J I T A A
Journal of the Indonesian Tropical Animal Agriculture
Accredited by Ditjen Riset, Teknologi dan Pengabdian
kepada Masyarakat No. 164/E/KPT/2021

J. Indonesian Trop. Anim. Agric.
pISSN 2087-8273 eISSN 2460-6278
http://ejournal.undip.ac.id/index.php/jitaa
48(3): 208-221, September 2023
DOI: 10.14710/jitaa.48.3. 208-221

A study on the evaluation of Indonesian local microbial phytase supplementation and its impact on broiler chicken performance, metabolic energy utilization, ileal histomorphology, and meat and bone mineralization

A. E. Suryani[1*], A. S. Anggraeni[1], M. F. Karimy[1], L. Istiqomah[1], and H. Herdian[2]
[1]*Research Center for Food Technology and Processing, National Research and Innovation Agency (BRIN), Yogyakarta 55861 Indonesia.*
[2]*Research Center for Animal Husbandry, National Research and Innovation Agency (BRIN), Cibinong, Bogor 16911, Indonesia.*
**Corresponding e-mail: adeermasuryani02@gmail.com*

Received April 04, 2023 ; Accepted July 07, 2023

ABSTRACT

The effect of local Indonesian microbial phytase, derived from *L. plantarum* A1-E extracted from the small intestine of Indonesian free-range chickens and *C. tropicalis* TKd-3 obtained from fermented Indonesian soybean tempe, on growth performance, metabolizable energy, ileal histomorphology, and meat and bone mineralization in broilers is the subject of evaluation in this study. A total of 140 day-old broiler chicks were divided into four treatments, five replicates, and seven chicks in each replicate. The research was carried out for 28 days. The experimental groups consisted of a basal diet without phytase (FA) serving as the negative control, a basal diet supplemented with 500 FTU/kg of *L. plantarum* A1-E phytase (FB), a basal diet supplemented with 500 FTU/kg of *C. tropicalis* TKd-3 phytase (FC), and a basal diet with commercial phytase as the positive control (FD). The results revealed that the broiler performance index increased by the FB and FC treatments ($P<0.05$), and the FC treatment exhibited a tendency to enhance metabolizable energy ($P=0.06$). The result of ileum histomorphology showed that the FB treatment increased villous height, the ratio of villous height to crypt depth, and villous surface area ($P<0.05$). The highest mineral content of phosphorus (P), magnesium (Mg), and iron (Fe) in broiler breast meat was obtained in the FB treatment ($P<0.05$). Furthermore, the highest mineral content of calcium (Ca), P, Mg, zinc (Zn), and Fe in thigh meat was found in the FC treatment ($P<0.05$). The mineralization of the tibia bone demonstrated that the FB treatment exerted a significant effect ($P<0.05$) on the P, Mg, and Fe mineral content. In conclusion, the performance index increased due to microbial phytase supplementation. Besides that, *L. plantarum* A1-E phytase improved the surface area of the villus, the absorption of ileum minerals, the mineral content of breast meat, and minerals deposition in the tibia.

Keywords: Broilers, Histomorphology, Mineralization, Performance, Phytase

INTRODUCTION

The availability of phosphorus (P) obtained from grain-based feedstuff has yet to be optimally utilized by poultry. This situation causes a P-available deficiency to support the poultry metabolic process (Krieg *et al*., 2021). Phosphorous 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*., 2018b).

Phytase, an enzyme capable of hydrolyzing phytate in grain-based feedstuff (Babatunde *et al*., 2020), has long been employed as an exogenous supplement in poultry feed. This practice aims to decrease the reliance on inorganic phosphorus (P) and calcium (Ca) additives while concurrently enhancing feed conversion ratio (FCR) and amino acid digestibility (Borda-Molina *et al*., 2019; Zanu *et al*., 2020). By liberating P and various minerals (e.g., Ca, Zn, Fe, Cu), exogenous phytase supplementation offers dual benefits by promoting animal health and mitigating environmental phosphorus (P) pollution (Beeson *et al*., 2017). Notably, P and Ca are vital constituents of the skeletal system, primarily stored in bone tissue as hydroxyapatite.

