Evaluation of the efficacy of a novel phytase in short-term digestibility and long-term egg production studies with laying hens

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ABSTRACT Three independent trials were conducted to evaluate the efficacy of a novel phytase in laying hens. Trial 1 used a total of 90 laying hens (Lohmann Brown, 33-wk-old) fed either a negative control (**NC**) diet with 0.09% non-phytate P (**NPP**) or NC supplemented with 187.5 or 375 FYT phytase/kg feed for 4 d before collection of excreta and ileal digesta to measure ileal digestibility and retention of Ca and P. In trial 2 and 3, a total of 108 laying hens (Hy Line Brown, 25-wk-old) and 360 hens (Lohman Brown, 25-wk-old) were used, respectively. In both trials, the hens were randomly assigned to 3 dietary treatments: NC with 0.1% NPP, positive control (**PC**) and NC plus 187.5 FYT phytase/kg feed, the experimental diets were fed for 12 wk, and egg production and bone mineralization were measured. The results showed that the ileal digestibility of P increased both linearly ($P = 0.012$) and quadratically ($P = 0.01$) with increasing supplementation of phytase in trial 1. In trial 2, phytase supplementation significantly improved egg production, egg weight, and feed conversion ratio and reduced the percentage of broken eggs during the overall trial duration compared with NC. In trial 3, phytase significantly improved egg production, egg weight, and feed intake and reduced the percentage of broken eggs during the entire trial duration. In addition, percentage and weight of bone Ca and P increased significantly with added phytase. In trial 2 and 3, there was no significant difference between PC and the phytase treatment. In conclusion, the novel phytase significantly increased the ileal digestibility of P in a short-term digestibility study and improved egg production and bone mineralization in a 12-wk laying cycle. Ileal digestibility of P rather than P retention in short-term digestibility studies as well as egg production and whole tibia mineralization in long-term studies should be measured to demonstrate the efficacy of phytase in laying hens.

Key words: digestibility, egg production, laying hen, phosphorus, phytase

INTRODUCTION

Good eggshell quality results from feeding diet high in Ca and low in P, however, both insufficient and excessive dietary P impair egg production and egg quality (El Boushy, 1979; Härtel, 1990; Leeson et al., 1993). Therefore, the P requirement in laying hens can be determined by optimizing the responses of egg production and egg quality. Egg production provides the best measure of adequacy of P nutrition (Scott et al., 2001). With 21-wk-old hens, feeding 0.1% non-phytate P (**NPP**) decreased egg production, feed consumption, egg weight, and egg specific gravity over the 17-wk trial

duration whereas no deficiency symptoms were observed in hens fed diets containing 0.2 to 0.5% NPP (Gordon and Roland, 1997). A diet containing 0.15% available P (**AP**) supported optimal egg production from 20 to 70 wk of age (Boling et al., 2000a,b). It is evident that the laying hens have very low P requirements but higher P equivalence to phytase when compared to broilers (van der Klis et al., 1997).

The efficacy of phytase in laying hens depends on strain and age of hens, lay rate, dietary Ca level, duration of P deprivation, dose of phytase, dietary composition, and phytate content (Gordon and Roland, 1997; Kamińska, 1997; van der Klis et al., 1997; Boling et al., 2000a,b; Nie et al., 2013). In terms of dose of phytase, satisfactory egg production appeared to have been achieved by low supplemental levels of phytase. In the trial by Gordon and Roland (1997), all the adverse effects of feeding hens the 0.1% NPP diet were completely overcame with the supplementation of 300 U/kg phytase. However, Simons et al. (1992)

investigated varying levels of phytase supplemented to a diet without added P and concluded that 200 U of phytase/kg feed were enough to improve egg performance and additional phytase supplementation gave no further benefits. Moreover, van der Klis et al. (1997) and Boling et al. (2000a) demonstrated that 100 U/kg phytase averted the deleterious effect of a corn-soybean meal diet containing 0.10% AP on egg performance for 40- to 50-wk laying cycles.

