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Original Article

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

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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 phytate destruction (dos Santos *et al.,* 2014; Gehring *et al.,* 2013; Walk *et al.,* 2014). In addition to the tangible commercial benefits, the use of higher doses of phytases helps reduce reliance on non-renewable inorganic phosphate reserves (Cordell 2016) and a further reduction in manure P (Kies *et al.,* 2006).

The current experiment was designed to study the effect of incremental doses of an enhanced *E. coli* phytase on the growth performance and bone mineralization of broilers fed diets gradually reduced on avP and Ca. Our main objective was to test the hypothesis that a higher dose (1500 FTU per kg) of an enhanced *E. coli* phytase should enable an all-vegetable, corn-soy diet void of supplemental inorganic phosphate to be fed without negatively affecting the growth performance of broilers.

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[A]	500	6.3	4.3	2.4
-0.19% AvP; -0.15% Ca[A]	1000	4.4	2.5	-
-0.23% AvP; -0.15% Ca[A]	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.

[A] assumed avP and Ca matrix for corresponding dose of phytase.

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 m*M* borate buffer at pH 10 and analysis was conducted at pH 4.5 and 60ºC 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 m^2 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)		
		1-10 d	11-25 d	26-42 d
PC	None	<LD	<LD	<LD
PC + +	None	<LD	<LD	<LD
-0.15% AvP; -0.15% Ca	500	381	492	370
-0.19% AvP; -0.15% Ca	1000	798	860	834
-0.23% AvP; -0.15% Ca	1500	1,150	1,200	1,080

<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 ($p<0.05$) low compared with the PC which was a result of numerically high weights of dried toes at all groups treated with phytase (Table 5).

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	330[a]	330[b]	0.998
PC + +	0	15.7	317[b]	320[c]	0.990
-0.15% avP	500	6.3	337[a]	336[ab]	1.004
-0.19% avP	1,000	4.4	340[a]	340[a]	0.995
-0.23% avP	1,500	2.6	340[a]	340[a]	0.999
Probability			*<0.001*	*<0.001*	*0.488*
Pooled SEM			*2.75*	*2.39*	*0.006*
				1-25 d	
PC	0	13.4/11.4	1949[ab]	1468[b]	1.316[b]
PC + +	0	15.7/13.7	1913[b]	1441[b]	1.318[b]
-0.15% avP	500	6.3/4.3	1951[ab]	1469[b]	1.326[b]
-0.19% avP	1000	4.4/2.5	1981[a]	1505[a]	1.277[a]
-0.23% avP	1500	2.6/0	1982[a]	1505[a]	1.312[ab]
Probability			*<0.001*	*<0.001*	*0.003*
Pooled SEM			*12.90*	*8.73*	*0.004*
				1-42d	
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	1000	4.4/2.5/0	5130	3182	1.601
-0.23% avP	1500	2.6/0/0	5127	3164	1.601
Probability			*0.501*	*0.498*	*0.820*
Pooled SEM			*47.56*	*64.25*	*0.01*

[a-c] Means within the same column with no common superscript differ significantly ($p<0.05$); *1-10 d/ 11-25 d/26-42 d.

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 a high phytate, corn-soy-rice 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 5 – Dried toe weight, toe ash weigh and toe ash percentage of broilers at 25 d.

Treatment	Added phytase, FTU	Added MCP kg per metric ton*	Dried toe weight, mg	Toe ash weight, mg	Toe ash, %
PC	0	13.4/11.4	2657	345	13.03[a]
PC + +	0	15.7/13.7	2782	351	12.62[ab]
-0.15% avP	500	6.3/4.3	2841	342	12.06[b]
-0.19% avP	1000	4.4/2.5	2786	342	12.26[b]
-0.23% avP	1500	2.6/0	2872	347	12.09[b]
Probability			*0.156*	*0.772*	*0.002*
Pooled SEM			*50*	*4*	*0.13*

[a-c] Means within column with no common superscript differ significantly ($p<0.05$)

1-10 d/11-25 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 clearance 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.

REFERENCES

Aftab U. Towards an inorganic phosphate free nutrition for broilers. Asian Feed 2017;(dec.):30-31.

Aftab U, Creswell. Optimizing phosphorus in broiler diets. Asian Poultry 2019;(mar.):32-35.

AOAC - Association of Official Agricultural Chemists. Official methods of analysis. 18th ed. Gaithersburg; 2005.

Augspurger NR, Webel DM, Lei XG, Baker DH. Efficacy of an *E. coli* phytase expressed in yeast for releasing phytate-bound phosphorus in young chicks and pigs. Journal of Animal Science 2003;81:474-483.

Cordell D. Global phosphorus scarcity: a food secure future. Proceedings of 27th Annual Australian Poultry Science Symposium; 2016 Feb. 14-17. Sydney (AUS): New South Wales; 2016. p.153-157.

Cowieson AJ, Wilcock P, Bedford MR. Super-dosing effect of phytase in poultry and other monogastrics. World's Poult. Sci. J. 2011; 67:225-236.