Phytase supplementation can break down phytate complexes through the addition of water and release the P and Ca bound-up for use by broilers. Therefore, phytase supplementation enhanced the birds' weight and tibia ash percentage on days 21 and 42 post-hatching (Babatunde *et al*., 2020). EL Enshasy *et al*. (2018) stated that 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 Indonesian free-range chickens and *Candida tropicalis* TKd-3 isolated from tempeh produced extracellular phytase *in vitro* (Istiqomah, 2015; Karimy *et al.,* 2020; Suryani *et al*., 2021). Heat stress can reduce intestinal performance and immune response during maintenance in broiler chickens. Therefore maintaining intestinal microstructure balance is an important thing to attend to (Elbaz *et al*., 2021). The ileum is 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 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. Enhancing 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 broilers' growth performance, nutrient digestibility, and bone mineralization (Handa *et al*., 2020). The benefit of microbial phytase in poultry nutrition has been extensively reviewed. However, discussing and exploring other response factors, such as energy utilization, digestive system morphology, and blood characteristics, will further enrich and deepen insights regarding adding microbial enzymes as a feed additive. For this purpose, this study investigates the response of adding microbial phytase from various microbes on the growth performance, metabolizable energy, ileal histomorphology, meat, and bone mineralization of broiler chickens.

MATERIALS AND METHODS

The experiment was conducted at the Poultry Closed House, 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), Universitas Gadjah Mada, 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 using a modified method (Mandviwala & Khire, 2000). Phytase enzyme activity was measured using the Vohra & Satyanarayana (2001) method.

Bird, Diet, and Sample Collection

A total of one hundred forty unsexed, day-old Cobb broiler chicks (DOC), that had been vaccinated against Newcastle Disease (ND), Infectious bronchitis-Newcastle Disease (IB-ND), and infectious bursal disease (IBD) were obtained from commercial hatcheries used as animal models. These DOC with a mean bodyweight of 44.65 g ± 2.04 were randomly allocated into four treatments, each consisted of five replicates with seven chicks in each replicate. The research design used a completely randomized design. The experiment consisted of 4 treatments as follows; 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 Standar Nasional, 2006), which was presented in Table 1. Chickens were housed in a closed system cage (closed cage) 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's arrival.

Feed intake (FI) and body weight (BW) were recorded weekly. Mortality was recorded daily, and the mortality percentage was calculated. The feed conversion ratio (FCR) was calculated as the total FI divided by the body weight gain (BWG) of the live chicken. Growth performance parameters measured consisted of FI, BWG, and FCR, and broilers' performance index (PI), evaluated at 28 days. The performance index was calculated based on Sofyan *et al*. (2012) using the following equation.

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

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 collect the excreta. The chickens underwent a three-day adaptation period to a phytase-free diet. On the 4th day, they were given the treatment feed at 150 g per chicken and were required to consume it within 24 hours. Following this, they were fasted 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 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 in ovens for 48 hours before being pulverized and analyzed for moisture content, crude protein, and gross energy based on the Al-mentafji (2006) method to determine Apparent and True metabolizable energy (AME and TME, respectively). The energy and nitrogen retentions were estimated, respectively, using amount of energy and nitrogen consumptions and excretions. Nitrogen excretion was determined according to calculation method of Sibbald and Wolynetz (1986), using the measurement of total metabolizable energy by nitrogen (TMEn) retention.

At the end of the experimental period (at 28 days old), three birds were randomly selected from each experimental unit and euthanized with decapitation. Mineral content analysis of the tibia was conducted by removing the left tibiae and extracting the meat from the bone. Os patella was removed, and the tibiae were weighed and frozen (−20°C) until further analysis (Hafeez *et al*., 2014). After the process of removing the skin, evisceration, and splitting carcass in respect of hygiene, 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 (breast and thigh meat) (Benamirouche *et al*., 2020).*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). Routine histological examination was performed by staining the tissue sections with hematoxylin-eosin and observing by optical microscope (Suvana *et al*., 2019). Villus height (VH) and crypt depth (CD) were measured as described by Abdel-Moneim *et al.* (2020).

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

Statistical Analysis

Statistical calculations were carried out to determine the effect of diets on growth performance, metabolizable energy, ileal histomorphology (villus height, villus width, and crypt depth), meat and tibia mineral concentration. One-way analysis of variance and analysis of difference in measurement response and the Hotelling-Lawley test were used to analyze data. Duncan's multiple range test was carried out to determine the significant differences among treatments. The data input and analysis process utilized R.64. 4.0.4. program (R Core Team, 2019).

