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Original Article

Evaluation of Phosphorus Storage and Performance of Broilers Using Phytase Synthetic Enzyme

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Abstract

Phytic acid is a stored form of phosphorus in cereals, 65 to 70% of phosphorus in plant sources is phytate, and broilers are only able to use part of the phosphorus in plant sources. To meet the needs of chickens, it is necessary to use other artificial resources, which not only impose part of the cost of the breeding period because of its presence in the manure but is one of the factors polluting the environment. This study aimed to use different levels of phytase enzyme to reduce dietary phosphorus levels. 600 Ross 308 broilers were used in this experiment with five treatments and six replications, and in each replication, 20 chickens were used in a completely randomized design (CRD). Experimental treatments include 1) basal diet (control) 2) basal diet with 15% less phosphorus 3) basal diet with 15% less phosphorus + 1250 (FTU) phytase enzyme 4) basal diet with 15% less phosphorus + 2500 (FTU) phytase enzyme 5) basal diet with 15% less phosphorus + 5000 (FTU) phytase enzyme. The evaluated traits included weekly feed intake, weekly weight gain, feed conversion ratio, carcass characteristics, ash, calcium, and bone phosphorus. The use of phytase enzyme in different diets had no significant effect on food intake, weight gain, and feed conversion ratio ($P>0.05$). However, the use of phytase in different diets significantly affected the percentage of Gizzard, Heart, Liver, Proventriculus, and Spleen ($P<0.05$). The most changes were the increase in the ratio of feed intake and weight gain in the fourth week compared to the third week so that the changes in the ratio of feed intake ranged from 1.85 to 1.91, and this ratio for weight gain also ranged from 3.12 to 3.86 was recorded, and the lowest feed conversion ratio was obtained at the same age. The percentage of raw ash in broiler chickens was significantly increased by adding dietary phytase. The lowest amount of ash, calcium, and phosphorus belonged to the second group (diets with low phosphorus and no enzyme). The difference between the other groups and the control was not significant. Feed intake, weight gain, and feed conversion ratio with the addition of phytase enzyme were not affected by phosphorus reduction and had no significant effect on carcass characteristics. Environmental pollution can be prevented by reducing the level of dietary phosphorus and reducing excreted phosphorus.

Keywords: Phosphorus, Phytase Synthetic Enzyme, Performance, Broiler

1. Introduction

Phosphorus is an essential element in poultry nutrition. In monogastric animals such as poultry, due to the lack of phytase production in the early parts of the intestine, the digestion of phosphorus in plants, most of which is bound to phytates (1). Therefore, to provide the phosphorus needed by the bird, high-digestibility phosphorus sources such as dicalcium phosphate (DCP) are typically used in diets (2). Commercial DCP is the most important inorganic source to supply phosphorus in livestock and poultry feed. However, these phosphate supplements are expensive, and the global resources required to produce them are limited (3). In addition, excessive phosphorus intake in areas where livestock are raised densely poses environmental risks. Therefore, responsible management of phosphorus sources and their careful use in the diet of animals requires proper knowledge of the differences between foods in terms of their digestibility of phosphorus (4, 5).

Animal manures from broiler farms contain large amounts of phosphorus due to these birds' low ability to use phosphorus phytate (6). Side effects of phosphorus disposal in the poultry industry and its harmful effects on the environment (7) on the one hand and efforts to increase the economic efficiency of this element have led to extensive efforts to reduce phosphorus disposal to the environment (8). Feeding the world's growing population accelerates the depletion of phosphate reserves in nature. On the other hand, due to rising world prices for cereals and protein crops, planting these crops is increasing, which also increases the demand for phosphorus (9).

In recent years, nutritionists have made great efforts to increase the efficiency of phosphorus usage. These experts have proposed several strategies to reduce phosphorus in poultry waste. One of these suggestions is to prepare diets in which the amount of phosphorus is close to the need to keep the animal. Also, these strategies have been supplementing the diet with microbial phytase and vitamin D_3 metabolites or developing cultivated plants with low amounts of phosphorus phytate. Most of these solutions, although practical, increase production costs.

