

Effect of a microbial phytase on growth performance, plasma parameters and apparent ileal amino acid digestibility in Youxian Sheldrake fed a low-phosphorus corn-soybean diet

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Objective: This study investigated the effect of microbial phytase supplementation on growth performance, tibia ash, plasma parameters, apparent ileal digestibility (AID) of amino acid (AA) and apparent digestibility of nutrients in Youxian Sheldrakes fed with low-phosphorus (P) corn-soybean diets.

Methods: A total of 350 Youxian Sheldrakes (7d old) were randomly divided into 5 treatment groups: positive control (PC) group has adequate available P diet (0.42% and 0.38%, starter and grower), negative control (NC) group were deficient in available P (0.32% and 0.28%, starter and grower) and NC diet was supplemented with 3 levels of microbial phytase (500, 750, and 1,000 U/kg).

Results: Dietary supplementation of phytase in NC diet improved the average daily gain, increased the levels of serum calcium (Ca), tibia Ca and P, AID of AA and apparent digestibility of energy and Ca in starter stage ($p < 0.05$). There was an increased ($p < 0.001$) in the utilization of P from 17.3% to 23.9%. Phytase supplementation (1,000 U/kg) has shown that the AID of His, Thr, Val, indispensable AA, Glu, Pro, and dispensable AA was higher ($p < 0.05$) than that of NC. Moreover, phytase supplementation improved ($p < 0.05$) serum and tibia Ca and P, AID of AA and apparent digestibility of dry matter, crude protein, energy, P and Ca, and reduced ($p < 0.05$) feed to gain ratio (F/G) and the levels of serum alkaline phosphatase in grower stage. Likewise, an increase ($p < 0.001$) in the utilization of P was noticed from 12.6% to 17.2%. Supplement phytase at 750 U/kg improved the AID of His, Thr, Asp, Cys, Pro, and Ser ($p < 0.05$).

Conclusion: The microbial phytase supplement could improve growth performance, AID of some AA and apparent utilization of other nutrients in Youxian Sheldrakes, and reduce excreta P load to environment.

Keywords: Youxian Sheldrake; Phytase; Apparent Nutrient Digestibility; Performance; Apparent Ileal Amino Acid

INTRODUCTION

Phytic acid (PA, myo-inositol hexakisphosphate, IP6) is a natural plant compound with a unique structure that is responsible for its characteristic properties. The PA is the primary phosphate storage compound in seeds, typically contributing 50% to 80% of total phosphate in plant seeds [1]. The bioavailability of phosphorus (P) present in phytate is generally poor [2,3]. As PA has 12 replaceable reactive sites, it is able to bind di- and trivalent minerals as well as amino acids (AAs) and proteins. Physiologically, the reason for this unavailability is due to the very low phytase activity found in the digestive tract of monogastric animals.

Phytase is an enzyme, chemically known as myoinositol (1, 2, 3, 4, 5, 6) -hexaphosphate phosphohydrolase, and mainly catalyses the hydrolysis of phytate rendering P available for absorption. Phytases are divided into two categories, 3-phytase (EC 3.1.3.8) and 6-phytase (EC 3.1.3.26),

based on the site where the hydrolysis of the phytate molecule is initiated [1]. Phytases are widely distributed among various life forms such as microorganisms. Phytases from fungal origin and bacterial origin are major sources of microbial phytases [4]. The application of microbial phytase is well established as an effective and practical method to improve the bioavailability of phosphorus in animal production. Many studies in broilers and pigs have demonstrated the effectiveness of phytases in improving the bioavailability of phosphorus [3]. However, there is no consensus as to the effect of phytase on ileal AA digestibility [5,6]. Effects of phytase on different AA digestibility are different [7].

Youxian Sheldrake is a native duck breed of Hunan province, China, which was listed in Chinese famous livestock and poultry genetic resources [8]. However, the application of phytase in Youxian Sheldrake diets is rarely reported. Thus the objective of this study was to investigate the effect of microbial 6-phytase on growth performance, apparent nutrient digestibility, chemical traits of tibia, plasma parameters and apparent ileal AA digestibility in Youxian Sheldrakes fed a low-phosphorus corn-soybean diet.

