Evaluation of Phytase Supplementation on Performance, Metabolic Energy, Ileal Histomorphology, Meat and Bone Mineralization of Broiler Chickens

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Abstract

Phytase as a feed additive has been shown to promote broiler performance and bone mineralization. This study evaluated the effects of microbial phytase (produced by *L. plantarum* A1-E and *C. tropicalis* TKd-3) on growth performance, metabolic energy, ileal histomorphology, and meat and bone mineralization in broilers. One hundred forty one-day-old broiler chicks were distributed into four treatments, five replicates, and seven chicks in each replicate. The experiment consisted of (FA) basal diet without phytase (negative control); (FB) basal diet with *L. plantarum* A1-E phytase (500 FTU/kg of feed); (FC) basal diet with *C. tropicalis* TKd-3 phytase (500 FTU/kg of feed); and (FD) basal diet with commercial phytase (500 FTU/kg of feed, positive control). Results revealed that FB and FC treatments improved the broiler performance index ($P < 0.05$), and FC treatment tended to increase metabolic energy ($P > 0.05$). Ileal histomorphology showed that FB treatment increased villus height, villi height to crypt depth ratio, and villus surface area ($P < 0.05$). Breast meat mineral content of broiler revealed the highest mineral content in FB treatment, mainly of the minerals P, Mg, and Fe ($P < 0.05$). Moreover, the FC treatment resulted in the highest mineral content in thigh meat, particularly for the minerals Ca, P, Mg, Zn, and Fe ($P < 0.05$). Tibia bone mineralization showed that FB treatment had the most significant effect ($P < 0.05$) on the mineral content of P, Mg, and Fe. Conclusively, microbial phytase (*L. plantarum* A1-E and *C. tropicalis* TKd-3) increased performance index, whereas *L. plantarum* A1-E phytase improved villus surface area, increasing the ileum mineral absorption, breast meat mineral content, and tibia mineral deposits.

Introduction

The availability of phosphorus (P) obtained from grain-based feedstuff has not been optimally utilized by poultry. This situation causes a P-available deficiency to support the poultry metabolic process (Krieg et al., 2021). Phosphorous that is contained in grain-based feedstuff is primarily bound in phytic acid [myo-inositol 1,2,3,4,5, 6- hexakis (dihydrogen phosphate); InsP6] and is present as phytate, which requires the enzyme hydrolysis to make P available to animals (Sommerfeld et al., 2018).

Phytase is an enzyme that can hydrolyze phytate in grain-based feedstuff (Babatunde et al., 2020a). Exogenous phytase supplementation has been used for many years to reduce the addition of inorganic phosphorus (P) and calcium (Ca) in poultry feed, improved the feed conversion ratio (FCR), and increase amino acid digestibility (Borda-Molina et al., 2019; Zanu et al., 2020), releasing P and another mineral (e.g., Ca, Zn, Fe, Cu), as well as reducing environmental P pollution (Beeson et al., 2017). Phosphorus and Ca are the skeletal system's main components and are primarily stored in the bone as hydroxyapatite. Phytase supplementation can hydrolyze the phytate complex and release the P and Ca bound-up to use broilers. Therefore, phytase supplementation enhanced the birds weight and tibia ash percentage at days 21 and 42 post-hatching (Babatunde et al., 2020a). EL Enshasy et al. (2018) stated the primary role of phytase is to increase phytate phosphorus utilization and increase the use of protein or amino acids, energy, calcium phosphorus, and some trace minerals. The most commonly used phytase dose as a feed additive for broilers is 500 FTU / kg of feed (Beeson et al., 2017).