It has long been known that broilers get accustomed to diets deficient in specific nutrients. Studies show that animals deficient in these nutrients try to absorb them more efficiently, reducing gastrointestinal excretion (10-12). The human ability to adapt to low-calorie diets was also identified in the 1950s (13, 14). This theory was tested on dogs during extensive and lengthy experiments. Phosphorus and calcium are closely related. It turned out that dogs fed low-calcium diets could maintain calcium balance without showing signs of calcium deficiency and bone disorders (15). Subsequent research by Morrissey and Wassermanin 1971 also confirmed this in broilers. The researchers found that chickens fed little calcium and phosphorus in the diet had more radioisotope calcium than chickens fed deficient diets (16). They absorbed and released less radioisotope calcium from the bones at older ages.

Studies in the United States have shown that calcium intake is likely at least 20 percent lower than that recommended by the National Research Association in 1984 and 1994. The minerals required by broilers in these publications are calculated approximately based on the required amounts of a bird weighing 2 kg and 2 feed units per unit weight gain, which at the age of 9 weeks weighs about 2.925 kg, and consumption of the cumulative feed was 6.65 kg. However, in 2004, a broiler at the same weight was about 6 weeks old and had a cumulative feed intake of 5.899 kg. The present experiment aimed to investigate the effect of phytase in diets with low levels of available phosphorus on the performance of broilers and to find the minimum required amounts of these elements.

2. Materials and Methods

Six hundred one-day-old broilers, including both sexes, were procured from the commercial broiler breed Ross 308 from the commercial hatchery and transported to the test site. After determining the sex, 20 chickens were assigned to each experimental unit (six replicates per treatment). The mean weight of chickens at the time of arrival was determined. Experimental diets based on corn and soybean meal were adjusted with the same energy and protein. The five experimental diets for the starter and finisher periods were as follows (Table 1):

1- Basaldiet (corn grain and soybean meal)

2- Basaldietwith 15% less phosphorus

3- Basaldietwith 15% less phosphorus, containing phytase enzyme at the rate of 1250 units (FTU) per kilogram

4- Basaldietwith 15% less phosphorus, containing phytase enzyme at the rate of 2500 units (FTU) per kilogram

5- Basaldietwith 15% less phosphorus contains phytase enzyme at the rate of 5000 (FTU) units per kilogram

Table 1. Dietary compositions based on dry matter (DM)

Diet	Groups	Starter	Finisher
		%	
Corn grain		56.50	61.00
Soybean meal		38.00	30.00
Oil		3.00	4.40
Limestone	Control	1.00	1.86
	LP_1, LP_2, LP_3	1.32	2.60
DCP	Control	0.75	1.37
	LP_1, LP_2, LP_3	0.42	0.59
Premix		0.75	1.37
Phytase enzyme	Control	0	0
	LP_1	1250	1250
	LP_2	2500	2500
	LP_3	5000	5000
Chemical analysis (%)			
Metabolizable Energy (Kcal/Kg)		3100	3050
Crude protein		22.00	19.04
Ash		5.14	4.98
Calcium		1.00	0.94
Phosphorous	Control	0.40	0.50
	LP_1, LP_2, LP_3	0.34	0.36

LP_1: LP+1250, LP_2: LP+2500, LP_3: LP+5000; Vitamin A 12 IU, Vitamin D_3 3 IU, Vitamin E 50 IU, Vitamin K_3 2.5 Gram, Vitamin B_1 1 Gram, Vitamin B_2 7 Gram, Panototic Acid 14 Gram, Niacin 37 Gram, Vitamin B_6 3 Gram, Vitamin B_{12} 10Mg, Folic Acid 1 Gram, Biotin 150 Mg, Cholin Chloride 200, Gram, Cobalt 0.20 Gram, Copper 15 Gram, Iron 20 Gram, Manganese 80 Gram, Iodine 1.20 Gram, Selenium 0.20 Gram, Zinc 50 Gram, Limestone 1897.09 Gram, Anilox 125 Gram, Methionine 2000 Gram, Lysine 1500 Gram

