METABOLISM AND NUTRITION

The adaptability of Hy-Line Brown laying hens to low-phosphorus diets supplemented with phytase

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ABSTRACT Body phosphorus homeostasis network allows laying hens to adapt to wide range of changes in dietary phosphorus levels. Phytase hydrolyzes phytate rendering phosphorus and reduces the laying hens' requirements for inorganic phosphate rock. Here, we demonstrate that there is no need to keep large safety margins in dietary phosphorus when hens are fed with phytase. Hy-Line Brown laying hens (n = 504) were randomly assigned to 7 treatments (6 replicates of 12 birds). A corn-soybean meal–based diet, with no inorganic phosphate rock, was formulated to contain 0.12% non-phytate phosphorus (**nPP**), 3.8% calcium, and 2,000 FTU/kg phytase. Inorganic phosphate rock (di-calcium phosphate) was supplemented into the basal diet to create 6 other diets containing 0.17, 0.22, 0.27, 0.32, 0.37, and 0.42% nPP. Levels of calcium carbonate and zeolite powder were adjusted to make sure all the 7 experimental diets contained the same nutrition levels (including calcium and phytase) except nPP. The diets were subjected to laying hens from 29 to 40 wk of age. As a result, when supplemented with 2,000 FTU/kg phytase, extra supplementation of inorganic phosphate rock had no effects ($P > 0.05$) on serum phosphorus levels, serum calcium levels, laying performance (laying rate, egg weight, feed intake, feed-to-egg ratio, and unqualified egg rate), egg quality (shell thickness, shell strength, albumen height, yolk color, and Haugh unit), and tibia quality parameters (breaking strength and ash, calcium, and phosphorus contents). Extra supplementation of inorganic phosphate rock linearly increased ($P < 0.01$) fecal phosphorus excretion and linearly decreased ($P = 0.032$) the apparent metabolizability of dietary phosphorus. While serum hormones and intestine gene expressions were varied within treatments, no consistent changes were found. In conclusion, the supplementation of inorganic phosphate rock (provided 0.05–0.30% extra nPP) to phytase-containing basal diets (2,000 FTU/kg; nPP = 0.12%) provided limited benefits to egg production performance in laying hens from 29 to 40 wk of age. Further investigating the body phosphorus homeostasis would help to understand the nutritional and physiological reasonability of formulating low-phosphorus diets in the laying hen industry.

Key words: adaptability, Hy-Line Brown laying hen, low phosphorus diet, phosphate rock, phytase

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INTRODUCTION

Dietary inorganic phosphate input of laying hens should be tightly controlled to reduce feed cost and avoid (or partially avoid) laying hen-related environmental concerns (Fink et al., 2018; Goyette et al., 2018). In the last few decades, a variety of technologies have been proposed to improve the bio-availabilities of dietary phosphate. Liao et al. (2017) reported that the perfusions of 1,25-dihydroxy-cholecalciferol [**$1,25(OH)_2D_3$**] or parathyroid hormone (**PTH**) fragment (1-34) increased intestinal expressions (both mRNA and protein levels) of type IIb sodium-phosphate cotransporter (**NaPi-IIb**) and thereby enhanced phosphorus absorption in broilers. A Wisconsin group found that antibodies to fibroblast growth factor 23 were effective in increasing renal phosphate resorption and decreasing phosphate requirements and excretions in laying hens and its progeny chicks (Ren et al., 2017a,b,c). In addition, phytase, the most widely used dietary exogenous enzyme in animal production (Adeola, 2011), breaks down phytate-phosphate from plant feed ingredients and dramatically reduces the animals' requirement for inorganic phosphorus (Fernández et al., 2019). Interestingly, while the aforementioned technologies have repeatedly been documented in the literature, it still remains unclear in the field that how much inorganic phosphate rock will need be

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supplemented into poultry diets when those technologies are applied (Li et al., 2015; Adhikari et al., 2020; Ren et al., 2020).