Phytase enzyme was found in many plants, microorganisms, and animals (Humer et al., 2015). Previous research resulted in *Lactobacillus plantarum* A1-E from the small intestine of Kampong chicken and *Candida tropicalis* TKd-3 isolated from tempeh produced extracellular phytase *in vitro* (Anggraeni et al., 2020; Istiqomah, 2015; Suryani et al., 2021). In broiler chickens, heat stress can reduce intestinal performance and immune response during the maintenance period. Therefore maintaining intestinal microstructure balance is an important thing to attend (Elbaz et al., 2021). The ileum is the part of the small intestine with the primer function to absorb water and minerals from feed ingredients that enter the digestive tract (Svihus, 2014). Haile et al. (2020) stated that the ileum is an intestinal segment that important for measuring the apparent mineral digestibility in poultry. Histomorphology of the ileum is a structure that can provide information about the mineral absorption activity in poultry. The enhancement of the villus-crypt structure improves the function of the digestive tract, especially the intestinal segment, and improves feed utilization (Elbaz et al., 2021).

Various phytases from different sources, concentrations, and application methods influence growth performance, nutrient digestibility, and bone mineralization of broiler (Handa et al., 2020). The benefit of microbial phytase in poultry nutrition has been extensively reviewed. However, the information impact of different sources of microbial phytase on broiler is still limited. Therefore, this research was conducted to evaluate microbial phytase administration from various sources on growth performance, metabolic energy, ileal histomorphology, meat and bone mineralization of broiler chickens**.**

Materials And Methods

The experiment was carried out at the Poultry Closed House, Laboratory of Bio-Feed Additive Technology, Research Center for Food Technology and Processing, National Research and Innovation Agency (BRIN). All the experimental techniques and animal trials were executed with the approval of the Commission of Ethical Clearance for Pre-clinical experiment (No. 00004/04/LPPT/III/2019) from the Integrated Laboratory of Research and Testing (LPPT), Gadjah Mada University, Yogyakarta. Indonesia.

Phytase enzyme production and measurement of phytase activity

Production of microbial phytase from *L. plantarum* A1-E and *C. tropicalis* TKd-3 was prepared by solid-state fermentation based on a modified method (Mandviwala & Khire, 2000). Phytase enzyme activity was measured based on the Vohra & Satyanarayana (2001) method.

Bird, diet, and sample collection

A total of one hundred forty, unsexed, day-old Cobb broiler chicks (DOC), vaccinated against Newcastle Disease (ND), Infectious bronchitis-Newcastle Disease (IB-ND), and infectious bursal disease (IBD) were obtained from commercial hatchery used as animal models. These DOC with a mean bodyweight of 44.65 g ± 2.04 were randomly allocated into four treatments, each consisting of five replicates with seven chicks in each replicate. The research design that was used was a Completely Randomized Design. The experiment consisted of 4 treatments as follow; FA treatment: basal diet without phytase administration (negative control); FB treatment: basal diet with *L. plantarum* A1-E phytase administration (500 FTU/kg of feed); FC: basal diet with *C. tropicalis* TKd-3 phytase administration (500 FTU/kg of feed); FD: basal diet with commercial phytase administration (500 FTU/kg of feed) (positive control) Diet and drinking water were provided *ad libitum* during the experimental period. The starter (0–14 days) and finisher (15– 28 days) basal diet was formulated based on the nutrient requirement of broiler chicken according to the Indonesian National Standard (Badan Standard Nasional, 2006), which was presented in Table 1. Chickens were housed in a closed system cage (closed cage) with a size of 10 x 10 meters, equipped with several standard supporting tools such as a cooling system, fan, Thermo hygrometer / temptron, 60-W tungsten lamp, and heated gas. During the first week, a constant temperature of 32ºC was maintained, then gradually dropped to a constant temperature of 26ºC. Cages were fumigated and disinfected before DOC arrival.