2.1. Enzyme Preparation

The enzymes used in this experiment included fungal origin (*Aspergillus niger*). In this study, fungal phytase enzymes are based on various purification methods, including centrifugation, precipitation, ammonium sulphate, dialysis, ion-nickel exchange chromatography, Q Sepharose, polyacrylamide electrophoresis, and freezer using fungal sources in the laboratory-produced (17).

2.2. Diets Preparation

All diets were adjusted using software UFFDA based on the amounts of metabolizable energy and crude protein recommended in the Ross 308 strain breeding guide. The food composition and digestibility of amino acids contained in them were extracted from the tables of the National Research Association (NRC, 1994). The purpose of correcting diets and considering the nutrients equivalent to the enzyme is to calculate the nutrients released by the free enzyme and available to the bird. Various researchers have estimated equivalent amounts of the enzyme, and in many of these studies, the apparent ileal digestibility of various nutrients was measured by the addition of an indigestible marker of chromium oxide or solute in the diet.

Throughout the period, chickens had free access to water and food, and all diets were prepared in mesh form. The studied traits included body weight gain, feed intake, feed conversion ratio (in the starter and finisher stages), bone ash percentage, calcium, and phosphorus percentage.

At the end of the experimental period, two chickens from each experimental unit (pen) with a weighted average close to each unit's average were selected and transferred to the slaughterhouse. After slaughter, carcass, breast, thighs, gizzard, heart, liver, proventriculus, crop, spleen, small intestine, large intestine, and cecum weights were measured. The tibia of each killed bird was removed and frozen for later Ca and P analysis. Acid-insoluble soils were measured in experimental diets and digested samples according to Scott and Balnave (18) method and hydrochloric acid digestion technique. Calcium and phosphorus digestibility was calculated using the Dilger and Adeola (19).

Finally, the collected data were analyzed using the SAS program's general linear model. The statistical model used in this study compared the mean of different experimental groups using Duncan's multiple range test and considering the 5% probability level.

3. Results

The effect of different levels of phytase enzyme on weekly feed intake is shown in figure 1. Feed consumption of broilers increased by a slight difference until the fifth week. However, the trend of feed consumption from 5 weeks to the end of the rearing period (week 6) in the group receiving less phosphorus without enzyme remained constant. The use of phytase enzyme improved feed intake in broilers, but this trend was not significant (P>0.05).

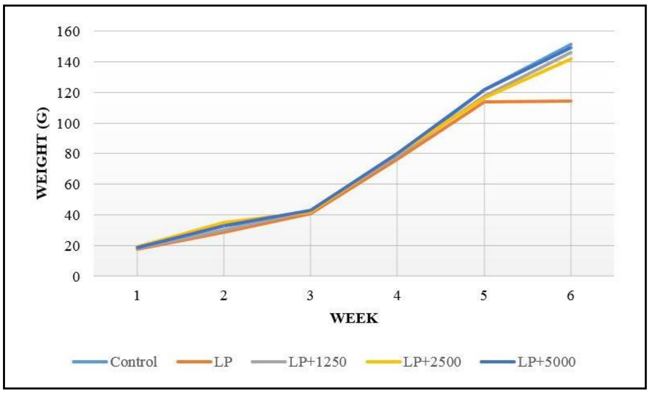

Figure 1. The effect of different levels of phytase enzyme on the average weekly food intake of broilers

The weekly weight gain of chickens receiving different treatments is shown in figure 2. The difference in weight gain of chickens up to 3 weeks of age was so slight that the weight gain of all groups showed overlapped. However, from 3 weeks of age until the end of the period, chickens receiving diets low in phosphorus and without enzymes revealed a more significant difference in weight gain, but this difference was not statistically significant.

