Phosphorus equivalency of phytase with various evaluation indicators of meat duck

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ABSTRACT The objective of the present experiment was to determine the efficacy and the phosphorus (P) equivalency of phytase in the corn-soybean meal-rape-seed meal diets of Cherry Valley ducks from 1 to 35 d of age. 320 ducks were randomly divided into 8 blocks of 5 cages with 8 ducks per cage. This experiment included eight treatments diets. The available P levels of I to IV treatments were respectively 0.25%, 0.32%, 0.39%, 0.46% (d 1−14) and 0.20%, 0.27%, 0.34%, 0.41% (d 15−35). And 4 levels of phytase added to low-P basal diet (treatment I) with 300, 600, 900, and 1,200 U/kg (treatment V to VIII). Among them, treatment IV was a P-adequate positive control, treatment I was a low-P negative control. The ratio of calcium (Ca) to P was 1.3:1 for all diets. The other nutritional indexes in all diets were basically the same. Ducks were provided ad libitum access to water and experimental diets. The negative control diet reduced (P < 0.05) body weight, carcase weight, eviscerated weigh, breast muscle weight, leg muscle weight, bone ash, tibia Ca and tibia P, and increasing levels of available P and supplementary phytase significantly (P < 0.05) improved the growth performance and slaughtering performance of meat ducks. Phytase supplementation at a dose of 900 U/kg in the low-P basal diet increased the growth performance of ducks to a level comparable to that of a P-adequate diet. The available P level of 0.39% (1−14 d) and 0.34% (15−35 d) could meet the nutritional needs of meat ducks for P, and the apparent P utilization rate was high, and the effective utilization effect of P was the best. In addition, with the evaluation indexes of feed intake, body weight gain, tibia ash, tibia Ca, tibia P, content of blood Ca and P, the addition of 500 U/kg phytase could release available P of 0.02%, 0.02%, 0.02%, 0.02%, 0.01%, 0.04%, and 0.03%, respectively. In the same way, the addition of 1,000 U/kg phytase could release available phosphorus of 0.14%, 0.04%, 0.04%, 0.05%, 0.02%, 0.12%, and 0.01%, respectively.

Key words: phosphorus, phytase, phosphorus equivalency, duck

INTRODUCTION

The main components of poultry diets are the plant origin feedstuffs (Rapp et al., 2001) and more than half of the total phosphorus (P) in plants is in the form of phytate P, which is poorly utilized by poultry (Waldroup et al., 2000). It has been shown that exogenous phytase effectively releases a portion of the phytate-bound P in a dose-dependent manner (Cowieson et al., 2006; Olukosi et al., 2007, 2008). Over the past 2 decades, the addition of exogenous phytases to broilers diets has become a standard practice. There are increasing environmental and economic concerns regarding P utilization, and therefore, how to efficiently use feed raw material P and phytase has become the key to the research of P nutrition at present.

In recent years, researchers have conducted many studies on the physicochemical properties of phytase and its application effects in diets, and a series of achievements have been made (Rao et al., 2009; Farzana et al., 2019; Liu et al., 2020). However, phytase activity is affected by many factors, such as dietary phytate P content, calcium (Ca) and P level, exogenous phytase addition level, endogenous phytase activity and dietary structure, and the evaluation indexes used in each study are different. Thus, the practical application effect of phytase is lack of comparability among different studies. So it is difficult to determine the optimal addition level of phytase under certain conditions. To solve this problem, Yi et al. (1996) have proposed the concept of phytase P equivalent that is adding exogenous phytase to the diet can equivalently replace inorganic P or effective P content under certain conditions.

By far, reports abound in the literature on the equivalency of phytase relative to P for broiler chickens.
Broz et al. (1994) stated that inclusion of phytase at a level of 500 U/kg allowed the omission of additional dietary inorganic P in birds fed low P diets. Since this study, phytases have developed and substantially improved. Adeola (2010) indicated that addition of 500, 1,000, and 1,500 units of phytase to basal diet for 7-day-old ducks released 0.453, 0.847, and 1.242 g inorganic P/kg of diet after 10 d, respectively. The findings are in agreement with Cowieson et al. (2015) who found that addition of 1,000 FYT/kg of microbial phytase was equivalent to 0.75 g/kg of P from KH$_2$PO$_4$. However, ducks differ in P digestion from other avian species (Rodehutscord and Dieckmann, 2005) and a paucity of data exists for ducks. Thus, the objective of this study was to evaluate the efficacy as well as the P equivalency value of phytase in the diets of Cherry Valley ducks aged from 1 to 35 d, when dicalcium phosphate is used as an inorganic P in the reference diets.

**MATERIALS AND METHODS**

The experiment was conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals of Huazhong Agricultural University, China. The protocol for this experiment was approved by the Institution Animal Care and Use Committee at Huazhong Agriculture University (Wuhan, China), and the animal trial was conducted in accordance with the National Institute of Health Guidelines for the Care and Use of Experimental Animals (Beijing, China), with the approval number of HZAUDU-2005-001.