To demonstrate the efficacy of phytase in layers, both short-term and long-term studies can be performed (EFSA, 2018). The short-term efficacy trials have the advantage of short trial duration and the resultant saving of resources. Their potential drawback could be the difficulty in showing significant improvement either in P retention or P digestibility plus P retention in bone as prescribed by EFSA (2018) considering the complexity of P metabolism in hens associated with egg production and the individual animal variability. The current study aimed to evaluate the efficacy of a novel phytase in laying hens in both short-term digestibility and long-term egg production trials. In addition, this would allow gaining an understanding about phytase efficacy demonstration with these 2 types of trials.

MATERIALS AND METHODS

The paper comprises 3 independent trials. Trial 1 and 2 were conducted at DSM Nutritional Products Research Center for Animal Nutrition and Health (CRNA, Village-Neuf, France). All applied procedures were approved by the CRNA ethical committee (CEEA-123) and complied with the official French regulation on use of animals for experimental purposes under the EU regulation (DIRECTIVE 2010/63/UE). Trial 3 was performed at Sichuan Agricultural University (Chengdu, China) with its protocol approved by Animal Care and Use Committee of Sichuan Agricultural University.

Animals

In trial 1, ninety laying hens (Lohmann Brown, 33 wk of age) were fed a standard diet for 3 wk prior to the commencement of trial. During this pre-trial period egg production and feed intake were recorded. The hens were randomly allocated in 90 cages to be fed the experimental diets for 7 d. Each experimental diet was offered to 30 cages of hens as replicates. In trial 2 and 3, one hundred eight laying hens (Hy Line Brown, 25 wk of age) and 360 hens (Lohman Brown, 25 wks of age) were used, respectively. The hens were housed individually in battery cages in trial 2 and in groups of 4 in trial 3. There were 36 and 30 cages/replicates for each treatment in trial 2 and 3, respectively. The hens in trial 2 weighed 1.97 ± 0.14 kg (mean ± standard deviation) at the start of trial and 2.03 ± 0.121 kg at the end, whereas the hens in trial 3 weighed 1.60 ± 0.06 kg and 1.74 ± 0.11 kg accordingly. In both trials 2 and 3, the experimental diets were fed for 84 d. The hens were randomly

assigned to 3 dietary treatments considering their initial body weight, recorded egg production, and the spatial distribution of cages. In all these trials, the animal houses were environmentally controlled to achieve a room temperature of 18 to 22°C. Water and feed were supplied ad libitum.

Experimental Diets

In trial 1, there were 3 experimental diets including a NC diet deficient in P and the NC supplemented with 187.5 or 375.0 phytase units (**FYT**)/kg phytase (HiPhorius, DSM Nutritional Products, Switzerland). In trials 2 and 3, there were 3 experimental diets including a NC diet deficient in P, a P adequate diet (**PC**) and the NC supplemented with 187.5 FYT/kg phytase. The PC diets of trial 2 and 3 included 1.30 and 0.59% dicalcium phosphate, respectively, and met the hen's nutrient requirement (NRC, 1994). The NC diets were formulated to be devoid of any mineral P supplement.

All diets were supplied in mash form. The ingredient and nutrient composition of the basal diets in trials 1 to 3 are presented in Table 1. Titanium dioxide was included at 1 g/kg feed as an indigestible marker in trial

Table 1. Ingredient and nutrient composition of the negative control diets (g/kg of feed, as-is basis).

Items	Trial 1	Trial 2	Trial 3
Ingredients			
Corn	648.0	557.0	617.4
Soybean meal	235.0	220.0	206.0
Sunflower meal		80.0	
Rapeseed meal			50.0
Soybean oil	17.5	30.0	20.0
Salt	1.0	1.0	2.1
$NaHCO_3$	1.0	1.0	
Na_2CO_3			2.0
Limestone	82.0	96.0	93.0
L-Lysine•HCl, 78%	0.5	0.5	0.8
DL-Methionine	2.0	1.5	2.4
Choline chloride	0.0	0.0	1.0
Vitamin-mineral premix[1]	10.0[1]	10.0[1]	5.3[2]
Sand	2.0	3.0	
TiO_2	1.0	0.0	0.0
Total	1,000.0	1,000.0	1,000.0
Calculated nutrients and energy			
Crude protein	15.8	17.1	16.7
ME, MJ/kg	2873	2811	2770
Total Ca	34.3	40.0	36.0
Total P	3.2	3.4	3.5
Non phytate P	0.9	1.0	1.1
Phytate P	2.3	2.4	2.4
Digestible lysine	7.6	7.7	8.6
Digestible methionine	4.2	4.1	5.0