MATERIALS AND METHODS

All trial procedures were carried out in accordance with the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and the experimental protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of Hunan Agricultural University.

Birds feeding and management

In this study, a total of 350 male Youxian sheldrakes (7 d old) were purchased from a local commercial hatchery (Elite hatchery of Youxian sheldrake, Zhuzhou, China). The sheldrakes were individually weighed and randomly allotted to 5 groups with 5 replicates for each group (14 sheldrakes per replicate). All of Youxian Sheldrakes were reared in pens with plastic-cages, housed in an environmentally controlled building with an auto-controlled lighting program. The temperature in the room was controlled by electrically heated battery brooders and ventilation fans. The room was maintained at 30°C and 25°C from 7 d (days of age) to 15 d and 16 d to 63 d respectively. Feed and water were provided *ad libitum*. Birds, feed, and water were checked three times daily and mortality was recorded. This feeding experiment was conducted in Animal Husbandry and Veterinary Institute of Hunan Province.

Experimental design and diets

The experimental period was divided into two stages, starter (7 to 28 d) and grower stage (29 to 63 d). The diets were corn-soybean meal based diet. All experimental diets were formulated to meet or exceed the nutritional requirement for Youxian Sheldrakes as recommended by the NRC [9], DB43/T 898-2014 (China) and

1993-Feeding Standard of laying ducks (Taiwan). The ingredients and calculated nutrients profile of the experimental diets are presented in Table 1. The positive control (PC) diet contain available P 4.2 g/kg (starter stage) and 3.8 g/kg (grower stage) of dry matter (DM), whereas a similarly formulated diet but with 1 g/kg less available P (starter and grower stage) served as the negative control (NC). The other groups were NC diet supplement with three levels of phytase (500, 750, 1,000 phytase units/kg), respectively. One unit of phytase activity is defined as the quantity of enzyme required to hydrolyze 1 μmol of inorganic P/min, at pH 5.5, from an excess of 1.5 mM sodium phytate at 37°C. In this study, phytase (*Escherichia coli* 6-phytase) was added at a concentration of 5,000 U/kg. A source of acid-insoluble ash (AIA) was used as an indigestible marker to determine nutrient digestibility. Sheldrakes were fed with crumbled starter diets from d 7 to 28 and pelleted grower diets from d 29 to 63.

Measurements

At 28 d and 63 d birds were weighted individually and feed intake per cage was recorded daily. The average daily gain (ADG), average daily feed intake (ADFI), and ratio of feed to gain (F/G) were

Table 1. Nutrient composition of diets (as fed basis)

Items (%)	Positive control diet		Low phosphorus diet	
	7 to 28 days of age	29 to 63 days of age	7 to 28 days of age	29 to 63 days of age
Corn	59.4	63	59.4	63
Soybean meal	26.3	17.8	26.3	17.8
Rice bran	5.5	8.6	5.5	8.6
Rapeseed meal	2.7	5.5	2.7	5.5
Soybean oil	1.77	0.17	1.77	0.17
Dicalcium phosphate	1.27	1.08	0.82	0.63
Calcium carbonate	1.07	1.1	1.42	1.46
Lysine	0.13	0.1	0.13	0.1
Methionine	0.15	0.13	0.15	0.13
Salt	0.31	0.3	0.31	0.3
Premix ¹⁾	1	1	1	1
Zeolite	0.4	1.22	0.5	1.31
Total	100	100	100	100
Nutrient composition ²⁾				
ME (kcal/kg)	2,900	2,800	2,900	2,800
CP	18	16	18	16
Ca	0.9	0.85	0.9	0.85
Total P	0.71	0.7	0.61	0.6
Available P	0.42	0.38	0.32	0.28
Lysine	1.05	0.85	1.05	0.85
Methionine	0.42	0.38	0.42	0.38
Met+Cys	0.71	0.65	0.71	0.65

ME, metabolizable energy; CP, crude protein.

¹⁾ The premix provides following for per kilogram diet: vitamin A 7,000 IU, vitamin D₃ 1,000 IU, vitamin E 18 IU, vitamin K₃ 2.2 mg, vitamin B₁ 1.3 mg, vitamin B₂ 3.1 mg, vitamin B₆ 2.2 mg, vitamin B₁₂ 0.02 mg, nicotinic acid 40 mg, pantothenic acid 11 mg, biotin 0.1 mg, folic acid 0.68 mg, Cu 10 mg, Fe 100 mg, Zn 80 mg, Mn 50 mg, Se 0.2 mg, I 0.5 mg.

