# Effects of graded levels of phytase supplementation on growth performance, serum biochemistry, tibia mineralization, and nutrient utilization in Pekin ducks

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**ABSTRACT** A total of 560 one-day-old Pekin ducks were randomly allocated to 7 treatments with 8 replicate cages of 10 ducks per cage. The treatments included a corn–rice bran–soybean meal–based diet with recommended nonphytate phosphorus (**NPP**) (0.40% for 1–14 D/0.35% for 15–35 D, positive control; **PC**), NPP-deficient diet (0.22% for 1–14 D/0.18% for 15–35 D, negative control; **NC**), and NC diets supplemented with different levels of phytase (500, 2,500, 5,000, 7,500, 10,000 FTU/kg). Compared with the PC diet, feeding the NC diet significantly decreased ($P < 0.05$) the bird growth performance, serum total protein, and albumin concentration as well as tibia bone mineralization and strength and increased ($P < 0.05$) serum calcium (**Ca**), urea content, and alkaline phosphatase activity throughout the experimental period. Phytase supplementation to NC diets at 5,000 to 10,000 FTU/kg restored ($P < 0.05$) growth performance, serum biochemical parameters, and tibia traits when compared with the levels of the PC. Moreover, the addition of phytase linearly increased ($P < 0.05$) dietary protein, Ca, and phosphorus (**P**) utilization as well as nitrogen output, and excreta iron, copper, manganese, and zinc concentration quadratically increased ($P < 0.05$) as well as P output. In conclusion, phytase at ≥5,000 FTU/kg was effective in ameliorating the negative effects of NC diets and reducing trace mineral supplementation in diet of Pekin ducks.

**Key words:** excreta mineral content, low nonphytate phosphorus diet, Pekin ducks, phytase

2020 Poultry Science 99:4845–4852
https://doi.org/10.1016/j.psj.2020.06.047

## INTRODUCTION

In recent years, a growing number of studies have shown that adding phytase to livestock and poultry feed improves phosphorus (**P**) utilization and reduces feed costs and P output to the environment, which is an important way to improve the performance of animal production and reduce environmental pollution (Viveros et al., 2002; Cowieson et al., 2004; Walk et al., 2013, 2014). It has been shown that exogenous phytase effectively releases a portion of the phytate-bound P in a dose-dependent manner (Ravindran et al., 1999; Cowieson et al., 2006; Olukosi et al., 2007, 2008). Some studies have shown that in diets with 0.23 and 0.14% nonphytate phosphorus (**NPP**), respectively, the addition of 1,000 and 4,000 FTU/kg phytase can be sufficient to obtain performance in broilers that is comparable to that of birds given diets adequate in available P (Taheri et al., 2015). Some previous studies have also found that phytase continued to improve performance, bone characteristics, as well as P and calcium (**Ca**) digestibility up to a dose of 10,000 (Harper et al., 1997; Augspurger and Baker, 2004) or 12,000 FTU/kg (Shirley and Edwards, 2003). Phytase reduces not only the amount of inorganic P added to finisher diet of ducks but also the amount of P excreted in manure by increasing duck P utilization (Orban et al., 1999). The effects of microbial phytase supplementation vary with the intrinsic phytase level (Carlson and Poulsen, 2003), the physiological status of the animals, the origin of the supplemented phytase (Paditz et al., 2004), and the total P concentration in the basal diets (Zeng et al., 2015). For meat duck production, several alternative feedstuffs are used in the commercial complete feed, which may result in higher total P levels than in a



Received January 23, 2020.
Accepted June 17, 2020.

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corn–soybean meal diet. Therefore, the proper use of phytase in a typical commercial diet of meat ducks is a very significant economic and environmental issue for the practical production of ducks.

Dietary microbial phytase was reported to increase the digestibility of Ca, manganese (**Mn**), zinc (**Zn**), copper (**Cu**), and iron (**Fe**) in pigs at a dose of 500 to 1,500 FTU/kg of feed (Pallauf et al., 1992; Adeola et al., 1995; Jongbloed et al., 1995). Kies et al. (2006) found that dietary phytase supplementation beyond the commercial dose of phytase (500 FTU/kg) could further improve mineral use and consequently reduce mineral output to the environment. Similarly, Walk et al. (2014) also found that the changes in broiler performance with phytase can be largely attributed to the release of P in P-deficient diets and the elimination of the adverse effects of phytate on the digestibility of various dietary nutrients such as Ca, Zn, Fe, starch, lipids, and certain amino acids. However, little is known about the high dose phytase supplementation in P-deficient diets on nitrogen (**N**), P, and trace element output in Pekin ducks. Therefore, the objective of this study was to evaluate the effect of adding graded levels of phytase (500–10,000 FTU/kg) to a NPP-deficient diet on growth performance, serum biochemical parameters, tibia bone mineralization, and nutrition utilization or output in Pekin ducks aged from 1 to 35 D.

## MATERIALS AND METHODS

The Institutional Animal Care and Use Committee of Sichuan Agricultural University approved all procedures used in the study (SAUPN-19-04).

### Birds, Diets, and Management

A total of 560 one-day-old Pekin ducks were obtained from a local hatchery. On the day of arrival, all birds were weighed and randomly allocated among 7 treatments, which were distributed across 56 cages (1.0 m × 1.0 m) in a completely randomized design. Each treatment was replicated 8 times (each with 10 birds per cage). Each cage was provided with a suspended plastic tube feeder and 2 nipple drinkers, both adjustable according to the size of the birds. The barn temperature was initially maintained at 32°C at the outset of the experiments and then gradually reduced to 22°C and maintained during the rest of the trial period. All ducks had free access to water and feed throughout the experimental period.

As presented in Table 1, the basal experimental diets were formulated to meet or exceed meat duck nutritional requirements (NRC, 1994) with the exception of the NPP levels.

Dietary treatments included a corn–rice bran–soybean meal–based diet with the recommended levels of NPP (0.40 and 0.35% of NPP during the starter [day 1–14] and grower [day 15–35] periods, respectively) as a positive control (**PC**), a NPP-deficient diet (0.22 and 0.18% of NPP during the starter and grower periods, respectively) as a negative control (**NC**), and NC diets that were supplemented with graded levels of microbial phytase (500, 2,500, 5,000, 7,500, and 10,000 FTU/kg). Correspondingly, the analyzed activities of phytase in starter/growth diets was 298/275, 1,490/1,373, 2,980/2,745, 4,470/4,118, and 5,950/5,290 FTU/kg. The activity of phytase is commonly expressed as FTU, which is defined as the amount of phytase that liberates 1 mmol of inorganic phosphate per minute from 0.0051 mol$L^{-1}$ sodium phytate at pH 5.5 and a temperature of 37°C. The phytase source was provided by Mianyang Habio Bio-tech Co., Ltd. (Mianyang, China) and added based on its analytical activity. All experimental diets were provided in a pellet form, and the diameter of the pellets was 2 mm in the starter feed and 3 mm in the grower feed, which use steam modulation, and the temperature is 75°C to 85°C.