[1]Vitamin-mineral premix provided per kilogram of diet: vitamin A: 10,000 IU; vitamin D_3: 3,000 IU; vitamin E: 30 IU; vitamin K_3: 2.5 mg; vitamin C: 100 mg; vitamin B_1: 2.00 mg; vitamin B_2: 6.00 mg; vitamin B_6: 4.00 mg; vitamin B_{12}: 0.02 mg; niacin: 30.0 mg; pantothenate acid: 8.0 mg; folic acid: 0.80 mg; biotin: 0.13 mg; choline: 260 mg; Na: 1.0 g; Cl: 1.6 g; Mn: 80 mg; Fe: 50 mg; Cu: 10 mg; Zn: 70 mg; I: 1.24 mg; Se: 0.2 mg; Ca: 2.4 g.
[2]Vitamin-mineral premix provided per kilogram of diet: vitamin A: 10,000 IU; vitamin D_3: 2,500 IU; vitamin E: 30 IU; vitamin K_3: 2.5 mg; vitamin B_1: 2.00 mg; vitamin B_2: 5.00 mg; vitamin B_6: 2.00 mg; vitamin B_{12}: 0.02 mg; niacin: 24.0 mg; pantothenate acid: 6.0 mg; folic acid: 1.00 mg; biotin: 0.12 mg; choline: 260 mg; Mn: 60 mg; Fe: 60 mg; Cu: 8 mg; Zn: 80 mg; I: 0.35 mg; Se: 0.2 mg.

1 to allow for measurement of digestibility and retention of Ca and P. The test phytase was encoded by a 6-phytase gene from *Citrobacter braakii* as described by Zhai et al. (2021).

Sampling and Measurement

In trial 1, each hen was a statistical unit. The hens were weighed at the beginning and the end of trial, and feed consumption was recorded. Excreta from individual hens were quantitatively collected during 3 consecutive days after an adaptation of 4 d to the experimental diets. The excreta collected from the 3 d was pooled per hen, homogenized and subsampled for freeze-drying and further analyses. Two eggs were randomly collected during the excreta collection period from each hen for the analysis of Ca and P. At the end of trial, 20 hens per treatment were selected and euthanized by cervical dislocation. The contents of the terminal part of the ileum, defined as the posterior 15-cm section between Meckel's diverticulum and 2 cm anterior to the ileocecal conjunction, and the right tibias were collected. Fifteen ileal samples per treatment were abundant and thus used for the analysis. The diaphysis of tibia from each of 20 hens per treatment was sawed, removed of the muscular tissues, and kept in a plastic bag at $-20°C$ until further processing.

In trial 2, the statistical analysis unit was the 3 stacked cages for egg production due to concern of feed spillage from top-tier cages to cages underneath. Individual hens were the statistical analysis units for bone parameters. Eggs were collected daily during the 3 successive 4-wk periods of the trial and the number of broken eggs was recorded for each hen. Egg production parameters (hen-day egg production, egg weight, feed conversion ratio [**FCR**], average daily feed intake [**ADFI**], broken eggs) were aggregated for each group of 3 stacked cages (n = 12 per treatment). All the collected eggs (≤ 28) per hen during each 4-wk period were stored in a fridge at 4°C and weighed at the end of each period. At the end of trial, 18 hens per treatment were randomly selected and euthanized to collect the bone samples as described in trial 1.

In trial 3, the statistical analysis units were the individual cages for both egg production and bone mineralization. Eggs were collected daily during the 3 successive 4-wk periods of the trial and the number of broken eggs was recorded for each cage. All the collected eggs (≤ 112) per cage during each 4-wk period were stored in a fridge at 4°C and weighed at the end of each period. At the end of trial, one hen per cage was randomly selected and euthanized to collect the right tibia. The whole tibia was removed of the soft tissues and cartilaginous caps before storage at $-20°C$ until further processing.