RESULTS AND DISCUSSION

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). Body weight gains of broilers fed with phytase *C. tropicalis* TKd-3 were higher than those of controls (P<0.05), but were not significantly different from other treatments. In addition, broilers that were given phytase *C. tropicalis* TKd-3 also had lower FCR values than controls (P<0.05), and the best PI values, but were not significantly different from those that received phytase *L. plantarun* A1-E. These results indicated a linear relationship between BWG, FCR, and PI values, so broiler chickens fed phytase of microbial origin *C. tropicalis* TKd -3, and *L. plantarum* A1-E had higher growth performance than negative controls (P<0.05). For the feed intakr parameter (FI), there was no significant difference between the control and treatment groups, as shown in Table 2. This insignificant result could be due to the same macro-structure of feed in chickens from the control and treatment groups. Abdullahi *et al.* (2018) stated that the macro-structural characteristics of pellets, such as the proportion of intact pellets, durability, hardness, length, and diameter can affect feed consumption in broiler chickens.

In evaluating feed additive supplementation of poultry, growth performance, and metabolizable nergy 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 hydrolyzed the phytate found in poultry feed components. The ability of phytase 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).

Some publications reported, the use of phytase improves growth performance, particularly BWG, and FCR (Amiri *et al.*, 2021; Babatunde *et al.*, 2020). 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.

Several factors affect the effectiveness of giving phytase to poultry, such as feed composition (i.e., composition of P, energy, and protein), mineral content in feed (which can function as activators and inhibitors), sources of phytase microbes, endogenous conditions of the gastrointestinal tract (including pH conditions and enzymes in the digestive tract), and the last one is the poultry species (El-Hack *et al.*, 2018). The part of the small intestine named jejunum has absorbing nutrient responsibility (carbohydrate, protein, and lipid) from digested feed into the bloodstream, and increasing the height of jejunal villi could help maximise the capacity of digestion and correlated with growth performance (Suryani *et al.*, 2019). Phytase activity was pH-dependent, all phytases have a pH optimum that is microbial source dependant,

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

Parameters	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} ± 96	1202^{A} ± 101	1099^{A} ± 186	1176^{A} ± 118	**
Zn (mg/kg)	32 ± 3	34 ± 3	33 ± 1	33 ± 3	ns
Fe (mg/kg)	27^{C} ± 5	98^{A} ± 15	70^{B} ± 7	19^{C} ± 3	**
Thigh meat minerals					
Ca (mg/kg)	797^{C} ±138	1045^{B} ±156	2049^{A} ±216	769^{C} ±80	**
P (mg/kg)	4674^{A} ±123	4545^{A}±198	4731^{A} ±282	2562^{B} ±418	**
K (mg/kg)	9673 ±540	9465 ±952	10930 ±1612	9128 ±1095	ns
Mg (mg/kg)	690^{B} ±80	747^{AB} ±66	904^{A} ±142	841^{AB} ±139	*
Zn (mg/kg)	60^{B} ±2	62^{B} ±2	71^{A} ±9	61^{B}±7	*
Fe (mg/kg)	20^{C} ±3	32^{B} ±5	44^{A} ±6	19^{C}±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}±154	4.090^{A} ±236	3.976^{AB} ±286	3.796^{B} ±255	*
K (mg/kg)	3.561±351	3.696 ±242	3.699 ±260	3.661 ±580	ns
Mg (mg/kg)	1.895^{B} ±0.351	2.252^{A} ±0.372	1.862^{B} ±0.349	2.203^{AB} ±409	*
Zn (mg/kg)	1390 ±39	1460 ±59	1270 ±35	1340 ±21	ns
Fe (mg/kg)	232^{B} ±30	3220^{A} ±59	1850^{C} ±35	1690^{C}±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, AME: Apparent Metabolic Energy, TME: True Metabolic Energy, TMEn: True metabolic energy with nitrogen correction, VH: Ileal villi height, VW: Ileal villi width, VH/CD: ratio value of ileal villi height and crypt dept.: Different Uppercase between rows indicate differences among experimental diets ($P<0.05$)

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

and it affects the capacity of phytase to perform within the GI tracts of animals (El-Hack *et al*., 2018). It is a big challenge in South Asia to meet nutritional needs during the first 1000 days because their diet is generally dominated by staple foods with low nutrient density and poor mineral bioavailability (Dewey, 2016).