### Sample Collection

All birds were weighed on arrival, at 14 and 35 D of age on a group basis. Feed intake was also recorded at the same time points during each growth period for the calculation of the feed to gain ratio (**F/G**) after adjusting for mortality. The average body weight (**BW**), body weight gain (**BWG**), and daily feed intake (**ADFI**) were then estimated from 1 to 14 D, 15 to 35 D, and 1 to 35 D of age. On day 14 and 35, one bird per replicate was randomly selected (its BW being approximately equal to the average replicate BW), weighed, bled via the jugular vein, and then euthanized by stunning with cervical dislocation. Blood was centrifuged at 3,500 × $g$ for 15 min at 4°C to obtain serum, which was stored at −20°C. Subsequently, the left tibia bones were removed, stripped of adherent soft tissues, and then stored at −20°C.

### Serum Biochemical Parameters

The concentrations of total protein (**TP**), albumin (**ALB**), urea, Ca, and P and the activity of alkaline phosphatase (**ALP**) were measured with commercial assay kits (Nanjing Jiancheng Biological Product Co., Ltd., China) according to the manufacturer's instructions.

### Tibia Bone Mineralization

The left tibia bones were dehydrated in ethanol for 72 h, defatted for 72 h in diethyl ether: methanol (9:1), and dried at 105°C for 24 h to a constant weight. The tibia bones were then ashed in a muffle furnace at 600°C overnight in porcelain crucibles, and the tibia ash percentage was expressed as grams of ash per 100 g of dry, fat-free weight of the tibia (AOAC, 2006). The concentrations of Ca and P in the tibia were analyzed according to Ren et al. (2016).

### Nutrient Utilization and Output

An endogenous indicator method (insoluble ash in hydrochloric acid) was used to determinate nutrient

**Table 1.** Composition and calculated analysis of experimental diets (as fed basis).

| Ingredient, % | Starter period (1–14 D ) | | Grower period (15–35 D) | |
|---|---|---|---|---|
| | Control (PC) | low NPP (NC) | Control (PC) | low NPP (NC) |
| Corn | 54.13 | 54.13 | 50.79 | 50.79 |
| Soybean meal | 28.86 | 28.86 | 16.84 | 16.84 |
| Soybean oil | 1.62 | 1.62 | 2.44 | 2.44 |
| Rice bran | 8.40 | 8.40 | 20.60 | 20.60 |
| Rapeseed meal | 3.20 | 3.20 | 6.00 | 6.00 |
| L-Lysine | 0.13 | 0.13 | 0.07 | 0.07 |
| DL-Methionine | 0.16 | 0.16 | 0.14 | 0.14 |
| Limestone | 1.34 | 1.62 | 1.20 | 1.47 |
| Monocalcium phosphate | 1.13 | 0.33 | 0.89 | 0.13 |
| Salt | 0.35 | 0.35 | 0.35 | 0.35 |
| Choline chloride | 0.15 | 0.15 | 0.15 | 0.15 |
| Vitamin premix[1] | 0.03 | 0.03 | 0.03 | 0.03 |
| Mineral premix[2] | 0.50 | 0.50 | 0.50 | 0.50 |
| Bentonite | 0.00 | 0.52 | 0.00 | 0.49 |
| Calculated nutrients levels | | | | |
| Metabolizable energy, kcal/kg | 2,850 | 2,850 | 2,900 | 2,900 |
| Crude protein, CP% | 19.50 | 19.50 | 16.50 | 16.50 |
| Ca, % | 0.80 | 0.80 | 0.70 | 0.70 |
| Nonphytate P, % | 0.40 | 0.22 | 0.35 | 0.18 |
| Analyzed nutrients levels | | | | |
| DM, % | 88.84 | 88.96 | 86.13 | 85.97 |
| Ca, % | 0.75 | 0.75 | 0.63 | 0.65 |
| TP, % | 0.73 | 0.53 | 0.78 | 0.57 |
| Cu, mg/kg | 12.68 | 9.68 | 9.97 | 12.69 |
| Fe, mg/kg | 282.94 | 345.16 | 346.06 | 356.97 |
| Mn, mg/kg | 622.99 | 608.44 | 624.71 | 697.45 |
| Zn, mg/kg | 136.84 | 129.56 | 133.05 | 134.65 |

Abbreviations: NC, negative control; NPP, nonphytate phosphorus; PC, positive control.

[1]Vitamin premix provides the following per kg of final diet: vitamin A 8,000 IU; vitamin $D_3$ 2,000 IU; vitamin E 5 mg; vitamin $K_2$ 1 mg; vitamin $B_1$ 0.6 mg; vitamin $B_2$ 4.8 mg; vitamin $B_6$ 1.8 mg; vitamin $B_{12}$ 0.009 mg; niacin 10.5 mg; DL-calcium pantothenate 7.5 mg; folic acid 0.15 mg.

[2]Mineral premix provides the following per kg of final diet: Fe ($FeSO_4 \cdot H_2O$) 80 mg; Cu ($CuSO_4 \cdot 5H_2O$) 8 mg; Mn ($MnSO_4 \cdot H_2O$) 70 mg; Zn ($ZnSO_4 \cdot H_2O$) 90 mg; I (KI) 0.4 mg; Se ($Na_2SeO_3$) 0.3 mg.

utilization, and the nutrient output was then calculated based on utilization. Briefly, 100 g per cage of fresh duck excreta was repeatedly collected on 3 connective days from 33 to 35 D of age, and the collected feces were the evenly mixed. The excreta were dried at 65°C ± 5°C for 24 h, weighed, and crushed to pass through a 40-mesh sieve for dry matter, Ca, P, protein, and energy availability according to Cowieson and Bedford (2009) and Zeng et al. (2015). The levels of the trace elements Fe, Cu, Mn, and Zn in feed and excreta were determined according to the methods of Jorhem et al. (1993).

### Statistical Analysis

All data were collected and analyzed using the GLM procedure in SAS (SAS, 9.2) in a randomized complete block design with pen as the experimental unit. Significant differences among all dietary treatments were determined at $P < 0.05$ by Tukey's tests. The dose–response effect of supplemental phytase was computed using an orthogonal polynomial contrast for linear and quadratic effects (SAS, 9.2). All results were expressed as the mean and the SEM.