Chemical Analyses

In trial 1, the ileal and excreta samples were freeze-dried to a constant weight and ground to pass through a 0.5-mm screen before analysis. The samples were dried at 105°C in an oven for 4 h for dry matter determination (method 934.01; AOAC International, 2006). The bone samples were oven-dried, incinerated and dissolved in sulfuric acid before the measurement of Ca and P. For analysis of Ca and P, the egg contents and eggshell were separated manually. The eggshell was dried in an oven at 105°C for 4 h for dry matter determination and then dissolved in sulfuric acid for the analysis of Ca and P. The egg contents were freeze-dried and then ashed at 550°C before the acid dissolution and chemical analysis. Titanium, Ca, and P were determined by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES; 5100 Dual View, Agilent, Santa Clara, CA or Optima TM 8000, PerkinElmer, Shelton, CT; method 985.01; AOAC International, 2006). Phytase activity was expressed in FYT. One FYT was defined as the amount of enzyme which liberated 1 μmol inorganic phosphate per minute from a 0.0051 M sodium-phytate solution at pH 5.5 and 37°C.

Statistical Analyses

In trial 1, the results were analyzed by one-way ANOVA. Digestibility of Ca and P were calculated using the index method. A polynomial contrast was constructed to test the linear effect of phytase, and a specific test was planned to compare control diet with the diets added with phytase.

In trials 2 and 3, the results were analyzed by one-way ANOVA and Tukey's test was used for multiple comparisons with the exception of percentage of broken eggs. The significance of differences in the percentage of broken eggs was examined using the Kruskal-Wallis test and the nonparametric comparisons were performed with the Steel-Dwass test.

All the statistical analyses were performed with JMP 15.1 (SAS Institute, Cary, NC). The statistical significance was defined at $P < 0.05$.

RESULTS

Analyzed Ca, P, and Phytase Activity

The analyzed Ca levels were generally higher compared to the formulated values but similar among the treatments in each trial (Table 2). The analyzed P levels were 90 to 103% of the target values. The analyzed phytase activities were about 20 to 30% higher than the targets in trial 1, but the increments in phytase activity met the purpose of this trial. In trial 2 and 3, the analyzed phytase activities were very close to the targets.

Digestibility and Retention (Trial 1)

The ileal digestibility of P increased both linearly ($P = 0.012$) and quadratically ($P = 0.01$) with increasing supplementation of phytase (Table 3). The phytase treatments gave significantly higher ileal digestibility of

Table 2. Analyzed nutrients in the experimental diets (as-is basis).

Items	Calcium, %	Phosphorus, %	Phytase, FYT/kg
Trial 1			
Negative control	3.6	0.33	LOD[1]
187.5 FYT/kg phytase	3.8	0.33	231.0
375 FYT/kg phytase	3.8	0.33	494.0
Trial 2			
Negative control	3.8	0.34	LOD
Positive control	4.1	0.58	LOD
187.5 FYT/kg phytase	4.1	0.33	176.0
Trial 3			
Negative control	4.1	0.32	LOD
Positive control	3.9	0.41	LOD
187.5 FYT/kg phytase	3.8	0.31	175.0

[1]LOD, under limit of detection.

P compared with the control. There was no significant difference in P retention based on index and total collection methods, and the total collection method generated negative retention results with greater variation (data not shown).

Mineral Deposition in Eggs (Trial 1)

The addition of phytase decreased the percentage of eggshell and increased the percentage of egg contents ($P < 0.05$; Table 3). The dry matter concentration of egg contents decreased linearly ($P < 0.001$) with added phytase, which contrasts with a linear increase in the concentrations of Ca and P ($P < 0.05$).

Egg Production (Trial 2 and 3)

In trial 2, phytase supplementation significantly improved egg production during the 1st four wk and the overall 12 wk, egg weight during all phases excluding the 2nd four wk, and FCR during all phases, and reduced the percentage of broken eggs during the 2nd four wk and the overall duration when compared with NC (Table 4). The PC showed significant improvement in egg production during the 3rd four wk, egg weight during the 2nd four wk, ADFI during the 3rd four wk and FCR during the 2nd wk and the overall 12 wk relative to NC.

In trial 3, dietary inclusion of DCP or phytase significantly improved egg production and egg weight and reduced the percentage of broken eggs during the 2nd and 3rd four wk and the overall 12 wk whereas an improvement in ADFI was observed in all trial phases (Table 5). Feed conversion ratio was significantly higher for phytase treatment than NC during the 1st four wk, but this relationship was reversed during the 3rd four wk.