Some yeast strains have high viability (more than 50%) at pH 2-3 in vitro assays (Lohith & Appaiah, 2014), and high microbial phytase viability under acidic conditions allows for maximum phytase hydrolysis activity (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*., 2020). In line with our study, *C. tropicalis* TKd-3 phytase allowed for improving phytate hydrolysis in the proximal segment of the gastrointestinal tract, particularly in the proventriculus segment, to promote weight gain and feed efficiency performance index. Now that super-doses of phytase have beneficial effects (i.e.,> 2500 FTU/kg), are consistent, substantial, and have implications not only for animal performance but for health and welfare and possibly meat and egg quality (Cowieson *et al*., 2011).

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 metabolizable energy value of broilers which included AME, TME, and TMEn (Tabel 2). Although there was no significant difference in metabolizable energy between treatments, there was a linear result between the values of AME, TME, and TMEn which tended to be higher in *C. tropicalis* TKd-3 phytase administration (FC treatment).

Metabolic energy was affected by the amount of energy-protein intake and excreted, and also the ability of animals to feed and 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 adding 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 the broiler. Babatunde *et al*. (2020), in their research result, stated that broiler chickens with microbial phytase administration could 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 improvement 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 (Wu *et al*., 2015).

Ileal Histomorphology

Ileal histomorphology measurement of broiler with phytase administration during the experimental period (from 0 to 28 days of age) was presented in Table 2. Administration of *L. plantarum* A1-E phytase into broiler chicken diets during the experimental period significantly resulted in the highest villus height (VH)

($P<0.05$) compared to the control and the FC treatment. In addition, it also resulted in a higher ratio of villus 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 chicken diets with *L. plantarum* A1-E phytase had the greatest villius area ($P< 0.05$).

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 villus structure of the intestine, the more comprehensive the area of absorption of nutrients (Brudnicki *et al*., 2017). Numerous studies have demonstrated that dietary phytase addition supplementation improved broiler chickens' intestinal morphometric indices (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 to 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 villus 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 villus of the ileum indicate that the primary function of the ileum in mineral absorption is functioning correctly (Svihus, 2014). In the present study, addition in broiler chicken diets with *L. plantarum* A1-E phytase increased the ileal villus surface area, which means that mineral and other nutrient absorption.

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 the FC treatment, it was not significantly different from the negative control group and only considerably different compared to the FD treatment (Table 2).

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 minerals 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*., 2019a; Sommerfeld *et al*., 2018a; 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.

Ali *et al.* (2019) stated, 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, treating 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 and oxidation-reduction processes. Fe collaborates with zinc in bone formation, nucleic acid metabolism, protein synthesis, and eggshell formation (Wang *et al*., 2015).

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, other treatments had no significant difference ($P>0.05$).

The tibia mineral content parameter indicated a linear relationship with the highest surface area of an ileal villus in the broiler from FB treatment that gave *L. plantarum* A1-E phytase. In this study, phytase administration at a dose of 500 FTU/kg significantly increased tibia mineral content, particularly for minerals P, Mg, and Fe. Bone development depends on the digestive tract's function, digestive enzymes' availability, and the number of nutrients absorbed. In addition, heat stress and hydration (electrolyte balance) also affect health performance and bone structure in poultry (Cruvinel *et al*., 2021). 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 in untreated raw seeds decrease nutrient digestibility and reduce calcium, phosphorus, or iron absorption (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 those 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 follow research from Broch *et al*. (2021), who state that broilers fed phytase has higher tibia Ca and P content than controls. In line with that, Nourmohammadi *et al*. (2012) found 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).

CONCLUSION

The research result indicated that the administration of microbial phytase, both *L. plantarum* A1-E and *C. tropicalis* TKd-3 as a feed additive was able to improve body the weight gain and performance index, and tended to increase the metabolizable energy of broilers. In addition, the administration of *L. plantarum* A1-E as a feed additive improved the morphology of the ileal villus. It caused the enhancement of mineral absorption of the ileum, followed by the enhancement of mineral deposits tibia.