Feed intake (FI) and body weight (BW) were recorded weekly. Mortality was recorded daily, and the mortality percentage was calculated. Feed conversion ratio (FCR) was calculated as the total FI divided by the final BW of the live chicken. Growth performance parameters measured consisted of FI, BWG, and FCR, as well as the performance index (PI) of broilers, which were evaluated at the age of 28 days. The performance index was calculated to refer to Sofyan et al. (2012) using the following equitation.

$$PI = \frac{[BWG \times (100 - \%\, mortality)]}{[FCR \times 100 \times period]}$$

Measurements of nutrient digestibility refer to the modified methods according to Farrell et al. (1982) when the chicken entered the finisher's phase (28 days of age). Five chickens from each experimental unit were randomly placed in a metabolic cage to assess nutrient digestibility. Plastic pads were provided in the metabolic cage to manage excreta. Before treatment, chickens were adapted for three days without phytase administration and fed to spend ± 30 g/head for 24 hours, then fastened from feed for 24 hours, while water was provided *ad libitum*. Each type of microbial phytase was given according to the previous treatment. Chicken excreta was accommodated to ensure no feed in the digestive tract and collected for 36 hours since the phytase treatment was stopped. After collecting the excreta, it was immediately sprayed with H_2SO_4 0.01 N to bind the nitrogen and prevent it from evaporating. Excreta samples were dried at 50 °C ovens for 48 hours before being pulverized and analyzed for moisture content, crude protein, and gross energy based on the Al-mentafji (2006) method. The value of energy retention includes energy consumption and energy excretion. In contrast, nitrogen retention contains nitrogen consumption, excretion nitrogen was measured based on (Sibbald & Wolynetz, 1986) calculations methods and measurement of nitrogen retention according to Albuquerque et al. (2003).

At the end of the experimental period (at 28 days old), three birds were randomly selected from each experimental unit and euthanized with the decapitation method. Mineral content analysis of the tibia was conducted by removing the left tibiae and defleshed, os patella was removed, and then the tibiae were weighed and frozen (−20°C) until further analysis (Hafeez et al., 2014). After skined, evisceration, and splitting in respect of hygienic rules, representative samples (≈ 100 g) of breast and thigh meat were collected and then put in identified plastic bags and frozen at - 20°C until mineral content analysis of both muscles (Benamirouche et al., 2020). Concentrations of Ca, P, K, Mg, Zn, and Fe were determined using an Agilent 240FS AA Fast Sequential atomic absorption spectrometer (F-AAS) (Agilent Technologies, Waldbronn, Germany) (Benamirouche et al., 2020). Histomorphology analysis was performed by collecting ileal samples of approximately 4 cm and fixed in a 10% neutral buffered formalin solution for 24 h. The trimmed cross-sections of the ileal samples were placed in cassettes and processed on a tissue processor overnight. The tissues were then embedded with paraffin and finally the samples were cut to a thickness of 5 microns using a rotary microtome (Yamato RV-240) and placed on slides (Suryani et al., 2019). Examination of routine histological was performed by staining the tissue sections with hematoxylin-eosin and observed by optical microscope (Suvana et al., 2019). Villus height (VH) and crypt depth (CD) were measured as described by Abdel-Moneim et al. (2020).

Statistical analysis

To determine the effect of diets on growth performance, metabolic energy, ileal histomorphology (villous height, villus width, and crypt depth), meat and tibia mineral concentration, statistical calculations were carried out using a one-way model analysis of variance (One Way ANOVA) followed by an analysis of differences in measurement responses using the Hotelling-Lawley test. Duncan's Multiple Range Test analysis is carried out to determine the significant differences among treatments. The data input and analysis process uses R.64. 4.0.4. program (R Core Team, 2019).

Results

Growth performance

The experiment treatment had a significant effect on the body weight gain (BWG), FCR, and performance index (PI) of broiler chickens (Table 2). Broilers added with *C. tropicalis* TKd-3 phytase in diets during the treatment period (from 0 to 28 days of age) showed higher BWG than control ($P < 0.05$), but not significantly different from other treatments. In addition, broilers were given *C. tropicalis* TKd-3 phytase also had lower FCR values than control ($P < 0.05$), but did not significantly different from other treatments. These results indicated a linear relationship between BWG, FCR, and PI value, so the value of broiler chickens fed with *L. plantarum* A1-E and *C. tropicalis* TKd-3 phytase was higher than the negative control ($P<0.05$) but not significantly different from other treatments. The feed consumption (FI) results between control and other treatments were not significantly different, as shown in Table 2.