There was no significant difference in any of the studied parameter between PC and the phytase treatment in both trials.

Bone Mineralization (Trial 1 to 3)

No significant difference in bone mineralization among treatments was observed in trial 1 (Table 3). In trial 2, significant improvement in percentage bone ash, Ca, and P were observed in hens of PC than phytase treatment with the results of NC being intermediate whereas the weight of tibia diaphysis as well as the

Table 3. Ileal digestibility and retention of calcium and phosphorus and their deposition in eggs and bones (trial 1)[1,2].

	Phytase				Significance level	
	0	187.5	375.0	SEM	Phytase	Linear
Ileal digestibility, %						
Dry matter	70.5	67.1	66.7	2.94	0.318	0.688
Calcium	86.5	81.1	77.3	4.35	0.181	0.877
Phosphorus	51.2	68.5	64.1	3.37	< 0.001	0.012
Retention, %						
Dry matter	70.6	69.8	69.1	0.94	0.339	0.999
Calcium	39.6	46.5	41.7	4.37	0.407	0.282
Phosphorus	12.3	13.1	18.7	2.78	0.290	0.487
Egg						
Egg weight, g	61.3	61.0	59.6	0.23	0.281	0.122
Eggshell, %	13.6	12.7	13.0	0.28	0.043	0.168
Egg contents, %	86.4	87.3	87.0	0.28	0.043	0.168
Eggshell, %						
Dry matter	71.4	72.8	73.0	0.94	0.187	0.214
Calcium	35.5	35.7	35.5	0.16	0.801	0.864
Phosphorus	0.11	0.11	0.10	0.01	0.239	0.065
Egg contents, %						
Dry matter	26.35	24.64	23.40	0.46	< 0.001	< 0.001
Calcium	0.19	0.20	0.21	0.01	0.010	0.013
Phosphorus	0.68	0.72	0.76	0.01	< 0.001	< 0.001
Bone, %						
Ash	64.2	64.1	64.2	0.54	0.863	0.955
Calcium	23.9	23.9	24.0	0.22	0.774	0.608
Phosphorus	10.7	10.9	11.0	0.09	0.570	0.397

[1]The control diet was formulated to provide 34.3 g/kg Ca and 0.9 g/kg non-phytate P; the phytase activity was analyzed as 231 and 494 FYT/kg feed for the phytase treatments.

[2]There were 30 replicates for retention and egg parameters, 20 replicates for bone parameters and 15 replicates for ileal digestibility.

Table 4. Egg production and bone mineralization in laying hens (trial 2)[1,2].

Items	NC[3]	PC[3]	187.5	SEM[3]	Significance level
		Treatment			
Egg production, %					
1st 4 wk[4]	89.0[b]	92.8[ab]	96.0[a]	1.56	0.012
2nd 4 wk[4]	86.5	90.2	93.7	2.52	0.139
3rd 4 wk[4]	81.8[b]	92.5[a]	90.4[ab]	2.68	0.019
Overall	85.7[b]	91.8[ab]	93.4[a]	1.85	0.015
Egg weight, g					
1st 4 wk	56.1[b]	57.3[ab]	58.7[a]	0.72	0.057
2nd 4 wk	57.2[ab]	61.3[a]	59.9[ab]	0.82	0.005
3rd 4 wk	58.1[b]	60.7[ab]	61.4[a]	0.75	0.011
Overall	57.1[b]	59.8[ab]	60.0[a]	0.65	0.006
ADFI, g					
1st 4 wk	126	126	126	2.5	0.995
2nd 4 wk	122	123	124	3.3	0.910
3rd 4 wk	114[b]	121[a]	117[ab]	1.8	0.037
Overall	121	123	122	1.9	0.605
FCR, g					
1st 4 wk	2.54[a]	2.38[ab]	2.24[b]	0.07	0.010
2nd 4 wk	2.58[a]	2.25[b]	2.21[b]	0.08	0.007
3rd 4 wk	2.44[a]	2.17[ab]	2.12[b]	0.08	0.016
Overall	2.51[a]	2.27[b]	2.19[b]	0.06	0.001
Broken eggs, %					
1st 4 wk	0.7	0.0	0.0	0.30	0.128
2nd 4 wk	2.5[a]	0.6[ab]	0.1[b]	0.57	0.002
3rd 4 wk	4.8	0.9	0.0	1.46	0.048
Overall	2.7[a]	0.5[ab]	0.0[b]	0.66	0.002
Bones, %					
Ash	61.4[ab]	62.7[a]	60.2[b]	0.53	0.006
Calcium	22.8[ab]	23.2[a]	22.3[b]	0.21	0.013
Phosphorus	10.4[ab]	10.7[a]	10.2[b]	0.10	0.002
Bones, g					
Tibia diaphysis	0.90[b]	1.10[a]	1.03[ab]	0.05	0.011
Ash	0.55[b]	0.69[a]	0.62[ab]	0.03	0.003
Calcium	0.20[b]	0.26[a]	0.23[ab]	0.01	0.004
Phosphorus	0.09[b]	0.12[a]	0.11[ab]	0.01	0.002

