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The Effect of Hydrolyzed Rations Using Cellulase and Mannanase Enzymes on Carcass Weight in Broiler Chickens

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ABSTRACT

This research aims to determine the effect of palm kernel cake-based diets hydrolyzed using cellulase and mannanase enzymes on carcass weight in broiler chickens. This research used 200 DOC heads produced by PT. Japfa was kept for 35 days in the Poultry Experiment Cage of the Animal Cultivation Laboratory, Faculty of Animal Husbandry, Jambi University. This research used a Completely Randomized Design (CRD) with five treatments and four replications. The variables observed in this research were feed consumption, slaughter weight, absolute carcass weight, and relative carcass weight. Data were analyzed using analysis of variance (ANOVA). If there was a significant effect, then continue with the Duncan test. The results of the study showed that giving rations based on palm kernel cake, which was hydrolyzed using cellulase and mannanase enzymes, had a significant effect (P<0.05) on feed consumption, slaughter weight and absolute carcass weight but had no significant effect (P>0.05) on body weight. Relative carcass. It was concluded that the 50 mL/Kg cellulase enzyme and 100 mL/Kg mannanase enzyme showed increased broiler chicken carcass weight.

Keywords: Carcass weight, cellulase enzyme, mannanase enzyme

INTRODUCTION

Broiler productivity is determined mainly by the availability of feed ingredients in terms of quality and quantity. However, this is often hampered by the high demand for feed ingredients in rations such as corn, soybean meal and fish meal, which causes production costs to increase, especially feed costs. Utilizing by-products from the agro-industry as substitute feed ingredients in preparing poultry rations, such as palm kernel cake (PKC), can solve the high cost of conventional feed because the price of PKC is quite affordable.

PKC is a by-product of palm kernel processing to produce palm oil from extraction or by *expeller*. (Mairizal and Akmal, 2020) Palm kernel cake reported a crude protein content of 16.89%, crude fiber of 17.86%, and 2780 Kcal/Kg metabolic energy content. The abundant availability of PKC and the crude protein and metabolic energy content of PKC provide an opportunity to be used as a non-conventional feed in preparing broiler rations.

PKC is less effective if added directly to poultry feed because the crude fiber content in PKC is relatively high, especially in the form of non-starch polysaccharides, thereby reducing the digestibility of food substances (Alshelmani et al., 2017). The main component of the PKC cell wall contains non-starch polysaccharides consisting of hemicellulose (50.37%) and cellulose (21.39%) components (Sembiring, 2006). Mannan is the main hemicellulose component in PKC, which reaches 78% of the total non-starch polysaccharides in PKC (Shukor et al., 2016). The high crude fiber content in broiler rations will reduce the digestibility of food substances, resulting in low broiler chicken carcass weight.

Conventional feeds such as corn and soybean meal are the main ingredients in broiler rations because they contain high sources of protein and energy; however, corn and soybean meal also contains crude fiber, which is included in non-starch polysaccharides (NSP), especially in the form of arabinoxylan, hemicellulose, cellulose, β-mannans, and pectin (Choct et al., 2010). Using corn, soybean meal and palm kernel cake in high quantities in the ration will undoubtedly increase the crude fiber content, especially the mannan and cellulose fractions so that their efficiency as feed decreases. Therefore, it is necessary to pre-treat rations containing high levels of crude fiber, namely by processing them by hydrolysis with cellulase and mannanase enzymes.

According to Chin (2002), hydrolysis of cellulose with cellulase enzymes will produce monosaccharides in the form of glucose, which will be easily absorbed in the digestive tract. Meanwhile, according to Latham et al. (2020), hydrolysis of mannan with the mannanase enzyme will reduce intestinal viscosity, break mannan into simple compounds, and allow digestive enzymes to move more freely, thereby increasing the absorption of food substances.