Metabolic energy

There was no significant difference ($P>0.05$) in phytase administration in broilers during the experimental period (from 0 to 28 days of age) on the metabolic energy value of broilers which included AME, TME, and TMEn (Tabel 2). Although there was no significant difference in the value of energy metabolism between treatments, there was a linear result between the values of AME, TME, and TMEn which tended to be higher in *C. trophicalis* TKd-3 phytase administration (FC treatment).

Ileal histomorphology

Ileal histomorphology measurement of broiler with phytase administration during the experimental period (from 0 to 28 days of age) was presented in Tabel 2. Administration of *L. plantarum* A1-E phytase into broiler chicken diets during the experimental period significantly resulted in the highest villous height (VH) ($P<0.05$) compared to the control and FC treatment, in addition, it also resulted in a higher ratio of villi height to crypt depth (VH/CD) ($P<0.05$). As a form of a linear association between the value of villus height and the ratio of villus height to crypt depth, broiler chickens diets with *L. plantarum* A1-E phytase had the greatest villi area ($P< 0.05$).

Meat mineral content

The mineral content in the breast and thigh meat of broiler chickens administrated phytase supplementation during the treatment period (from 0 to 28 days of age) is shown in Table 2. Table 2 showed a significant effect of *L. plantarum* A1-E phytase administration (FB treatment) on the mineral content of P, Mg, and Fe in breast meat compared to the control group. However, the significant difference between the FB treatment with the other phytase treatments was only shown in the mineral content of P and Fe.

In contrast to the mineral content of the breast meat, the mineral content of the thigh meat showed that the administration of *C. tropicalis* Tkd-3 phytase (FC treatment) produced the highest mineral content of Ca, Zn, and Fe compared to the control and FB treatment. While for phosphorus minerals, although the highest content was in FC treatment, it was not significantly different from the negative control group and only considerably different when compared to the FD treatment (Table 2).

Tibia mineral content

The effects of the four experimental diets with phytase administration on broiler chickens during the experimental period are summarized in Table 2. The results indicated that the treatment with the administration of *L. plantarum* A1-E phytase had a significant effect ($P<0.05$) on the mineral content of P, Mg, and Fe. Likewise, the mineral content of Ca and Zn also tended to be higher in broiler chickens treated with *L. plantarum* A1-E phytase. However, there was no significant difference between other treatments ($P>0.05$).

Discussion

In evaluating feed additive supplementation of poultry, growth performance and energy metabolism were essential parameters that were determined. The phytase enzyme is one of the most extensively utilized feed additives in the commercial poultry business. The enzyme phytase was responsible for hydrolyzing the phytate found in poultry feed components. Phytase's ability to improve growth performance and nutrient digestibility in poultry was dependent on the feed composition, mineral content, endogenous microbiota that affects pH range in the gastrointestinal tract, source of phytase, species of birds, and age of the birds (El-Hack et al., 2018; EL Enshasy et al., 2018).

According to numerous publications, the use of phytase improves growth performance, particularly BWG and FCR (Amiri et al., 2021; Babatunde et al., 2020b; Beeson et al., 2017; Y. Dersjant-Li et al., 2018; Khan et al., 2019; Pieniazek et al., 2017). According to the growth performance data, this study confirmed Amiri *et al.* (2021), who stated that adding phytase (500 FTU/Kg) to experimental diets during the trial period increased broiler body weight gain and improved feed efficiency compared to the control group. Following that, Khan et al. (2019) found that administration of broilers basal diets with phytase (500, 1000, and 1500 FTU/kg) for three weeks (1-21 days old) improved live body weight and feed efficiency (1.4) compared with control treatment without phytase addition.