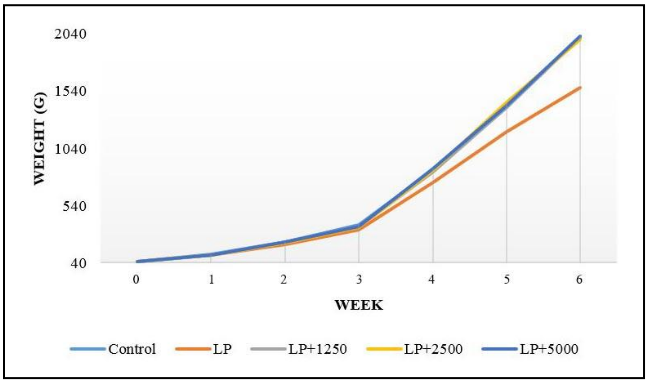

Figure 2. The effect of different levels of phytase enzyme on the average weekly weight gain of broilers

The trend of feed conversion ratio fluctuated in different groups (Figure 3). From the first week of the experiment to the third week, the range of differences in the feed conversion ratio changed from 1.91 to 2.26; this coefficient dropped significantly in the fourth week of the experiment and then resumed its routine.

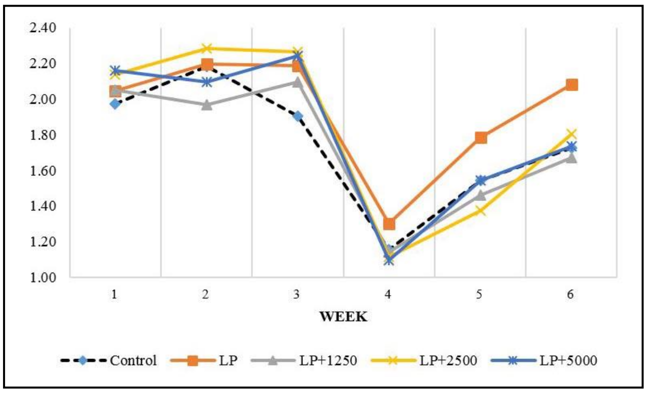

Figure 3 The effect of different levels of phytase enzyme on the weekly feed conversion ratio of broilers

The ratio of weight gain, feed intake, and feed conversion per week to the previous week is shown in table 2. With increasing the ratio of consumed feed each week compared to the previous week, the weight gain ratio also increased, and similarly, the ratio of feed conversion decreased. However, the most changes were the increase in the ratio of feed intake and weight gain in the fourth week compared to the third week; therefore, the changes in the ratio of feed intake ranged from 1.85 to 1.91, and this ratio for weight gain also ranged from 3.12 to 3.86 was recorded, and the lowest feed conversion ratio was obtained at the same age.

The results showed that the hearts of birds fed phosphorus-deficient diets had the highest weight percentage (*P*<0.05). The hearts of broilers fed phosphorus-deficient diets had the highest percentage but were similar to broilers fed phytase-fed broilers. The highest percentage of the liver was observed in chickens fed a phosphorus deficiency diet, which was higher than in birds that received the lowest level of phytase (*P*<0.05) but was similar in other treatments (Table 3). The highest percentage of gizzard belonged to the first group receiving the enzyme (1250 FTU), and the lowest percentage was determined in the two groups receiving the enzyme (2500 and 5000 FTU). The group receiving less phosphorus and no enzyme-free diets showed the lowest percentage of the proventriculus. The test results showed that the highest percentage of spleen belonged to the group receiving the lowest enzyme level (1250 FTU), and the lowest percentage belonged to the fourth group (5000 FTU). The use of different treatments in this experiment did not have a significant effect on other carcass characteristics of broilers.

The effect of phytase supplementation on low phosphorus diets on the percentage of minerals is shown in figure 4. The percentage of raw ash in broiler chickens was significantly increased by adding dietary phytase. The lowest amount of ash, calcium, and phosphorus belonged to the second group (diets with low phosphorus and no enzyme). The difference between the other groups and the control was not significant.