## RESULTS

### Growth Performance

Feeding the NC diet (0.18–0.17% less NPP vs. PC) to ducks reduced ($P < 0.01$) BWG and ADFI compared with the PC diet, regardless of whether it was day 1 to 14, 15 to 35, or 1 to 35 (Table 2). In addition, the birds fed the NC diet had a significantly higher F/G during 1 to 14 D of age. Phytase at 500 FTU/kg significantly improved performance compared with NC but was still significantly lower compared with the PC group ($P < 0.05$). Interestingly, the supplementation of NC diets with higher doses of phytase, particularly above 5,000 FTU/kg, improved ($P < 0.05$) BW at 14 and 35 D of age and BWG from 1 to 14 and 1 to 35 D of age ($P < 0.05$). There were positive linear and quadratic responses in ($P < 0.05$) BW and BWG and linear increase ($P < 0.05$) in ADFI during both the starter and grower periods with graded levels of phytase supplementation. The F/G improved linearly ($P < 0.05$), during the starter phase. The study indicated that 5,000 to 10,000 FTU/kg phytase supplementation can replace 0.18% NPP in a practical diet.

**Table 2.** Effects of graded levels of phytase supplementation on growth performance in Pekin ducks fed phosphorus-deficient diets.

| | | Negative control (NC) with phytase supplementation (FTU/kg) | | | | | | | $P$-value | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Item | Positive control (PC) | 0 | 500 | 2,500 | 5,000 | 7,500 | 10,000 | SEM | ANOVA | Linear | Quadratic |
| Body weight, BW (g) | | | | | | | | | | | |
| 1 D | 57.0[1] | 57.1 | 57.1 | 57.0 | 57.0 | 57.1 | 57.0 | 0.13 | 0.942 | 0.846 | 0.975 |
| 14 D | 758.4$^{a}$ | 442.2$^{d}$ | 514.9$^{c}$ | 708.6$^{b}$ | 731.6$^{a,b}$ | 751.1$^{a,b}$ | 731.0$^{a,b}$ | 15.4 | <0.001 | 0.0001 | 0.0001 |
| 35 D | 2,699$^{a}$ | 1,634$^{c}$ | 1,843$^{b}$ | 2,634$^{a}$ | 2,612$^{a}$ | 2,631$^{a}$ | 2,644$^{a}$ | 61.7 | <0.001 | 0.0001 | 0.0001 |
| Body weight gain, BWG (g) | | | | | | | | | | | |
| 1–14 D | 701.4$^{a}$ | 385.2$^{d}$ | 457.8$^{c}$ | 651.6$^{b}$ | 674.7$^{a,b}$ | 694.1$^{a,b}$ | 674.0$^{a,b}$ | 15.4 | <0.001 | 0.0001 | 0.0001 |
| 15–35 D | 1,940$^{a}$ | 1,183$^{c}$ | 1,329$^{b}$ | 1,926$^{a}$ | 1,880$^{a}$ | 1,881$^{a}$ | 1,913$^{a}$ | 59.8 | <0.001 | 0.0001 | 0.0001 |
| 1–35 D | 2,641$^{a}$ | 1,577$^{c}$ | 1,787$^{b}$ | 2,578$^{a}$ | 2,555$^{a}$ | 2,575$^{a}$ | 2,587$^{a}$ | 59.3 | <0.001 | 0.0001 | 0.0001 |
| Feed to gain ratio, F/G ( g/g ) | | | | | | | | | | | |
| 1–14 D | 1.38$^{c}$ | 1.56$^{a}$ | 1.46$^{b}$ | 1.41$^{b,c}$ | 1.42$^{b,c}$ | 1.38$^{c}$ | 1.41$^{b,c}$ | 0.03 | <0.001 | 0.001 | 0.234 |
| 15–35 D | 2.01$^{a}$ | 2.10$^{a}$ | 1.79$^{b}$ | 2.05$^{a}$ | 2.13$^{a}$ | 2.11$^{a}$ | 2.09$^{a}$ | 0.08 | <0.001 | 0.238 | 0.001 |
| 1–35 D | 1.83$^{a}$ | 1.86$^{a}$ | 1.68$^{b}$ | 1.87$^{a}$ | 1.93$^{a}$ | 1.89$^{a}$ | 1.90$^{a}$ | 0.05 | <0.001 | 0.052 | 0.003 |
| Average daily feed intake, ADFI (g) | | | | | | | | | | | |
| 1–14 D | 69.17$^{a}$ | 42.99$^{d}$ | 47.71$^{c}$ | 65.39$^{b}$ | 68.53$^{a,b}$ | 68.14$^{a,b}$ | 68.03$^{a}$ | 1.25 | <0.001 | <0.001 | 0.757 |
| 15–35 D | 185.0$^{a}$ | 117.3$^{b}$ | 113.3$^{b}$ | 187.4$^{a}$ | 190.8$^{a}$ | 188.6$^{a}$ | 190.6$^{a}$ | 3.92 | <0.001 | <0.001 | 0.103 |
| 1–35 D | 137.7$^{a}$ | 83.6$^{b}$ | 85.8$^{b}$ | 137.5$^{a}$ | 140.6$^{a}$ | 139.2$^{a}$ | 140.4$^{a}$ | 2.34 | <0.001 | <0.001 | 0.202 |

[a-d]Means within column and under each main effect with no common superscript differ significantly ($P < 0.05$).
Abbreviation: ANOVA, analysis of variance.
[1]Data are means of 8 replicate cages with 10 birds per cage.

## Serum Parameters

The effects of graded levels of phytase supplementation on the serum metabolite concentrations of ducks are presented in Table 3. The ducks fed the NC diet had significantly higher ($P < 0.05$) serum Ca, P, ALB, urea, TP levels, as well as ALP activity compared with the PC group. The ducks fed the NC diet supplemented with 2,500 to 5,000 FTU/kg phytase had the highest serum P level. Feeding the NC diet with graded levels of phytase supplementation to ducks linearly responses ($P < 0.05$) serum ALB and TP levels and linearly decreases ($P < 0.05$) in serum urea, Ca levels, and ALP activity at 14 and 35 D of age. In addition, there were quadratic effect ($P < 0.05$) on Ca, ALB levels, and ALP activity at 14 D of age and P level at 14 or 35 D of age with phytase addition to low-P diet.