Phytase activity was pH-dependent, all phytases have a pH optimum that is microbial source dependant, and it affects the capacity of phytase to perform within the GI tracts of animals (El-Hack et al., 2018). Some yeast strains have high viability (more than 50%) at pH 2-3 in vitro assays (Chen et al., 2010; Lohith & Appaiah, 2014), high microbial phytase viability under acidic conditions allows for maximum phytase hydrolysis activity (Yueming Dersjant-Li et al., 2015). Sommerfeld et al. (2018) report that the presence of phytase in the proventriculus (pH 2,5-3,5) and distal ileum (pH 6,5-7,5) improves phytate hydrolysis and the appearance of myo-inositol (Svihus, 2014). The presence of phytase in digesta of the proventriculus and ventriculus suggests that phytase has begun hydrolyzing phytate and releasing nutrients for use by poultry. Therefore, phytase administration may promote weight gain and feed efficiency (Babatunde et al., 2020a). In line with our study, *C. tropicalis* TKd-3 phytase allowed to improve phytate hydrolysis in the proximal segment of the gastrointestinal tract, particularly in the proventriculus segment, to promote weight gain and feed efficiency performance index.

Metabolic energy was affected by the amount of energy-protein intake and excreted, and also the ability of animals to feed metabolize in the body (Sibbald & Wolynetz, 1986). These results show a positive correlation with the results of the growth performance parameters. The better growth performance associated with the addition of phytase may be related to the increased energy released from the diet due to nutrient digestibility improvement via the release of nutrients bound to phytic acid and increased phosphorus utilization efficiency (El-Hack et al., 2018). He et al. (2017) report that phytase administration in the diet increased daily body weight gain (BWG), serum Ca levels and P tibia level, and apparent digestion of energy in the starter period of broiler. Babatunde et al., (2020a), in their research result, stated that broiler chickens with microbial phytase administration were able to increase their productivity in terms of growth performance, energy and nutrient utilization, and bone mineralization. Santos et al. (2008) found that adding a phytase enhanced ME value by 65–195 kcal/kg in 21-day broilers fed meals containing 500–1000 FTU/kg, and only by about 195 kcal/kg in 22–42-day broilers fed diets containing 750 and 1000 FTU/kg phytase.

The energy improvements mechanism associated with phytase addition may result from the enhancement in protein absorption or increased carbohydrate and fat digestibility due to the dissolution of the phytate complexes (Humer et al., 2015). The ability to counteract endogenous losses by phytase administration may also enhance metabolizable energy by lowering the energy required for maintenance, thereby allowing significantly more energy for growth (D. Wu et al., 2015).

In broiler chickens, adding phytase enzymes to the diet improves the VH and the ratio of VH to CD (Amiri et al., 2021). The capacity for nutritional absorption is highly influenced by the morphology of the small intestine tissue structures, one of which is the villus height structure. The higher the villi structure of the intestine, it is mean the more comprehensive the area of absorption of nutrients (Adil et al., 2010; Brudnicki et al., 2017). Numerous studies have demonstrated that phytase addition supplementation in diet improved the intestinal morphometric indices of broiler chickens (Y. B. Wu et al., 2004; Zaefarian et al., 2013). This experiment confirmed the resulting study of Sajadi Hezaveh et al. (2020), who demonstrated a rise in VH in the small intestine of broiler chickens following feed treatment with phytase.

Karami et al. (2020) also demonstrated consistent results with those obtained in the study. They stated that phytase addition into the diet with a dose of 500 FTU/kg significantly raised VH ($P<0.01$) of broiler chickens at 28 days of age and significantly increased the value of VH to CD ratio ($P<0.01$). According to Mohammadagheri et al. (2016), phytase raised VH, lowered CD, and significantly increased VH/CD ratio in broilers. A high VH/CD ratio indicates that broiler chickens have optimally mature enterocytes at the tips of the villi, resulting in increased surface area and absorption. Shallower crypts and longer villi are related to an increased enterocyte lifespan, decreased cell turnover, faster repair of damaged enterocytes, and enhanced intestinal function (Awad et al., 2009). Increased surface area and absorption in the villi of the ileum indicate that the primary function of the ileum in mineral absorption is functioning correctly (Svihus, 2014). According to the present study, addition in broiler chicken diets with *L. plantarum* A1-E phytase increased the ileal villi surface area, which means that mineral and other nutrient absorption occurred optimally.