ACKNOWLEDGEMENTS

The authors would like to thank Indonesia Toray Science Foundation for funding this research and thanks to my institution, National Research and Innovation Agency for giving all the support system**.**

REFERENCES

Abdel-Moneim, A. M. E., A. M. Elbaz, R. E. S. Khidr, and F. B. Badri. 2020. Effect of in Ovo Inoculation of Bifidobacterium spp. on Growth Performance, Thyroid Activity, Ileum Histomorphometry, and Microbial Enumeration of Broilers. Probiotics and Antimicrobial Proteins, 12(3): 873-882.

Abdollahi, M. R., F. Zaefarian, and V. Ravindran. 2018. Feed intake response of broilers: Impact of feed processing. Anim. Feed Sci. Technol., 237:154-165.

Albuquerque, R. de, D.E. de Faria, OM Junqueira, D. Salvador, D.E. de Faria Filho, and MF Rizzo. 2003. Effects of energy level in finisher diets and slaughter age of on the performance and carcass yield in broiler chickens. Braz. J. Poult. Sci., 5(2): 99-104.

Al-mentafji, H. N. 2006. Official Methods of Analysis. AOAC International. Assoc. Off. Anal. Chem., Arlington.

Ali, M., S. Y. Lee, J. Y. Park, S. Jung, C. Jo, and K. C. Nam. 2019. Comparison of functional compounds and micronutrients of chicken breast meat by breeds. Food Sci. Anim., 39 (4): 632-642.

Amiri, M. Y. A., M. A. Jafari, and M. Irani. 2021. Growth performance, internal organ traits, intestinal morphology, and microbial population of broiler chickens fed quinoa seed–based diets with phytase or protease supplements and their combination. Trop. Anim. Health Prod. 53, 535: 1-8.

Awad, W. A., K. Ghareeb, S. Abdel-Raheem, and J. Böhm. 2009. Effects of dietary inclusion of probiotic and synbiotic on growth performance, organ weights, and intestinal histomorphology of broiler chickens. Poult. Sci., 88(1): 49-56.

Babatunde, O. O., A. J. Cowieson, J. W. Wilson, and O. Adeola. 2019a. Influence of age and duration of feeding low-phosphorus diet on phytase efficacy in broiler chickens during the starter phase. Poult. Sci., 98(6): 2588-2597.

Babatunde, O. O., A. J. Cowieson, J. W. Wilson, and O. Adeola. 2019b. The impact of age and feeding length on phytase efficacy during the starter phase of broiler chickens. Poult. Sci., 98(12): 6742-6750.

Babatunde, O. O., J. A. Jendza, P. Ader, P. Xue, S. A. Adedokun, and O. Adeola. 2020. Response of broiler chickens in the starter and finisher phases to 3 sources of microbial phytase. Poult. Sci., 99(8): 3997-4008.

Badan Standar Nasional. 2006. Pakan Anak Ayam Ras Pedaging Masa Akhir *(broiler finisher)*. SNI 01-3931-2006.

Beeson, L. A., C. L. Walk, M. R. Bedford, and O. A. Olukosi,. 2017. Hydrolysis of phytate to its lower esters can influence the growth performance and nutrient utilization of broilers with regular or super doses of phytase. Poult. Sci., 96(7): 2243-2253.

Benamirouche, K., D. Baazize-Ammi, N. Hezil, R. Djezzar, A. Niar, and D. Guetarni. 2020. Effect of probiotics and Yucca schidigera extract supplementation on broiler meat quality. Acta Scientiarum - Anim. Sci., 42 (1): 1-9.

Borda-Molina, D., T. Zuber, W. Siegert, A. Camarinha-Silva, D. Feuerstein, and M. Rodehutscord. 2019. Effects of protease and phytase supplements on small intestinal microbiota and amino acid digestibility in broiler chickens. Poult. Sci., 98(7): 2906-2918.

Broch, J., V. D. L. Savaris, L. Wachholz, E. H. Cirilo, G. L. S. Tesser, W. J. Pacheco, C. Eyng, G. M. Pesti, and R. V. Nunes. 2021. Influence of phytate and phytase on performance, bone, and blood parameters of broilers at 42 days old. South Afr. J. Anim. Sci., 51(2): 160-171.

