Effect of Graded Levels of an Enhanced E. coli Phytase with Step-Wise Reduction of Supplemental Inorganic Phosphate on Growth Performance of Broilers Fed Corn-Soy Diet

ABSTRACT

This study evaluated the potential of incremental doses of an enhanced Escherichia coli-derived phytase to support step-wise reduction of supplemental inorganic phosphate in an all-vegetable broiler diet. Corn-soybean meal-based diets containing 0.40/0.80%, 0.35/0.70%, and 0.30/0.60% avP and Ca, respectively from 0-10, 10-25, and 26-42 days posthatch served as experimental control (PC). Three test diets were formulated with 500, 1000, and 1500 FTU/kg of phytase assigned respectively an avP matrix of 0.15, 0.19, and 0.23% and a fixed Ca matrix of 0.15%. An additional diet (PC++) containing extra avP and Ca (+0.05% avP/+0.1% Ca) to that of PC was included to test if avP and Ca were not limiting in PC. Each diet was offered to 16 replicates of straight-run broilers kept in floor pens (30 birds per pen). PC++ had lower (p<0.05; 10 and 25 d) or similar (42 d) BW and toe ash compared with PC confirming the avP and Ca set in PC were sufficient to support optimal growth and bone mineralization. Compared to the PC, diets containing 1000 and 1500 FTU phytase had higher BW (p<0.05) at 10 and 25 d. For the overall period of 0-42 d, FI, BW and FCR did not differ across treatments. Percentage-, but not the absolute-, toe ash at phytase treated groups was significantly (p<0.05) low compared with the PC. The experiment demonstrated that 1500 FTU/kg of enhance E. coli phytase supports optimal BW and FCR of broilers fed corn-soy diet largely void of supplemental inorganic phosphate.

INTRODUCTION

The effect of supplementary microbial phytase in improving the availability of phytate-bound phosphorus (PP) has been well documented in poultry and swine nutrition. Improved availability of PP in the diet by phytase allows reduction in the supplemental inorganic phosphates. This replacement value, often referred to as P-equivalency, typically follows a log-linear curve and is a function of the phytase in question (Augspurger et al., 2003; Dersjant-Li et al., 2015). The log-linear curves offer the flexibility to choose the most economical dosage under variable nutritional and price conditions. However, the maximum potential of phytases to replace inorganic phosphates and thus reduce feed costs is far from being completely exploited – e.g. a common approach is to use phytases to replace 0.10-0.15% AvP, despite the evidence that modern phytases can potentially offer more complete phytate destruction and much higher P-release than the former counterparts (Van der Klis & Star, 2013; Walk et al., 2013; dos Santos et al., 2014; Zeller et al., 2015). Higher doses of phytase can potentially offer, 1) direct savings in the feed cost through replacing more of an expensive inorganic phosphate, and 2) improved technical performance associated with more complete
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effect of graded levels of an enhanced e. coli phytase with step-wise reduction of supplemental inorganic phosphate on growth performance of broilers fed corn-soy diet

Materials and Methods

Two thousand and four hundred (2,400) newly hatched commercial straight-run broiler chicks (Lohman Indian River) were randomly allocated to 5 treatments, with 16 replicate pens per treatment, in a randomized complete block design using 30 chicks per experimental unit (floor pen). Table 1 presents a summary of the dietary treatments highlighting the phytase dose rates and also the inclusions of monocalcium phosphate by feeding phase. A corn-soybean meal-based diet with avP/Ca levels of 0.40/0.80%, 0.35/0.70%, and 0.30/0.60%, respectively for 0-10, 10-25, and 26-42 days posthatch served as experimental control (PC); ingredient composition and calculated and analyzed nutrient content of PC are presented in Table 2. Three

Table 1 – Diet design and treatments.

| Treatment     | Added Phytase (FTU/kg) | Supplemental level of monocalcium phosphate, kg per metric ton of feed |
|---------------|------------------------|---------------------------------------------------------------|
|               | 1-10 d                 | 11-25 d | 26-42 d |
| PC            | None                   | 13.4    | 11.4 | 9.5 |
| PC ++         | None                   | 15.7    | 13.7 | 11.8 |
| -0.15% avP; -0.15% Ca | 500 | 6.3 | 4.3 | 2.4 |
| -0.19% avP; -0.15% Ca | 1000 | 4.4 | 2.5 | - |
| -0.23% avP; -0.15% Ca | 1500 | 2.6 | - | - |

Diets were formulated to contain 0.40, 0.35, and 0.30% avP, respectively for 0-10, 11-25, and 26-42 d; the corresponding levels of total Ca were set to 0.80, 0.70, and 0.60% respectively.