**Experimental Design, Birds, and Diets**

Three hundred and twenty 1-day-old Cherry Valley ducks (50% males, 50% females) were randomly assigned to 8 dietary treatment groups, ensuring that there were no significant differences between different treatment groups. There were 5 cages with 8 ducks per cage for each group. Test diets were administered to ducks from 1-day-old until 35-day-old.

The experimental diets were prepared according to the nutrition standard of meat duck put forward by British Cherry Valley Company, and combined with actual production. Corn-soybean meal-rapeseed meal diets were prepared according to the available P content of raw materials. There were 8 kinds of dietary treatments, which were expressed by I to VIII in turn. The feeding program consisted of 2 diets: starter diets supplied from d 1 to 14 (Table 1), followed by a grower diet from d 15 to 35 (Table 2). The available phosphorus levels of I to IV groups were respectively 0.25%, 0.32%, 0.39%, 0.46% (d 1 to 14) and 0.20%, 0.27%, 0.34%, 0.41% (d 15 to 35). Low-P basal diet (treatment I) supplemented with 300, 600, 900 and 1,200 U/kg phytase (Enzymatic activity 5,000 U/kg, Xinhuayang, Wuhan, China) respectively formed treatment V to VIII. The other nutritional indexes in all diets were basically the same (except calcium and phosphorus), and the ratio of Ca to P in the diet was about 1.3:1.

The use of phytase in low non-phytate P (nPP) diets benefits phytate hydrolysis, and has been demonstrated to improve the availability of P, body weight gain (BWG), bone mineralization, and nutrient utilization in ducks (Rodehutscord et al., 2006; Adeola, 2010). These benefits have been observed in a range of doses.

### Table 1. Composition and calculation analysis of the trial diets in starter period (% dry basis).

| Items                        | I      | II     | III    | IV     | V      | VI     | VII    | VIII    |
|-----------------------------|--------|--------|--------|--------|--------|--------|--------|--------|
| Dietary treatments          |        |        |        |        |        |        |        |        |
| Ingredients (%)             |        |        |        |        |        |        |        |        |
| Wheat bran                  | 5      | 5      | 5      | 5      | 5      | 5      | 5      | 5      |
| Corn                        | 58.74  | 58.34  | 57.94  | 57.54  | 57.54  | 58.74  | 58.74  | 58.74  |
| Soybean meal                | 27     | 27     | 27     | 27     | 27     | 27     | 27     | 27     |
| Rapeseed meal               | 6      | 6      | 6      | 6      | 6      | 6      | 6      | 6      |
| Limestone                   | 1.2    | 1.2    | 1.2    | 1.2    | 1.2    | 1.2    | 1.2    | 1.2    |
| Dicalcium phosphate         | 0.3    | 0.7    | 1.1    | 1.5    | 0.3    | 0.3    | 0.3    | 0.3    |
| Premix feed$^1$             | 1.0    | 1.0    | 1.0    | 1.0    | 1.0    | 1.0    | 1.0    | 1.0    |
| Salt                        | 0.4    | 0.4    | 0.4    | 0.4    | 0.4    | 0.4    | 0.4    | 0.4    |
| Synthetic lysine            | 0.2    | 0.2    | 0.2    | 0.2    | 0.2    | 0.2    | 0.2    | 0.2    |
| Synthetic methionine        | 0.16   | 0.16   | 0.16   | 0.16   | 0.16   | 0.16   | 0.16   | 0.16   |
| Phytase (U/kg)              | 0      | 0      | 0      | 0      | 300    | 600    | 900    | 1200   |
| Proximate compositions (%)  |        |        |        |        |        |        |        |        |
| Metabolic energy (Mcal/kg)  | 2.91   | 2.90   | 2.89   | 2.87   | 2.91   | 2.91   | 2.91   | 2.91   |
| Crude protein               | 19.6   | 19.5   | 19.5   | 19.5   | 19.5   | 19.6   | 19.6   | 19.6   |
| Total Ca                    | 0.65   | 0.74   | 0.83   | 0.92   | 0.65   | 0.65   | 0.65   | 0.65   |
| Available P                 | 0.25   | 0.32   | 0.39   | 0.46   | 0.25   | 0.25   | 0.25   | 0.25   |
| Total P                     | 0.49   | 0.57   | 0.64   | 0.71   | 0.49   | 0.49   | 0.49   | 0.49   |
| CaP                         | 1.31   | 1.31   | 1.31   | 1.31   | 1.31   | 1.31   | 1.31   | 1.31   |

$^1$Vitamin and mineral premixes supplied per kilogram diet: vitamin A, 13,000 IU; vitamin D$_3$, 6,600 IU; vitamin E, 83 mg; vitamin B$_6$, 10 mg; vitamin K$_3$, 2 mg; pantothenic acid, 8 mg; niacin, 42 mg; folic acid, 1.6 mg; biotin, 0.05 mg; Fe, 80 mg; Mn, 80 mg; Cu, 5 mg; Zn, 70 mg; I, 5 mg; Co, 1 mg; Se, 0.2 mg.
from 500 to 1,000 U/kg of phytase in diets, with the industry using a uniform inclusion level around 500 U/kg mark. Aureli et al. (2011) showed that with the same phytase and similar diets that apparent total P utilization begins to plateau at a dietary phytase activity of approximately 1,000 U/kg. Previous studies described that the type, amount of phytase in a practical diet might be supplemented at 500 or 1,000 U/kg (Manobhavan et al., 2015). Therefore, in the following analysis of the phosphorus equivalent of phytase in meat ducks, the phytase of 500 U/kg and 1,000 U/kg were taken as examples.