The results of previous research (Mairizal and Akmal, 2020) show that hydrolysis of rations containing 30% palm kernel cake using the mannanase enzyme from *Bacillus cereus* V9 of 100 mL or the equivalent of 2,950 UI/Kg ration did not show a significant difference to rations without palm kernel cake, but showed a tendency to reduce body weight. It is suspected that part of the crude fiber fraction, namely cellulose, in palm kernel cake and other feed ingredients has not been degraded because cellulase enzymes are not added in the hydrolysis process. Research results (Navidshad et al., 2016) show that hydrolysis of PKC with cellulase enzyme as much as 35 units/gram PKC and mannanase enzyme as much as five units/gram PKC can reduce crude fiber content from 203 grams/Kg to 105 grams/Kg.

Enzymes work based on the substrate they are going to break down, so it is said that the enzyme works on a specific substrate according to the activity of the enzyme it produces. Therefore, using cellulase and mannanase enzymes simultaneously will undoubtedly have the opportunity to improve the quality of palm kernel cake-based broiler rations so that food digestibility will also increase.

The carcass is the final product and the clean product (net product) from the beef livestock business, so carcass can also be used to measure beef livestock's productivity, including broiler chickens (Putra, 2017). The carcass is influenced by animal ration consumption and slaughter weight; Fahrudin et al. (2016) stated that the factors that influence carcass are feed consumption, including chicken body size, daily activities, environmental temperature, quality and quantity of feed. Based on the description above, a study has been conducted to see the effect of providing rations based on palm kernel cake hydrolyzed using cellulase and mannanase enzymes on broiler chicken carcass weight.

MATERIALS AND METHODS

This research was carried out for 35 days in a poultry experimental pen at the Faculty of Animal Husbandry, Jambi University. The time for carrying out this research starts from September 21 to October 26 2022.

Materials and Equipment

The material used in this research was 200 DOC produced by PT Japfa Comfeed Indonesia Tbk. Then, the feed ingredients used for the research basal ration consisted of palm kernel cake (30%), corn (29.50%), soybean meal (20%), fine bran (3%), fish meal (12%), and coconut oil. (4 %), premium (0.50 %), Dl-Methionine (0.25 %), L-Lysine (0.25 %), and $CaCO_3$ (0.50 %). The cellulase and mannanase enzymes used are research products from (Mairizal et al., 2018).

There are 20 cages equipped with feed and drink containers and 40-watt lamps for heating and lighting. Each cage unit houses ten broilers. The equipment used is one thermometer, a 5 kg scale and a 2 kg scale for weighing carcasses, sawdust, tarpaulin, black plastic, newspapers, buckets, brushes, knives, pans and gas stoves.

Research methods

Research methods carried out during the research included the preparation stage, preparation of research tools, preparation of rations, implementation and maintenance, and sampling. The treatment given in this study was the level of administration of mannanase and cellulase enzymes in palm kernel cake-based rations, namely P0 = basal ration without the addition of cellulase and mannanase enzymes, P1 = basal ration based on palm kernel cake, which was hydrolyzed using 50 mL of cellulase enzyme/Kg ration + 50 mL mannanase enzyme/Kg ration, P2 = basal ration based on palm kernel cake which is hydrolyzed using 100 mL cellulase enzyme/Kg ration + 50 mL mannanase enzyme/Kg ration, P3 = basal ration based on palm kernel cake which is hydrolyzed using 50 mL cellulase enzyme/Kg ration + 100 mL mannanase enzyme/Kg ration, P4 = basal ration based on palm kernel cake which is hydrolyzed using 100 mL cellulase enzyme/Kg ration + 100 mL mannanase enzyme/Kg ration.

Preparation phase

This research began with the isolate rejuvenation of *Bacillus cereus V9,* which will be used to produce cellulase and mannanase enzymes. Isolate *Bacillus cereus* V9 is rejuvenated by growing it again in a specific medium (NA). Then, incubate the media containing the isolate in a shaker or rocking incubator with a rotation speed of 130 rpm for 24 hours at a temperature of 30 ˚C. Next is the bacteria *Bacillus cereus.* The grown V9 was planted in solid media containing 0.5% *locus bean gum* for mannanase production and 0.5% CMC for cellulase production and added 1.5% bacto agar, 0.05% yeast extract, 0.075% peptone and minerals, respectively (Mendels and Sternberg, 1976). Next, incubate in an incubator at 30 ºC for 24 to 32 hours, and the grown bacteria are ready to be used as isolate propagation stock during the research.

