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-rapeseed 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

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INTRODUCTION

The main components of poultry diets are the plant origin feedstuffs (Rapp et al., 2001) and more than half of the total phosporous (**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.

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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_2PO_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 Experiment 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).

	Dietary treatments							
Items	I	II	III	IV	V	VI	VII	VIII
Ingredients (%)								
Wheat bran	5	5	5	5	5	5	5	5
Corn	58.74	58.34	57.94	57.54	58.74	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	19.6	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
Ca:P	1.3:1	1.3:1	1.3:1	1.3:1	1.3:1	1.3:1	1.3:1	1.3:1

The available phosphorus levels of I to IV groups were respectively 0.25%, 0.32%, 0.39%, 0.46% . Low-P basal diet (treatment I) supplemented with 300, 600, 900 and 1,200 U/kg phytase respectively formed treatment V to VIII.

[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_2, 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, 3 mg; Co, 1 mg; Se, 0.2 mg.

Table 2. Composition and calculation analysis of the trial diets in grower period (% dry basis).

Items	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 feed[1]	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	1.3:1	1.3:1	1.3:1	1.3:1	1.3:1	1.3:1	1.3:1	1.3:1

The available phosphorus levels of I to IV groups were respectively 0.20%, 0.27%, 0.34%, 0.41% . Low-P basal diet (treatment I) supplemented with 300, 600, 900 and 1,200 U/kg phytase respectively formed treatment V to VIII.

[1]Vitamin and mineral premixes supplied per kilogram diet: vitamin A, 9,000 IU; vitamin D_3, 6,000 IU; vitamin E, 79 mg; vitamin B_2, 8 mg; vitamin K_3, 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.

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 ashed 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 (Cr_2O_3) 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 ratio of Ca and P was calculated using the following equation.

Apparent utilization of Ca or P (%) = 100-[Ca or P in feces × Cr_2O_3 in feed/(Ca or P in feed × Cr_2O_3 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 $y=a(1\text{-}be^{-kx})$ 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:

$$AIC_C = \left[N \times Ln\left(\frac{SS}{N}\right) + 2 \times K\right] + \frac{2 \times K(K+1)}{N-K-1}$$

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:

$$\mathrm{Pr}obability = \frac{e^{-0.5\times\Delta}}{1+e^{-0.5\times\Delta}}$$

where Δ 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 ≥ 0.5, it indicates that this set of data is suitable for the use of nonlinear curve; when the probability ≤ 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.

	Dietary treatments									
Items	I	II	III	IV	V	VI	VII	VII	SEM	P-value
FI (g)										
0−14 d	314.3[e]	451.0[c]	491.9[b]	534.0[a]	367.5[d]	390.9[d]	448.0[c]	443.9[c]	11.3	<0.0001
15−35 d	2100.8[c]	2250.6[bc]	2451.0[ab]	2625.5[a]	1591.1[d]	2336.5[abc]	2383.0[abc]	2605.0[a]	59.6	<0.0001
0−35 d	2415.1[d]	2701.7[cd]	2942.9[abc]	3159.6[a]	1958.6[e]	2727.5[cd]	2831.0[bc]	3048.9[ab]	66.8	<0.0001
BW (g)										
0 d	53.4	53.7	52.8	51.3	53.5	52.1	52.3	52.1	0.20	——
14 d	177.8[d]	294.7[b]	301.8[b]	337.4[a]	236.8[c]	275.3[b]	291.4[b]	299.9[b]	7.84	<0.0001
35 d	816.3[d]	1430.9[abc]	1458.1[ab]	1529.1[a]	842.6[d]	1265.5[c]	1321.3[bc]	1393.7[abc]	44.6	<0.0001
BWG (g)										
0−14 d	124.4[d]	241.0[b]	249.0[b]	286.0[a]	183.3[c]	223.2[b]	239.1[b]	247.8[b]	7.92	<0.0001
15−35 d	638.5[c]	1136.1[ab]	1156.3[ab]	1191.7[a]	605.8[c]	990.2[b]	1029.9[ab]	1093.9[ab]	38.6	<0.0001
0−35 d	762.6[d]	1377.3[abc]	1405.3[ab]	1477.8[a]	789.0[d]	1213.4[c]	1269·0[bc]	1341.6[abc]	44.0	<0.0001
FCR										
0−14 d	2.59[a]	1.88[bc]	1.99[b]	1.87[bc]	2.02[b]	1.76[c]	1.88[bc]	1.80[c]	0.04	<0.0001
15−35 d	3.31[a]	1.98[c]	2.13[c]	2.20[c]	2.98[ab]	2.39[bc]	2.32[bc]	2.39[bc]	0.09	<0.0001
0−35 d	3.19[a]	1.96[c]	2.10[c]	2.14[c]	2.65[ab]	2.27[bc]	2.24[bc]	2.28[bc]	0.07	<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.
[a-d]Means within the same row without similar superscripts are significantly different ($P < 0.05$).

