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Biotechnology Application of Individual and Multienzyme in Feed, and Its Effect on Broiler Growth Performance

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ABSTRACT

Bromelain as a single enzyme is widely used as a meat tenderizer; however this can damage the meat cell's. The effect of adding multienzyme Phyprozyme HP in feed is not yet known, so it needs to be investigated. This study aimed to evaluate the growth performance of broilers with the addition of single and multienzymes. This study used 42 Lohman MB 202 Grade Platinum broilers aged 21 to 35 days, using 6 treatments and 4 replications. P0 as the control feed. Bromelin was added in feed at the dose of P1: 600 GDU/kg, P2: 1200 GDU/kg, P3: 1800 GDU/kg, P4: 2400 GDU/kg, and P5: 3000 GDU/kg. Phyprozyme HP was added at P1: 0.19 g/kg, P2: 0.26 g/kg, P3: 0.33 g/kg, P4: 0.40 g/kg, and P5: 0.47 g/kg. Parameters measured included body weight, body weight gain, and carcass percentage. The results were analyzed using One-Way ANOVA. The addition of bromelain in feed decreased body weight, while the addition of multienzyme significantly improved broiler performance up to a dose of 0.33 g/kg feed. The addition of bromelain decreased carcass percentage to 11.07%, while the addition of multienzyme increased carcass percentage to 8.83%, but decreased it to 7.68% at the highest dose.

1. INTRODUCTION

International Broiler production in the livestock industry plays an important role in providing the world's animal protein supply (Nkukwana 2019). Broilers are highly favored by consumers due to several advantages, such as a short harvest period, lower costs compared to native chickens, more tender meat, and ease of maintenance in tropical climates. However, there are some drawbacks in raising broilers, including the limited nutritional value of feed and the health status of the animals against diseases (Chuka 2014). Several studies have also indicated that broiler productivity is still low and needs to be increased to meet the market demand for broiler meat (Tavárez and de los Santos 2016). Because of this problem, currently research is still being carried out on efforts to improve broiler performance, one of which is using vegetable ingredients, high temperature processing and adding enzymes.

Efforts to improve the quality and cost of feed can be done by utilizing vegetable ingredients that are rich in protein and phosphate (especially grains). The use of vegetable raw materials is also widely available in nature because Indonesia is an agricultural country. Apart from that, the use of vegetable waste such as fine rice bran and soybean meal is also more beneficial for the environment and can be obtained at a cheaper price. The plant-based feed ingredients that have been used reach a presentation of 90% of the total feed formulation regardless of the region, however the high use of plant-based feed is also feared to result in an increase in anti-nutritional

compounds which interfere with the absorption of nutrients in the feed (Syed Fazal ur Rahim and Muhammad Abdullah Bin Masood 2022). Antinutrients in the form of phytate have quite a large negative effect on most animals, namely strong binding to minerals such as Fe, Zn, Ca, Mg, Mn, Cu, Se, and Ni, as well as protein and starch (Sahu et al. 2024). Therefore, enzyme supplementation in feed is one solution and is useful in increasing the nutritional value of feed (Kari et al. 2022). Enzymes are defined as proteins that facilitate certain chemical reactions (Lovelock et al. 2022). Enzymes work on a substrate specifically by catalyzing reactions to convert complex substances into substances that can be absorbed (Khan 2021). Several studies state that the use of enzymes in feed can help nutrient digestibility, maintain intestinal health, reduce the possibility of nutrient waste, and can be done conventionally, thereby saving costs (Mahmoud Alagawany et al. 2022).

The application of enzymes as biocatalysts plays a role in accelerating the occurrence of a reaction. Enzymes that are capable of hydrolyzing protein substrates in feed ingredients are protease enzymes, one of which is bromelain. The bromelain enzyme can hydrolyze proteins as complex protein compounds into amino acids as simpler compounds. The use of bromelain in the food and livestock sectors is to make meat more tender when consumed. However, several studies also state that adding bromelain to feed can cause cell damage in meat (Grassin and Coutel 2009). Enzyme research on broilers still needs further research to find the right dosage and its impact on broilers.