The rejuvenated bacteria were aseptically inoculated three times in culture media and then incubated in a shaker incubator for 80 hours (Mairizal et al., 2018). The mannanase enzyme is obtained by centrifuging the culture sample at a speed of 12,000 rpm for 15 minutes at a temperature of 4ºC to obtain a supernatant, which is a crude extract of the mannanase enzyme which is ready to be used to hydrolyze palm kernel cake-based rations. Meanwhile, cellulase enzyme production follows the same procedure as mannanase enzyme production, but the substrate used is 0.5% CMC, and the incubation time is 48 hours (Mairizal, 2020 Unpublish)

Making Rations

The ingredients for the ration consist of corn, soybean meal, fine bran, fish meal, palm oil, premium, methionine, lysine, and $CaCO_3$, as well as palm kernel cake. According to the recommendations of Leeson and Summers (2005), the ration is made based on nutrient-feed ingredients in isocaloric and isoprotein in the form of crumbles (small granules). The rations used in this research are rations prepared by yourself using the composition of the basalt ration ingredient in Table 1, the nutritional content of basal rations treated in Table 2, and the cost of ingredients making up basal rations in Table 3.

Table 1. Composition of Basal Ration Ingredients.

Feed Ingredients	%
Corn	29.50
Fine Bran	3.00
Soybean Meal	20.00
Palm kernel cake	30.00
Fish meal	12.00
Coconut oil	4.00
$CaCO_3$	0.50
Movement	0.50
Dl-Methionine	0.25
L-Lysine	0.25
Total	100

Table 2. Nutrient Content of Treatment Basal Rations.

Nutritional Content	Treatment Rations				
	P0	P1	P2	P3	P4
Dry materials (DM) (%)*	90.15	89.85	89.95	89.9	89.65
Net protein (NP) (%)*	21.80	21.85	21.92	21.92	21.94
Crude fiber (CF) (%)*	8.6	6.2	6.0	5.4	5.2
Crude Fat/Extract ether (EE) (%)*	3.82	3.28	3.25	3.22	3.05
Ash (%)*	5.12	5.22	5.49	5.55	5.58
Nitrogen free-extract (NFE) (%)**	50.81	53.3	53.29	53.81	53.88
ME (Kcal/Kg)***	3008.94	3057.39	3056.99	3073.80	3062.61

Information : * Results of laboratory analysis of the Faculty of Animal Husbandry, Jambi University (2022).
** Calculation Results NFE = DM - (Ash+NP+CF+EE)
*** Calculation Results ME = 36.21 x Net protein + 85.44 x Crude fat +37.26 x NFE (NRC, 1994)

Data analysis

The data obtained was further analyzed using multivariate analysis according to the plan. If there is a significant influence between the treatments, proceed with Duncan's multiple-range test (Steel and Torrie, 1991).

RESULTS AND DISCUSSION

Ration Consumption

The effect of providing rations based on palm kernel cake hydrolyzed using cellulase and mannanase enzymes on broiler chicken ration consumption can be seen in Table 3.

The variance analysis shows that the provision of rations based on palm kernel cake, which is hydrolyzed using cellulase and mannanase enzymes, significantly affects ration consumption ($P<0.05$). Duncan's further test showed that treatment P0 was not significantly different ($P>0.05$) from treatment P1 but was significantly different ($P<0.05$) from P2, P3, and P4. The P2 treatment was not significantly different ($P>0.05$) from the P4 treatment but was significantly different ($P<0.05$) from the P3 treatment.

Table 3. Average Ration Consumption

Treatment	Ration Consumption (grams/head/week)
P0	$409.41^{a} \pm 10.63$
P1	$420.86^{a} \pm 14.38$
P2	$456.17^{b} \pm 17.41$
P3	$483.56^{c} \pm 8.23$
P4	$465.18^{bc} \pm 13.70$

Note: P0 = Without addition of cellulase and mannanase enzymes, P1 = 50 mL cellulase enzyme/Kg ration + 50 mL mannanase enzyme/Kg ration, P2 = 100 mL cellulase enzyme/Kg ration + 50 mL mannanase enzyme/Kg ration, P3 = 50 mL cellulase enzyme/Kg ration + 100 mL mannanase enzyme/Kg ration, P4 = 100 mL cellulase enzyme/Kg ration + 100 mL mannanase enzyme/Kg ration. Different superscripts in the same column indicate significant differences (P<0.05).