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

Growth performance	Regression equation[1] L	Regression equation[1] Q	r^2 L	r^2 Q	*P*-value L	*P*-value Q	Probability[2]
Available phosphorus (%)							
FI,g	Y=2535X+1583	$Y=650384.28\ (1\text{-}0.9976e^{-0.003912X})$	0.9972	0.9972	0.0014	0.0532	0.0092
BWG,g	Y=2399.6X+298.8	$Y=1177.30\ (1\text{-}599.3223e^{-35.89X})$	0.6820	0.9974	0.1735	0.0508	0.9999
Supplemental phytase (U/kg)							
FI,g	Y=0.600X+1843	$Y=268839.75\ (1\text{-}0.9931e^{-0.000002X})$	0.5431	0.5430	0.1554	0.4570	0.0473
BWG,g	Y=0.444X+604.7	$Y=929.94\ (1\text{-}0.3133e^{72.70X})$	0.8340	0.3182	0.0302	0.6818	0.0004

L: linear regression equation; Q: nonlinear regression equation.
[1]Y=predicted performance for a give criteria (g); X=levels of available phosphorus (%) or levels of supplemental phytase (U/kg).
[2]Probability that the data fits the nonlinear regression equation (probability ≥ 0.5, data fits nonlinear regression equation; probability ≤ 0.5, data fits linear regression equation).

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 with FI 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, and leg muscle weight of low-P basal diet (treatment I) were significantly or extremely significantly lower than those of other treatments (except treatment V) ($P < 0.05$; $P < 0.01$), and the left tibia weight of treatment I was significantly lower than that of other treatments ($P < 0.05$).

It can be seen from annexed table 1 that the linear or nonlinear equations of the slaughter performance index and dietary available P level of meat ducks were 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 ($r^2 > 0.78$, $P < 0.05$).

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

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

Items	Equivalency equation[1]	Phytase[2],U/kg 500	Phytase[2],U/kg 1,000
Growth performance			
FI	$Y_{AP}=0.000237X_{phy}+0.1026$	0.02	0.14
BWG	$Y_{AP}=Ln\ (0.0008115 - 6.2927*10^{-7}X_{phy})\ /\text{-}35.89$	0.02	0.14
Tibia parameters			
Tibia ash	$Y_{AP}=Ln\ (0.0228 - 1.1499*10^{-5}X_{phy})\ /\text{-}18.63$	0.02	0.04
Tibia Ca	$Y_{AP}=Ln\ (0.0393 - 2.0138*10^{-5}X_{phy})\ /\text{-}16.34$	0.02	0.05
Tibia P	$Y_{AP}=Ln\ (5.672*10^{-9} - 2.162*10^{-12}X_{phy})\ /\text{-}94.93$	0.01	0.02
Serum parameters			
Ca (mmol/L)	$Y_{AP}=Ln\ (0.0856 - 6.33*10^{-5}X_{phy})\ /\text{-}12.06$	0.04	0.12
P (mmol/L)	$Y_{AP}=\text{-}0.0001X_{phy}+0.2782$	0.03	0.01

[1]Equivalency equation created by setting the regression equations for available phosphorus and supplemental phytase equal to each other, Y_{AP}= levels of available phosphorus (%), X_{phy}= levels of supplemental phytase (U/kg).
[2]Calculated available phosphorus equivalency values (%) of phytase.