## Tibia Bone Mineralization

Table 4 summarize the effect of microbial phytase on bone mineralization. Compared with the PC group, the percent ash, Ca, P, and the strength (day 14) of the tibia in birds fed NC diets significantly decreased ($P < 0.05$). The addition of phytase in NC diets up to 7,500 to 10,000 FTU/kg improved these parameters to a level comparable to or higher than those of the PC group. There were linear increases ($P < 0.05$) in tibia

**Table 3.** Effects of graded levels of phytase supplementation on serum metabolites concentration in Pekin ducks fed phosphorus-deficient diets.

| | | Negative control (NC) with phytase supplementation (FTU/kg) | | | | | | | $P$-value | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Item | Positive control (PC) | 0 | 500 | 2,500 | 5,000 | 7,500 | 10,000 | SEM | ANOVA | Linear | Quadratic |
| Total protein, TP (g/L) | | | | | | | | | | | |
| 14 D | 31.3$^{b}$ | 36.3$^{a}$ | 36.4$^{a}$ | 30.3$^{b}$ | 32.6$^{a,b}$ | 33.0$^{a,b}$ | 33.0$^{a,b}$ | 0.40 | 0.0246 | 0.0283 | 0.526 |
| 35 D | 26.4$^{b,c}$ | 31.0$^{a}$ | 29.6$^{a,b}$ | 27.8$^{ab}$ | 22.7$^{c}$ | 23.5$^{c}$ | 22.8$^{c}$ | 1.23 | <0.0001 | <0.0001 | 0.220 |
| Albumin, ALB (g/L) | | | | | | | | | | | |
| 14 D | 13.8$^{b}$ | 16.5$^{a}$ | 17.1$^{a}$ | 16.1$^{a}$ | 13.5$^{b}$ | 12.9$^{b}$ | 12.7$^{b}$ | 0.51 | <0.0001 | <0.0001 | 0.001 |
| 35 D | 9.58$^{b,c}$ | 12.06$^{a}$ | 11.69$^{a}$ | 11.05$^{a,b}$ | 8.54$^{c}$ | 8.88$^{c}$ | 8.48$^{c}$ | 0.55 | <0.0001 | <0.0001 | 0.103 |
| Urea (mmol/L) | | | | | | | | | | | |
| 14 D | 0.70$^{b}$ | 1.07$^{a}$ | 1.00$^{a}$ | 0.91$^{a}$ | 0.69$^{b}$ | 0.63$^{b}$ | 0.60$^{b}$ | 0.06 | <0.0001 | <0.0001 | 0.054 |
| 35 D | 0.59$^{b}$ | 0.96$^{a}$ | 0.94$^{a}$ | 0.80$^{a}$ | 0.57$^{b}$ | 0.55$^{b}$ | 0.53$^{b}$ | 0.06 | <0.0001 | <0.0001 | 0.039 |
| Calcium, Ca (mmol/L | | | | | | | | | | | |
| 14 D | 2.13$^{b}$ | 2.87$^{a}$ | 2.60$^{a}$ | 2.61$^{a}$ | 2.00$^{b}$ | 1.40$^{c}$ | 1.92$^{b}$ | 0.12 | <0.0001 | <0.0001 | <0.001 |
| 35 D | 1.68$^{c}$ | 2.51$^{a}$ | 2.19$^{b}$ | 2.11$^{b}$ | 1.62$^{c}$ | 1.54$^{c}$ | 1.60$^{c}$ | 0.09 | <0.0001 | <0.0001 | 0.108 |
| Phosphorus, P (mmol/L) | | | | | | | | | | | |
| 14 D | 3.36$^{c}$ | 4.58$^{b,c}$ | 5.40$^{b}$ | 6.72$^{a}$ | 4.52$^{b,c}$ | 3.59$^{c}$ | 3.60$^{c}$ | 0.44 | <0.0001 | 0.2528 | 0.002 |
| 35 D | 2.35$^{c}$ | 3.40$^{b,c}$ | 4.48$^{a,b}$ | 5.44$^{a}$ | 2.96$^{b,c}$ | 2.31$^{c}$ | 2.21$^{c}$ | 0.53 | 0.0002 | 0.1830 | 0.001 |
| Alkaline phosphatase, ALP (U/L) | | | | | | | | | | | |
| 14 D | 886$^{c}$ | 2,039$^{a}$ | 1,956$^{a}$ | 1,398$^{b}$ | 969$^{c}$ | 880$^{c}$ | 847$^{c}$ | 112.3 | <0.0001 | <0.0001 | 0.027 |
| 35 D | 620$^{c}$ | 1,542$^{a}$ | 1,379$^{a}$ | 975$^{b}$ | 583$^{c}$ | 534$^{c}$ | 507$^{c}$ | 89.59 | <0.0001 | <0.0001 | 0.059 |

Data are means of 8 replicates with 1 bird per replicate.
[a–c]Means within column and under each main effect with no common superscript differ significantly ($P < 0.05$).
Abbreviation: ANOVA, analysis of variance.

**Table 4.** Effects of graded levels of phytase supplementation on tibia bone mineralization in Pekin ducks fed phosphorus-deficient diets.

| Item | Positive control (PC) | Negative control (NC) with phytase supplementation (FTU/kg) | | | | | | SEM | *P*-value | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 500 | 2,500 | 5,000 | 7,500 | 10,000 | | ANOVA | Linear | Quadratic |
| Ash ( %) | | | | | | | | | | | |
| 14 D | 50.2[1]$^{a,b}$ | 36.7$^{d}$ | 37.7$^{d}$ | 45.2$^{c}$ | 48.8$^{b}$ | 51.5$^{a}$ | 50.9$^{a,b}$ | 0.49 | <0.0001 | 0.0045 | 0.223 |
| 35 D | 54.0$^{a,b}$ | 42.8$^{c}$ | 43.3$^{c}$ | 52.4$^{b}$ | 55.3$^{a}$ | 54.0$^{a,b}$ | 55.6$^{a}$ | 1.07 | <0.0001 | 0.0019 | 0.207 |
| Calcium, Ca ( %) | | | | | | | | | | | |
| 14 D | 17.7$^{a}$ | 12.7$^{c}$ | 13.0$^{c}$ | 16.1$^{b}$ | 17.3$^{a,b}$ | 18.5$^{a}$ | 17.8$^{a}$ | 4.46 | <0.0001 | 0.7328 | 0.735 |
| 35 D | 19.9$^{b,c}$ | 16.0$^{d}$ | 16.4$^{d}$ | 19.3$^{c}$ | 21.4$^{a}$ | 21.0$^{a,b}$ | 21.3$^{a,b}$ | 0.47 | <0.0001 | <0.0001 | 0.092 |
| Phosphorus, P ( %) | | | | | | | | | | | |
| 14 D | 8.97$^{a}$ | 5.90$^{c}$ | 5.90$^{c}$ | 6.60$^{b,c}$ | 7.22$^{b,c}$ | 7.59$^{a,b}$ | 8.88$^{a}$ | 0.44 | <0.0001 | <0.0001 | 0.007 |
| 35 D | 8.72$^{b}$ | 7.14$^{c}$ | 7.08$^{c}$ | 9.08$^{a,b}$ | 9.46$^{a}$ | 9.44$^{a}$ | 9.70$^{a}$ | 0.24 | <0.0001 | <0.0001 | 0.113 |
| Tibia bone strength, TBS (kg) | | | | | | | | | | | |
| 14 D | 26.5$^{a,b}$ | 10.9$^{c}$ | 13.9$^{c}$ | 27.3$^{a,b}$ | 20.9$^{b}$ | 30.9$^{a}$ | 33.2$^{a}$ | 0.83 | <0.0001 | <0.0001 | 0.001 |
| 35 D | 35.7$^{a,b}$ | 37.0$^{a,b}$ | 35.0$^{a,b}$ | 31.9$^{b}$ | 38.8$^{a,b}$ | 37.5$^{a,b}$ | 43.9$^{a}$ | 3.13 | 0.0001 | <0.0001 | 0.267 |

