Evaluation of Commercial 6-Phytases on Growth Performance, Bone Mineral Content, and Feed Digestibility of Broiler Chicks

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We evaluated the effects of 6-phytases on the growth performance of broilers (UK Chunky) under the recommended supplier-application dosage of each phytase. A nutritionally sufficient standard diet was administered as the base diets in the positive control feed. The diet in the negative control feed was designed by reducing total phosphorous, non-phytate phosphorus, and calcium by 0.1% to evaluate the effect of the nutrient restriction on broilers. Four 6-phytases were added to negative control feeds at the level of the recommended dosage of each product to compare the effect of phytases on broiler technical performance, tibia ash, and feed digestibility. Nine hundred one-day-old broiler chicks (males and females) were distributed in a completely randomized design composed of six treatments and three replicates of 50 chicks each. Chicks were fed ad libitum for 49 days. Body weight gain and feed intake were recorded on days 21 and 49, tibia ash was measured on day 21, and apparent ileal digestibility of dry matter, crude protein, and total phosphorus were analyzed on day 49. Birds reared with test feeds supplemented with phytase showed higher body weight gain and feed intake compared to those of the negative control birds. No significant differences in traits were observed among different phytase treatments. Similarly, the percentage of tibia ash increased when phytase was supplemented, resulting in higher bone levels compared to that of the positive control. The apparent ileal digestibility of crude protein and total phosphorus was enhanced by supplementing negative control diets with phytases.

Key words: broiler, ileal digestibility, phytase, tibia ash

Introduction

Plant materials in poultry feed store phosphorus (P) in the form of phytate-P (pP), which is largely unavailable to them and results in a reduction in nutrient digestibility and excretion of inorganic P into the environment (Kerr et al., 2010; Dersjant-Li et al., 2015). The enzyme phytase (myo-inositol hexakisphosphate phosphohydrolase) catalyzes phytate hydrolysis and releases P, myo-inositol, and phytate-bound nutrients. It has been reported that 30%–40% of the excretion of inorganic P can be reduced without harming animal growth by the addition of phytase into feeds (NARO, 2012). Consequently, microbial phytase is commonly added to monogastric animal diets as a feed additive and accounts for 60% of the market share of enzymes for animal feeds (Adeola and Cowieson, 2011; Fuji Keizai Osaka market office, 2020). Since the first generation of commercial 3-phytase from Aspergillus niger was launched in 1996 in Japan, the number of registered products has increased (MAFF 2021). Recently, four 6-phytases have become commercially available in Japan as an alternative to 3-phytase (Fuji Keizai Osaka market office, 2020); however, the differences among the performance of 6-phytase products have not been evaluated. In this study, the effects of four 6-phytase products (administered at the supplier-recommended inclusion levels into feeds) on bird performance and ileal digestibility of nutrients were evaluated.
Materials and Methods

Experimental Chicks
One-day-old broiler chicks (UK chunky) were used in this study. Three hundred chicks (average body weight 42 ± 0.19 g, 150 and 150 females, respectively) were classified into six groups. Each group contained 25 male and female chicks, and the total body weight of each group was similar between treatments. Fifty chicks were reared in a 3.3 m² pen with floor heating and 24 h lighting in a forced ventilation-type windowless poultry house. Feeds were provided ad libitum, and the floor was covered with sawdust. The experiments were repeated three times, and a total of 900 birds were used (6 treatments × 3 repetitions × 50 chicks).

The animals were reared according to the Guide for the Care and Use of Experimental Animals (Japan Scientific Feeds Association, ethical approval number: 819).

Feeds
Base diets were prepared by modifying the standard mixed feeds provided by the Japan Chunky Association (2014) (positive control; PC). The negative control (NC), quantity of total phosphorous (TP), non-phytate phosphorus (npP), and calcium (Ca) were reduced by 0.1% from the base diets by reducing dicalcium phosphate (Table 1).

Feeds for 1–22-day-old chicks contained 0.66% TP, 0.44% npP, 0.87% Ca, 21.5% CP, 1.15% av. Lys, and 3010 kcal/kg metabolizable energy (PC). The NC feed was similar, although it contained 0.56% TP, 0.33% npP, and 0.77% Ca. Feeds for 23–50-day-old chicks contained 0.58% TP, 0.37% npP, 0.73% Ca, 18.5% CP, 1.01% av. Lys, and 3200 kcal/kg metabolizable energy. The NC feed was similar although it contained 0.48% TP, 0.27% npP, and 0.63% Ca.