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

Item	Dietary treatments								SEM	*P*-value
	I	II	III	IV	V	VI	VII	VIII		
Live body weight (g)	813.0^{e}	1373.2^{bc}	1467.6^{ab}	1560.4^{a}	897.4^{e}	1253.0^{d}	1309.0^{cd}	1437.4^{b}	41.81	<0.0001
Carcase weight (g)	748.0^{e}	1250.0^{b}	1317.6^{ab}	1382.4^{a}	794.6^{e}	1123.4^{d}	1157.0^{cd}	1269.0^{b}	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)	676.4^{e}	1128.4^{bc}	1200.0^{ab}	1272.4^{a}	718.4^{e}	1016.6^{d}	1085.2^{cd}	1175.8^{abc}	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)	600.8^{e}	1013.0^{bc}	1091.2^{ab}	1156.6^{a}	640.0^{e}	907.0^{d}	981.6^{cd}	1016.2^{bc}	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)	20.5^{b}	54.5^{a}	62.1^{a}	61.6^{a}	25.5^{b}	46.8^{a}	51.6^{a}	58.5^{a}	2.89	<0.0001
Breast muscle rate (%)	3.4^{b}	5.4^{a}	5.6^{a}	5.3^{a}	3.7^{b}	5.1^{a}	5.2^{a}	5.5^{a}	0.19	0.0070
Leg muscle weight (g)	96.8^{e}	170.8^{bcd}	177.7^{ab}	193.4^{a}	108.8^{e}	152.0^{d}	173.7^{bc}	157.1^{cd}	5.41	<0.0001
Leg muscle rate (%)	16.1	16.9	16.3	16.8	17.1	16.8	17.7	14.8	0.24	0.1351
Sebum thickness (mm)	5.2^{bc}	5.7^{abc}	5.5^{abc}	5.8^{ab}	5.0^{c}	6.1^{ab}	5.6^{abc}	5.8^{ab}	0.09	0.0481
Left tibia weight (g)	8.4^{b}	11.1^{a}	11.4^{a}	12.1^{a}	11.0^{a}	11.6^{a}	12.8^{a}	11.5^{a}	0.26	0.0006

[a-d]Means 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.

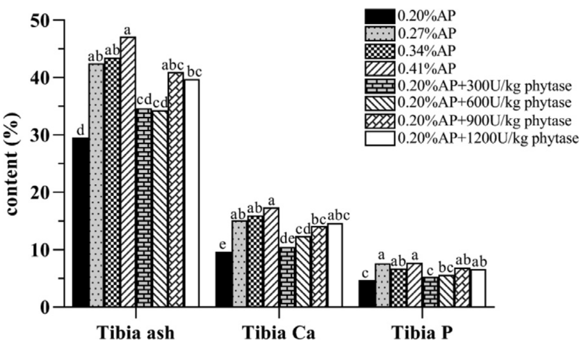

Figure 1. Effects of available phosphorus and phytase on tibia parameters of ducks at 35 d of age. [a-e]Means within the same row without similar superscripts are significantly different ($P < 0.05$). Abbreviations: Ca, calcium; P, phosphorus; AP, available phosphorus.

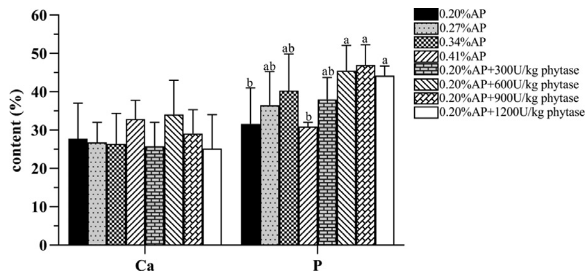

Figure 2. Effects of available phosphorus and supplemental phytase on apparent utilization of calcium and phosphorus of ducks (15−35 d). [a,b]Means within the same row without similar superscripts are significantly different ($P < 0.05$). Abbreviations: Ca, calcium; P, phosphorus; AP, available phosphorus.

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.