Table 2. The effect of different levels of phytase enzyme on relative changes in mean weight, feed intake and feed conversion ratio of broilers

Week	Body weight (g)					Feed intake (g)					Feed conversion				
	Control	LP	LP_1	LP_2	LP_3	Control	LP	LP_1	LP_2	LP_3	Control	LP	LP_1	LP_2	LP_3
2	1.56	1.54	1.73	1.73	1.83	1.73	1.65	1.67	1.85	1.78	1.10	1.07	0.96	1.07	0.97
3	1.42	1.43	1.30	1.21	1.22	1.24	1.43	1.39	1.20	1.30	0.87	1.00	1.07	0.99	1.07
4	3.16	3.12	3.41	3.86	3.78	1.91	1.86	1.86	1.90	1.85	0.61	0.60	0.54	0.49	0.49
5	1.15	1.09	1.17	1.18	1.08	1.55	1.49	1.50	1.46	1.52	1.34	1.37	1.28	1.24	1.41
6	1.11	0.86	1.09	0.93	1.09	1.24	1.01	1.24	1.22	1.22	1.12	1.17	1.14	1.31	1.12

LP_1: LP+1250, LP_2: LP+2500, LP_3: LP+5000

Table 3 Effect of different levels of phytase enzyme on different carcass characteristics of broilers

Characteristics	Control	LP	LP_1	LP_2	LP_3
Live weight (g)	2038.50	1785.60	2035.00	2160.00	2096.85
Carcass weight (g)	1485.55	1287.43	1450.38	1530.58	1531.20
Gizzard (%)	2.65^{ab}	2.75^{ab}	2.95^{a}	2.38^{b}	2.50^{b}
Heart (%)	0.63^{ab}	0.72^{a}	0.56^{b}	0.65^{ab}	0.53^{b}
Liver (%)	2.20^{ab}	2.45^{a}	2.10^{b}	2.40^{ab}	2.18^{ab}
Proventriculus (%)	0.77^{a}	0.51^{b}	0.67^{ab}	0.65^{ab}	0.53^{ab}
Crop (%)	0.69	0.63	0.81	0.96	0.74
Spleen (%)	0.13^{ab}	0.11^{ab}	0.15^{a}	0.11^{ab}	0.08^{b}
Small intestine (%)	4.15	4.22	4.08	4.63	4.10
Large intestine (%)	0.43	0.52	0.48	0.43	0.45
Cecum (%)	0.88	0.84	0.93	0.86	0.87
Thighs (%)	20.43	21.34	20.88	20.65	20.38
Breast (%)	25.67	24.75	24.56	24.43	26.55

LP_1: LP+1250, LP_2: LP+2500, LP_3: LP+5000
a-b: dissimilar letters in each column indicate a significant difference ($P<0.05$)

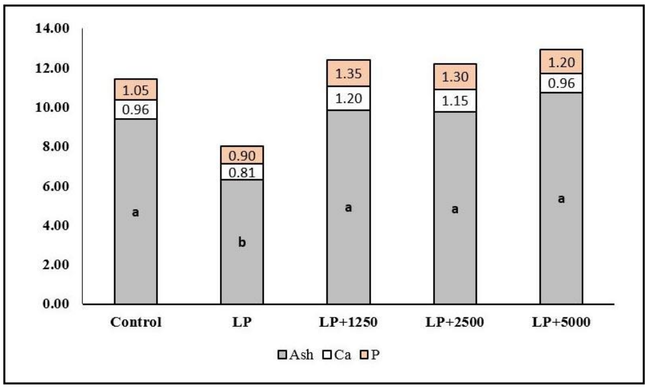

Figure 4 Effect of different levels of phytase on ash, calcium and phosphorus of broiler bones
a-b: dissimilar letters in each column indicate a significant difference (*P*<0.05)

4. Discussion

Regarding the effect of the enzyme on the average daily feed intake in different periods of this experiment, no significant differences were observed between enzyme consuming and control groups. Warden and Schaible (20) reported no significant difference in the amount of feed consumed by adding phytase alone or with citric acid compared to the control group. Hassanabadi, Moghadam (21) reported that the addition of phytase enzyme at different levels to the diet did not have a significant effect on increasing feed intake. While Juanpere, Pérez-Vendrell (22) reported that phytase improves food intake by increasing starch digestibility and protein productivity, Mousavi, Niknafs (23) also stated that phytase significantly increases food intake and body weight in the whole course.