Ahmadi and Rodehutscord (2012) meta-analyzed 14 trials where the nonphytate phosphorus (**nPP**) requirements of laying hens were determined in the presence of phytase. As a result, the optimal dietary nPP levels for laying hens were 0.18, 0.15, and 0.14% when per kg diet contained 150, 300, and 400 phytase unit (FTU), respectively. Generally, the nPP content of corn-soybean meal laying hen basal diet (with no inorganic phosphate supplementation) is around 0.12%. That means, to meet the layers' requirements, a laying hen nutritionist only need to add 0.11% di-calcium phosphate ($2H_2O$, contains 18% phosphorus), which can supply 0.02% nPP, when the basal diet contains 0.12% nPP and 400 FTU/kg phytase. Indeed, to our knowledge, dietary phytase supplementation levels in the laying hen industry are much higher than 400 FTU/kg (Boney and Moritz, 2017; Gautier et al., 2018). In recent years, a lot of studies showed that high-does phytase supplementation (>1,000 FTU/kg) could further increase the utilizations of phytate phosphorus (Taheri et al., 2015; Taheri et al., 2016; Gautier et al., 2018). According to a field survey conducted by Guo et al. (2018), phytase is supplemented at around 2,000 FTU/kg in laying hen diets in China. However, even with this high-dose phytase supplementation, dietary nPP level is set as much as 0.36% (that means 0.36–0.12% = 0.24% nPP is supplemented in the form of extra inorganic phosphate rock) in laying hen diets (Guo et al., 2018). In recent decades, efforts to conquer phosphorus disorders in humans have led to the illustration of a complex phosphorus homeostasis network that enables the body to adjust to very different levels of phosphorus consumptions (Michigami et al., 2018). These phosphorus nutrition advances bring up new arguments regarding the nutritional and physiological values of large phosphorus safety margins in laying hen diets (Ahmadi and Rodehutscord, 2012). The present study was conducted to investigate whether or not extra inorganic phosphate rock should be supplemented into a commercial corn-soybean meal-based laying hen diet that contains 0.12% nPP and 2,000 FTU/kg phytase.

MATERIALS AND METHODS

All the animal protocols used in this study were reviewed and approved by the Animal Care and Use Committee of the College of Animal Science and Technology at the Northwest A&F University (Yangling, Shaanxi, China).

Birds and Diets

Hy-Line Brown layers (29 wk old, n = 504) were randomly allotted into 7 treatments with 6 replicates of 12 birds. The experimental diets were prepared using a commercial corn-soybean meal–based laying hen basal diet (Table 1) that is widely used in Northwest China.

Table 1. Composition and nutrient contents of the basal diet.

Item	%, unless mentioned
Corn	60.53
Soybean meal, 46%	15.09
Corn germ meal, 23%	4.00
Corn gluten meal, 60%	3.00
DDGS, 27.5%	4.00
Soybean oil	0.47
Sodium chloride	0.20
Choline chloride, 50%	0.08
Di-calcium phosphate, $2H_2O$	-
Calcium carbonate	9.60
L-Lysine-H_2SO_4, 65%	0.26
DL-Methionine, 99%	0.16
L-Tryptophan, 99%	0.08
Premix[1]	0.53
Zeolite powder	2.00
In total	100
Nutrient contents	
Metabolic energy (Kcal/kg)	2,650
Crude protein	15.80
Calcium	3.80
Total phosphorus (analyzed)	0.34
nPP	0.12
Lysine	0.80
Methionine + cysteine	0.69

Abbreviations: DDGS, distillers dried grains with soluble; nPP, nonphytate phosphorus.

[1]Supplied per kg of diet: vitamin A, 8050 IU; vitamin D_3, 2415 IU; vitamin E, 24.2 IU; vitamin K_3, 2.0 mg; vitamin B_1, 1.7 mg; vitamin B_2, 5.2 mg; vitamin B_6, 3.7 mg; niacin, 10.6 mg; copper (from $CuSO_4 \cdot 5H_2O$), 8.4 mg; manganese (from $MnSO_4 \cdot H_2O$), 70.5 mg; zinc (from $ZnSO_4 \cdot 6H_2O$), 49.5 mg; iron (from $FeSO_4 \cdot 6H_2O$), 54.0 mg; phytase, 2,000 FTU; sodium bicarbonate, 2 g.