²⁾ Partial nutrient were calculated values.

calculated based on the recorded date. Excreta samples were collected daily from 26 d to 28 d and 61 d to 63 d; waxed plastic paper was placed in trays, pooled within a cage, sub-sampled and stored at -20°C for the determination of apparent nutrient digestibility. On 28 d and 63 d, two sheldrakes were randomly selected from each pen and slaughtered by cervical dislocation after venous blood collection from the wing. Blood samples were centrifuged for 10 min at 3,000 rpm at 4°C . Serum was collected and frozen at -20°C until subsequent analysis for the content of Ca, P, total protein (TP), alkaline phosphatase (ALP), uric acid (UA), and blood urea nitrogen (BUN). The left tibias were collected to determine bone ash, P and Ca. The tibias were subsequently dried at 105°C for 12 h, extracted with diethyl ether, dried again, and weighed. The dry fat-free bones were ashed in a muffle furnace at 550°C for 6 h. The ash was used to determine concentrations of Ca, P [10]. Ileal contents from sheldrakes in the same cage were flushed with distilled water into plastic containers and stored at -20°C until dried and ground.

Chemical analysis

All chemical analyses were performed in duplicate. Feed and excreta samples were ground to pass a 0.5 mm screen and were ashed as described above for bone samples before the analysis. The diets and excreta samples were analyzed for dry matter (DM), crude protein (CP), and ether extract, energy and minerals (Ca and P). Energy was determined from an increased temperature when the sample was ignited in an oxygen-rich atmosphere in a bomb calorimeter. Calcium in the ash were determined with an atomic absorption spectrophotometer; whereas, P was analyzed by the vanadate colorimetric method with a UV spectrophotometer, according to the AOAC International [11]. AA in diets and digesta were determined using ion-exchange chromatography as according to Wang et al [12]. Plasma Ca, P, ALP, UA, and BUN were determined with a Full Automation Biochemistry Analyzer (T600-020, RiLi, Tokyo, Japan).

Calculations and statistical analysis

The apparent digestibility of nutrient and the apparent ileal digestibility (AID) of AA were calculated by the following formula using AIA as the indigestible marker [13].

$$\text{Apparent nutrient digestibility (\%)} \\ = \{[(\text{NT}/\text{AIA})_{\text{d}} - (\text{NT}/\text{AIA})_{\text{i}}] / (\text{NT}/\text{AIA})_{\text{d}}\} \times 100$$

Where (NT/AIA)_d = ratio of nutrient and AIA in diet, and (NT/AIA)_i = ratio of nutrient and AIA in excreta or ileal digesta (NT, nutrient; d, in diet; i, in excreta or ileal digesta).

Data were analyzed by one-way analysis of variance in SPSS 21.0 (SPSS, Inc., Chicago, IL, USA) and used a cage mean as an experimental unit. A probability value of $p < 0.05$ was described to be statistically significant. Polynomial contrasts were used to assess the significance of linear or quadratic models in response to the

dependent variable (with or without the addition of phytase).

RESULTS

Growth performance

As shown in Table 2, dietary treatments exhibited no significant effect ($p > 0.05$) on ADFI during 7 to 28 d and 29 to 63 d, but during the entire experimental period (7 to 63 d), the phytase (1,000 U/kg) supplemented diet showed a higher ADFI ($p > 0.05$). As for ADG, there existed no notable difference between PC and phytase supplementation groups (500 to 1,000 U/kg), but the low-P without phytase supplementation group exhibited lower ($p < 0.05$) ADG than that in PC during the period 7 to 28 d. Intriguingly, there was no significant difference in the ADG during period 29 to 63 and 7 to 63 days. Moreover, there were no significant differences in F/G during period 7 to 28 days, but during the period 29 to 63 days, the lowest ($p < 0.05$) F/G was observed in the group supplemented with phytase at 1,000 U/kg. Notably, during the whole experimental period 7 to 63 days, low-P diet without phytase group had a higher F/G, and the group supplemented with phytase at 1,000 U/kg diet exhibited more favorable ($p < 0.05$) F/G than all other groups excluding the group supplemented with phytase at 750 U/kg.