Brudnicki, A., W. Brudnicki, R. Szymeczko, M. Bednarczyk, D. Pietruszyńska, and K. Kirkiłło-Stacewicz. 2017. Histo-morphometric adaptation in the small intestine of broiler chicken, after embryonic exposure to a – Galactosides. J. Anim. Plant Sci., 27(4): 1075-1082.

Cowieson, A. J., P. Wilcock, and M. R. Bedford. 2011. Super-dosing effects of phytase in poultry and other monogastrics. Worlds Poult. Sci. J., 67: 225-235.

Cruvinel, J. M., P. M. G. Urayama, T. S. dos Santos, J. C. Denadai, E. M. Muro, L. C. Dornelas, G. A. M. Pasquali, A. C. C. Neto, L. H. Zanetti, R. G. F. Netto, J. R. Sartori, and A. C. Pezzato. 2021. Different dietary electrolyte balance values on performance, egg, and bone quality of Japanese quail (*Coturnix Coturnix Japonica*) under heat stress. Trop. Anim. Health Prod. 53, 17: 1-8.

Dersjant-Li, Y., A. Awati, H. Schulze, and G. Partridge. 2015. Phytase in non-ruminant animal nutrition: A critical review on phytase activities in the gastrointestinal tract and influencing factors. J. Sci. Food Agric., 95(5): 878-896.

Dewey, K. G., 2016. Reducing stunting by improving maternal, infant and young child nutrition in regions such as South Asia: Evidence, challenges and opportunities. Matern. Child Nutr., 12: 27-38.

El-Hack, M. E. A., M. Alagawany, M. Arif, M. Emam, M. Saeed, M. A. Arain, F. A. Siyal, A. Patra, S. S. Elnesr, and R. U. Khan. 2018. The uses of microbial phytase as a feed additive in poultry nutrition - A review. Ann. Anim. Sci., 18(3): 639-658.

EL Enshasy, H., D. J. Dailin, N. H. Abd Manas, N. I. Wan Azlee, J. Eyahmalay, S. S. Yahaya, R. Abd Malek, V. Siwapiragam, and D. Sukmawati. 2018. Current and Future Applications of Phytases in Poultry Industry: A Critical Review. J. Advan. VetBio Sci. Techn., 3(3): 65-74.

Elbaz, A. M., N. S. Ibrahim, A. M. Shehata, N. G. Mohamed, and A. M. E. Abdel-Moneim. 2021. Impact of multi-strain probiotic, citric acid, garlic powder or their combinations on performance, ileal histomorphometry, microbial enumeration and humoral immunity of broiler chickens. Trop. Anim. Health Prod., 53, 115: 1-8.

Farrell, D. J., S. I. Atmamihardja, and R. A. E. Pym. 1982. Calorimetric Measurements of the Energy and Nitrogen Metabolism of Japanese Quail. Br. Poult. Sci., 23(5): 375-382.

Gautier, A. E., C. L. Walk, and R. N. Dilger. 2017. Influence of dietary calcium concentrations and the calcium-to-non-phytate phosphorus ratio on growth performance, bone characteristics, and digestibility in broilers. Poult. Sci., 96(8): 2795-2803.

Gautier, A. E., C. L. Walk, and R. N. Dilger. 2018. Effects of a high level of phytase on broiler performance, bone ash, phosphorus utilization, and phytate dephosphorylation to inositol. Poult. Sci., 97(1): 211-218.

Geldenhuys, G., L. C. Hoffman, and N. Muller. 2015. The fatty acid, amino acid, and mineral composition of Egyptian goose meat as affected by season, gender, and portion. Poult. Sci., 94(5): 1075-1087.

Hafeez, A., A. Mader, F. G. Boroojeni, I. Ruhnke, I. Röhe, K. Männer, and J. Zentek. 2014. Impact of thermal and organic acid treatment of feed on apparent ileal mineral absorption, tibial and liver mineral concentration, and tibia quality in broilers. Poult. Sci., 93(7): 1754-1763.

Haile, T. H., C. Hyuie, W. Zhengke, C. Jiang, S. A. Prizado, A. Purba, C. Guilan, and L. Guohua. 2020. Ileal digestibility of phosphorus in plant origin feedstuffs fed for broiler chickens: The effect of microbial phytase. Poult. Sci. J., 8(2): 201-210.