Several research results show that enzymes working together are more effective when compared to individual enzyme systems (Hwang and Lee 2019) (Ellis et al. 2019) (Dubey and Tripathi 2021). These enzymes are usually referred to as multi-enzyme systems. Several studies have shown that enzymes that work synergistically are more effective than those that work individually. These enzymes are usually referred to as multi-enzyme systems. Several enzymes commonly used in animal feed include protease, xylanase, hemicellulase and phytase. These enzymes are available in a multi-enzyme system, such as Phyprozyme HP, which is used to feed pigs, various poultry and fish (Mohammed and Budihargo 2020). The raw material components of these enzymes are derived from dried extracts obtained from the fermentation of fungi (*Aspergillus niger, Aspergillus oryzae*) and bacteria (*Bacillus subtilis, Escherichia coli*). To date, there has been no research into the application of this multi-enzyme system in broilers. Therefore, more research is needed to determine the appropriate dosage and its impact when adding this multi-enzyme system to broiler feed.

2. MATERIALS AND METHODS

2.1. Materials

Day-old broiler chicks (DOC) of 42 Lohman MB 202 Grade Platinum strain were weighed using digital scales. The weighed DOC were randomly divided into 6 groups, each consisting of 3 broiler chicks, with 4 replicates per group. This study was conducted using a litter-based cage system (with rice husk as the base). The cage system consisted of 24 compartments, corresponding to 6 treatments and 4 replications. The dividers for each compartment were made of bamboo. Each compartment measured 100 cm x 40 cm x 40 cm, thus meeting the minimum size requirements for cages.

2.2. Methods

The research was conducted at the Pekuncen broiler farming unit, Banyumas, Central Java, Indonesia. Proximate analysis of feed and carcasses was carried out at the Food Engineering

Laboratory, Sebelas Maret University and the Biochemistry Laboratory, Faculty of Mathematics and Natural Sciences, Jendral Soedirman University. The research method used was a completely randomized design (CRD). Broiler chickens were reared from day 14 until harvest on day 35. Feed was given twice a day (at 07.00 and 17.00 WIB). The amount of feed given was adjusted to the age of the chickens, following broiler rearing management guidelines. Drinking water was provided ad libitum. This experiment used a uniform feed formulation and consisted of 6 different dose treatments: P0, P1, P2, P3, P4, and P5. For single bromelain enzyme, P0 was set as the control group without enzyme supplementation, while the dose variations were P1 with 600 GDU/kg feed, P2 with 1200 GDU/kg feed, P3 with 1800 GDU/kg feed, P4 with 2400 GDU/kg feed, and P5 with 3000 GDU/kg feed. In the Phyprozyme HP multi-enzyme system, P0 was the control group without enzyme supplementation, P1 was dosed with 0.19 g/kg feed, P2 was dosed with 0.26 g/kg feed, P3 was dosed with 0.33 g/kg feed, P4 was dosed with 0.40 g/kg feed, and P5 was dosed with 0.47 g/kg feed. The sampling method was carried out by purposive sampling where one chicken was taken to represent a group with normal and healthy growth conditions.