Briefly, the basal diet contained 15.8% crude protein, 3.8% calcium, 0.12% nPP, 2415 IU/kg vitamin D_3, and 2,000 FTU/kg phytase. The phytase (commercial name: Habio phytase; 6-phytase produced by *Escherichia coli*) was purchased from Habio Biological Engineering Co., Ltd., Mianyang, Sichuan, China. Di-calcium phosphate ($2H_2O$) was added into the basal diet to provide 0, 0.05, 0.10, 0.15, 0.20, 0.25, and 0.30% inorganic phosphorus and made the final nPP levels of the experimental diets to 0.12, 0.17, 0.22, 0.27, 0.32, 0.37, and 0.42%, respectively. The levels of calcium carbonate and zeolite powder were adjusted accordingly to make sure all the experimental diets had the same level of calcium. The animal trial was conducted under proper management and laboratory conditions to avoided unnecessary discomfort to laying hens. Layers were exposed to a 16- to 8-h (light-to-dark) cycle. Feed and water were provided *ad libitum*. The feeding trail was conducted for 12 wk.

Laying Performance and Egg Quality

Eggs were daily collected, and data on total eggs, egg weight, dirty eggs, broken eggs, soft eggs, and malformed eggs were recorded. Feed consumption was weekly recorded. Day laying rate, average egg weight, feed intake, average egg mass, feed-egg mass ratio, and unqualified egg rate were calculated accordingly. At 40 wk of age, 84 eggs were randomly selected (2 eggs per replicate, 12 eggs per treatment) for the determination of egg quality parameters. Briefly, eggshell strength

was measured using a texture analyzer (EFG-0503; Robotmation, Co., Ltd., Tokyo, Japan); eggshell thickness was measured using a dial pipe gauge (ETG-1061; Robotmation, Co., Ltd., Tokyo, Japan); egg yolk color, albumen height, and Haugh units were tested using a multifunction egg quality analyzer (EMT-5200; Robotmation, Co., Ltd., Tokyo, Japan).

Serum Analysis

At 40 wk of age, 1 bird per replicate (randomly selected) was stunned and bled (10 mL per bird, via left wing vein). Serum samples were obtained from the blood samples and were stored at −80°C for future analysis. Serum levels of calcium, phosphorus, and alkaline phosphatase were analyzed at Yangling Demonstration District Hospital (Yangling, Shaanxi, China) using a fully automatic biochemical analyzer (ADVIA2400; Siemens, Berlin, Germany). The levels of calcitonin, PTH, and $1,25(OH)_2D_3$ were determined using commercial kits (IDs for kits were CK-E94743 C, CK-E60009 C, and CK-E95254 C, respectively), purchased from Shanghai Yuanye Bioengineering Institute (Shanghai, China), according to the manufacturer's instructions.

Tibia Quality

Immediately after being bled, the birds were euthanized (cervical dislocation) for tibia and intestine samples. As previously described in the study by Ren et al. (2016c), adherent tissues were removed, and the left tibia was subjected to breaking strength measurement based on a three-point bending test (supporting distance, 40 mm; test speed 10 mm/min) using an electronic universal mechanical test machine (CMT5504; Shenzhen SANS Material Testing Co., Ltd., Guangdong, China). The right tibia was defatted (ethyl ether, 16 h), oven dried (105°C, 24 h), and ashed (muffle furnace, 600°C, 8 h) as reported in the study by Ren et al. (2016b). The tibia ash samples were analyzed for calcium (using a flame atomic absorption spectrophotometer, Z-2000; Hitachi Ltd., Tokyo, Japan) and phosphorus (ammonium metavanadate colorimetric method). Ash, calcium, and phosphorus contents of the defatted-and-oven-dried tibia were calculated accordingly.