This investigation revealed that the highest mineral content in breast meat was detected in samples of meat from FB treated with *L. plantarum* A1-E phytase. Particularly in kinds of the mineral P, Mg, and Fe. Moreover, the treatment with *C. tropicalis* Tkd-3 phytase resulted in the highest mineral content in thigh meat, particularly for the minerals Ca, P, Mg, Zn, and Fe. The results showed that the mineral content of the breast meat positively related to the results obtained on the performance of the ileal villi. The high surface area in the FB treatment allowed the optimum mineral absorption, so that the mineral content in the breast meat was higher than the control group. Numerous studies have demonstrated that phytase administration can increase myo-inositol concentrations in the gut and blood (Babatunde et al., 2019; Schmeisser et al., 2017; Sommerfeld et al., 2018; Walk et al., 2019), thereby increasing this molecule availability for peripheral tissue metabolism. Due to the presence of myo-inositol in the blood, the chelate-mineral release effect is enhanced, resulting in the greater availability of minerals that can be absorbed.

According to Ali et al. (2019), the mineral composition of meat was affected by various factors, including species, breeding conditions, age, and nutrient supplementation. Geldenhuys et al. (2015) also stated that the main determinants that affect the mineral content of meat are genetic, gender, environmental, and nutritional factors. In this study, the treatment of different types of phytase gave different responses to the affected broiler meat. An important result in this study was the highest concentration of Fe minerals in the breast meat of the broiler group that received *L. plantarum* A1-E phytase and thigh meat from the broiler group that received *C. tropicalis* TKd-3 phytase. These two mineral elements play an essential role in the body of broiler chickens. In this role, Fe contributes to hemoglobin synthesis, oxidation-reduction processes, Fe collaborates with zinc in bone formation, nucleic acid metabolism and protein synthesis, and eggshell formation (Wang et al., 2015).

The result of the tibia mineral content parameter indicated a linear relationship with the highest surface area of an ileal villus in broiler from FB treatment that gave L. *plantarum* A1-E phytase. In this study, phytase administration at a dose 500 FTU/kg significantly increased tibia mineral content, particularly for minerals P, Mg, and Fe. Bone development depends on the function of the digestive tract, the availability of digestive enzymes, and the number of nutrients absorbed (Palander et al., 2006). In addition, heat stress and hydration (electrolyte balance) also affect health performance and bone structure in poultry. According to Cruvinel et al. (2021), heat stress and electrolyte imbalance affect the bone mineral density of birds (*Japanese quail*). Antinutritive factors such as phytates found in untreated raw seeds decrease nutrient digestibility and reduce calcium, phosphorus, or iron absorption (Hassan et al., 2003; Muszyński et al., 2018).

Phosphorus is an essential component in broiler diets for proper growth and development. It is the second most of the body prevalent mineral, and a lack of it can lead to rickets, growth retardation, and other skeletal abnormalities. In addition to increasing feed costs, excessive P administration in feed was not used by animals and will be released so that it can damage the environment (Gautier et al., 2017). Rao et al. (2013) stated that the group fed an organic trace mineral supplement diet had higher concentrations of Ca, P, and other trace minerals in their tibia than the group fed an inorganic trace mineral supplement diet. In addition, they also stated that phytase supplementation at the level of 500 FTU/kg in broiler chicken feed would improve the performance of the ileal villi and increase the number of organic trace minerals that could be absorbed.

The results of this study accordance with research from Broch et al.(2021) which states that broilers fed phytase has higher tibia Ca and P content than controls. In line with that, Nourmohammadi et al. (2012) found that there was a 6 percent increase in Mg digestibility in broiler chickens fed phytase supplementation in the diet. The increase in tibia mineral content in the treatment group with phytase supplementation could be caused by the increased availability of Ca, P, Fe, and other minerals released from the complex phytate minerals (Gautier et al., 2018).