Ash, Ca, and P concentration in tibia

In this study, in terms of tibia ash content, there were no significant difference ($p > 0.05$) between treatment groups on day 28 and day 63 respectively (Table 3). On 28 days of age, the tibia P content for Sheldrake fed low-P supplement phytase 1,000 U/kg was observed to be the highest ($p < 0.001$). In addition, the obtained data for 63 days of age supplemented with phytase 750 or 1,000 U/kg were higher ($p < 0.001$) in tibia P content compared with other groups. Moreover, the tibia Ca showed the same trend as tibia P.

Table 2. Effects of different phytase on performance in Youxian sheldrake

Items	PC	Phytase (U/kg)				SEM	p-value
		0 ¹⁾	500	750	1,000		
7 to 28 d							
ADG (g)	17.75 ^a	16.11 ^b	17.14 ^{ab}	17.18 ^{ab}	17.32 ^{ab}	0.19	0.033
ADFI (g)	40.77	39.99	41.06	38.99	41.36	0.33	0.149
F/G	2.30	2.49	2.41	2.27	2.39	0.35	0.316
29 to 63 d							
ADG (g)	17.77	17.67	18.39	17.17	19.12	0.29	0.481
ADFI (g)	75.12	75.23	77.07	73.56	76.88	0.96	0.807
F/G	4.23 ^a	4.26 ^a	4.20 ^a	4.16 ^a	4.03 ^b	0.02	0.004
7 to 63 d							
ADG (g)	17.76	17.09	17.92	17.51	18.44	0.18	0.210
ADFI (g)	61.64	61.50	63.01	60.02	63.05	0.68	0.636
F/G	3.47 ^{ab}	3.60 ^a	3.52 ^{ab}	3.43 ^b	3.42 ^b	0.21	0.033

PC, positive control; SEM, standard error of the mean; ADG, The average daily gain; ADFI, average daily feed intake; F/G, ratio of feed to gain.

¹⁾ Negative control, similar to PC except available P.

^{ab} Values within a row with different superscript letters differ significantly at $p < 0.05$.

Groups supplemented with phytase 750 or 1,000 U/kg exhibited significant difference ($p < 0.05$) compared with group supplement phytase 0 and 500 U/kg.

Plasma parameters

As shown in Table 4, phytase supplementation had no effect ($p > 0.05$) on the levels of TP and BUN. Phytase supplementation significantly decreased the levels of serum UA by 38% to 48% ($p < 0.001$) and significantly increased the levels of P ($p < 0.05$); whereas, there was no effect on the levels of serum ALP and Ca on 28 days of age. On 63 days of age, groups supplemented with phytase 500 and 750 U/kg had increased levels of serum ALP ($p < 0.05$) compared with low-P group without supplementation of phytase. Moreover, group supplemented with phytase 1,000 U/kg had the highest ($p < 0.05$) levels of serum Ca, except for group supplemented with phytase 500 U/kg ($p > 0.05$). As with Ca, the plasma P concentration was increased by phytase supplementation at 750 or 1,000 U/kg ($p < 0.05$), and the group supplemented

with phytase 750 U/kg was the highest. However, dietary supplementation with phytase had no effects ($p > 0.05$) on the levels of UA in this period.

Apparent ileal amino acid digestibility

After the starter trial period, on d 28, the apparent ileal AA digestibility was determined for each of the 5 dietary treatments (Table 5). There was a significant difference ($p < 0.05$) among treatments for the AID of His, Thr, Val, indispensable AA (IAA), Cys, Glu, Pro, Ser, and dispensable AA (DAA). The AID of the AA had no significant difference ($p > 0.05$) among the PC, low-P without phytase and low-P with 500 U/kg phytase group. In sheldrakes fed with low-P Phytase supplemented at 1,000 U/kg, the apparent ileal AA digestibility of His, Thr, Val, IAA, Glu, Pro, and DAA was 4.8%, 8.7%, 11.2%, 7.5%, 7.0%, 7.4%, and 7.4% higher ($p < 0.05$) than low-P without phytase, respectively. Moreover, for Thr and Glu, the apparent ileal AA digestibility of low-P supplemented with phytase 750 U/kg was also 8.2% and 5.8% higher ($p < 0.05$)