Intestinal mRNA Expressions

Mucosa samples were collected from the middle sections of duodenum and jejunum and stored at −80°C for the determination of mRNA expressions (NaPi-IIb; calbindin-d28k, **CaBP-D28k**). RT-PCR analysis was conducted as described in a previous publication (Liu et al., 2016). Briefly, total RNA of the samples was extracted using TRIZOL reagent (Invitrogen, Carlsbad, CA). The concentration and purity of the extracted RNA were determined using a NanoDrop 2000c spectrophotometer (Thermo Scientific, Wilmington, DE). Qualified RNA samples were subjected to cDNA synthesis using a Primer Script RT Reagent Kit (TaKaRa, Dalian, China). Then, mRNA expression was analyzed using a SYBR Premix Ex Taq kit (TaKaRa, Dalian, China). Sequences (5′ to 3′) of the primers used in the present study were β-actin, accession number L08165, forward ATTGTCCACCGCAAATGCTTC, reverse AAATAAAGCCATGCCAATCTCGTC; NaPi-IIb, accession number NM204474.2, forward CTGGATGCACTCCCTAGAGC, reverse TTATCTTTGGCACCCTCCTG; CaBP-D28k, accession number NM205513.1, forward GGCAGGCTTGGACTTAACAC, reverse GCTGCTGGCACCTAAAGAAC. Relative mRNA expression was calculated to β-actin using $2^{-\Delta\Delta\ Ct}$ method.

Metabolic Experiment

At 40 wk of age, 35 birds (5 birds per treatment) were randomly selected for the metabolic experiment. After a 3-day adapting period, the birds were fasted for 12 h. Then, the birds were fed with the experimental diets for 60 h, and continuous excreta samples were collected for 72 h. Water was provided *ad libitum*. Feed intake was daily recorded. HCl (10%) was sprayed onto the surface of the excreta to avoid nitrogen loss. Oven dried (65°C, overnight) excreta samples were weighed and subjected to analyses for gross energy (using an adiabatic oxygen bomb calorimeter, model 6100, Calorimeter, Germany), crude protein (under a Kjeldahl procedure, model 8400, Kjeltec, Denmark), crude fat (method 991.36, AOAC, 2005), calcium (same as tibia calcium analysis), and phosphorus (same as tibia phosphorus analysis). The apparent metabolizable energy of the experimental diets and the apparent metabolizability of crude protein, crude fat, calcium, and phosphorus were calculated accordingly.

Statistical Analysis

One-way ANOVA was conducted under the GLM procedure of SPSS 21.0 (IBM Corp., Chicago, IL). Duncan's test was applied when the one-way ANOVA showed significant results. Linear and quadratic regressions were carried out using SPSS 21.0. The statistical unit was each replicate for egg production performance, each egg for egg quality parameters, and each hen for all other measurements. Data are listed as the means ± standard error of the mean. Significance was considered with a P value of <0.05, and a trend was considered with $0.05 \leq P < 0.1$.

RESULTS AND DISCUSSION

Here we demonstrate that the supplementation of extra inorganic phosphate rock (in the form of di-calcium phosphate $[2H_2O]$) had no effects on laying performance (Table 2; day laying rate, $P = 0.726$; egg weight, $P = 0.505$; feed intake, $P = 0.281$; egg mass, $P = 0.747$; feed-to-egg mass ratio, $P = 0.784$; and unqualified egg rate, $P = 0.727$) and egg quality parameters (Table 2; eggshell thickness, $P = 0.631$; eggshell strength, $P = 0.432$; albumen height, $P = 0.173$; yolk color, $P = 0.217$; and Haugh unit, $P = 0.183$) in Hy-Line Brown

Table 2. Effects of dietary inorganic phosphate supplementation on laying performance and egg quality in hens fed 2,000 FTU/kg phytase[1].