**Bird and Management**

Birds were allowed ad libitum access to the treatment diets and water for the duration of the trial. At the age of 35 d, a duck close to the average weight was taken from per cage for slaughtering experiment. Institutional and national guidelines for the care and use of animals were followed.

**Sampling and Measurement**

At d 35, after feed withdrawal for 12 h, the ducks and feed in each cage were weighed. BWG, cumulative feed intake (FI), and feed-to-gain ratio (FCR) were calculated, taking into consideration mortalities. Then, one duck with a weight closest to the cage average was selected and bled through the jugular vein. The blood samples were centrifuged at 3000 rpm for 10 min in a refrigerated centrifuge at about 4°C. Serum was collected and stored at −20°C until biochemical parameters were assayed. Spectrophotometer and standard kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) were used to measure serum Ca, P, and alkaline phosphatase (ALP) activities.

After blood collection, the birds were euthanized by cervical dislocation. The body weight at slaughter, carcass weight, semi-eviscerated weight, eviscerated weight, breast muscle weight and leg muscle weight were measured, and the dressing percentage, semi-eviscerated percentage, eviscerated percentage, breast muscle rate and leg muscle rate were calculated. Carcass yield was determined as the percentage of carcass weight in relation to total body weight, whereas the yield of the breast meat and leg muscle were expressed as percentages of the carcass weight.

After the slaughtering experiment, the left tibias were removed to measure tibia weight and ash content. The right tibia bone samples were analyzed for Ca and P contents by potassium permanganate method (GB/T6436-2002) and phosphovanadomolybdic acid colorimetric method (GB/T6437-2002), respectively. For the detailed determination method, please refer to the article by Yu and Zhang (2008).

Metabolic tests were conducted on 35 to 40 d and divided into a preliminary (3 d) and a formal (3 d) phase. A dietary indicator of 0.3% chromium trioxide (Cr₂O₃) was added, and each duck was fed a diet of 3% of its body weight every day. Five ducks of the similar weight were selected from each treatment, with a total of 40 ducks. Each was fed in a single cage and its excrement was collected in plate. Feces during the formal period were collected, mixed and sampled. Add 10% hydrochloric acid 10 mL for per 100 g of fresh fecal sample, then dry and smash the sample. Ca, P, and chromium contents in feed samples and dried feces samples of metabolic test were determined respectively. The apparent utilization

| Table 2. Composition and calculation analysis of the trial diets in grower period (% dry basis). |
|-----------------------------------------------|
| **Dietary treatments** | I | II | III | IV | V | VI | VII | VIII |
| **Ingredients (%)** | | | | | | | | |
| Wheat bran | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| Corn | 64.25 | 63.85 | 63.45 | 63.05 | 64.25 | 64.25 | 64.25 | 64.25 |
| Soybean meal | 17 | 17 | 17 | 17 | 17 | 17 | 17 | 17 |
| Rapeseed meal | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| Limestone | 1.2 | 1.2 | 1.2 | 1.2 | 1.2 | 1.2 | 1.2 | 1.2 |
| Dicalcium phosphate | 0 | 0.4 | 0.8 | 1.2 | 0 | 0 | 0 | 0 |
| Premix feed¹ | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Salt | 0.35 | 0.35 | 0.35 | 0.35 | 0.35 | 0.35 | 0.35 | 0.35 |
| Synthetic lysine | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Synthetic methionine | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Phytase (U/kg) | 0 | 0 | 0 | 0 | 300 | 600 | 900 | 1200 |
| **Proximate compositions (%)** | | | | | | | | |
| Metabolic energy (Mcal/kg) | 2.95 | 2.94 | 2.93 | 2.91 | 2.95 | 2.95 | 2.95 | 2.95 |
| Crude protein | 16.9 | 16.9 | 16.9 | 16.8 | 16.9 | 16.9 | 16.9 | 16.9 |
| Total Ca | 0.57 | 0.66 | 0.75 | 0.85 | 0.57 | 0.57 | 0.57 | 0.57 |
| Available P | 0.20 | 0.27 | 0.34 | 0.41 | 0.20 | 0.20 | 0.20 | 0.20 |
| Total P | 0.43 | 0.51 | 0.58 | 0.65 | 0.43 | 0.43 | 0.43 | 0.43 |
| Ca:P | 1.31 | 1.31 | 1.31 | 1.31 | 1.31 | 1.31 | 1.31 | 1.31 |

¹Vitamin and mineral premixes supplied per kilogram diet: vitamin A, 9,000 IU; vitamin D₃, 6,000 IU; vitamin E, 79 mg; vitamin B₁₂, 8 mg; vitamin K₃, 2 mg; pantothenic acid, 3.2 mg; niacin, 11 mg; folic acid, 1.5 mg; biotin, 0.05 mg; Fe, 60 mg; Mn, 50 mg; Cu, 6 mg; Zn, 60 mg; I, 2 mg; Co, 1 mg; Se, 0.18 mg.
ratio of Ca and P was calculated using the following equation.