There was no significant difference between the body weight of the enzyme-consuming and control groups. Hassanabadi, Moghadam (24) reported that the addition of phytase enzyme at any of the levels used did not significantly affect body weight and daily weight gain. Santos, Hruby (25) reported that adding phytase increased body weight and daily weight gain by increasing the utilization of phosphorus, protein, and amino acids and increasing the digestibility of starch. The researchers found that the enzyme phytase significantly increased the energy released from the diet by significantly increasing the digestibility of nutrients by releasing nutrients bound to phytic acid.

As can be seen, no significant difference was observed between the feed conversion ratio of the enzyme group and the control group. Tang, Gao (26) reported no significant difference between the feed conversion ratio in the microbial phytase consuming group and the positive control group. In a study, Shaw, Blake (27) investigated the effect of increasing the two types of phytase enzymes with two levels of 500 and 750 on performance-related traits. In a study by Żyła, Koreleski (28), the addition of phytase significantly improved the feed conversion ratio but did not significantly reduce weight gain. Ceylan, Cangiri (29) reported that adding phytase significantly increased growth performance during the starter and growth period.

The results of broiler carcass characteristics are consistent with previous findings by Angel, Saylor (30). However, it contradicts the results of Pillai, O'Connor-Dennie (31), who showed that phytase supplementation significantly improved carcass characteristics compared to phosphorus-deficient diets.

Augspurger, Webel (32) investigated the effect of using different levels of commercial enzymes Natuphos and Ronozyme and phytase extracted from the bacterium on the percentage of tibial bone ash. They showed that the highest percentage of ash was related to the addition of Natuphos and Ronozyme. The researchers also reported that the combined effect of enzymes could be effective due to the possibility of starting to separate phosphorus from different sites in the phytic acid molecule. Shirley and Edwards reported that as the amount of phytase in the diet increased, the weight of tibial ash increased (33). During an experiment conducted by these researchers, the highest weight of tibial ash was reported due to adding 1200 units of phytase enzyme. Santos, Hruby (25) showed that adding levels of 750, 500, and 1000 units of Phyzyme to diets increased the percentage of tibial ash compared to the control group, while there was no statistically significant difference between the levels of the tibia.

5. Conclusion

According to the results of this study, the enzyme phytase used in the starter and finisher diets of broilers increased the bioavailability of phosphorus and improved the growth performance of broilers. The dietary phosphorus levels can be reduced by 0.44 and 0.56%, respectively, without affecting growth performance. Feed intake, weight gain, and feed conversion ratio with the addition of phytase enzyme were not affected by phosphorus reduction and had no significant effect on carcass characteristics. Environmental pollution can be prevented by reducing the level of dietary phosphorus and reducing excreted phosphorus.

Authors' Contribution

Study concept and design: H. H. and M. M. S.
Acquisition of data: Y. F. M. and J. B.
Analysis and interpretation of data: F. M.
Drafting of the manuscript: T. A. H.
Critical revision of the manuscript for important intellectual content: I. I., M. M. K. and K. A. Z.
Statistical analysis: J. B.
Administrative, technical, and material support: H. H. and M. M. S.

Ethics

The study design was approved by the ethics committee of the Universitas Islam Negeri Alauddin Makassar, Sultan Alauddin Street, Gowa 92118, Indonesia.

Conflict of Interest

The authors declare that they have no conflict of interest.

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