Table 2 – Ingredient and nutrient composition of control (PC) diets.

| Ingredients                  | 1-10 d | 11-25 d | 26-42 d |
|------------------------------|--------|---------|---------|
| Corn                         | 59.4   | 62.5    | 68.1    |
| Corn gluten meal             | 3.2    | 3.9     | 5.9     |
| Soybean meal                 | 31.1   | 26.4    | 19.4    |
| Palm oil                     | 1.6    | 2.9     | 3.0     |
| Limestone                    | 1.25   | 1.10    | 0.96    |
| MDCP                         | 1.34   | 1.14    | 0.95    |
| Salt                         | 0.30   | 0.27    | 0.19    |
| Sodium bicarbonate           | 0.34   | 0.28    | 0.28    |
| L-lysine HCl                 | 0.31   | 0.31    | 0.29    |
| DL-methionine                | 0.32   | 0.28    | 0.16    |
| L-threonine                  | 0.10   | 0.10    | 0.03    |
| Choline Chloride             | 0.06   | 0.06    | 0.07    |
| Vitamin/Mineral mix          | 0.30   | 0.30    | 0.30    |
| Builders sand                | 0.40   | 0.40    | 0.40    |
| Total                        | 100    | 100     | 100     |

Calculations and analysis

| AMEn, kcal per kg | 2950 | 3080 | 3175 |
|-------------------|------|------|------|
| CP, %             | 22.1 (22.1) | 20.5 (20.4) | 18.6 (19.4) |
| Digestible lysine, % | 1.24 | 1.12 | 0.94 |
| Available P, %    | 0.40 | 0.35 | 0.30 |
| Ca, %             | 0.80 (0.74) | 0.70 (0.66) | 0.60 (0.61) |
| Total P, %        | 0.67 (0.66) | 0.61 (0.58) | 0.54 (0.54) |
| Phytic P, %       | 0.24 (0.26) | 0.23 (0.25) | 0.23 (0.25) |
| Na, %             | 0.22 (0.21) | 0.19 (0.16) | 0.16 (0.14) |

*Analyzed nutrients in the parenthesis
test diets were formulated with 500, 1000, and 1500 FTU/kg of an *Escherichia coli*-derived phytase (AB Vista, Marlborough, UK) assigned respectively an avP matrix of 0.15, 0.19, and 0.23%; the Ca matrix was set at 0.15% for all doses of phytases. An additional diet containing extra avP and Ca (+0.05% avP; +0.1% Ca) to that of PC was included to make sure the PC was not deficient in avP and Ca. Test diets were steam-pelleted to 3 mm diameter with a conditioning temperature of 85°C and were offered ad-libitum as fine-crumbles (1-10 d), coarse-crumbles (11-25 d) or pellets (26-42 d).

Feed samples were analyzed for proximate constituents and total P and Ca were analyzed using induction coupled plasma method (AOAC 2005). Milled sample of test feeds were scanned using a FOSS NIR spectrophotometer for the prediction of phytate-P (Enzyme Services and Consultancy, UK). The phytase activity (Engelen et al., 1994; Engelen et al., 2001) in the test diets was analyzed in Enzyme Services and Consultancy, UK. Briefly, feed samples were extracted for 30 min in 25 mM borate buffer at pH 10 and analysis was conducted at pH 4.5 and 60°C using sodium phytate as substrate. The phytase activity was based on the endpoint determination of phosphate using molybdovanadate color system. One phytase unit (FTU) is therefore defined as the amount of enzyme which liberates 1 micromole inorganic phosphorus per minute from sodium phytate at pH 5.50 and 37.0°C and under the specific conditions of the assay as described herein.

The experiment was conducted in a close-sided house with evaporative cooling system and concrete floor pens using rice hull as bedding material. Each pen measured 2.448 m² and was equipped with a tabular-self-feeder and tubular water drinkers. Feed and water were provided *ad libitum*. Birds were maintained under the lighting and management programs according to the supplier's management manual. The max/min temperature and relative humidity in the experimental house were 34.3/27.3°C and 57.5% during 0-7 days of age, 30.6/26.0°C and 67.1% during 7-14 days of age, 28.2/24.9°C and 72.5% during 14-28 days of age and 28.1/25.1°C and 72.5% during 28-42 days of age. All birds were vaccinated for Newcastle, Infectious Bronchitis, and Gumboro diseases at the hatchery.