[1]The control diet was formulated to provide 40.0 g/kg Ca and 1.0 g/kg non-phytate P; the phytase activity was analyzed as 176 FYT/kg feed for phytase treatment.

[2]12 replicates (3 hens per replicate) per treatment for egg production parameters and 18 replicates for bone parameters.

[3]NC: negative control; PC: positive control.

[4]1st, 2nd, and 3rd four wk indicate hen's age from wk 25 to 29, 29 to 33, and 33 to 37, respectively.

[a-b]Means in the same row and not sharing superscript letters differ significantly.

weights of bone ash, Ca, and P were significantly higher in hens of PC than NC with the results of phytase treatment being intermediate (Table 4). In trial 3, PC and phytase treatment gave significantly higher percentage and weight of bone Ca and P than NC whereas the bone ash was significantly improved only by PC (Table 5).

DISCUSSION

Ileal Digestibility and Retention of Calcium and Phosphorus

The laying hens are well-recognized for their ability to exploit their bone reservoir for Ca to lay eggs. The Ca deposited in an egg was estimated at 2.0 g whereas the retained Ca per day and per hen was approximately 1.7 g in the current study, which indicates that the retained Ca could not meet the hen's requirement for eggshell formation. This gap therefore needs to be filled via bone mineral mobilization (Taylor, 1970). The medullary bone is a labile source of Ca for hens to mobilize for eggshell calcification, resulting in an inevitable loss of structural bone during the laying period. This can be minimized, but not prevented by good nutrition

(Whitehead and Fleming, 2000). Bone Ca resorption resulted in an excess of P because of the high Ca: P ratio of eggshell compared with that in hydroxyapatite of bone (Clunies et al., 1992). Consequently, the elevated concentration of P in plasma during shell formation increased P excretion (Miles et al., 1984), and this agreed with the discovery by Hurwitz and Griminger (1961) that 19% of the excreted Ca and 62% of the excreted P in excreta originated from urine in laying hens. The urinary P excretion makes it difficult to determine the availability of P in hens based on balance measurements (Rodehutscord et al., 2002). In trial 1, the scale of phytase effect on P retention appeared to be smaller than on ileal P digestibility, which partly explains the observed significant effect of phytase on ileal P digestibility but not on P retention. Physiologically this could be attributed to the fact that as available P intake increases, a great amount of P will be excreted into the post-ileal digestive tract or excreted via the urine (Rodehutscord et al., 2002). There is no denying that the effect of phytase on P retention in layers is multifactorial. In a meta-analysis, duration of the trial, age of hens, and dietary Ca content were identified as the main factors (Bougouin et al., 2014).

Table 5. Egg production and bone mineralization in laying hens (trial 3)[1,2].