The increase in ration consumption in treatments P2, P3, and P4 was due to the addition of cellulase and mannanase enzymes. However, treatment P1 also received additional enzymes at 50 mL cellulase enzyme/Kg ration + 50 mL mannanase enzyme/Kg ration. However, the addition of enzymes in treatment P1 was not optimal, so feed consumption was still low or not significantly different (P>0.05) from treatment P0. Treatments P2, P3 and P4 showed an increase in feed consumption along with a decrease in the crude fiber content of the feed from 8.6% (P0) to 6% (P2), 5.4% (P3) and 5.2 (P4) (can be seen in Table 3). The lower the crude fiber content in the ration, the higher the ration consumption. A high crude fiber content can result in low ration consumption because crude fiber is bulky or filling, so the cache fills quickly, and as a result, ration consumption will stop. It is in line with the opinion of Wahju (2004) that crude fiber has bulky properties. It was confirmed by Prawitasari et al. (2012) stated that the higher the crude fiber content in the ration causes, the lower the crude fiber digestibility and vice versa.

The increase in feed consumption in treatments P2, P3 and P4 was in line with the increase in the administration of cellulase and mannanase enzymes in the hydrolysis of palm kernel cake-based diets. Palm kernel meal-based rations contain high levels of crude fiber, as shown in the P0 treatment ration (8.6%), mainly in mannan and cellulose, which are difficult for poultry to digest. The addition of cellulase and mannanase enzymes will be able to reduce the crude fiber content of the ration because these enzymes will break down cellulose into glucose and mannan into mannose and mannan oligosaccharides, which are more easily digested and absorbed in the broiler's digestive tract. The decrease in crude fiber content due to the addition of cellulase and mannanase enzymes was seen in the crude fiber content of treatment diets P1 to P4 (6.2% to 5.2%). However, the addition of cellulase enzyme at 50 mL/Kg ration and 100 mL/Kg ration and mannanase enzyme at 100 mL/Kg ration was more effective in reducing the crude fiber content of the ration. The results of this research are in line with research by Mairizal and Akmal (2020) that hydrolysis of rations containing 30% palm kernel cake using the mannanase enzyme from *Bacillus cereus* 100 mL of V9 or the equivalent of 2,950 UI/Kg can reduce the crude fiber content in the ration. In line with Latham's et al. (2020) opinion, hydrolysis of mannan with the mannanase enzyme will reduce intestinal viscosity, break mannan into simple compounds, and allow digestive enzymes to move more freely, thereby increasing the absorption of food substances.

Meanwhile, according to Chin (2002), hydrolysis of cellulose with the cellulase enzyme will produce monosaccharides in the form of glucose, which will be easily absorbed in the digestive tract. This condition causes the crude fiber of the ration to decrease and will affect the consumption of broiler chicken rations. Crude fiber is bulky, so its presence in poultry rations is also limited to no more than 6%, as the NRC (1993) recommends. If the crude fiber content is high in the ration, it will cause the livestock to feel full quickly so that the livestock will stop eating and ultimately reduce ration consumption.

The average feed consumption in this study ranged from 409.41 - 483.56 grams/head/week (Table 3). The results of this research are lower than the research (Rahmadani et al., 2020), which states that the average ration consumption is around 537.34 grams/head/week. Due to the amount of crude fiber in the study (Rahmadani et al., 2020) for each treatment ranges between 2 - 4%, it will affect broiler chicken ration consumption.

Slaughter Weight

The effect of providing a ration based on palm kernel cake hydrolyzed using cellulase and mannanase enzymes on the slaughter weight of broiler chickens can be seen in Table 4. The analysis of variance showed that the provision of rations based on palm kernel cake which was hydrolyzed using cellulase and mannanase enzymes, significantly effect (P<0.05) the slaughter weight of broiler chickens.