6-phytase
Four commercially available 6-phytase products were used in this study: Ronozyme HiPhos granule (DSM, Netherlands) as phytase A, Natuphos E granule (BASF, Germany), phytase B; Axtra PHY TPT2 (Dupont, USA) as phytase C, and OptiPhos G (Huvepharma, Belgium) as phytase D (Table 2). The recommended dose of each phytase was added to the NC feed.

Performance Test
Chicks were grown for 49 days from May 13, 2020, with feeds as described above. In the test feeds, each 6-phytase was added to the NC feed at the supplier-recommended dose. Chromium oxide (Cr₂O₃) was added as an indigestible marker for ileal digestibility analysis in feeds of 23–50-day-old chicks at 0.1%. The body weight gain (BWG) and feed intake (FI) of the chicks in each pen were recorded every seven days during the trial. The data were then summed as the initial body weight and body weight on days 21 and 49. The feed conversion ratio (FCR) was calculated as FI/BWG.

Tibia Ash and Apparent Ileal Digestibility of TP
The left tibia was collected from four chicks with average

| Table 1. Composition and nutrient density of experimental diets |
|---------------------------------------------------------------|
| Ingredients %                                                 | Starter feed 1–22 days of age | Grower feed 23–50 days of age |
|                                                              | PC (NC)                        | PC (NC)                        |
| Corn                                                 | 54.84 (55.88)                  | 61.04 (62.16)                  |
| Soybean meal                                           | 33.67 (33.40)                  | 26.10 (25.80)                  |
| Rapedseed meal                                         | 4.00 (4.00)                    | 4.00 (4.00)                    |
| Soybean oils                                           | 3.30 (3.00)                    | 5.30 (4.95)                    |
| Dicalcium Phosphaté                                    | 1.47 (0.90)                    | 1.19 (0.62)                    |
| Calcium carbonate                                      | 1.02 (1.10)                    | 0.88 (0.95)                    |
| Salt                                                  | 0.36 (0.36)                    | 0.37 (0.37)                    |
| Vitamin, mineral premix                                 | 0.25 (0.25)                    | 0.25 (0.25)                    |
| Choline chloride                                       | 0.28 (0.28)                    | 0.24 (0.24)                    |
| L-lysine hydrochloride                                  | 0.12 (0.13)                    | 0.17 (0.18)                    |
| DL-methionine                                          | 0.33 (0.33)                    | 0.23 (0.23)                    |
| L-threonine                                            | 0.07 (0.07)                    | 0.02 (0.03)                    |
| L-arginine                                             | 0.18 (0.19)                    | 0.17 (0.18)                    |
| L-isoleucine                                           | 0.06 (0.06)                    | 0.03 (0.03)                    |
| L-valine                                               | 0.05 (0.05)                    | 0.01 (0.01)                    |
| Total                                                 | 100.00 (100.00)                | 100.00 (100.00)                |
| Nutrient                                               |                                |                                |
| CP (%)                                                 | 21.550 (21.537)                | 18.520 (18.506)                |
| ME (Mcal/kg)                                           | 3.006 (3.007)                  | 3.202 (3.201)                  |
| Ca (%)                                                 | 0.870 (0.773)                  | 0.730 (0.630)                  |
| P (%)                                                  | 0.659 (0.559)                  | 0.577 (0.477)                  |
| NpP (%)                                                | 0.435 (0.334)                  | 0.366 (0.265)                  |

The composition is calculated from the Standard Tables of Feed Composition in Japan (2009). CP: crude protein, ME: metabolizable energy, Ca: calcium, P: phosphate, NpP: non-phytate phosphorus.
weights (two male and two female each) from each pen on the day 21st of rearing. Then, all bones were defatted by soaking in ethanol for 20 h, further petroleum ether for 20 h, drying for 4 h, and weight and ash contents measured following AOAC (2000). On day 49, the ileum from the vitelline diverticulum to a point 40 mm proximal to the ileocecal junction was dissected. The apparent ileal digestibility (AID) was estimated for 18 chicks (8 males and 10 females). DM, CP, and TP were measured following the AOAC (2000). Cr₂O₃ was measured according to the method of Takemasa (1992). AID was calculated using the following formula:

\[ AID = \frac{\text{Nutrients}_{\text{feed}} - \text{Nutrients}_{\text{ileal sample}}}{\text{Cr}_2\text{O}_3_{\text{feed}}} \times \frac{\text{Cr}_2\text{O}_3_{\text{ifeal sample}}}{100} \]

**Statistical Analysis**
Data were analyzed by ANOVA and significant (\( p < 0.05 \)) results were analyzed further by using the Tukey-Kramer HSD test (Yoshida, 1998).