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 ($P > 0.05$),

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

Tibia parameters	Regression equation[1]		r^2		*P*-value		Probability[2]
	L	Q	L	Q	L	Q	
Available phosphorus,%							
Tibia ash,%	Y=76.78X+17.23	$Y=46.37\ (1-15.0036e^{-18.63X})$	0.8168	0.9749	0.0963	0.1584	0.9639
Tibia Ca,%	Y=34.11X+4.101	$Y=17.25\ (1-11.5138e^{-16.34X})$	0.8415	0.9862	0.0827	0.1176	0.9938
Tibia P,%	Y=11.42X+3.197	$Y=7.34\ (1-63025911.16e^{-94.93X})$	0.5486	0.8939	0.2593	0.3257	0.7551
Supplemental phytase,U/kg							
Tibia ash,%	Y=0.008X+30.51	$Y=45.25\ (1-0.3440e^{-0.00098X})$	0.8453	0.8723	0.0271	0.1277	0.1148
Tibia Ca,%	Y=0.004X+9.454	$Y=12.87\ (1-0.2491e^{2.45X})$	0.9598	0.4106	0.0035	0.5894	0.0001
Tibia P,%	Y=0.001X+4.716	$Y=9.95\ (1-0.5336e^{-0.00045X})$	0.8925	0.8998	0.0155	0.1002	0.0660

L: linear regression equation; Q: nonlinear regression equation.
[1]Y=predicted performance for a give criteria (g); X=levels of available phosphorus (%) or levels of supplemental phytase (U/kg).
[2]Probability that the data fits the nonlinear regression equation (probability ≧ 0.5, data fits nonlinear regression equation; probability ≦ 0.5, data fits linear regression equation).

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 ($P > 0.05$).

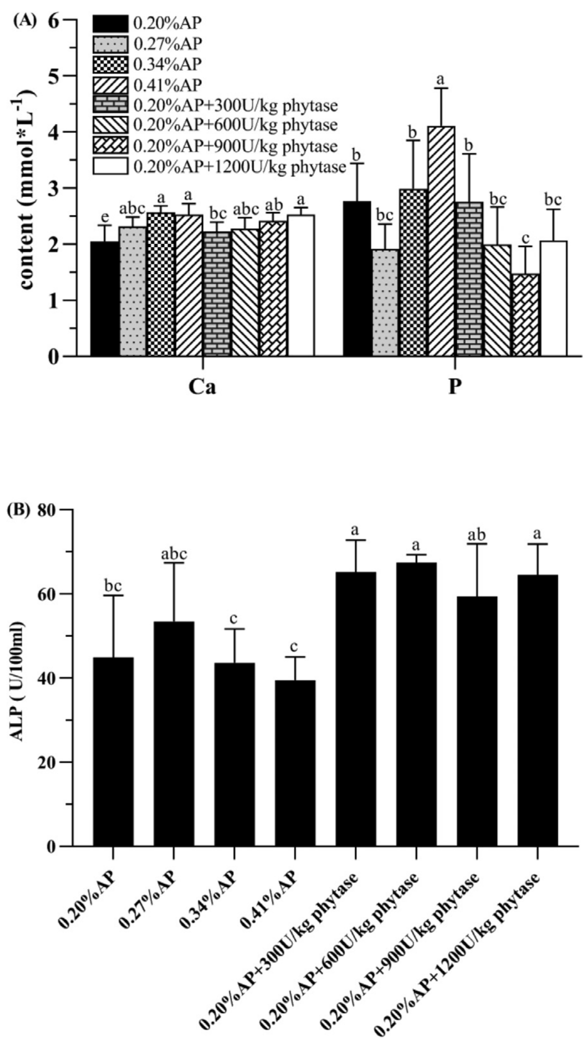

Figure 3. Effects of available phosphorus and supplemental phytase on serum parameters of ducks at 35 d of age. [a-c]Means within the same row without similar superscripts are significantly different ($P < 0.05$). Abbreviations: AP, available phosphorus; ALP, alkaline phosphatase.