Item	Inorganic phosphorus supplementation levels (%)							SEM	*P* value
	0	0.05	0.10	0.15	0.20	0.25	0.30		
Laying performance									
Day laying rate (%)	94.8	93.9	96.0	91.4	95.6	93.6	93.8	0.7	0.726
Average egg weight (g)	58.7	58.8	59.2	59.2	59.8	58.8	59.3	0.2	0.505
Feed intake (g/hen/d)	120.2	121.5	123.5	122.1	123.6	120.3	121.6	0.5	0.281
Average egg mass (g/hen/d)	55.7	55.5	57.0	54.3	57.2	55.2	55.8	0.5	0.747
Feed-to-egg mass ratio (kg:kg)	2.16	2.20	2.18	2.27	2.16	2.19	2.20	0.02	0.784
Unqualified egg rate (%)[2]	0.09	0.20	0.15	0.17	0.09	0.09	0.20	0.02	0.727
Egg quality									
Eggshell thickness (mm)	0.409	0.393	0.393	0.399	0.388	0.400	0.397	0.003	0.631
Eggshell strength (g of force)	4,495	4,719	4,675	4,744	4,297	4,725	4,750	64	0.432
Albumen height (mm)	7.5	8.2	8.1	7.4	7.3	8.1	8.0	0.1	0.173
Yolk color	8.7	7.9	8.0	8.3	8.0	7.9	7.8	0.1	0.217
Haugh units	86.2	90.3	89.4	85.3	84.8	89.6	88.1	0.7	0.183

[1]All the data were subjected to linear and quadratic regression analyses, but no significance was recorded ($P > 0.05$).
[2]Unqualified egg rate = (total number of dirty, broken, softshell, and malformed eggs)/(total egg number).

laying hens (from 29–40 wk of age) fed with commercial diets containing 2,000 FTU/kg phytase, 0.12% nPP, 3.8% calcium, and 2145 IU/kg vitamin D_3. These results are consistent with Ahmadi and Rodehutscord (2012)'s calculation that the optimal dietary nPP level is 0.14% for laying hens fed 400 FTU/kg phytase (a meta-analysis assembled from 14 trials conducted with 6 strains of laying hens in the range of 36 to 76 wk of age; 88% of the experimental diets contained 3.7 to 3.8% calcium). In this sense, the currently used dietary nPP levels (0.36% as surveyed by Guo et al. (2018)) in the laying hen industry are much higher than the layers' requirements for maintaining laying performance and egg quality. Assuming raising 1 billion laying hens and the average daily feed intake is 110 g, based on our results, there will be 264,000 kg (1 billion × 0.11 × [0.36%–0.12%]) inorganic phosphorus (even more if calculated to phosphate rock) to be supplemented into laying hen diets every single day but will cause no (or very limited) benefit effects on the production and quality of eggs. We do realize that, to make solid conclusions, more criteria will need to be involved to evaluate the laying performance and egg quality, and of course, long-term field studies with a larger scale will need to be conducted. However, the current results do bring up an argument that laying hens may not need large safety margins for phosphorus in the diet and suggest that poultry nutritionists should pay serious attentions to dietary nPP levels when technologies such as phytase, vitamin D_3, and fibroblast growth factor 23 antibody are used. In addition, in diets containing high-does phytase (>1000 FTU/kg), the utilization of other nutrients (e.g., minerals) is also increased (Viveros et al., 2002; Troesch et al., 2013). Yang et al. (2012) pointed out that some of the minerals such as iron and manganese are overdosed in commercial laying hen diets. It has been warned that mineral overdose

Table 3. Effects of dietary inorganic phosphate supplementation on serum parameters, tibia quality, and intestinal gene expressions in hens fed 2,000 FTU/kg phytase[1].