Pen feed intake (FI) and body weight (BW) were recorded at the end of every feeding phase, and feed conversion ratio (FCR) corrected for mortality was calculated. At 25 d two birds (one male and one female) close to the pen average weight, from each pen were chosen for the measurement of toe ash. Body weight, feed intake, feed conversion ratio, livability, and toe ash were calculated and subjected to analysis of variance as a randomized complete block design using SPSS. Means were compared using Tukey’s test using *p*<0.05 as conventional significance.

The study was conducted in Indonesia following the Law of the Republic of Indonesia No. 18, 2009 regarding the animal welfare and use in veterinary research.

### RESULTS AND DISCUSSION

The analyzed total P, Ca, and phytic P were in close agreement with the calculated values (Table 2). The phytase activity in starter and grower diets was close to the expected while the finisher diets showed lower than expected activity (Table 3). The average growth performance in this experiment was 3132 g BW and 1.60 FCR at 42 d which was marginally exceeding to the breed standard (Aviagen, 2014a).

| Table 3 – Analyzed activity of phytase enzyme in test diets. |
| --- |
| Treatment | Expected Phytase (FTU/kg) | Analyzed Phytase (FTU/kg) |
| |
| |
| PC | None | <LD |
| PC + | None | <LD |
| -0.15% AvP; -0.15% Ca | 500 | 381 |
| -0.19% AvP; -0.15% Ca | 1000 | 798 |
| -0.23% AvP; -0.15% Ca | 1500 | 1,150 |

<LD = below limit of detection (< 50 FTU/kg).

Variability in the published estimates of the avP and Ca requirements of broilers (Yan et al., 2003; Yan et al., 2004; Fritts & Waldroup 2006; Rousseau et al., 2012) makes it relevant to question the avP and Ca level in PC. It becomes more crucial in experiments aimed at establishing the ‘mineral-sparing’ effect of phytase since an over-formulation of avP in PC would likely lead to an erroneous overestimation of this effect. In view of this, we set avP and Ca level in our PC that was lower than those suggested by the major genetic suppliers as well as those practiced in the most commercial scenarios. In order to test if PC in our experiment was sufficient in...
avP and Ca, an extra treatment (PC++) with additional avP and Ca (+0.05% AP/+0.1% Ca added to PC) was included. Compared with the PC, the treatment group with additional avP and Ca had lower (p<0.05; 10 and 25 d) or similar (42 d) BW (Table 4) and toe ash (Table 5). Depressed growth performance at PC++ appears to relate to reduced FI mediated by higher Ca (PC++ had 0.1% higher Ca than PC); similar effects of high dietary Ca have recently been reported in other studies using broiler (Ravindran, 2016) and pig (González-Vega, 2016) models. These data suggests that avP/ Ca level of 0.4/0.80, 0.35/0.70, and 0.30/0.60%, as were set respectively for 1-10, 11-25, and 26-42 d in our PC were sufficient to support the optimal growth performance and bone mineralization of broilers (Aftab & Creswell, 2019).

Test diets were formulated with 500, 1000, and 1500 FTU/kg of phytase assigned respectively an avP/ Ca matrix of 0.15/0.15, 0.19/0.15, and 0.23/0.15. As a result of the increasing dosages of phytase, a stepwise reduction in the supplemental monocalcium phosphate (MCP) was seen until all added MCP was removed and hence no further reduction in the calculated avP was possible. As a consequence, the final two diets of the finisher series i.e. -0.19% and -0.23% AP were exactly the same except that the latter had higher phytase than the former (Table 1).

Compared to the PC, diets containing 1000 and 1500 FTU phytase had higher BW (p<0.05) at 10 and 25 d (Tables 4). Improved BW at higher doses of phytase may be a result of the release of nutrients and provision of inositol associated with more complete phytate hydrolysis in the gut (Walk et al., 2014; Cowieson et al., 2011); this apparent advantage however did not continue through to the advance age and no across treatment differences were observed for 42 d BW and FCR. Percentage-, but not the absolute-, toe ash at phytase treated groups was significantly lower compared with the PC which was a result of numerically high weights of dried toes at all groups treated with phytase (Table 5).