2.3. Data analysis

Growth parameters were measured every seven days throughout the experimental period to assess broiler growth performance. Performance measurements included average body weight, weight gain, and feed conversion ratio. The average body weight of broilers was calculated using the following formula:

$$\text{Average body weight} = \frac{\text{Total body weight (g)}}{\text{Number of broilers}}$$

Weight gain was used as a criterion for growth and the ability of livestock to convert nutrients from feed. Chicken body weight was measured in grams using an electronic scale at the start of the experiment, every seven days during the experiment, and at the end of the experiment. Weight gain was calculated using the formula as follows:

$$\text{Weight gain} = \frac{(Wt - Wo)}{Wo} \text{ x } 100\%$$

description:

Wo = Initial body weight at the start of the experiment (g)

Wt = Final body weight at the end of the experiment (g)

Carcass weight is defined as the weight of the chicken meat without feathers, feet, internal organs, neck, and head. Carcass weight determines the economic value of the meat. The carcass percentage was calculated using the following formula:

$$\% \text{ Carcass} = \frac{\text{Carcass weight (g)}}{\text{Live body weight (g)}} \text{ x } 100\%$$

Data were analyzed using One-Way Analysis of Variance (ANOVA) with a 95% confidence level to determine the effect of different doses of Phyprozyme HP supplementation. Statistical analysis was conducted using SPSS software (Version 22, IBM Corporation, New York, USA).

3. RESULTS AND DISCUSSION

3.1. Results

The research results on broiler growth performance using individual and multienzyme enzymes at different doses are detailed in Table 1. The highest final body weight was observed in treatment P1 with the addition of a multienzyme dose of 0.16g/kg feed, while the lowest final body

weight was recorded in treatment P3 with the addition of bromelain enzyme dose of 1800 GDU/kg feed.

Table 1. Comparison of final broiler weights using different types and doses of enzymes.

Treatment	Body Weight (g)	
	Bromelin	Phyprozhyme HP
P0	1655.86 ±114.06*	1533.50 ± 15.40
P1	1641.09 ± 127.02	1716.63 ± 19.65*
P2	1655.43 ± 167.14	1689.75 ± 6.91
P3	1417.42 ±159.49**	1664.50 ± 11.55
P4	1562.57 ± 215.21	1571.38 ± 9.28
P5	1462.57 ± 125.34	1568.13 ± 3.97**

Notes:
* Highest final broiler body weight before harvesting
** Lowest final broiler body weight before harvesting

The final body weight results of broilers using individual enzymes and multienzymes at different doses are detailed in Figure 1.

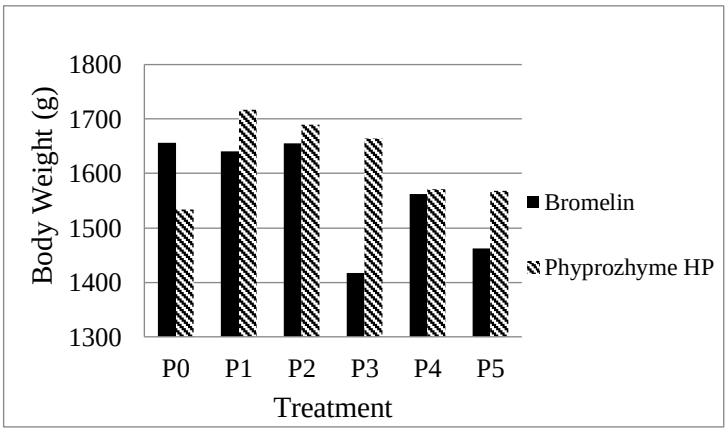

Figure 1. Comparison of final body weight using different enzymes

The research findings on the weekly weight gain of broilers using individual and multienzyme enzymes at different doses are detailed in Table 2.

Table 2. Comparison of broiler weight gain with different enzyme supplementation

Treatment	Weight Gain (g)					
	Bromelin (Day)			Phyprozhyme HP (Day)		
	21	28	35	21	28	35
P0	291.73	433.98	442.07	312.18	333.41	414.25
P1	230.31	447.57	475.13	357.26	347.01	488.75
P2	221.16	448.72	497.47	372.55	350.64	423.84
P3	193.45	393.43	340.46	351.06	336.06	419.05
P4	208.45	461.10	404.94	338.38	331.71	414.13
P5	163.45	359.10	451.94	303.83	323.78	418.50

Notes:
* Highest broiler weight gain every week
** Lowest final broiler weight gain every week

The research results in Table 2 indicate that the weight gain of broiler chickens increases with the addition of multienzyme doses ranging from 0.19 g/kg to 0.33 g/kg of feed and decreases with doses exceeding 0.33 g/kg of feed. The increase in chicken weight is a positive effect of enzyme supplementation in feed, while negative effects of enzyme supplementation may occur with high enzyme doses.