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[1] L	Regression equation[1] Q	r^2 L	r^2 Q	*P*-value L	*P*-value Q	Probability[2]
Available phosphorus,%							
Ca,mmol/L	Y=2.381X+1.633	$Y=2.60\ (1\text{-}2.4301e^{-12.06X})$	0.8321	0.9600	0.0878	0.2000	0.7432
P,mmol/L	Y=7.328X+0.705	$Y=2986.49\ (1\text{-}0.9998e^{-0.0025X})$	0.5339	0.5338	0.2693	0.6828	0.0093
ALP,U/100mL	——	$Y=48.66\ (1\text{-}0.00013e^{17.905X})$	——	0.5238	——	0.6901	
Supplemental phytase,U/kg							
Ca,mmol/L	Y=0.0004X+2.059	$Y=5.14\ (1\text{-}0.6004e^{-0.00014X})$	0.9797	0.9803	0.0120	0.0197	0.0498
P,mmol/L	Y=-0.0008X+2.744	$Y=2482.62\ (1\text{-}0.9989e^{-0.000001X})$	0.5924	0.5923	0.1280	0.4077	0.0112
ALP,U/100 mL	——	$Y=64.18\ (1\text{-}0.3007e^{-0.3117X})$	——	0.8985	——	0.1015	

L: linear regression equation; Q: nonlinear regression equation.
[1]Y=predicted performance for a give criteria (g); X=levels of available phosphorus (%) or levels of supplemental phytase (U/kg).
[2]Probability that the data fits the nonlinear regression equation (probability ≧ 0.5, data fits nonlinear regression equation; probability ≦ 0.5, data fits linear regression equation).

effect on improving the bone performance of meat ducks under low-P basal diet condition.

Similar to that observed in the present study, Majeed et al. (2020) reported that inclusion of phytase improved both Ca and P digestibility at 14 d while only P digestibility was affected at 35 d. Phytase degrades phytate in poultry diets, thus improves Ca and P availability, which can then result in better live performance (Bougouin et al., 2014; Bradbury et al., 2016; Scholey et al., 2017). Supplementation of low-P basal diet with phytase improved P use in the current study, which is interpreted as a phytase-induced release of phytate-bound P. The ability of phytase to improve P availability by hydrolyzing phytate-bound P in poultry diets is well documented (Dilger et al., 2004; Onyango et al., 2004; Jondreville et al., 2007; Walk et al., 2014).

Serum mineral concentration is often used as an important indicator of mineral status of birds. Serum P concentrations were affected by dietary P levels and increased linearly depending on dietary P levels. 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. Previous studies reported that dietary phytase supplementation had lower contents of serum Ca and P in poultry (Gautier et al., 2018). However, during the study, dietary phosphorus levels and phytase supplementation had little effect on serum ALP and Ca levels. This variation is largely due to differences in experimental design, analytical methods, diet composition and processing, feeding and management level, the age and breed of the ducks, and available P gradient.

Average bioequivalence nPP for each phytase level was dependent on the evaluated response. Vieira et al. (2015) observed that the addition of 500 FYT of phytase to basal diet released 0.077 and 0.145g inorganic P/kg of diet for toe P and femur Ca, respectively. And average bioequivalence nPP for 1,000 FYT supplementation was evaluated with lowest values of 0.143 based on BWG as the evaluation index and highest values 0.194 on toe ash. Jendza et al. (2006) indicated that 500 FTU/kg of ECP (Escherichia coli-derived phytase) was determined to be equivalent to the addition of 0.49 or 1.00 g of P per kg when using WG and bone ash, respectively. In our study, the P equivalent values of phytase, which takes BWG and P utilization as the response index, were higher than that of bone ash, even up to twice. These results agreed with previous studies, which found the P equivalent using BWG was more than double that using bone ash suggesting that the P equivalent values of phytase were different for different evaluation indexes (Radcliffe and Kornegay, 1998).

CONCLUSIONS

Overall, this study demonstrated that the phytase was effective at improving growth performance, bone performance and P utilization. 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 P-equivalence values of phytase in different response parameters vary greatly. Based on corn-soybean-rapeseed meal diet, with the evaluation indexes of FI, BWG, tibia ash, tibia Ca, tibia P, content of blood Ca and P, the addition of 500 U/kg phytase could release available phosphorus 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. It is instructive that in our study, the evaluation for determining P bioavailability in ducks led to the conclusion that growth performance and bone ash are more sensitive than other response criteria.

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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.

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