Based on the results of Duncan's further test, the cutting weight showed that treatment P0 was not significantly different (P>0.05) from P1 but significantly different (P<0.05) from P2, P3 and P4. The increase in slaughter weight in treatments P2, P3 and P4 is in line with the increase in feed consumption, whereas the slaughter weight will also increase as feed consumption increases. This statement is under the opinion of Amrullah (2004), which states that slaughter weight is influenced by the amount of ration consumed and the quality of the ration.

Table 4. Average Slaughter Weight

Treatment	Slaughter weight (grams/head)
P0	$1145.00^{a} \pm 85.83$
P1	$1153.75^{a} \pm 77.20$
P2	$1291.25^{b} \pm 52.02$
P3	$1408.13^{b} \pm 81.48$
P4	$1371.88^{b} \pm 88.26$

Note: P0 = Without addition of cellulase and mannanase enzymes, P1 = 50 mL cellulase enzyme/Kg ration + 50 mL mannanase enzyme/Kg ration, P2 = 100 mL cellulase enzyme/Kg ration + 50 mL mannanase enzyme/Kg ration, P3 = 50 mL cellulase enzyme/Kg ration + 100 mL mannanase enzyme/Kg ration, P4 = 100 mL cellulase enzyme/Kg ration + 100 mL mannanase enzyme/Kg ration. Different superscripts in the same column indicate significant differences (P<0.05).

The low slaughter weight in the P0 treatment was caused by the high crude fiber content, namely 8.6%, so it would affect the digestibility of food substances because the digestibility of food substances would decrease if the crude fiber were too high. It is in line with Suciani's et al. (2011), who said that the efficiency of using food substances would decrease because broiler chickens cannot digest too much crude fiber. It was confirmed by Syamsuhaidi (2004), who said that the rate of digestion and absorption of nutrients would be slower if the level of crude fiber in the ration were high. A decrease in the digestibility of food substances will affect the availability of nutrients, especially the crude protein content that livestock will use for production. It aligns with Gultom's et al. (2014) that adequate protein intake in meat and amino acids in the body and the normal metabolism of cells in the body are influenced by high protein consumption. The administration of cellulase and mannanase enzymes is considered effective in reducing crude fiber, especially with cellulase enzymes at 50 mL/Kg ration and 100 mL/Kg ration and mannanase enzymes at 100 mL/Kg ration. The addition of cellulase and mannanase enzymes to reduce crude fiber content can be seen in Table 2 above.

The average slaughter weight in this study ranged from 1145.00 - 1408.13 grams/head (Table 4). This study's results were higher than those of the research reported by Sinuraya (2015), who reported that the average slaughter weight of broiler chickens reared for 35 days with multi-enzyme treatment on rations containing palm kernel cake was around 927.75 grams/head. Compared with the results of Sinuraya's research, the results of this research are better because the effect of hydrolysis of palm kernel cake-based rations using cellulase and mannanase enzymes in this research can improve the quality and quantity of rations along with the level of administration. Rasyaf (2010) believes that the body weight of poultry is influenced by the quality and quantity of food provided.

Absolute Carcass Weight

The effect of providing a ration based on palm kernel cake that is hydrolyzed using cellulase and mannanase enzymes on broiler chickens' absolute carcass weight of broiler chickens can be seen in Table 5.

Table 5. Average Absolute Carcass Weight

Treatment	Absolute Weight (gram/head)
P0	$847.50^{a} \pm 48.95$
P1	$829.38^{a} \pm 19.51$
P2	$920.63^{ab} \pm 60.98$
P3	$1013.13^{b} \pm 80.53$
P4	$966.25^{b} \pm 78.17$

Note: P0 = Without addition of cellulase and mannanase enzymes, P1 = 50 mL cellulase enzyme/Kg ration + 50 mL mannanase enzyme/Kg ration, P2 = 100 mL cellulase enzyme/Kg ration + 50 mL mannanase enzyme/Kg ration, P3 = 50 mL cellulase enzyme/Kg ration + 100 mL mannanase enzyme/Kg ration, P4 = 100 mL cellulase enzyme/Kg ration + 100 mL mannanase enzyme/Kg ration. Different superscripts in the same column indicate significant differences (P<0.05).