Apparent utilization of Ca or P (%) = 100-[Ca or P in feces × Cr₂O₃ in feed/(Ca or P in feed × Cr₂O₃ in feces)] × 100

Statistical Analysis

The regression equations of evaluation index value and available P and phytase levels can be expressed by linear and nonlinear models: \( y = a + bx \) and \( y = a(1 - be^{-kt}) \) respectively, where \( x \) was the available P level (%) or the addition level of phytase (U/kg) in the diet, and \( y \) was the corresponding evaluation index value.

The second-order Akaike’s Information Criterion (AICc) was used to determine whether the linear or nonlinear regression was a better fit to the data (Motulsky and Christopoulos, 2004). The AICc is calculated for each line using the equation:

\[
AICc = N \times \ln\left(\frac{SS}{N}\right) + 2 \times K + \frac{2 \times K(K + 1)}{N - K - 1}
\]

where \( N \) was the number of samples, \( Ln \) was the natural logarithm, \( K \) was the number of parameters in the regression equation plus 1, and \( SS \) is the sum of squares of errors. Then the probability that the data fits the nonlinear regression equation was calculated using the following formula:

\[
\text{Probability} = \frac{e^{-0.5 \times \Delta}}{1 + e^{-0.5 \times \Delta}}
\]

where \( \Delta \) is the difference between the AICc results of the nonlinear equation and the linear equation. Suppose the probability sum of two equations explaining the same set of data is 1. When the probability \( \geq 0.5 \), it indicates that this set of data is suitable for the use of nonlinear curve; when the probability \( \leq 0.5 \), it indicates that it is more suitable for the use of linear curve; when the probability = 0.5, it is considered as uncertain.

All data were analyzed using one-way ANOVA in SAS8.0 software. Differences were considered significant at \( P < 0.05; P < 0.01 \) was considered extremely significant. Data are expressed as means and standard error of the mean (SEM). The linear regression model of the data was analyzed by the REG procedure in the SAS8.0 software. The nonlinear regression model was analyzed by the JMP procedure in the SAS8.0 software.

RESULTS

Growth Performance

The effect of dietary treatment on growth performance is presented in Table 3. With the increase of the level of available P, the FI and BWG increased in varying degrees. When the available P level was increased to the treatment II, the FI was significantly different from the highest P level (treatment IV \( P < 0.05 \)), but there was no significant difference between the BWG and treatment IV \( P > 0.05 \), and the FCR was the lowest. The addition of phytase to the low-P basal diet could improve the growth performance of meat ducks. With the increase of the level of phytase, the FI, BWG, and FCR basically decreased. During the grower phases, no significant differences were found in FI between treatment VI to VIII and treatment IV, no significant differences were found in BWG between treatment VII, VIII and treatment IV \( P > 0.05 \).

It can be seen from Table 4 that during the whole experiment, the regression analysis of dietary available P level and FI was suitable for the linear regression equation, and the regression analysis of BWG was suitable for nonlinear regression equation. The regression analysis of dietary phytase addition level and FI and BWG

Table 3. Effects of available phosphorus and supplemental phytase on growth performance of ducks.

| Items   | Dietary treatments | SEM | P-value |
|---------|--------------------|-----|---------|
| FI (g)  |                    |     |         |
| 0–14 d  | 314.3<sup>a</sup>  | 11.3| <0.0001 |
| 15–35 d | 2100.8<sup>b</sup> | 59.6| <0.0001 |
| 35 d    | 2415.1<sup>c</sup> | 66.8| <0.0001 |
| BW (g)  |                    |     |         |
| 0 d     | 53.4 <sup>a</sup>  | 0.20|         |
| 14 d    | 177.8<sup>d</sup>  |    |         |
| 35 d    | 816.3<sup>e</sup>  |    |         |
| BWG (g) |                    |     |         |
| 0–14 d  | 124.4<sup>a</sup>  | 7.92| <0.0001 |
| 15–35 d | 638.5<sup>b</sup>  | 38.6| <0.0001 |
| 0–35 d  | 762.6<sup>c</sup>  | 44.0| <0.0001 |

Abbreviations: BWG, body weight gain; FCR, feed-to-gain ratio; FI, cumulative feed intake.

At d 35, after feed withdrawal for 12 h, the ducks and feed in each cage were weighed.

Means within the same row without similar superscripts are significantly different \( P < 0.05 \).
were suitable for the linear regression equation, and the probability was higher than 95%.

When the growth performance index was used as the evaluation index, the equivalent equation of phytase to replace the available P content was shown in Table 5. The results showed that under the condition of corn-soybean meal-rapeseed meal diet, 500 U/kg and 1,000 U/kg phytase in diet could release 0.02% and 0.14% available P respectively, and the regression analysis was conducted separately when BWG was taken as response index; when BWG was taken as response index, 500 U/kg and 1,000 U/kg phytase in diet could release 0.02% and 0.04% available P respectively.