Items	NC[3]	PC[3]	187.5	SEM[3]	Significance level
		Treatment			
Egg production, %					
1st 4 wk[4]	97.9	98.5	98.1	0.57	0.767
2nd 4 wk[4]	92. 9[b]	98.9[a]	98.6[a]	0.90	< 0.001
3rd 4 wk[4]	79.6[b]	95.6[a]	95.0[a]	1.65	< 0.001
Overall	90.1[b]	97.6[a]	97.3[a]	0.79	< 0.001
Egg weight, g					
1st 4 wk	56.3	56.8	55.4	0.84	0.476
2nd 4 wk	57.2[b]	61.3[a]	59.9[a]	0.82	< 0.001
3rd 4 wk	56.8[b]	60.8[a]	60.7[a]	0.34	< 0.001
Overall	56.8[b]	58.9[a]	58.5[a]	0.35	< 0.001
ADFI, g					
1st 4 wk	108[b]	112[a]	113[a]	0.8	< 0.001
2nd 4 wk	102[b]	113[a]	112[a]	0.8	< 0.001
3rd 4 wk	96[b]	114[a]	113[a]	1.4	< 0.001
Overall	102[b]	113[a]	113[a]	0.8	< 0.001
FCR, g					
1st 4 wk	1.97[b]	2.01[ab]	2.04[a]	0.02	0.030
2nd 4 wk	1.93	1.92	1.92	0.02	0.907
3rd 4 wk	2.16[a,a]	1.97[ab]	1.96[b]	0.03	< 0.001
Overall	2.00	1.97	1.99	0.02	0.384
Broken eggs, %					
1st 4 wk	1.0	0.9	0.4	0.30	0.084
2nd 4 wk	2.4[a]	0.3[b]	0.4[b]	0.21	< 0.001
3rd 4 wk	3.5[a]	0.1[b]	0.4[b]	0.32	< 0.001
Overall	2.2[a]	0.4[b]	0.4[b]	0.17	< 0.001
Bones, %					
Ash	49.0[b]	50.6[a]	50.3[ab]	0.40	0.021
Calcium	14.9[b]	17.7[a]	17.0[a]	0.31	< 0.001
Phosphorus	5.4[b]	5.9[a]	5.7[a]	0.10	< 0.001
Bones, g					
Tibia	4.76	4.91	4.87	0.08	0.333
Ash	2.33[b]	2.48[a]	2.45[ab]	0.04	0.038
Calcium	0.71[b]	0.86[a]	0.82[a]	0.02	< 0.001
Phosphorus	0.25[b]	0.29[a]	0.28[a]	0.01	< 0.001

[1]The control diet was formulated to provide 36 g/kg Ca and 1.1 g/kg non-phytate P; the phytase activity was analyzed as 175 FYT/kg feed for the phytase treatment.

[2]30 replicates per treatment; 4 birds per replicate.

[3]NC: negative control; PC: positive control.

[4]1st, 2nd, and 3rd four wk indicate hen's age from wk 25 to 29, 29 to 33, and 33 to 37, respectively.

[a-b]Means in the same row and not sharing superscript letters differ significantly.

Deposition of Calcium and Phosphorus in Eggs

Plasma P concentration, when not influenced strongly by shell formation, reflects dietary P content (Miles et al., 1983; Boorman and Gunaratne, 2001), and thus an elevation in plasma P concentration and greater influx of P toward liver due to more ileal digestible P in connection with phytase addition can be assumed. Consequently, a reduction in the proportion of eggshell was observed in the current study, which was supported by the depression in shell quality such as shell percentage and thickness with increasing level of P in feed/plasma (El Boushy, 1979) and the steady decrease in egg specific gravity with increasing daily intake of P (Miles et al., 1983). In addition, higher concentrations of Ca and P were observed in egg contents of hens supplemented with phytase. Phytase supplementation tends to lead to higher yolk weights (Żyła et al., 2012), and most minerals in egg contents are concentrated in egg yolk, which happens in liver, the organ for the production of egg yolk (Réhault-Godbert et al., 2019). A greater ratio of yolk to albumin could cause an uplift in the concentrations of Ca and P in egg contents. This speculation, however, contradicts the observed reduction in dry matter content in egg contents with phytase supplementation in trial 1. The reduction in dry matter content in egg contents could occur only with a smaller ratio of yolk over albumin considering approximately 12% total solids content in egg albumin and 50% in egg yolk (Ahn et al., 1997). In the literature, no strong evidence exists in support of phytase affecting egg quality. Taylor et al. (2018) reported that there was no significant effect of 300 and 1,500 FYT phytase/kg feed supplementation for 24 wk on egg quality parameters including egg weight, shell weight, yolk weight, shell strength and Haugh unit. Even with super-dosing phytase for 5 wk, no significant effect on egg quality (egg weight, eggshell strength and thickness, and Haugh unit) was observed (Kim et al., 2017).