The variance analysis shows that the provision of rations based on palm kernel cake, which is hydrolyzed using cellulase and mannanase enzymes, has a significant effect (P<0.05) on the absolute weight of broiler chickens. The results of the Duncan further test showed that treatment P0 was not significantly different (P>0.05) from P1 and P2 but was significantly different (P<0.05) from P3 and P4. Meanwhile, treatment P3 was not significantly different (P>0.05) from P2 and P4 but significantly different (P<0.05) from P0 and P1 in terms of the absolute weight produced.

Cellulase and mannanase enzymes can improve the quality of palm kernel cake-based rations by reducing crude fiber content to increase food digestibility. Reducing the crude fiber content from 8.6% (P0) to 5.2% (P4) increases the digestibility of food substances so that the slaughter weight will also increase and ultimately will increase the absolute carcass weight. It aligns with Despal's (2000) opinion that crude fiber negatively affects food digestibility. The lower the crude fiber, the higher the digestibility of food substances.

The increase in absolute carcass weight in treatments P2, P3, and P4 was in line with the increase in slaughter weight in each of the treatments above. Slaughter weight is directly proportional to absolute weight, where an increase in slaughter weight will also cause an increase in absolute carcass weight. It is in line with the opinion of Marwandana and Augustine (2013) that there is no difference in slaughter weight, which causes the carcass weight not to be different because the slaughter weight is directly proportional to the carcass weight. Nahashon et al. (2005) stated that carcass weight is greatly influenced by the live weight produced. The higher the live weight, the higher the carcass weight and vice versa.

Relative Carcass Weight

The effect of providing a ration based on palm kernel cake hydrolyzed using cellulase and mannanase enzymes on the relative carcass weight of broiler chickens can be seen in Table 6.

The variance analysis shows that the provision of rations based on palm kernel cake hydrolyzed using cellulase and mannanase enzymes has no significant effect (P>0.05) on relative carcass weight.

One of the factors that causes the carcass weight to be relatively the same between treatments is that the decrease in absolute carcass weight is in line with the decrease in slaughter weight for each treatment. According to Juniarti et al. (2019), factors that influence relative carcass weight are race, gender, age, ration quality, and slaughter weight because relative carcass weight is obtained from a comparison of absolute carcass weight with slaughter weight multiplied by 100%.

Table 6. Average Relative Carcass Weight

Treatment	Relative weight (%)
P0	74.10 ± 2.43
P1	72.13 ± 4.74
P2	71.25 ± 2.16
P3	71.92 ± 1.56
P4	70.38 ± 2.36

Note: P0 = Without addition of cellulase and mannanase enzymes, P1 = 50 mL cellulase enzyme/Kg ration + 50 mL mannanase enzyme/Kg ration, P2 = 100 mL cellulase enzyme/Kg ration + 50 mL mannanase enzyme/Kg ration, P3 = 50 mL cellulase enzyme/Kg ration + 100 mL mannanase enzyme/Kg ration, P4 = 100 mL cellulase enzyme/Kg ration + 100 mL mannanase enzyme/Kg ration.

Carcass weight is a reflection of the growth of tissue and bones and is closely related to the quality of the ration, where the higher the quality of the ration, the higher the growth of meat and bone tissue will be. The percentage of carcass weight produced between treatment rations P0, P1, P2, P3, and P4 ranged from 70.38 – 74.10% (Table 6). The results of this study are still within the normal range because, according to Amrullah (2004), the average chicken carcass weight ranges from 65-75% of live weight.

CONCLUSION

Based on the results of this research, it can be concluded that giving a ration based on palm kernel cake, which is hydrolyzed using the cellulase enzyme at a level of 50 mL/Kg of ration to 100 mL/Kg of ration combined with a mannanase enzyme at a level of 100 mL/Kg of ration can increase carcass weight in broilers chickens.

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