Carcass weight refers to the weight of chicken meat without feathers, feet, internal organs, neck, and head. Carcass weight is significant in the trading industry due to its economic value. The carcass percentage is calculated by comparing the carcass weight to the live weight of broilers. The carcass percentages of broilers using different enzyme supplementation are presented in Table 3.

Table 3. Comparison of broiler carcass percentage with different enzyme supplementation

Treatment	Carcass Weight (g)	Body Weight (g)	Carcass Percentage (%)
Enzim Bromelin			
T0	1220.00	1655.86	73.68*
T1	1076.67	1641.09	65.61
T2	1133.00	1655.43	68.44
T3	1042.00	1417.42	73.51
T4	1127.34	1562.57	72.15
T5	958.34	1462.57	65.52**
Enzim Phyprozyme HP			
T0	1018.25	1508.50	67.50
T1	1310.25	1716.63	76.33*
T2	1151.00	1689.75	68.12
T3	1018.75	1564.50	65.12
T4	955.75	1496.38	63.87
T5	878.25	1468.13	59.82**

Notes:
* Highest carcass percentage every treatment
** Lowest carcass percentage every treatment

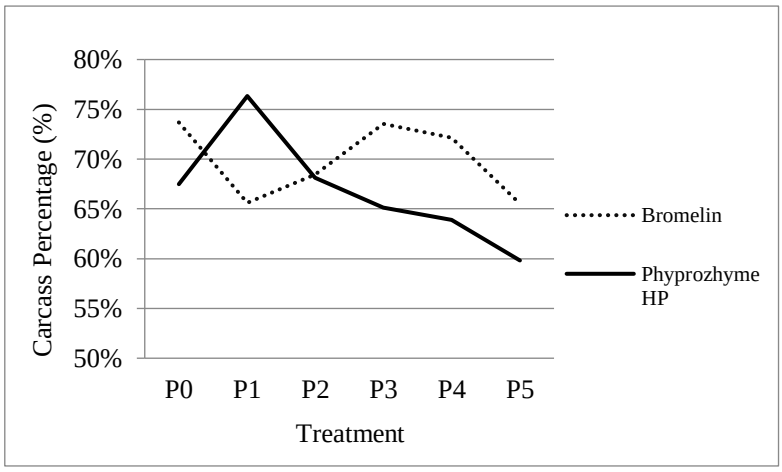

Figure 2. The carcass and non-carcass percentages (Phyprozyme HP)

3.2. Discussion

Broilers are among the most extensively farmed livestock due to several advantages, such as rapid harvesting and suitability for cultivation in tropical environments like Indonesia. Broiler meat remains one of the largest contributors to animal protein worldwide. Research on the effects of various enzyme doses as a biotechnology application in broiler feed is still necessary to determine accurate dosages and their impacts. Previous studies have indicated that bromelain enzyme, when used singularly, is widely employed as a livestock feed supplement to produce tender broiler meat (Juárez et al. 2012) (Naveena, Kiran, and Mendiratta 2013). However, other research suggests that bromelain usage may result in cellular damage within the meat (Guevara and Daleo 2018)(Humayan Kabir et al. 2021). The use of Phyprozyme HP enzyme as a multienzyme has not been previously studied in broilers. Therefore, this study examines the effects

of both types of enzymes on broiler growth performance, considering final body weight, weight gain, and carcass percentage, which represents the economically valuable part of the broiler.