$^{a–c}$Means within column and under each main effect with no common superscript differ significantly ($P < 0.05$).
Abbreviation: ANOVA, analysis of variance.
[1]Data are means of 8 replicates with 1 bird per replicate.

bone ash, Ca (day 35), and P content as well as bone strength with phytase supplementation. In addition, the concentration of P and the strength (day 14) of the tibia exhibited a quadratic change ($P < 0.05$) with the increase in phytase dose.

## Nutrient Utilization and Output

There was no difference between the PC and NC diet in apparent availability of dry matter, protein, Ca, and P except for energy in which apparent availability was greater ($P < 0.05$) in the NC than the PC diet during the grower period (Table 5). Data showed a linear response ($P < 0.05$) for apparent energy, Ca, and P utilization with phytase supplement, and effect of commercial dose of phytase (500 FTU/kg) showed comparable to that of other doses of phytase supplementation. Besides, cumulative N and P output during 15 to 35 D were lower ($P < 0.05$) in ducks fed the NC group or NC diet with phytase addition than in those fed the PC diet. Moreover, the addition of phytase linearly increased ($P < 0.05$) the N output and quadratically increased ($P < 0.05$) the P output.

Feeding the NC diet to ducks significantly decreased ($P < 0.05$) Zn and Cu content in excreta compared with the PC diet (Table 6), but the excreta Fe content was markedly increased ($P < 0.05$). There were linear increases ($P < 0.05$) in the Mn, Cu, and Fe content in excreta with phytase supplementation. In addition, the supplementation of phytase up to 7,500 to 10,000 FTU/kg significantly increased ($P < 0.05$) the excreta Cu and Fe content when compared with the other 4 treatments.

## DISCUSSION

Diets of nonruminant animals are supplemented with phytase to improve the utilization of phytate P with the consequence of reducing the amount of P flowing into the environment from animal waste (Adeola, 2010). Indices of improved P utilization include improved growth performance, increased bone mineralization, and greater digestibility of P. Some reports have shown that the inclusion of high levels of phytase 1,500 FTU/kg and above can bring further improvements in performance compared with 500 FTU/kg (Karadas et al.,

**Table 5.** Effects of graded levels of phytase supplementation on nutrient utilization and nitrogen and phosphorus emissions in Pekin ducks fed phosphorus-deficient diets.

| Item | Positive control(PC) | Negative control (NC) with phytase supplementation (FTU/kg) | | | | | | SEM | *P*-value | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 500 | 2,500 | 5,000 | 7,500 | 10,000 | | ANOVA | Linear | Quadratic |
| Dry matter, % | 77.29[1] | 78.41 | 75.78 | 80.96 | 81.52 | 81.13 | 80.74 | 1.86 | 0.211 | 0.0893 | 0.354 |
| Energy, % | 80.4$^{b}$ | 83.0$^{a}$ | 83.4$^{a}$ | 83.8$^{a}$ | 84.6$^{a}$ | 84.6$^{a}$ | 84.6$^{a}$ | 0.76 | 0.0024 | 0.0082 | 0.590 |
| Protein, % | 70.6$^{b}$ | 73.8$^{a,b}$ | 76.3$^{a}$ | 78.7$^{a}$ | 78.2$^{a}$ | 76.1$^{a}$ | 76.5$^{a}$ | 1.72 | 0.0272 | 0.1391 | 0.951 |
| Calcium, % | 45.8$^{b,c}$ | 41.0$^{c}$ | 50.7$^{a,b}$ | 56.3$^{a}$ | 59.5$^{a}$ | 58.9$^{a}$ | 59.9$^{a}$ | 3.01 | <0.0001 | 0.0085 | 0.1335 |
| Phosphorus, % | 24.9$^{b}$ | 17.9$^{b}$ | 38.7$^{a}$ | 42.0$^{a}$ | 45.9$^{a}$ | 43.4$^{a}$ | 42.3$^{a}$ | 3.32 | <0.0001 | <0.0001 | 0.010 |
| Cumulative N output[2] (g) | 5494.6$^{a}$ | 3229.2$^{d}$ | 3436.8$^{c,d}$ | 4133.3$^{b,c,d}$ | 4310.0$^{a,b,c,d}$ | 4773.0$^{a,b}$ | 4558.9$^{a,b,c}$ | 411.52 | 0.0077 | 0.0009 | 0.3596 |
| Cumulative P output[2] (g) | 6667.7$^{a}$ | 4100.5$^{b}$ | 2,487.2$^{c}$ | 3865.1$^{b,c}$ | 3728.8$^{b,c}$ | 4128.7$^{b}$ | 4097.3$^{b,c}$ | 458.04 | 0.0002 | 0.7407 | 0.0314 |

$^{a–d}$Means within column and under each main effect with no common superscript differ significantly ($P < 0.05$). Abbreviation: ANOVA, analysis of variance.
Abbreviation: ANOVA, analysis of variance.
[1]Data are means of 8 replicate cages with 10 birds per cage.
[2]Cumulative N (nitrogen) or P (phosphorus) output = Feed intake (day 15–35) × dietary N or P concentration × (100- N or P utilization).

**Table 6.** Effects of graded levels of phytase supplementation on excreta trace elements content in Pekin ducks fed phosphorus-deficient diets.

| Item | Positive Control (PC) | Negative control (NC) with phytase supplementation FTU/kg) | | | | | | SEM | P-value | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 500 | 2,500 | 5,000 | 7,500 | 10,000 | | ANOVA | Linear | Quadratic |
| Zn (mg/kg) | 685.0[a] | 604.2[b] | 697.0[a] | 632.7[a,b] | 686.6[a] | 671.1[a] | 673.2[a] | 55.80 | 0.0347 | 0.0548 | 0.250 |
| Mn (mg/kg) | 581.1[c] | 590.1[c] | 597.6[c] | 669.2[b] | 723.3[a] | 695.9[a,b] | 710.3[a,b] | 38.29 | <0.0001 | <0.0001 | 0.612 |
| Cu (mg/kg) | 42.87[b,c] | 30.99[d] | 37.49[c] | 41.69[b,c] | 46.31[a,b] | 48.95[a] | 50.94[a] | 4.75 | <0.0001 | <0.0001 | 0.077 |
| Fe (mg/kg) | 806.4[d] | 1110.7[c] | 1264.2[b,c] | 1363.7[b] | 1599.6[a] | 1706.3[a] | 1696.1[a] | 195.09 | <0.0001 | 0.0012 | 0.528 |

[a–d]Means within column and under each main effect with no common superscript differ significantly ($P < 0.05$).
Abbreviations: ANOVA, analysis of variance; Cu, copper; Fe; iron; Mn, manganese; Zn, zinc.