Egg Production

Insufficient dietary P depresses egg production (Härtel, 1990) and phytase supplementation could alleviate the status of P deficiency as purposefully induced to evaluate phytase efficacy in the current study and

thus improve egg production. The depression on egg production by insufficient P supply depends on the severity and duration of P shortage. Feeding 0.1% NPP decreased egg production by 8.1% over the entire 17-wk trial duration and by 29.6% over the last 4 wk, relative to diets with 0.2 to 0.5% NPP and the reduction in feed consumption followed a similar pattern (Gordon and Roland, 1997). In the same vein, greater reductions -in egg production and feed intake of hens fed control diets with 0.1% NPP were recorded during the last 4 wk in the current study. In the trial by Gordon and Roland (1997), all the adverse effects due to feeding hens the 0.1% NPP diet were completely overcame with the supplementation of 300 U/kg phytase, whereas in our study the supplementation of 187.5 FTU/kg feed corrected the deficiency symptoms and improved egg production up to the level of PC. Similar corrective effects of phytase at 100 to 200 U/kg feed were reported by Simons et al. (1992), van der Klis et al. (1997), and Boling et al. (2000a). In addition, the law of diminishing return is also true in layers. In a meta-analysis by Ahmadi and Rodehutscord (2012), the optimal NPP levels were 0.18, 0.15, and 0.14% in the presence of 150, 300, and 400 U phytase/kg feed, respectively, which shows marginal improvement beyond 300 U phytase /kg feed.

Long-term egg production and short-term ileal digestibility of P appeared to be very responsive to the supplementation of phytase. The development of more severe P deficiency symptoms of hens on P deficient diet with the progression of trial favors the adoption of long-term egg production studies to demonstrate the efficacy of phytase. The diminished ability to maintain egg production driven by the continuous depletion of bone of the hens on P deficient diet means a greater drop in egg production and a greater chance to show the efficacy of phytase. The phytase liberates more Ca and P bound by phytate in layers (Liu et al., 2007) to enrich the plasma which could be partly used in the production of eggs or to signal to bones to retard mobilization (Miles et al., 1983; Bougouin et al., 2014). To demonstrate the efficacy of phytase in a short term with laying hens, a shorter adaptation period and ileal digestibility of P should be advocated. A meta-analysis shows that prolonging phytase supplementation duration and age of hens had negative effects on phytase efficacy (Bougouin et al., 2014). In broilers, it has been clearly demonstrated that a shorter feeding period such as 2 d can give greater phytase efficacy due to homeostatic adaptations which occurs when the feeding goes beyond 2 d (Li et al., 2015b; Babatunde et al., 2019).

Bone Mineralization

The phytase supplementation improved the amounts of ash, Ca, and P deposited in bone up to levels commensurate with the hens of PC. It was well-recognized that increasing dietary P level significantly increases the concentrations of plasma P and P in tibia (El Boushy, 1979). On the contrary, the plasma and bone Ca levels were not influenced by variations in dietary supply of Ca and P (Keshavarz and Nakajima, 1993). Considering the strong relationship between dietary P supply, plasma P enrichment, and P deposition in tibia in laying hens (El Boushy, 1979; Boorman and Gunaratne, 2001), it is plausible that the liberated P by phytase narrows the Ca to P ratio in plasma towards an optimal ratio for greater bone mineralization. In addition, the P enrichment in plasma in association with added phytase might serve as a signal to retard the catabolic response of bones. The weights of bone ash, Ca, and P appeared to be more correlated with phytase addition than the percentage of bone ash, Ca, and P in the current study. This agrees with the finding by Li et al. (2015a) that ash weight better reflects the amount of bone mineralization as compared to ash percentage and using ash percentage may lead to an underestimation of phytase efficacy.

CONCLUSIONS

The current study showed that the novel phytase can improve the ileal digestibility of P in a short-term digestibility study and improve egg performance and bone mineralization in long-term studies. In short-term digestibility studies, ileal digestibility of P should be measured to demonstrate the efficacy of phytase, whereas egg production parameters and the mineralization of whole tibias are very responsive to phytase supplementation in long-term studies.

DISCLOSURES

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service, and/or company that could be construed as influencing the content of this paper.

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