The research findings demonstrate that the addition of different enzyme doses, whether using individual or multienzymes, does not significantly affect broiler growth performance. Surprisingly, the addition of bromelain as a singular enzyme may decrease the formed carcass percentage by up to 11.07%. Some studies suggest that adding bromelain at specific doses to broiler feed can indeed impart tenderness to the meat and reduce fat content (Chandrasekaran 2015). However, it may decrease carcass percentage compared to the use of multienzymes and control treatments. Giving bromelain can also reduce liver lipid disorders, especially free cholesterol, total cholesterol, fatty acids, triglycerides and glycerol by 15-30% (Achilonu et al. 2018). The fatty acid content in chicken meat is also an indicator of meat quality. Bromelain has a function as an anti-obesity drug by inhibiting the process of adipose cell differentiation (Hasoon, Kadhim, and Rahmah 2022). The number and size of adipose cells determine the mass of fat tissue formed. High-fat diets cause reversible changes in the gut microbiota, but can further lead to inflammation, obesity and cancer if poor diets persist (Cândido et al. 2018). Foods high in glucose and fructose also cause increased endotoxin levels, inflammation, loss of microbial diversity, and increased intestinal permeability (Jamar, Ribeiro, and Pisani 2021).

The carcass portion of broilers comprises the parts of the chicken without feathers, blood, internal organs, head, neck, and claws. Carcass percentage can be influenced by several factors such as chicken genetics, feed quality, gender, slaughter age, and environment. In this case, carcass percentage is affected by the addition of enzymes compared to the control group. If the nutrition and digestibility of the feed entering the chicken's body decrease, the carcass weight will also decrease. The research results show that although bromelain may impart a more savory and tender taste to the meat, it can decrease the carcass percentage. This research outcome provides a distinct evaluation for stakeholders in the broiler carcass market. If traders prioritize taste, then bromelain can be a solution. However, if traders require a higher broiler carcass percentage, then bromelain cannot be used due to its resulting impact.

The research findings indicate that the supplementation of multienzymes in feed can help improve the growth performance of broiler chickens by increasing their weight gain at doses ranging from 0.19 g/kg^{-1} to 0.33 g/kg^{-1} of feed (P1, P2, and P3). The highest growth performance based on weight gain data is observed in chickens given an enzyme dose of 0.26 g/kg^{-1} of feed (P2), while the lowest weight gain is observed in chickens given the highest enzyme dose compared to other enzyme dose treatments, namely treatment P5 with an enzyme dose of 0.47 g/kg^{-1}. This suggests that multienzyme supplementation is beneficial for chicken growth but can also decrease chicken weight at high doses. Phyprozyme HP enzyme has a positive impact on broiler chicken growth (0.19-0.33 g/kg^{-1} of feed), but it can have negative effects, such as weight loss, when supplemented with high doses (0.4-0.47 g/kg^{-1} of feed). The research results show that the amount of feed consumed by chickens significantly affects broiler chicken growth performance, but it is not the sole determining factor for improving chicken growth performance. This is evidenced by the fact that the amount of feed consumed by chickens in the control treatment is higher than in all other treatments, yet the weekly weight gain of chickens in the control treatment is lower than that in P1, P2, and P3. Other factors affecting growth performance may include the presence of certain plant proteins in feed that are not well digested in the chicken's digestive tract (Qaisrani et al. 2015).

The growth performance response of broiler chickens based on the research findings demonstrates that the digestibility of plant materials can be tolerated up to a certain limit with the addition of multienzymes. This is evidenced by the addition of multienzyme doses up to 0.33 g/kg of feed, resulting in an increase in carcass percentage, while doses exceeding that can decrease carcass percentage by up to 7.68%. The research results also prove that the multi-enzyme system can enhance growth performance and protein digestibility better than adding enzymes individually. These findings are consistent with various previous studies (Shekarabi et al. 2022) (Lu et al. 2016)

(K.A. et al. 2011). The research results indicate that multienzymes, which can enhance growth performance better than individual enzymes, will also have negative impacts if excessive enzyme doses are used. Some research facts also show that broiler chicken growth performance may decline if the enzyme doses added to the feed are too high (Raza, Bashir, and Tabassum 2019) (M. Alagawany, Elnesr, and Farag 2018). Therefore, it can be concluded that enzyme concentration plays a crucial role in feed.