Slaughtering Performance

The results showed that different levels of dietary available P and phytase all affected the carcass quality of meat ducks (Table 6). At 35 d of age, the live weight, carcass weight, semi-eviscerated weight, eviscerated weight, breast muscle weight, leg muscle weight and dietary phytase supplementation level of meat ducks were well evaluated (r2 ≥ 0.5, data fits nonlinear regression equation; probability ≤ 0.5, data fits linear regression equation).

Table 4. Regressions of growth performance on available phosphorus and supplemental phytase in diets of 1–35 d old ducks.

| Growth performance | Regression equation1 | r2 | P-value | Probability2 |
|--------------------|----------------------|----|---------|--------------|
| Available phosphorus (%) | Y = −2535X + 1583 | 0.9972 | 0.0014 | 0.0992 |
| FIg | Y = 650384.28 (1-0.99976e−0.003902X) | 0.9572 | 0.0014 | 0.0992 |
| BWG,g | Y = 2399.6X + 298.8 | 0.6820 | 0.1735 | 0.9999 |
| Supplemental phytase (U/kg) | Y = 0.0005X + 1843 | 0.5431 | 0.1554 | 0.0473 |
| FI,g | Y = 2399.6X + 298.8 | 0.8340 | 0.0302 | 0.0004 |
| BWG,g | Y = 6380.4X + 604.7 | 0.9299 | 0.0039 | 0.0004 |

1Y = predicted performance for a given criteria (g); X = levels of available phosphorus (%) or levels of supplemental phytase (U/kg).

2Probability that the data fits the nonlinear regression equation (probability ≥ 0.5, data fits nonlinear regression equation; probability ≤ 0.5, data fits linear regression equation).

3Calculated available phosphorus equivalency values (%) of phytase.

4Equivalency equation created by setting the regression equations for available phosphorus and supplemental phytase equal to each other, Y = 0.444X + 604.7 = 929.94 (1-0.3133e−72.70X) = 0.8340 = 0.3182 = 0.0302 = 0.6818 = 0.0004

The results showed that different levels of dietary available P content reached a significant level (P < 0.05). However, the linear equations of live weight, carcass weight, semi-eviscerated weight, eviscerated weight, breast muscle weight, leg muscle weight and dietary phytase supplementation level of meat ducks were well fitted (r2 > 0.78, P < 0.05).

Tibia Parameters

The results showed that the tibia indexes basically increased with the increase of dietary available P level or phytase addition level (Figure 1). Compared with the low-P basal diet, the addition of 300 U/kg and 600 U/kg phytase did not significantly increase the tibia ash and P content of growing ducks (P > 0.05), while the addition of 900 U/kg phytase basically tibia ash and P content can reach the highest P level (P > 0.05). Adding 1,200 U/kg phytase can basically achieve the highest P level Ca deposition in tibia (P > 0.05).

The tibia index was used as the evaluation index, and the regression analysis was conducted separately with the available P and phytase levels (Table 7). At 35 d of age, the regression analysis of tibia index value and dietary available P level was suitable for nonlinear regression equation, and the probability was higher than 75%. The regression analysis of tibia index value and dietary phytase addition level was basically not significant (P > 0.05), only the linear equation between breast muscle weight and dietary available P content reached a significant level (P < 0.05). However, the linear equations of live weight, carcass weight, semi-eviscerated weight, eviscerated weight, breast muscle weight, leg muscle weight and dietary phytase supplementation level of meat ducks were well fitted (r2 > 0.78, P < 0.05).

Table 5. Equivalency of phytase relative to available phosphorus for the evaluation indexes of ducks.

| Items | Equivalency equation1 | Phytase2, U/kg |
|-------|------------------------|----------------|
| Growth performance | | 500 | 1,000 |
| FI | Y2P = 0.00023X + 0.1026 | 0.02 | 0.14 |
| BWG | Y2P = Ln (−0.0008115 - 6.2927 X10−7 Xph)/−35.89 | 0.02 | 0.14 |
| Tibia parameters | | | |
| Tibia ash | Y2P = 0.0228 - 1.4399 X10−7 Xph)/−18.63 | 0.02 | 0.04 |
| Tibia Ca | Y2P = 0.0393 - 2.0138 X10−7 Xph)/−16.34 | 0.02 | 0.04 |
| Tibia P | Y2P = 0.0102 - 2.1621 X10−7 Xph)/−94.93 | 0.01 | 0.02 |
| Serum parameters | | | |
| Ca (mmol/L) | Y2P = 0.0856 - 6.32 X10−7 Xph)/−12.06 | 0.04 | 0.12 |
| P (mmol/L) | Y2P = 0.0001 Xph + 0.2782 | 0.03 | 0.01 |

1Equivalency equation created by setting the regression equations for available phosphorus and supplemental phytase equal to each other, Y2P = levels of available phosphorus (%), Xph = levels of supplemental phytase (U/kg).

2Calculated available phosphorus equivalency values (%) of phytase.
When the tibia index was used as the evaluation index, the equivalent equation of phytase to replace the available P content was shown in Table 5. The results showed that under the condition of corn-soybean meal-rapeseed meal diet, when 500 U/kg and 1,000 U/kg phytase were added to the growing duck diet, 0.02% and 0.04% available P could be released respectively with bone ash content as response index; 0.02% and 0.05% available P could be released respectively with bone Ca content as response index; 0.01% and 0.02% available P can be released respectively with bone P content as response index.