**Results**

**Performance**

BWG and FI were significantly higher in the PC and all phytase-administered groups than those in the NC group. There was no significant difference between the PC and phytase-supplemented groups. All phytases improved growth performance, although there was no significant difference in BWG, yet there were considerable numerical differences among phytases (Table 3). FCR did not differ between the treatments.

**Tibia Bone Ash**

Tibia ash content was significantly higher in the PC than in the NC. Similarly, tibia bone ash was significantly higher in phytase C and D groups than that in the PC group (Table 3, **Table 3. Results of growth performance and apparent ileal digestibility**

| Product name          | Distributer                | Recommended dose |
|-----------------------|----------------------------|------------------|
| Phytase A             | Ronozyme HiPhos            | DSM Japan K.K (Tokyo, Japan) 1000 (FYT/kg)* |
| Phytase B             | Nataphos E granule         | BASF Japan Ltd. (Tokyo, Japan) 500 (FTU/kg)** |
| Phytase C             | Astra Phy 20000 TPT2       | DuPont Japan Ltd. (Tokyo, Japan) 500 (FTU/kg)** |
| Phytase D             | OptiPhos 4000 G            | Huvepharma Japan Inc. (Kyoto, Japan) 250 (FTU/Phytex/kg)*** |

*One FYT is the amount of enzyme that liberates 1 μmol inorganic phosphate from phytate per minute under reaction conditions with a phytate concentration of 5.0 mM at pH 5.5, and 37°C.
**One FTU is the amount of enzyme which liberates 1 μmol inorganic phosphate per minute from sodium phytate at pH 5.5 and 37°C.
***One FTU-phytase is the amount of enzyme that catalyzes the release of 1 μmol inorganic phosphate per minute from 5.1 mM sodium phytate in pH 5.5 citrate buffer at 37°C, measured as the blue P-molybdate complex color at 820 nm.
All phytase groups showed a higher tibia ash percentage than that of the NC group.

**Apparent Ileal Digestibility of DM, CP and TP**

Adding 6-phytase to the diet significantly increased the AID of DM, CP, and TP ($p<0.05$) compared to the PC, except for the phytase B group (Table 3). No significant differences were observed among the phytase groups or within the phytase groups and NC for AID of DM and CP. All phytase treatments improved the AID of TP by 13% compared to that of the NC.

**Discussion**

The effects of 6-phytases on broiler growth performance and DM, CP, and P digestibility were evaluated using four commercially available products in Japan at the recommended dose. All 6-phytase in feeds improved BWG and FI, but not FCR, of broiler chickens when phytase was added to the NC diet. No statistical difference in relative tibia weights indicated the well-balanced growth of the chicks in this study, and the dry weight of the tibia ash response supported the increase in AID of P observed (Table 3). This study demonstrated that the supply of 6-phytase accelerated the utility of phytic acid in feeds and effectively improved growth performance and P digestibility. In this study, we evaluated the effects of four 6-phytase products on growth performance. The recommended dose of each 6-phytase was as follows: phytase A, 1000 FYT/kg; phytase B and C, 500 FTU/kg; and phytase D, 250 FTU-phytex/kg. Although a different value with a unique unit was officially registered as a recommended dose, this was regulated to release 1 μmol of inorganic phosphate from phytate per minute under reaction conditions (Table 1) (MAFF 2021). Thus, it is natural that the results showed supplementation with phytases was efficient in restoring growth performance and tibia ash to the same level as those observed in the PC; however, no significant difference in any criterion among the 6-phytase-addition groups was observed. Alternatively, some variations were noted in the AID of DM, CP, and tibia ash among the 6-phytase groups, which reflect the features of the products. The negative effect of reducing dietary TP, npP, and Ca on growth was alleviated via FI, AID of TP, and tibia ash.

**Conflicts of Interest**

The authors declare no conflicts of interest.

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