Based on the result, it can be concluded that administration of microbial phytase both *L. plantarum* A1-E and *C. tropicalis* TKd-3 as a feed additive was able to improve body weight gain, performance index, and tend to increase metabolic energy of broilers. In addition, administration of *L. plantarum* A1-E as a feed additive improved the morphology of the ileal villus that caused the enhancement of mineral absorption of the ileum, followed by enhancement of mineral deposits tibia.

Declarations

Funding:

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Conflict of interest:

The authors certify that we have no financial, personal, or other connections with other people or organizations relevant to the subject discussed in the manuscript.

Availability of data and material:

The collected data of this study were deposited at the Indonesian Scientific Repository (RIN) https://data.lipi.go.id/dataset.xhtml?persistentId=hdl%3A20.500.12690%2FRIN%2FS0R178&version=DRAFT). The data might be shared upon reasonable request to the corresponding author.

Ethical approval:

All the experimental techniques and animal trials were executed with the approval of the Commission of Ethical Clearance for Pre-clinical experiment (No. 00004/04/LPPT/III/2019) from the Integrated Laboratory of Research and Testing (LPPT), Gadjah Mada University, Yogyakarta. Indonesia.

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STATEMENT OF ANIMAL RIGHTS:

All the experimental techniques and animal trials were executed with the approval of the Commission of Ethical Clearance for Pre-clinical experiment (No. 00004/04/LPPT/III/2019) from the Integrated Laboratory of Research and Testing (LPPT), Gadjah Mada University, Yogyakarta. Indonesia.

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Tables

Table 1. Ingredient and composition of the basal diet of broiler starter period (0-14 day old) and finisher period (15-28 day old)

Ingredients	Composition (%)	
	Starter diet	Finisher diet
Corn	60.50	62.30
Rice bran	0.00	2.30
Soybean meal	30.00	26.00
Meat bone meal	1.70	2.20
Palm oil	2.30	2.90
Premix	0.50	0.50
Di-Calcium Phosphate (DCP)	0.90	0.50
Salt (NaCl)	0.10	0.10
Limestone	1.30	1.40
L-Lysin	1.70	1.20
DL-Methionine	1.00	0.60
Total	100.00	100.00
Nutrient content (%)		
Moisture	8.83	8.98
Crude protein	22.95	21.89
Crude fat	4.23	3.75
Crude fiber	2.75	5.98
Ash	4.92	5.21
Calcium	2.24	2.63
Total fosfor (P)	0.74	0.80
Gross energy (kal/g)	4197	4134

Table 2. The effects of experimental diets with phytase administration from different sources on growth performance, metabolic energy, ileal histomorphology, meat and bone mineralization of broiler chickens from 1-28 days of age

Parameter	Experimental diets[a]				
	FA	FB	FC	FD	P[c]
Growth performance[b]					
BWG (g/bird)	1009.37[B] ± 67.71	1076.78[AB] ± 48.17	1116.76[A] ± 39.17	1044.86[AB] ± 41.19	*
FI(g/bird)	1798.69 ± 70.48	1754.62 ± 46.97	1748.82 ± 94.74	1726.01 ± 76.26	ns
FCR	1.79[A] ± 0.06	1.63[B] ± 0.07	1.57[B] ± 0.09	1.65[B] ± 0.07	**
PI	231.24[C]± 21.94	267.49[AB]± 20.36	289.11[A] ± 23.41	257.37[BC]± 16.85	**
Metabolic Energy[b]					
AME (cal/g)	3504.04 ± 168.88	3617.35 ± 202.60	3660.86 ± 263.02	3624.15 ± 55.15	ns
TME (cal/g)	3576.11 ± 177.08	3689.42 ± 197.07	3730.69 ± 264.08	3690.49 ± 63.57	ns
TMEn (cal/g)	3582.44 ± 178.48	3695.84 ± 197.13	3736.64 ± 264.95	3696.02 ± 64.54	ns
Ileal histomorphology[b]					
VH (μm)	761.714[B] ±99.98	875.814[A] ±170.83	741.573[B] ±138.25	614.816[C] ±52.51	**
CD (μm)	148.930[A] ±27.55	127.005[B] ±19.79	127.079[B] ±12.84	127.619[B] ±22.84	*
VH/CD	5.290[AB] ±1.15	7.077[A] ±70.62	5.877[AB] ±1.29	4.975[C] ±0.97	**
VW (μm)	126.829[C] ±20.86	143.873[B] ±17.42	125.335[C] ±18.55	178.575[A] ±22.05	**
Area of villus (μm^2)	9.717[B] ±2.22	12.649[A] ±3.29	9.392[B] ±2.27	10.985[AB] ±1.64	**
Breast meat minerals					
Ca (mg/kg)	519 ± 50	607 ± 86	531 ± 48	569 ± 97	ns
P (mg/kg)	2461[C] ± 270	3911[A] ± 185	3294[B] ± 229	3632[AB] ± 658	**
K (mg/kg)	10727 ± 855	11485 ± 1311	11249 ± 691	10894 ± 448	ns

