	4.54±0.68	16.37±2.15^{b}

^{a,b,c} The same lowercase superscripts in the same column present no significant difference at the level (P>0.05), while the different lowercase superscripts in the same column present a significant difference at the level (P<0.05).

T0 = Feed without hydrolyzed PKC, T1 = feed with 6% hydrolyzed PKC, T2 = feed with 12% hydrolyzed PKC, T3 = feed with 18% hydrolyzed PKC, T4 = feed with 24% hydrolyzed PKC, and T5 = feed with 30% hydrolyzed PKC.

The Tukey test result on the length of the small intestine showed that the length of the small intestine in treatments T1 and T3 was not significantly different from treatment T0 (control). However, the treatments of T2, T4, and T5 were significantly longer than T0 (control); and among the treatments of T2, T3, T4, and T5, the length of the small intestine showed no significant difference. This meant that the use of hydrolyzed PKC up to 6%, the length of the intestine being produced would be relatively the same, but those higher than 6% would increase the relative length of the small intestine. It was assumed that there is an increase in the work of the small intestine in digesting and absorbing nutrient contents. It was assumed that a higher content of PKC resulted in a higher amount of crude fiber and the number of shells or coconut shells that would be in affecting digestion and absorption, thereby would increase the relative length of the small intestine. Chickens were also unable to digest crude fiber because they did not have enzymes that were able to degrade fiber into simpler compounds that could be absorbed into the digestive tract of poultry (Pasaribu, 2018).

Conclusions

It can be concluded that the incubation of buffalo rumen fluid enzymes at a level of 2.25% could improve the quality of PKC by decreasing the crude fiber content and increasing the dissolved glucose total content. The use of hydrolyzed PKC after addition by rumen fluid enzyme of buffalo could be applied up to 18% in the feed without affecting the performance of the broilers.

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