2010; Pirgozlev et al., 2012; Dos Santos et al., 2013; Walk et al., 2014; Manobhavan et al., 2016). Similarly, in current study, we found that phytase supplementation at a dose of approximately 5,000 to 10,000 FTU/kg in NC diets with a reduced NPP level (0.18% [day 1–14] or 0.17% [day 15–35] less NPP vs. the PC) increased the growth performance of ducks to a level comparable to that of the PC group, which indicated that 5,000 to 10,000 FTU/kg phytase supplementation can replace 0.18% NPP in a practical diet. These results are consistent with those of many previous studies, which have shown that supplemental phytase, particularly at high doses (≥4,000 FTU/kg), increases the growth rate of broiler chickens to a level comparable to that of the PC group (Walk et al., 2014; Manobhavan et al., 2016). Pirgozlev et al. also found that birds fed high levels of phytase (12,500 FTU/kg) have an improved FCR when compared with birds fed diets sufficient in available P.

Increased ALP activity and Ca levels in the serum are associated with bone disorders (Brenes et al., 2003) and may be related to Ca or P deficiency or an excess Ca: P ratio in the diet. Consistent with this, we found that ducks fed NC diets had higher serum ALP activity and Ca levels in comparison with the PC group or high-dose phytase supplementation (approximately 5,000–10,000 FTU/kg). Huff et al. (1998) and Viveros et al. (2002) reported decreased ALP activity in response to phytase addition to low-P diets probably because of the downregulation of this enzyme as a result of the increased availability of P (CatalάGregori et al., 2006). In the present study, the addition of phytase (>5,000 FTU/kg) in P-deficient diets could decrease the concentrations of P, Ca, and ALP activity in the serum.

Some studies have reported an increase in serum P and a decrease in Ca in response to the addition of phytase to poultry diets (Viveros et al., 2002; Shirley and Edwards, 2003; Onyango et al., 2004; Hanczakowska et al., 2009). However, the decrease in the serum P level indicates that phytate hydrolysis in the present study was decreased when doses of ≥5,000 FTU/kg were used. The addition of high levels of phytase may change Ca: P ratio. Qian et al. (1997) showed that the increase observed in P retention by phytase addition was negatively influenced by increasing the dietary Ca: P ratio, this effect being stronger at lower levels of available P.

During the whole period of the study, P contents of serum were quadratic to phytase supplement. It may be because the formulated Ca: P rate was not held constant, and the release of inorganic P in diet supplemented with higher phytase concentration was at a higher rate relative to the diet with zero phytase supplementation. The improvements in duck performance and tibia bone mineralization from phytase can be largely attributed to the release of P in P-deficient diets. The reduced P intake of ducks fed the low-P diet, along with Ca, decreased the synthesis and deposition of hydroxyapatite in the tibia and hence the reduction in bone mineralization (Adeola, 2018). Even though P reduction in the diet negatively influences the process of bone mineralization, which is reversed by phytase supplementation (Kozłowski et al., 2010). In a series of broiler studies, Adeola showed that phytase was efficacious in hydrolyzing phytate P for bone mineralization and growth of ducks through the starter and grower periods (Adeola, 2018). In an earlier study, phytase supplementation at 10,000 FTU/kg produced tibia ash responses that were greater than those achieved with supplementation of 0.20% NPP (Augspurger and Baker, 2004). Orban et al. (1999) used a variety of bone dimensional measurements evaluated the response of White Pekin ducks to dietary supplementation with phytase and reported linear increases in these response criteria to addition of phytase to low-P diets. In the current study, the responses of ducks in ash, Ca, and P contents of the tibia as well as tibia bone strength to increasing dietary levels of supplemental phytase were positive and linear.

Changes in the amount of phytase to the animal diet also affect its P and protein available. Phytase supplements were found to positively affect P utilization in ducks (Farrell et al., 1993). There were both linear and quadratic increases in P utilization with phytase addition to the low-P NC diet (Adeola, 2018). Augspurger and Baker (2004) demonstrated that high dietary levels of phytase enzymes (5,000 and 10,000 FTU/kg) can release most of the P from phytate. The results of the present study are similarly to this study. The addition of phytase can partially ameliorate the detrimental effects of inositol hexaphosphate on protein utilization (Cowieson et al., 2006). The current result is consistent with the study showed decreased TP in the serum of birds fed a NC diet and a subsequent increase when fed phytase (Viveros et al., 2002).

Phytase supplementation of the diets in nonruminant animals improves the utilization of phytate P and ultimately reduces the amount of P flowing into the environment from animal waste. A 49% reduction in excreta P content was achieved by feeding hens low NPP diets supplemented with phytase, without compromising performance (Francesch et al., 2005). Adhikari et al. (2016) indicated that supplementation with phytase (2,500 FTU/kg) reduced fecal P output (g/D). Bingol et al. (2009) demonstrated that the addition of phytase linearly decreased the amount of P excreted in feces. Similarly, the results also showed that addition of phytase to NC diets decreased P output.

In the study, the standard reference for trace element addition is NRC (1994) and Chinese breeding standard (NY/T, 2012). The level of calculation is close to standard. But the addition amount of feeding standard did not consider the content of trace elements contained in the raw materials. At the same time, the content of trace elements in the corn miscellaneous meal diet was higher than that in the corn and soybean meal diet. Phytase hydrolyzes phytate to release inorganic P, which is used by the duck to meet its requirement for the mineral (Adeola, 2010). Phytic acid also reduces the absorption of Fe (McCance et al., 1943), Mn (Roberts and Yudkin, 1960), and Zn (O'Dell and Savage, 1960). It probably chelates, to some extent, all of the cations required by animals, which led to the increase of accounts in excreta. We found that addition of phytase to NC diets linearly increased the content of Zn, Mn, Cu, and Fe in excreta of ducks. The study indicated that 5,000 to 10,000 FTU/kg phytase supplementation can replace 0.18% NPP in a practical diet. Honestly, we are going to further study how much Zn and other trace minerals can be released by high dose phytase.