Dersjant-Li Y, Awati A, Schulze H, Partridge G. Phytase in non-ruminant animal nutrition: a critical review on phytase activities in the gastrointestinal tract and influencing factors. Journal of the Science Food and Agriculture 2015;95:878-896.

Engelen AJ, Heeft FC van der , Randsdorp PHG, Smit ELC. Simple and rapid determination of phytase activity. Journal of AOAC International 1994;77:760-764.

Engelen AJ, Heef FC van der , Randorp PHG, Somer WAC. Determination of phytase activity in feed by a colorimetric enzymatic method: Collaborative inter laboratory study. Journal of AOAC International 2001;84:629-633.

Fritts CA, Waldroup PW. Modified feeding program for broilers based on commercial feeding intervals to sustain live performance and reduce total and water soluble phosphorus in litter. Journal of Applied Poultry Research 2006;15:207-218.

Gehring, CK, Bedford MR, Dozier III WA. Extra-phosphoric effects of phytase with or without xylanase in corn-soybean meal-based diets fed to broilers. Poultry Science 2013;92:979-991.

González-Vega JC, Stein H. Calcium transporters and gene expression and absorption of calcium in pigs. In: Walk CL, Kuhn I, Stein HH, Kidd MT, Rodehutscord M, editors. Phytate destruction - consequences for precision animal nutrition. Wagening: Academic Publisher, 2016.

Kies, AK, Kemme PA, Sebek LBJ, Piepen JM, Jongbloed AW. Effect of graded doses and a high dose of microbial phytase on the digestibility of various minerals in weaner pigs. Journal of Animal Science 2006;84:1169-1175.

Mohammed A, Gibney MJ, Taylor TG. The effects of dietary levels of inorganic phosphorus, calcium and cholecalciferol on the digestibility of phytate phosphorus by the chick. British Journal Nutrition 1991;66:251–259.

Ravindran V. Measurement of calcium digestibility in feed ingredients in poultry: methodology and challenges. In: Walk CL, Kuhn I, Stein HH, Kidd MT, Rodehutscord M, editors. Phytate destruction - consequences for precision animal nutrition. Wagening: Academic Publisher, 2016.

Rodehutscord, M. Interactions between minerals and phytate degradation in poultry – challenges for phosphorus digestibility assays. In: Walk CL, Kuhn I, Stein HH, Kidd MT, Rodehutscord M, editors. Phytate destruction - consequences for precision animal nutrition. Wagening: Academic Publisher, 2016

Rousseau, X, Lotourneau-Montmin MP, Meme N, Magnin M, Nys Y, Narcy A. Phosphorus utilization in finishing broiler chickens: effects of dietary calcium and microbial phytase. Poultry Science 2012; 91:2829-2837.

Santos TT, Walk CL, Srinongkot S. Influence of phytate level on broiler performance and the efficacy of two microbial phytase from 0 to 21 days of age. Journal of Applied Poultry Research 2014;23:181-187.

Tamim NM, Angel R, Christman M. Influence of dietary calcium and phytase on phytate phosphorus hydrolysis in broiler chickens. Poultry Science 2004;83:1358-1367.

Truong HH, Yu S, Moss AF, Liu SY, Selle PH. Phytase degradation in gizzard is pivotal to phytase response in broiler chickens. Proceedings of 27th Annual Australian Poultry Science Symposium; 2016 Feb. 14-17. Sydney (AUS): New South Wales; 2016. p.174-177.

Van der Klis JD, Star L. Efficacy of different phytase products in broilers. Proceedings of the 18th European Symposium Poultry Science; 2013; Çesme (TUR).

Walk CL, Wilkinson SJ, Bedford MR, Cowieson AJ. Influence of conditioning temperature on post-pellet efficacy of three microbial phytases for broilers from d 0 to 21. Proceedings of the 18th European Symposium Poultry Science; 2013; Çesme (TUR).

Walk. C, Santos TT, Bedford MR. Influence of superdoses of a novel microbial phytase on growth performance, tibia ash, and gizzard phytate and inositol in young broilers. Poultry Science 2014;93:1172-1177.

Yan F, Fritts CA, Waldroup PW. Evaluation of modified phosphorus levels with and without phytase supplementation on live performance and fecal phosphorus levels in broiler diets. 1. Full-term feeding recommendation. Journal Applied Poultry Research 2003;12:174-182.

Yan. F, Fritts CA, Waldroup PW. Evaluation of modified phosphorus levels with and without phytase supplementation on live performance and fecal phosphorus levels in broiler diets. 2. Modified early phosphorus levels. Journal Applied Poultry Research 2004;13:394-400.

Zeller, E, Schollenberger M, Kuhn I, Rodehutscord M. Hydrolysis of phytate and formation of inositol phosphate isomers without or with supplemental phytases in different segments of digestive tract of broilers. Journal of Nutritional Science 2015;4:1-12.