The observation that 1500 FTU/kg of enhanced E. coli phytase supported optimal growth performance of broilers in the diets largely void of inorganic phosphates was in line with a previous study at our lab which using high phytate, corn-soy-bran diet, demonstrated a complete substitution of supplemental inorganic phosphate in the grower (15-28 d) and finisher (29-42 d) diets with 1500 FTU of phytase; the starting (0-14 d) diet in this experiment had marginal (1.2 kg per metric ton) added dicalcium phosphate (Aftab 2017). These findings corroborate the earlier mechanistic

### Table 4 – Growth performance of broilers for 1-10, 1-25, and 1-42 d, posthatch.

| Treatment | Added phytase, FTU | Added MCP kg per MT* | Feed intake, g | Body weight, g | FCR |
|-----------|--------------------|----------------------|----------------|----------------|-----|
| **1-10 d** |                    |                      |                |                |     |
| PC        | 0                  | 13.4                 | 330a           | 330b           | 0.998 |
| PC ++     | 0                  | 15.7                 | 317b           | 320c           | 0.990 |
| -0.15% avP| 500                | 6.3                  | 337b           | 336a           | 1.004 |
| -0.19% avP| 1,000              | 4.4                  | 340a           | 340a           | 0.995 |
| -0.23% avP| 1,500              | 2.6                  | 340a           | 340a           | 0.999 |
| Probability | <0.001          |                      |                |                |     |
| Pooled SEM| 2.75               |                      | 2.39           | 0.006          |     |
| **1-25 d** |                    |                      |                |                |     |
| PC        | 0                  | 13.4/11.4            | 1949ab         | 1468b          | 1.316b |
| PC ++     | 0                  | 15.7/13.7            | 1913b          | 1441b          | 1.318b |
| -0.15% avP| 500                | 6.3/4.3              | 1951b          | 1469b          | 1.326b |
| -0.19% avP| 1,000              | 4.4/2.5              | 1981b          | 1505b          | 1.277b |
| -0.23% avP| 1,500              | 2.6/0                | 1982b          | 1505b          | 1.312b |
| Probability | <0.001          |                      | <0.001         | 0.003          |     |
| Pooled SEM| 12.90              |                      | 8.73           | 0.004          |     |
| **1-42 d** |                    |                      |                |                |     |
| PC        | 0                  | 13.4/11.4/9.5        | 5136           | 3142           | 1.591 |
| PC ++     | 0                  | 15.7/13.7/11.8       | 5003           | 3080           | 1.607 |
| -0.15% avP| 500                | 6.3/4.3/2.4          | 5056           | 3094           | 1.605 |
| -0.19% avP| 1,000              | 4.4/2.5/0            | 5130           | 3182           | 1.601 |
| -0.23% avP| 1,500              | 2.6/0/0              | 5127           | 3164           | 1.601 |
| Probability | 0.501           |                      | 0.498          | 0.820          |     |
| Pooled SEM| 47.56              |                      | 64.25          | 0.01           |     |

* Means within the same column with no common superscript differ significantly (p<0.05); **1-10 d/11-25 d/26-42 d.
work showing a complete elimination of Inositol 6- and 5-phosphate, coupled with a significant rise in the free-inositol in the gizzard content of young broiler chickens with 1500 FTU (Walk et al., 2014). Indeed, an extensive clearing of the higher phytic esters in the gastric phase is fundamental to the extent of phytate hydrolysis across the entire gastrointestinal tract (Walk et al., 2014; Troung et al., 2016). It is important to highlight, however, that several other nutritional factors influence in-vivo phytate hydrolysis and hence the optimization of these factors including e.g. dietary Ca (Tamim et al., 2004), avP (Rodehutscord, 2016), and vitamin D3 (Mohammed et al., 1991) need to be considered in addition to the proper dose of phytase when target is to seek a complete elimination of supplemental inorganic phosphates in broiler diets.

In conclusion, the results of the current experiment demonstrated that increasing doses of phytase could help reduce significantly the reliance on inorganic phosphates. Our results showed that 1500 FTU/kg of enhance E. coli phytase supported optimal BW and FCR of broilers fed corn-soy diet largely void of supplemental inorganic phosphate i.e. 2.6 kg per metric ton for starter (1-10 d) diet with no added phosphate for grower (11-25 d) or finisher (26-42 d) diets. This would have marked implications for sparing of non-renewable phosphate reserves and excretion of phosphorus in poultry manure.

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