Items	Inorganic phosphorus supplementation levels (%)							SEM	*P* value
	0	0.05	0.10	0.15	0.20	0.25	0.30		
Serum parameters									
Calcium (mmol/L)	3.57	3.50	3.51	3.56	3.59	3.57	3.54	0.02	0.631
Phosphorus (mmol/L)	1.42	1.61	1.44	1.56	1.44	1.46	1.49	0.04	0.905
ALP (U/L)	415	329	318	387	363	271	335	22	0.321
CT (pg/mL)	47.0^{b}	45.0^{b}	42.0^{b}	94.0^{a}	56.8^{b}	52.5^{b}	42.4^{b}	3.5	0.001
PTH (pg/mL)	222^{a}	$151^{b,c}$	$154^{b,c}$	$201^{a,b}$	$176^{a,b}$	$188^{a,b}$	120^{c}	8	0.005
1,25-$(OH)_2$-D_3 (pg/mL)	$82.8^{b,c}$	55.6^{c}	$74.3^{b,c}$	148.7^{a}	93.2^{b}	$65.2^{b,c}$	$65.2^{b,c}$	5.6	<0.001
Tibia quality									
Breaking strength (g of force)	17.559	19.057	19.051	17.780	18.225	20.201	18.242	709	0.966
Ash content (%)	48.9	47.0	45.2	47.5	47.4	46.9	46.2	0.5	0.712
Calcium (%)	21.9	23.5	23.0	22.3	21.5	21.3	22.6	0.3	0.358
Phosphorus (%)	7.59	7.53	7.03	7.48	7.28	7.08	6.91	0.12	0.601
Intestinal gene expressions									
NaPi-IIb (duodenum)	1.00^{d}	$2.17^{a,b,c}$	$1.39^{c,d}$	$2.58^{a,b}$	$2.64^{a,b}$	2.87^{a}	$1.84^{b,c,d}$	0.15	0.001
NaPi-IIb (jejunum)	1.00^{a}	$0.67^{b,c}$	$0.48^{b,c}$	1.05^{a}	0.40^{c}	$0.73^{a,b}$	$0.78^{a,b}$	0.05	0.001
CaBP-D28k (duodenum)	1.00^{c}	1.23^{c}	1.14^{c}	2.69^{a}	1.97^{b}	1.29^{c}	$1.42^{b,c}$	0.12	<0.001
CaBP-D28k (jejunum)	1.00	1.12	1.06	1.44	0.83	1.19	1.17	0.06	0.199

[a–d]Means with different superscripts within a row were significantly different ($P < 0.05$).
Abbreviations: 1,25$(OH)_2D_3$, 1,25-dihydroxy-cholecalciferol; ALP, alkaline phosphatase; CaBP-D28k, calbindin-d28k; CT, calcitonin; NaPi-IIb, type IIb sodium-phosphate cotransporter; PTH, parathyroid hormone.
[1]All the data were subjected to linear and quadratic regression analyses, but no significance was recorded ($P > 0.05$).

may let to increased abundance of antibiotic resistance genes in animal wastes (Baker-Austin et al., 2006), which could transfer to humans and cause unexpected health concerns. Future studies will need to document reliable data for avoiding mineral overdose in laying hen diets.

The linear correlation between dietary nPP and plasma phosphate levels has repeatedly been reported in poultry species (Viveros et al., 2002). Interestingly, in this study, the blood samples were collected from 9:00 to 9:30 am in the morning, and serum levels of calcium ($P = 0.631$) and phosphorus ($P = 0.905$) were not affected by the dietary supplementation of inorganic phosphate (Table 3). Similarly, in the laying hen trial (from 25–36 wk of age) performed by Boorman and Gunaratne (2001), plasma calcium and phosphorus levels were not affected by dietary nPP levels (0.16, 0.21, 0.31, and 0.39%) when the blood samples were collected at the end of the dark period (All the hens were bled at the same time.). However, they found that when the blood samples were collected immediately after oviposition (Each hen was bled based its own egg-laying cycle.), plasma phosphorus was tended to be increased with the increase of dietary nPP levels. Interestingly, in the same study (Boorman and Gunaratne, 2001), the plasma phosphorus level of hens under very high nPP (1.19%) was much higher than that of hens under 0.30% nPP; even the blood samples were collected at the end of the dark period. Seemingly, to correctly measure the plasma or serum phosphorus levels in laying hens, the time point for blood sampling should be seriously considered (e.g., bleeding based on the oviposition cycle) if the dietary phosphorus levels are not very different (e.g., 0.30% vs. 1.19%). Unfortunately, in most of the existing laying hen (or breeder hen) publications, blood samples of all the hens were collected just at the same time point, but not based on the egg-laying cycle of each hen, for phosphorus measurement (Mirakzehi et al., 2013; Ren et al., 2016a; Ren et al., 2018). This would also explain why we observed no consistent differences in plasma hormone levels (Table 3; alkaline phosphatase, calcitonin, PTH, and 1,25-$(OH)_2$-D_3) and intestinal gene expressions (NaPi-IIb and CaBP-D28k). In future laying hen phosphorus nutrition studies, the serum phosphorus (and hormone) dynamics during the egg-laying cycle will need to be illustrated (Ren et al., 2019), and the optimal sample-collecting time points will need to be investigated.