The addition of exogenous enzymes such as xylanase and hemicellulase in feed is highly recommended to increase the digestibility of NSP which initially cannot be digested properly (Morgan et al. 2022). Starch as a polysaccharide is important as an energy source in feed ingredients. However, there are several things that make starch indigestible in poultry digestion. First, the content of new feed energy sources can be increased if NSP which is found in significant amounts in some vegetable ingredients (soybean meal) can also be utilized (Aftab and Bedford 2018) (Nguyen, Bedford, and Morgan 2021). Apart from that, poultry, as a monogastric animal, cannot utilize this energy source because it does not have the enzymes used to digest complex NSP (Musigwa et al. 2021). One of the enzymes that contains xylanase and hemicellulase enzymes is Phprozyme HP. The Phprozyme HP enzyme acts as an anti-NSP enzyme whose role is to break down NSP into sugar and oligosaccharides so that the energy source used for growth can be increased. The use of several enzymes in feed can work in different locations and target different substrates so that it can increase the digestibility of feed nutrients in the gastrointestinal tract (Bedford 2018).

Research on the addition of Phyprozyme HP has so far only been carried out on tilapia (*Oreochromis niloticus*) (Mohammed and Budihargo 2020). The research results showed that there was a significant difference in the average final weight of tilapia due to the effect of adding Phyprozyme HP to the feed. Phyprozyme HP can be a supplement to tilapia fish feed without having a negative effect on tilapia carcass composition. The growth response parameters of *Oreochromis niloticus* increased at a dose of 0.05-0.4 g/kg feed, but could reduce fish weight at a high dose, namely at 0.47 g/kg feed. The results of this research are a very good start and can be developed again in the future because there has been no further research regarding Phyprozyme HP supplementation in animals, including broilers, which has shown the same results. In general, some plant-based feed ingredients used as feed ingredients contain anti-nutritional factors. These anti-nutritional factors play a negative role in the gastrointestinal tract and cause the digestibility of nutrients in feed to be disturbed. The Phprozyme HP enzyme which contains several enzymes that act as anti-NSP can break down NSP so that the digestibility of nutrients in feed can increase. The Phprozyme HP enzyme also plays a role in increasing the efficiency of the gastrointestinal tract and maintaining intestinal health in livestock.

The negative impact of Phyprozyme HP enzyme supplementation was found at high dose concentrations in the research results. This may be due to the excessive release of monosaccharides. The presence of excessive monosaccharides can lead to hyperglycemia (Arnone et al. 2022). Research on carbohydrate tolerance in experimental animals suggests that high concentrations of galactose in the blood can cause galactosemia, which cannot be tolerated in the bodies of broiler chickens (Haskovic et al. 2020) (Van Laar, Grootaert, and Van Camp 2021) (Md Benzamin et al. 2020). Prolonged galactosemia in chickens can lead to decreased growth performance and feed intake.

4. CONCLUSIONS

The research findings demonstrate that the addition of single enzyme bromelain and multi-enzyme Phyprozyme HP did not significantly differ in terms of broiler growth performance. The addition of bromelain in all treatments could lead to weight loss, while the addition of multi-enzyme could increase weight gain up to a dosage of 0.33g/kg of feed. Adding Phyprozyme HP at doses exceeding 0.33 g/kg of feed may decrease broiler weight. Bromelain addition could decrease

carcass percentage by up to 11.07%, while multi-enzyme addition could increase carcass percentage by up to 8.83%, but could decrease carcass percentage by up to 7.68% at the highest dose.

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