This result is in contrast to the results of Kies et al. (2006), who demonstrated that dietary phytase supplementation beyond 500 FTU/kg could further improve mineral use and consequently reduce mineral output to the environment. Under normal physiological conditions, phytate is a negatively charged Fe that is able to bind cations such as Ca, Mg, Zn, and proteins (Ravindran et al., 1995; Bebot-Brigaud et al., 1999). Phytase hydrolyzes the orthophosphate groups from phytate and phytate bound nutrients are liberated as well (Kies et al., 2006). These inconsistent results may occur because higher doses of phytase can hydrolyze more trace elements, which then results in a dietary available trace element content beyond the requirement of the ducks. Therefore, whether higher doses of phytase supplementation can reduce the addition of trace element needs to be considered further.

## CONCLUSIONS

On the basis of these results, it is concluded that supplementation with high doses (approximately 5,000–10,000 FTU/kg) of phytase in a P-deficient corn–rice bran–soybean meal diet (0.18% less NPP for the starter period and 17% less NPP for the grower period vs. the PC) was able to improve growth performance, serum biochemical parameters, and tibia bone mineralization, which may be attributed to the extraphosphoric effects of high-dose phytase in duck nutrition. However, high doses (approximately 5,000–10,000 FTU/kg) of phytase increased Fe, Cu, Mn, and Zn output, which suggests that the addition of high doses of phytase could save on trace element supplementation, and this requires further research.

## ACKNOWLEDGMENTS

This research was supported by grants from the National Key R & D Program of China (2017YFD0502004), the Modern Agri-industry Technology Research System of China (CARS-42-10), the "111" project of Foreign Experts Affairs of China, and the Sichuan Agricultural University 211 Foundation.

Conflict of Interest Statement: The authors did not provide any conflict of interest statement.

## REFERENCES

Augspurger, N. R., and D. H. Baker. 2004. High dietary phytase levels maximize phytate-phosphorus utilization but do not affect protein utilization in chicks fed phosphorus- or amino acid-deficient diets. J. Anim. Sci. 82:1100–1107.

Adeola, O., B. V. Lawrence, A. L. Sutton, and T. R. Cline. 1995. Phytase-induced changes in mineral utilization in zinc-supplemented diets for pigs. J. Anim. Sci. 11:3384–3391.

Adeola, O. 2010. Phosphorus equivalency value of an Escherichia coli phytase in the diets of White Pekin ducks. Poult. Sci. 89:1199–1206.

Adeola, O. 2018. Phytase in starter and grower diets of White Pekin ducks. Poult. Sci. 97:592–598.

Adhikari, P. A., J. M. Heo, and C. M. Nyachoti. 2016. High dose of phytase on apparent and standardized total tract digestibility of phosphorus and apparent total tract digestibility of calcium in canola meals from brassica napus, black and brassica juncea, yellow fed to growing pigs. Can. J. Anim. Sci. 96:121–127.

AOAC. 2006. Official Methods of Analysis. 17th ed. Assoc. Off. Anal. Chem. Int., Gaithersburg, MD.

Bebot-Brigaud, A., C. Dange, N. Fauconnier, and C. Gérard. 1999. 31p nmr, potentiometric and spectrophotometric studies of phytic acid ionization and complexation properties toward co2+, ni2+, cu2+, zn2+ and cd2+. J. Inorg. Biochem. 75:71–78.

Bingol, N. T., M. A. Karsli, D. Bolat, I. Akca, and T. Levendoglu. 2009. Effects of microbial phytase on animal performance, amount of phosphorus excreted and blood parameters in broiler fed low non-phytate phosphorus diets. Asian J. Anim. Vet. Adv. 4:160–166.

Brenes, A., A. Viveros, I. Arija, C. Centeno, M. Pizarro, and C. Bravo. 2003. The effect of citric acid and microbial phytase on mineral utilization in broiler chicks. Anim. Feed Sci. Technol. 110:201–219.

Carlson, D., and H. D. Poulsen. 2003. Phytate degradation in soaked and fermented liquid feed—effect of diet, time of soaking, heat treatment, phytase activity, pH and temperature. Anim. Feed Sci. Technol. 103:141–154.

CataláGregori, P., V. García, F. Hernández, J. Madrid, and J. J. Cerón. 2006. Response of broilers to feeding low-calcium and phosphorus diets plus phytase under different environmental conditions: body weight and tibiotarsus mineralization. Poult. Sci. 85:1923.

Cowieson, A. J., and M. R. Bedford. 2009. The effect of phytase and carbohydrase on ileal amino acid digestibility in monogastric diets: complimentary mode of action. Worlds Poult. Sci. J. 65:609.

Cowieson, A. J., T. Acamovic, and M. R. Bedford. 2004. The effects of phytase and phytic acid on the loss of endogenous amino acids and minerals from broiler chickens. Br. Poult. Sci. 45:101–108.

Cowieson, A. J., D. N. Singh, and O. Adeola. 2006. Prediction of ingredient quality and the effect of a combination of xylanase, amylase, protease and phytase in the diets of broiler chicks. 1.

Growth performance and digestible nutrient intake. Br. Poult. Sci. 47:477–489.

Dos Santos, T. T., S. Srinongkote, M. R. Bedford, and C. L. Walk. 2013. Effect of high phytase inclusion rates on performance of broilers fed diets not severely limited in available phosphorus. Asian-Aust. J. Anim. Sci. 26:227–232.

Francesch, M., J. Broz, and J. Brufau. 2005. Effects of an experimental phytase on performance, egg quality, tibia ash content and phosphorus bioavailability in laying hens fed on maize- or barley-based diets. Br. Poult. Sci. 46:340–348.

Farrell, D. J., E. Martin, J. J. DuPreez, M. Bongarts, M. Betts, A. A. Sudaman, and E. Thomson. 1993. The beneficial effects of a microbial feed phytase in diets of broiler chickens and ducklings. J. Anim. Physiol. Anim. Nutr. 69:278–283.

Harper, A. F., E. T. Kornegay, and T. C. Schell. 1997. Phytase supplementation of low-phosphorus growing-finishing pig diets improves performance, phosphorus digestibility, and bone mineralization and reduces phosphorus excretion. J. Anim. Sci. 75:3174–3186.

Hanczakowska, E. W. A., M. Świątkiewicz, and I. Kühn. 2009. Effect of microbial phytase supplement to feed for sows on apparent digestibility of P, Ca and crude protein and reproductive parameters in two consecutive reproduction cycles. Med. Weter. 65:250–254.

Huff, W. E., P.A. Moore, Jr, P. W. Waldroup, A. L. Waldroup, J. M. Balog, G. R. Huff, N. C. Rath, T. C. Daniel, and V. Raboys. 1998. Effect of dietary phytase and high non-phytate phosphorus corn on broiler chicken performance. Poult. Sci. 77:1899–1904.