The phosphorus excreted by agricultural animals can sometimes enter the water system and cause serious environmental problems (Mallin, 2000). When applied as a fertilizer, animal excreta may cause excess soil phosphorus to meet the nitrogen needs of the plants (Smil, 2000). In this study, while no difference was observed on laying performance and egg quality, the decreasing dietary nPP levels linearly decreased (Figure 1A; $P < 0.01$) phosphorus excretion and linearly increased (Figure 1B; $P = 0.032$) the apparent metabolizability of dietary phosphorus. Based on the calculation of Cordell et al. (2009), domestic animals excrete about 15 million tons of phosphorus into the environment

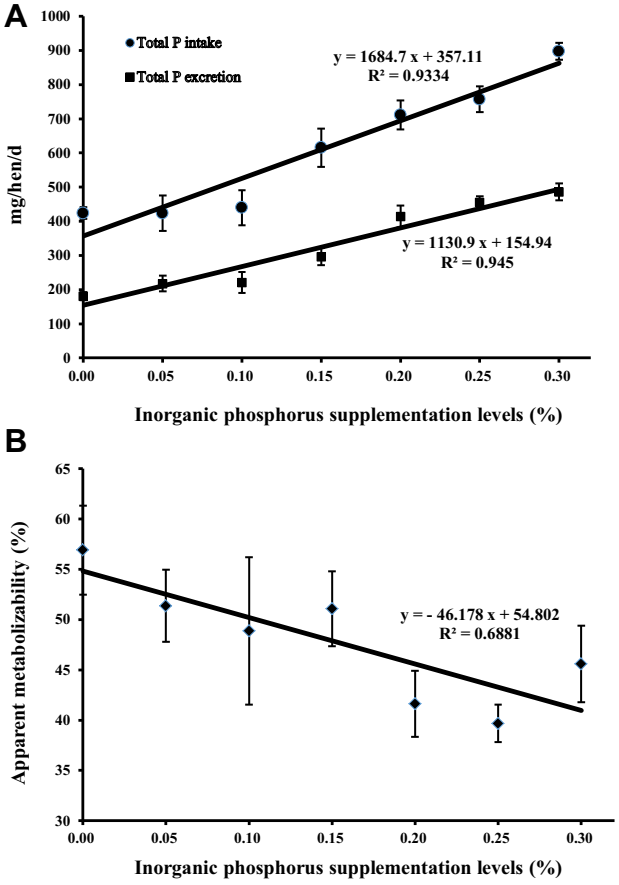

Figure 1. The intake, excretion (A), and apparent metabolizability (B) of phosphorus in laying hens fed 2,000 FTU/kg phytase. Data were presented as mean ± standard error of the mean. Significant linear (but not quadratic) regressions were recorded in phosphorus intake ($P < 0.01$), excretion ($P < 0.01$), and apparent metabolizability ($P = 0.032$).