Handa, V., D. Sharma, A. Kaur, and S. K. Arya. 2020. Biotechnological applications of microbial phytase and phytic acid in food and feed industries. Biocatal. Agric. Biotechnol., 25, 101600.

He, S., R. F. Medrano, Q. Yu, Y. Cai, Q. Dai, and J. He. 2017. Effect of a microbial phytase on growth performance, plasma parameters and apparent ileal amino acid digestibility in Youxian Sheldrake fed a low -phosphorus corn-soybean diet. Asian-Australas J. Anim .Sci., 30(10): 1442-1449.

Humer, E., C. Schwarz, and K. Schedle. 2015. Phytate in pig and poultry nutrition. J. Anim. Physiol. Anim. Nutr., 99(4): 605-625.

Istiqomah, L. 2015. Isolation and Characterisation of Lactic Acid Bacteria as Phytase Producer from Digestive Tract of Poultry and The Phytase Characterisation. Thesis. Universitas Gadjah Mada, Yogyakarta, Indonesia.

Karami, M., A. Karimi, A. Sadeghi, J. Zentek, and F. G. Boroojeni. 2020. Evaluation of interactive effects of phytase and benzoic acid supplementation on performance, nutrients digestibility, tibia mineralisation, gut morphology and serum traits in male broiler chickens. Ital. J. Anim. Sci., 19(1): 1428-1438.

Karimy, M. F., E. Damayanti, A. E. Suryani, E. Prasetyo, R. Nurhayati, M. Anwar, and A. S. Anggraeni. 2020. A simple method for analysis of *Saccharomyces cerevisiae* morphology by applying a high vacuum mode of the scanning electron microscopy and without chemical fixatives. IOP Conference Series: Earth and Environmental Science, 462, 012048. The 3rd International Conference on Natural Products and Bioresource Sciences, Tangerang, Indonesia, October 23-24, 2019. P. 1-13.

Khan, K., H. Zaneb, Z. U. Rehman, H. Maris, and H. ur. Rehman, H. 2019. Effect of phytase supplementation on growth performance in broiler chickens. Pak. J. Zool., 51(2): 731-735.

Krieg, J., D. Borda-Molina, W. Siegert, V. Sommerfeld, Y. P. Chi, H. R. Taheri, D. Feuerstein, A. Camarinha-Silva, and M. Rodehutscord. 2021. Effects of calcium level and source, formic acid, and phytase on phytate degradation and the microbiota in the digestive tract of broiler chickens. Anim. Microbiome, 3: 1-18.

Lohith, K., and K. A. A. Appaiah. 2014. In Vitro Probiotic Characterization of Yeasts of Food and Environmental Origin. Int. J. Probiotics Prebiotics, 9(3): 1-6.

Mandviwala, T. N., and J. M. Khire. 2000. Production of high activity thermostable phytase from thermotolerant Aspergillus niger in solid state fermentation. J. Ind. Microbiol. Biotechnol., 24(4): 237-243.

Mohammadagheri, N., R. Najafi, G. Najafi, and R. N. Dvm. 2016. Effects of dietary supplementation of organic acids and phytase on performance and intestinal histomorphology of broilers. Vet. Res. Forum, 7(3): 189-195.

Muszyński, S., E. Tomaszewska, M. Kwiecień, P. Dobrowolski, and A. Tomczyk-Warunek. 2018. Subsequent somatic axis and bone tissue metabolism responses to a low-zinc diet with or without phytase inclusion in broiler chickens. PLoSONE, 13(1): 1-21.

Nourmohammadi, R., S. M. Hosseini, H. Farhangfar, and M. Bashtani. 2012. Effect of citric acid and microbial phytase enzyme on ileal digestibility of some nutrients in broiler chicks fed corn-soybean meal diets. Ital. J. Anim. Sci., 11(1): 36-40.

R Core Team. 2019. An introduction to dplR. Industrial and Commercial Training, 10(1), 11-18.

Rao, S. V. R., B. Prakash, K. Kumari, M. V. L. N. Raju, A. K. and Panda. 2013. Effect of supplementing different concentrations of organic trace minerals on performance, antioxidant activity, and bone mineralization in Vanaraja chickens developed for free range farming. Trop. Anim. Health Prod., 45(6): 1447-1451.