Jongbloed, A. W., P. A. Kemme, Z. Mroz, and R. Bruggencate. 1995. Apparent total tract digestibility of organic matter, N, Ca, Mg and P in growing pigs as affected by levels of Ca, microbial phytase and phytate. Pages 198–204 in Proc. 2nd Eur. Symp. Feed Enzymes. W. van Hartingsveldt, M. Hessing, J. P. van der Lugt, and W. A. C. Somers, eds. TNO, Zeist, The Netherlands.

Jorhem, L. 1993. Determination of metals in foodstuffs by atomic absorption spectrophotometry after dry ashing:nmkl interlaboratory study of lead, cadmium, zinc, copper, iron, chromium, and nickel. J. AOAC Int. 76:798–813.

Karadas, F., V. Pirgozliev, A. C. Pappas, T. Acamovic, and M. R. Bedford. 2010. Effects of different dietary phytase activities on the concentration of antioxidants in the liver of growing broilers. J. Anim. Physiol. Anim. Nutr. (Berl.) 94:519–526.

Kies, A. K., P. A. Kemme, L. B. Sebek, J. T. Diepen, and A. W. Jongbloed. 2006. Effect of graded doses and a high dose of microbial phytase on the digestibility of various minerals in weaner pigs. J. Anim. Sci. 84:1169–1175.

Kozowski, K., J. Jankowski, and H. Jeroch. 2010. Efficacy of different levels of escherichia coli phytase in broiler diets with a reduced p content. Pol. J. Vet. Sci. 13:431–436.

Manobhavan, M., A. V. Elangovan, M. Sridhar, D. Shet, S. Ajith, D. T. Pal, and N. K. S. Gowda. 2016. Effect of super dosing of phytase on growth performance, ileal digestibility and bone characteristics in broilers fed corn–soya-based diets. J. Anim. Physiol. Anim. Nutr. 100:93–100.

Mccance, R. A., and E. M. Widdowson. 1943. Iron excretion and metabolism in man. Nature 152:326–327.

National Research Council. 1994. Nutrient Requirements of Poultry. 9th rev. ed. Natl. Acad. Press, Washington, DC.

Olukosi, O. A., A. J. Cowieson, and O. Adeola. 2008. Energy utilization and growth performance of broilers receiving diets supplemented with enzymes containing carbohydrase or phytase activity individually or in combination. Br. J. Nutr. 99:682–690.

Onyango, E. M., M. R. Bedford, and O. Adeola. 2004. The yeast production system in which Escherichia coli phytase is expressed may affect growth performance, bone ash, and nutrient use in broiler chicks. Poult. Sci. 83:421–427.

Olukosi, O. A., A. J. Cowieson, and O. Adeola. 2007. Age-related influence of a cocktail of xylanase, amylase, and protease or phytase individually or in combination in broilers. Poult. Sci. 86:77–86.

O'Dell, B. L., and J. E. Savage. 1960. Effect of phytic acid on zinc availability. Pro. Sci. Biol. Med. 103:304–306.

Orban, J. I., O. Adeola, and R. Stroshine. 1999. Microbial phytase in finisher diets of White Pekin ducks: effects on growth performance, plasma phosphorus concentration, and leg bone characteristics. Poult. Sci. 78:366–377.

Pallauf, J., D. Höhler, and G. Rimbach. 1992. Effect einer Zulage an mikrobieller Phytase zu einer Mais-Soja-Diät auf dieschein bare Absorption von Mg, Fe, Cu, Mn und Zn sowie auf Parameter des Zink status beim Ferkel. J. Anim. Physiol. Anim. Nutr. 68:1–9.

Paditz, K., H. Kluth, and M. Rodehutscord. 2004. Relationship between graded doses of three microbial phytases and digestible phosphorus in pigs. Anim. Sci. 78:429–438.

Pirgozliev, V., M. R. Bedford, O. Oduguwa, T. Acamovic, and M. Allymehr. 2012. The effect of supplementary bacterial phytase on dietary metabolizable energy, nutrient retention and endogenous losses in precision fed broiler chickens. J. Anim. Physiol. Anim. Nutr. 96:52–57.

Qian, H., E. T. Kornegay, and D. M. Denbow. 1997. Utilization of phytate phosphorus and calcium as influenced by microbial phytase, cholecalciferol, and the calcium: total phosphorus ratio in broiler diets. Poult. Sci. 76:37–46.

Ravindran, V., W. L. Bryden, and E. T. Kornegay. 1995. Phytates: occurrence, bioavailability and implications in poultry nutrition. Poult. Avian Biol. 6:125–143.

Ravindran, V., P. H. Selle, and W. L. Bryden. 1999. Effects of phytase supplementation, individually and in combination, with glycanase, on the nutritive value of wheat and barley. Poult. Sci. 78:1588–1595.

Ren, Z. Z., S. Z. Jiang, Q. F. Zeng, X. M. Ding, S. P. Bai, J. P. Wang, Y. H. Luo, Z. W. Su, Y. Xuan, and K. Y. Zhang. 2016. Effects of dietary canthaxanthin and 25-hydroxycholecalciferol supplementation on the antioxidant status and tibia quality of duck breeders and newly hatched ducklings. Poult. Sci. 95:2090–2096.

Roberts, A. H., and J. Yudkin. 1960. Dietary phytate as a possible cause of magnesium deficiency. Nature 185:823–825.

Shirley, R. B., and H. M. Edwards. 2003. Graded levels of phytase past industry standards improves broiler performance. Poult. Sci. 82:671–680.

Taheri, H. R., A. Heidari, and M. H. Shahir. 2015. Effect of high-dose phytase supplementation in broilers from 22 to 42 days post-hatch given diets severely limited in available phosphorus. Br. Poult. Sci. 56:330–336.

Viveros, A., A. Brenes, I. Arija, and C. Centeno. 2002. Effects of microbial phytase supplementation on mineral utilization and serum enzyme activities in broiler chicks fed different levels of phosphorus. Poult. Sci. 81:1172–1183.

Walk, C. L., M. R. Bedford, T. S. Santos, D. aiva, J. R. Bradley, H. Wladecki, C. Honaker, and A. P. McElroy. 2013. Extra phosphoric effects of super-doses of a novel microbial phytase. Poult. Sci. 92:719–725.

Walk, C. L., T. T. Santos, and M. R. Bedford. 2014. Influence of superdoses of a novel microbial phytase on growth performance, tibia ash, and gizzard phytate and inositol in young broilers. Poult. Sci. 84:248–255.

Zeng, Q. F., P. Cherry, A. Doster, R. Murdoch, O. Adeola, and T. J. Applegate. 2015. Effect of dietary energy and protein content on growth and carcass traits of Pekin ducks. Poult. Sci. 94:384–394.