### Table 6. Effects of available phosphorus and supplemental phytase on slaughter performance of 35-d old ducks.

| Item                              | I     | II    | III   | IV    | V     | VI    | VII   | VIII  | SEM  | P-value |
|-----------------------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|---------|
| Live body weight (g)              | 813.0a| 1373.2bc| 1467.6ab| 1560.4a| 897.4e| 1253.0d| 1309.4cd| 1437.4b| 41.81 | <0.0001 |
| Carcase weight (g)                | 748.0bc| 1290.0b| 1371.6ab| 1382.4d| 794.6c| 1123.4d| 1157.0cd| 1269.0d| 36.77 | <0.0001 |
| Dressing percentages (%)          | 92.0  | 91.1  | 89.8  | 88.6  | 88.4  | 89.7  | 88.4  | 88.3  | 0.36  | 0.0560  |
| Semi-eviscerated weight (g)       | 676.4b| 1128.4bc| 1209.6ab| 1272.4d| 718.4c| 1016.6d| 1065.2cd| 1175.8bc| 34.51 | <0.0001 |
| Semi-eviscerated percentage (%)   | 83.2  | 82.2  | 81.8  | 81.5  | 79.8  | 81.1  | 83.0  | 81.8  | 0.39  | 0.4992  |
| Eviscerated weight (g)            | 600.8a| 1013.0b| 1091.2ab| 1156.4c| 640.0d| 907.0d| 1016.2cd| 1175.8bc| 32.04 | <0.0001 |
| Eviscerated percentage (%)         | 74.0  | 73.8  | 74.4  | 74.1  | 71.0  | 72.4  | 75.0  | 73.9  | 0.39  | 0.2281  |
| Breast muscle weight (g)          | 20.5b | 54.5a | 62.1a | 61.6a | 25.5b | 46.8a | 51.6a | 58.5a | 2.89  | <0.0001 |
| Breast muscle rate (%)            | 3.4b  | 5.4a  | 5.6a  | 5.3a  | 3.7b  | 5.1a  | 5.2a  | 5.5a  | 0.19  | 0.0070  |
| Leg muscle weight (g)             | 16.1a | 16.9  | 16.3  | 16.8  | 17.1a | 16.8  | 17.7  | 14.8  | 0.24  | 0.1351  |
| Leg muscle rate (%)               | 16.1  | 16.9  | 16.3  | 16.8  | 17.1a | 16.8  | 17.7  | 14.8  | 0.24  | 0.1351  |
| Sebnum thickness (mm)             | 5.2a  | 5.7b  | 5.5abc| 5.8ab | 5.0c  | 6.1ab | 5.6bc | 5.8ab | 0.09  | 0.0481  |
| Left tibia weight (g)             | 8.4b  | 11.1a | 11.4a | 12.1a | 11.0a | 11.6a | 12.8a | 11.5a | 0.26  | 0.0006  |

a-dMeans within the same row without similar superscripts are significantly different 
\((P < 0.05)\).

At the age of 35 d, a duck close to the average weight was taken from per cage, a total of 40 ducks, for the slaughter experiment.

**Figure 1.** Effects of available phosphorus and phytase on tibia parameters of ducks at 35 d of age. **a-e**Means within the same row without similar superscripts are significantly different \((P < 0.05)\). Abbreviations: Ca, calcium; P, phosphorus; AP, available phosphorus.

**Table 7.** Regressions of tibia parameters on available phosphorus and supplemental phytase in diets of 35-d old ducks.

| Regression equation | r² | P-value |
|---------------------|----|---------|
| Y=76.78X+17.23      | 0.8168 | 0.0963 | 0.1584 | 0.9639 |
| Y=46.37 (1-15.0036e^-18.63X) | 0.9749 | 0.0035 | 0.1002 | 0.9938 |
| Y=34.11X+4.101      | 0.8415 | 0.0852 | 0.1176 | 0.7551 |
| Y=11.42X+3.197      | 0.5486 | 0.8939 | 0.3257 | 0.7551 |
| Y=7.34 (1-63025911.16e^-94.93X) | 0.5894 | 0.3257 | 0.2579 | 0.7551 |
| Y=0.008X+30.51      | 0.8453 | 0.8723 | 0.0271 | 0.1148 |
| Y=45.25 (1-0.3440e^-0.00009X) | 0.9584 | 0.0035 | 0.5894 | 0.0001 |
| Y=0.004X+9.454      | 0.9598 | 0.4106 | 0.0035 | 0.5894 |
| Y=12.87 (1-0.2491e^-0.0004X) | 0.8925 | 0.8998 | 0.0155 | 0.0600 |
| Y=0.001X+4.716      | 0.8925 | 0.8998 | 0.0155 | 0.0600 |

L: linear regression equation; Q: nonlinear regression equation.

**Figure 2.** Effects of available phosphorus and supplemental phytase on apparent utilization of calcium and phosphorus of ducks (15−35 d). **a-e**Means within the same row without similar superscripts are significantly different \((P < 0.05)\). Abbreviations: Ca, calcium; P, phosphorus; AP, available phosphorus.

available P could be released respectively with bone Ca content as response index; 0.01% and 0.02% available P can be released respectively with bone P content as response index.