Sajadi Hezaveh, M. S., H. A. Ghasemi, I. Hajkhodadadi, and M. H. Moradi. 2020. Single and combined effects of phytase and citric acid on growth performance, nutrient digestibility, bone characteristics, intestinal morphology, and blood components in meat -type quails fed low-phosphorous diets. Anim. Feed Sci. Technol., 269, 114677: 1-10.

Santos, F. R., M. Hruby, E. E. M. Pierson, J. C. Remus, and N. K. Sakomura. 2008. Effect of phytase supplementation in diets on nutrient digestibility and performance in broiler chicks. J. Appl. Poult. Res., 17(2): 191-201.

Sibbald, I. R., and M. S. Wolynetz. 1986. Comparison of three methods of excreta collection used in estimation of energy and nitrogen excretion. Poult. Sci., 65(1): 78-84.

Sofyan, A., M. Angwar, H. Herdian, E. Damayanti, L. Istiqomah, A. Febrisiantosa, H. Julendra, M. H. Wibowo, and T. Untari. 2012. Performance enhancement and immunity profile of broiler treated feed additive containing lactic acid bacteria and *Ganoderma lucidum*. Media Peternakan, 35 (3): 201-206.

Sommerfeld, V., S. Künzel, M. Schollenberger, I. Kühn, and M. Rodehutscord. 2018a. Influence of phytase or myo-inositol supplements on performance and phytate degradation products in the crop, ileum, and blood of broiler chickens. Poult. Sci., 97(3): 920-929.

Sommerfeld, V., S. Künzel, M. Schollenberger, I. Kühn, and M. Rodehutscord. 2018b. Influence of phytase or myo-inositol supplements on performance and phytate degradation products in the crop, ileum, and blood of broiler chickens. Poult. Sci., 97(3): 920-929.

Suryani, A. E., A. S. Anggraeni, L. Istiqomah, E. Damayanti, and M. F. Karimy. 2021. Isolation and identification of phytate-degrading yeast from traditional fermented food. Biodiversitas, 22(2): 866-873.

Suryani, A. E., H. Wuryastuty, R. Wasito, and M. F. Karimy. 2019. The effect of the water additive KimchiStock® as an herbal growth promoter on the jejunal histological and ultrastructural changes of broiler chickens. Pak J Nutr., 18(10): 983-988.

Suvana, S. K., C. Layton, and J. D. Bancroft. 2019. Bancroft's Theory and Practice of Histological Techniques. Elsevier Ltd.

Svihus, B. 2014. Function of the digestive system. J. Appl. Poult. Res., 23(2): 306–314.

Vohra, A., and T. Satyanarayana. 2001. Phytase production by the yeast, *Pichia anomala*. Biotechnol. Lett., 23(7): 551-554.

Walk, C. L., K. Juntunen, M. Paloheimo, and D. R. Ledoux. 2019. Evaluation of novel protease enzymes on growth performance and nutrient digestibility of poultry: enzyme dose response. Poult. Sci., 98(11): 5525-5532.

Wang, L., C. Wang, X. Gao, N. Xu, L. Lin, H. Zhao, S. Jia, and L. Jia. 2015. Purification, characterization and anti-aging capacity of mycelia zinc polysaccharide by Lentinus edodes SD-08. BMC Complement. Altern. Med., 15: 1-11.

Wu, D., S. B. Wu, M. Choct, and R. A. Swick. 2015. Comparison of 3 phytases on energy utilization of a nutritionally marginal wheat-soybean meal broiler diet. Poult. Sci., 94 (11): 2670-2676.

Zaefarian, F., L. F. Romero, and V. Ravindran. 2013. Influence of a microbial phytase on the performance and the utilisation of energy, crude protein and fatty acids of young broilers fed on phosphorus-adequate maize- and wheat-based diets. British Poult. Sci., 54(5): 653-660.

Zanu, H. K., S. K. Kheravii, N. K. Morgan, M. R. Bedford, and R. A. Swick. 2020. Interactive effect of 2 dietary calcium and phytase levels on broilers challenged with subclinical necrotic enteritis: part 1—broiler performance, gut lesions and pH, bacterial counts, and apparent ileal digestibility. Poult. Sci., 99(10): 4861-4873.