Mg (mg/kg)	$656^{B} \pm 96$	$1202^{A} \pm 101$	$1099^{A} \pm 186$	$1176^{A} \pm 118$	**
Zn (mg/kg)	32 ± 3	34 ± 3	33 ± 1	33 ± 3	ns
Fe (mg/kg)	$27^{C} \pm 5$	$98^{A} \pm 15$	$70^{B} \pm 7$	$19^{C} \pm 3$	**
Thigh meat minerals					
Ca (mg/kg)	$797^{C} \pm 138$	$1045^{B} \pm 156$	$2049^{A} \pm 216$	$769^{C} \pm 80$	**
P (mg/kg)	$4674^{A} \pm 123$	$4545^{A} \pm 198$	$4731^{A} \pm 282$	$2562^{B} \pm 418$	**
K (mg/kg)	9673 ±540	9465 ±952	10930 ±1612	9128 ±1095	ns
Mg (mg/kg)	$690^{B} \pm 80$	$747^{AB} \pm 66$	$904^{A} \pm 142$	$841^{AB} \pm 139$	*
Zn (mg/kg)	$60^{B} \pm 2$	$62^{B} \pm 2$	$71^{A} \pm 9$	$61^{B} \pm 7$	*
Fe (mg/kg)	$20^{C} \pm 3$	$32^{B} \pm 5$	$44^{A} \pm 6$	$19^{C} \pm 3$	**
Tibia minerals					
Ca (mg/kg)	69.606 ±10.443	76.668 ±9.625	65.077 ±6.676	69.446±12.510	ns
P (mg/kg)	$3.794^{B} \pm 154$	$4.090^{A} \pm 236$	$3.976^{AB} \pm 286$	$3.796^{B} \pm 255$	*
K (mg/kg)	3.561±351	3.696 ±242	3.699 ±260	3.661 ±580	ns
Mg (mg/kg)	$1.895^{B} \pm 0.351$	$2.252^{A} \pm 0.372$	$1.862^{B} \pm 0.349$	$2.203^{AB} \pm 409$	*
Zn (mg/kg)	1390 ±39	1460 ±59	1270 ±35	1340 ±21	ns
Fe (mg/kg)	$232^{B} \pm 30$	$3220^{A} \pm 59$	$1850^{C} \pm 35$	$1690^{C} \pm 21$	**

[a] FA: basal diet without phytase, FB : basal diet with phytase from *L. plantarum A1-E* (500 FTU/kg), FC : basal diet with phytase from *C. tropicalis TKd-3* (500 FTU/kg), FD = basal diet with commercial phytase (500 FTU/kg)

[b] BWG: Bodyweight, FI: Feed intake, FCR: Feed conversion ratio, IP: Index value of performance, EMS:EMM :EMMn:AME: TME: TMEn: VH: VH/CD: VW :

Different Uppercase between rows indicate differences among experimental diets (P<0.05)

[c] Response significances level measurement by Hotelling-Lawley test (ns P>0.05, * P <0.05, ** P <0.01)