### Apparent Utilization of Ca and P

The effect of dietary treatment on apparent utilization of Ca and P is presented in Figure 2. During the grower phases, no significant differences were found in apparent Ca utilization among treatments \((P > 0.05)\), suitable for linear regression equation, and the probability was higher than 88%.

When the tibia index was used as the evaluation index, the equivalent equation of phytase to replace the available P content was shown in Table 5. The results showed that under the condition of corn-soybean meal-rapeseed meal diet, when 500 U/kg and 1,000 U/kg phytase were added to the growing duck diet, 0.02% and 0.04% available P could be released respectively with bone ash content as response index; 0.02% and 0.05% available P could be released respectively with bone Ca content as response index; 0.01% and 0.02% available P can be released respectively with bone P content as response index.
and no significant differences were found in apparent P utilization among the treatments with the increase of the level of available P ($P > 0.05$). The apparent P utilization of low-P basal diet was improved by adding phytase. When the phytase addition exceeded 600 U/kg, the apparent P utilization was significantly higher than the low-P basal diet ($P < 0.05$), and there was no significant difference among the three treatments (treatment VI to VIII) ($P > 0.05$).

**Serum Parameters**

The results for serum parameters of ducks at 35 d of age are presented in Figure 3. With the increase of available P level, the serum Ca content of treatment III and IV was significantly higher than that of treatment I, and the blood P content of treatment IV was significantly higher than that of treatment I, II and III ($P < 0.05$). There was no significant difference in ALP activity among treatments ($P > 0.05$). With the increase of phytase level, the blood Ca content showed an increasing trend, while the blood P content basically showed a trend of first decreasing and then increasing. No significant differences were found in ALP activity among treatments ($P > 0.05$).

The serum index was used as the evaluation index, and the regression analysis was conducted separately with the available P and phytase levels (Table 8). At 35 d of age, the regression analysis of blood Ca content and dietary available P level was suitable for nonlinear regression equation, and the probability was higher than 74%. The regression analysis of available P level and blood P content was more suitable for the linear regression equation. The regression analysis of phytase addition level and blood Ca and P content were suitable for the linear regression equation.

When the contents of blood Ca and P were used as the evaluation index, the equivalent equation of phytase to replace the available P content was shown in Table 5. The results showed that under the condition of corn-soybean meal-rapeseed meal diet, when 500 U/kg and 1,000 U/kg phytase were added to the growing duck diet, 0.04% and 0.12% available P could be released respectively with blood Ca content as response index; 0.03% and 0.01% available P could be released respectively with blood P content as response index.

**DISCUSSION**

In our study, supplementation of dietary available P or phytase improved performance and carcass traits in ducks. These results agreed with previous studies on the growth performance of broilers (Shirley and Edwards, 2003; Majeed et al., 2020). But there is controversy about whether phytase can improve the FCR. Johnston and Southern (2000) reported that the weight gain of broilers supplemented with phytase was due to the increase of FI, rather than the improvement of FCR. Walk et al. (2013) observed significant improvements in the FCR of 49-day-old broilers fed phytase compared with broilers fed a nutrient-adequate control diet. The results of this experiment showed that the growth performance of meat ducks could reach the normal level when dietary available P content was 0.32% (1−14 d) and 0.27% (15−35 d). And the addition of phytase more than 600 U/kg to the low-P basal diet could significantly improve the growth and FCR of meat ducks.

The increase of available P and phytase in diet had significant effects on the bone performance of meat ducks (Xie et al., 2009; Liu et al., 2020). The use of phytase in maize-soybean meal-base diets has been demonstrated to improve the availability of P and bone mineralization in ducks (Fan et al., 2019). In this study, feeding low-P basal diet (0.20%) with increasing phytase levels from 300 to 1200 U/kg in ducks increased the content of bone ash, bone Ca and bone P. When the phytase addition reached 900 U/kg, the tibial index was significantly higher than that of the low-P basal diet ($P < 0.05$), indicating that dietary phytase had a significant
Table 8. Regressions of serum parameters on available phosphorus and supplemental phytase in diets of ducks at 35 d of age.

| Serum parameters | Regression equation | r² | P-value | Probability² |
|------------------|---------------------|----|---------|--------------|
| Ca, mmol/L       | Y=2.381X+1.633      | 0.8321 | 0.0878 | 0.2000 | 0.7432 |
| P, mmol/L        | Y=7.326X+0.705      | 0.5339 | 0.2693 | 0.6828 | 0.0093 |
| ALP, U/100 mL    | Y=48.66e(1-0.00015X) | 0.5238 |       |        |        |
| Supplemental phytase, U/kg |                     |       |        |        |        |
| Ca, mmol/L       | Y=0.004X+2.059      | 0.9797 | 0.0120 | 0.0197 | 0.0498 |
| P, mmol/L        | Y=-0.0008X+2.744   | 0.5924 | 0.1280 | 0.4077 | 0.0112 |
| ALP, U/100 mL    | Y=64.18e(1-0.3007X) |       |        |        |        |

L: linear regression equation; Q: nonlinear regression equation.