every year. For raising 1 billion laying hens, if the average daily phosphorus excretion of each hen can be decreased from 471 to 198 mg (according to the current results, with no effect on laying performance and egg quality), it is possible to decrease phosphorus excretion by 273,000 kg (1 billion × [471-198] × 10^{-6}) every day. Interestingly, this number is very closed to the number of the daily overdosed inorganic phosphorus input as calculated in the first paragraph of the Results and discussion section (264,000 kg). In other words, the overdosed dietary inorganic phosphorus was basically excreted, but not kept, by the laying hens. Also, the apparent metabolizability of calcium ($P = 0.089$; Table 4) was tended to be decreased by the increasing dietary nPP. Similar result was reported by Nahashon et al. (1994) where calcium retention was decreased when dietary nPP level was increased from 0.25 to 0.45%. Masuyama et al. (2003) and Kemi et al. (2006) found that high phosphorus intake can decrease intestinal calcium absorption and increase bone calcium resorption and finally cause calcium excretion. In the present study, the apparent metabolizable energy ($P = 0.346$) and the apparent metabolizability of crude protein ($P = 0.452$) and crude fat ($P = 0.252$) were not affected by dietary nPP levels.

Table 4. AME and the apparent metabolizability of calcium, crude protein, and crude fat and in hens fed 2,000 FTU/kg phytase[1].

	Inorganic phosphorus supplementation levels (%)								
Items	0	0.05	0.10	0.15	0.20	0.25	0.30	SEM	*P* value
AME (MJ/kg)	11.3	11.4	11.7	11.7	11.8	11.5	11.6	0.1	0.346
Crude protein (%)	86.0	86.1	81.4	85.3	84.1	85.6	85.9	0.7	0.452
Crude fat (%)	55.4	50.7	48.1	44.9	48.7	45.5	49.9	1.1	0.252
Calcium (%)	78.1	69.6	71.8	66.0	61.3	66.7	63.2	1.6	0.089

Abbreviation: AME, apparent metabolizable energy.
[1]All the data were subjected to linear and quadratic regression analyses, but no significance was recorded ($P > 0.05$).

Either phosphorus deficiency (Bai et al., 2004) or phosphorus excess (Katsumata et al., 2005; Huttunen et al., 2006) can cause bone problems. Tibia quality has long been used to evaluate the phosphorus requirement of poultry species because it is a more sensitive indicator of phosphorus sufficiency than productive performance (Waldroup et al., 2000; Dhandu and Angel, 2003). In the current trial, no leg problem was observed in laying hens, and the breaking strength ($P = 0.966$), ash content ($P = 0.712$), calcium content ($P = 0.358$), and phosphorus content ($P = 0.601$) of tibia were not affected by dietary nPP levels. These results indicate that 0.12% nPP is sufficient for tibia health (at least for the currently measured criteria) in laying hens (from 29–40 wk of age) fed 2,000 FTU/kg phytase. Long-term studies with a large-scale sample will need to be conducted to verify these results in the whole laying period. In addition, the average daily feed intake of the hens was 120 g in this study. It is highly possible that these findings are more applicable to laying hens with a similar feed intake because the phosphorus intake changes a lot because of feed intake.

In conclusion, dietary supplementation of extra inorganic phosphate rock (supplemented with 0.05, 0.10, 0.15, 0.20, 0.25, and 0.30% extra nPP) had no effects on laying performance, egg quality, and tibia quality parameter in Hy-Line Brown laying hens (from 29–40 wk of age; average daily feed intake was 120 g) fed with a corn-soybean meal–based commercial diet (which is widely used in Northwest China) containing 0.12% nPP, 3.8% calcium, and 2,000 FTU/kg phytase. While more studies will need to be conducted and more economic or physiological criteria will need to be involved, our results do suggest the current industry nPP levels are higher than the laying hens' requirements and will need to be decreased for economic and environmental goals. The nutritional and physiological values of large dietary phosphorus safety margins should be reconsidered in laying hens, especially when phosphorus-reducing technologies (such as phytase) are applied.

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