1Y=predicted performance for a given criteria (g); X=levels of available phosphorus (%) or levels of supplemental phytase (U/kg).

2Probability that the data fits the nonlinear regression equation (probability ≥ 0.5, data fits nonlinear regression equation; probability ≤ 0.5, data fits linear regression equation).

The authors declare that there is no conflict of interest.

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DISCLOSURES

The authors declare that there is no conflict of interest.
SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.psj.2021.101216.

REFERENCES

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

Aureli, R., M. Umar Faruk, I. Cechova, P. B. Pedersen, S. G. Elvig-Joergensen, F. Fri, and J. Broz. 2011. The efficacy of a novel microbial 6-phytase expressed in Aspergillus oryzae on the performance and phosphorus utilization in broiler chickens. Int. J. Poult. Sci. 10:160–168.

Bougouin, A., J. A. D. R. N. Appuhamy, E. Kebreab, J. Dijkstra, Bradbury, E. J., S. J. Wilkinson, G. M. Cronin, C. L. Walk, and C. Simoes Nunes. 1994. Effects of supplemental phytase on performance and phosphorus utilisation in broiler chickens. Anim. Feed Sci. Technol. 110:201–219.

Gautier, A. E., C. L. Walk, and R. N. Dilger. 2018. Effects of a high level of phytase on broiler performance, bone ash, phosphorus utilization, and phosphorus requirement and phosphorus excretion of broilers fed diets containing phytase. Poult. Sci. 97:409–416.

Johnston, S. L., and L. L. Southern. 2000. The Effects of varying mix uniformity (simulated) of phytase on growth performance, mineral retention, and bone mineralization in chicks. Poult. Sci. 79:1485–1490.

Jondreville, C., P. Leescoat, M. Magnin, D. Feuerstein, B. Gruenberg, and Y. Nys. 2007. Sparing effect of microbial phytase on zinc supplementation in maize-soya-bean meal diets for chickens. Animal 1:804–811.

Liu, Y. F., K. Y. Zhang, Y. Zhang, S. P. Bai, X. M. Ding, J. P. Wang, H. W. Peng, Y. Xuan, Z. W. Su, and Q. F. Zeng. 2020. Effects of graded levels of phytase supplementation on growth performance, serum biochemistry, tibia mineralization, and nutrient utilization in Pekin ducks. Poult. Sci. 99:1485–1482.

Majeed, S., R. Qudsieh, F. W. Edens, and J. Brake. 2020. Limestone particle size, calcium and phosphorus levels, and phytase effects on live performance and nutrients digestibility of broilers. Poult. Sci. 99:1502–1514.

Manoharavan, M., A. V. Elangovan, M. Sridhar, D. Shet, S. Ajith, D. T. Pal, and N. K. Gowda. 2015. 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.

Motulsky, H., and A. Christopoulos. 2004. Page 352 in Fitting Models to Biological Data Using Linear and Nonlinear Regression: A Practical Guide to Curve Fitting. Oxford Univ. Press, Oxford, UK.

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.

Olukosi, O. A., A. J. Cowieson, and O. Adeola. 2008. Energy utilization and growth performance of broilers receiving diets supplemented with enzymes containing carbohydrates 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.

Radcliffe, J. S., and E. T. Kornegay. 1998. Phosphorus equivalency value of microbial phytase in weaning pigs fed a corn-soybean meal based diet. J. Anim. Feed. Sci. 7:197–211.

Rao, E. C. S., K. V. Rao, T. P. Reddy, and V. D. Reddy. 2009. Molecular characterization, physicochemical properties, known and potential applications of phytases: an overview. Crit. Rev. Biotechnol. 29:182–198.

Rapp, C., H. J. Lantzych, and W. Drochner. 2001. Hydrolysis of phytic acid by intrinsic plant and supplemented microbial phytase (Aspergillus niger) in the stomach intestine of omniv pigs fitted with re-entrant cannulas: 3. Hydrolysis of phytic acid (IP6) and occurrence of products (IP5, IP4, IP3 and IP2). J. Anim. Physiol. Anim. Nutr (Berl). 85:420–430.

Rodelhutscord, M., and A. Dieckmann. 2005. Comparative studies with three-week-old chickens, turkeys, ducks, and quails on the response in phosphorus utilization to a supplementation of monobasic calcium phosphate. Poult. Sci. 84:1252–1260.

Rodelhutscord, M., R. Hempel, and P. Wendl. 2006. Phytase effects on the efficiency of utilisation and blood concentrations of phosphorus and calcium in Pekin ducks. Br. Poult. Sci. 47:311–321.

Schmey, D., E. Burton, N. Morgan, C. Sanni, C. K. Madsen, G. Dionisio, and H. Brinch-Pedersen. 2017. PI and Ca digestibility is increased in broiler diets supplemented with the high-phytase HIPHIHYP wheat. Animal 11:1457–1463.

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

Vieira, S. L., D. L. Anschau, N. C. Seranini, L. Kindleins, A. J. Cowieson, and J. B. O. Serbera. 2015. Phosphorus equivalency of a Citrobacter braakii phytase in broilers. 24:335–342.