Table 3. Effect of phytase on tibia characteristics in Youxian Sheldrake

Items	PC	Phytase (U/kg)				SEM	p-value
		0 ¹⁾	500	750	1,000		
28 d							
Ash (%)	45.74	44.49	47.26	45.81	48.18	0.71	0.569
Phosphorus (%)	6.80 ^{cd}	6.39 ^d	7.13 ^{bc}	7.33 ^b	7.82 ^a	0.14	<0.001
Calcium (%)	13.74 ^c	13.22 ^c	14.90 ^b	15.24 ^b	15.90 ^a	0.27	<0.001
63 d							
Ash (%)	42.56	40.37	41.79	46.47	45.82	0.83	0.054
Phosphorus (%)	7.56 ^b	6.74 ^c	7.04 ^c	8.02 ^a	8.18 ^a	0.16	<0.001
Calcium (%)	14.22 ^{ab}	13.60 ^b	13.61 ^b	15.16 ^a	15.10 ^a	0.21	0.006

PC, positive control; SEM, standard error of the mean.

¹⁾ Negative control, similar to PC except available P.

^{abcd} Values within a row with different superscript letters differ significantly at $p < 0.05$.

Table 4. Effect of phytase on plasma parameters in Youxian Sheldrake

Items	PC	Phytase (U/kg)				SEM	p-value
		0 ¹⁾	500	750	1,000		
28 d							
TP (g/L)	38.72	37.68	38.50	39.22	39.80	0.39	0.540
BUN (mmol/L)	0.93	1.02	0.94	0.98	0.92	0.03	0.940
UA (μ mol/L)	233.10 ^{bc}	385.18 ^a	238.60 ^{bc}	260.24 ^b	199.18 ^c	14.72	<0.001
ALP (U/L)	518.98	542.14	472.40	460.22	507.66	13.42	0.294
Ca (mmol/L)	2.67	2.57	2.61	2.73	2.64	0.03	0.336
P (mmol/L)	2.35 ^a	1.56 ^b	2.23 ^a	2.45 ^a	2.52 ^a	0.11	0.018
63 d							
TP (g/L)	42.00	41.64	43.66	42.14	42.08	0.35	0.440
BUN (mmol/L)	1.09	1.31	1.00	1.05	0.96	0.05	0.130
UA (μ mol/L)	427.42	439.36	447.46	416.62	392.00	12.39	0.690
ALP (U/L)	358.60 ^{ab}	409.90 ^a	309.48 ^b	327.64 ^b	351.76 ^{ab}	11.48	0.048
Ca (mmol/L)	2.51 ^b	2.50 ^b	2.61 ^{ab}	2.51 ^b	2.70 ^a	0.02	0.008
P (mmol/L)	1.67 ^b	1.49 ^c	1.69 ^b	1.90 ^a	1.88 ^a	0.04	<0.001

PC, positive control; SEM, standard error of the mean; TP, total protein; BUN, blood urea nitrogen; UA, uric acid; ALP, alkaline phosphatase; P, phosphorus.

¹⁾ Negative control, similar to PC except available P.

^{abc} Values within a row with different superscript letters differ significantly at $p < 0.05$.

Table 5. Effect of phytase on apparent ileal digestibility of amino acid in 28-d old Youxian Sheldrake

Items	PC	Phytase (U/kg)				SEM	p-value
		0 ¹⁾	500	750	1,000		
Arg	88.01	87.07	86.46	90.60	90.18	0.58	0.074
His	84.19 ^b	84.65 ^b	84.53 ^b	87.98 ^{ab}	88.71 ^a	0.63	0.035
Ile	76.85	76.01	76.46	81.83	82.31	0.97	0.070
Leu	80.76	80.21	81.29	85.74	85.98	0.87	0.061
Lys	80.42	80.38	78.11	83.80	83.39	0.81	0.131
Met	65.79	68.39	69.39	74.05	78.52	1.72	0.133
Phe	81.79	81.12	82.03	86.41	86.73	0.84	0.060
Thr	74.71 ^{ab}	73.66 ^a	73.05 ^a	79.69 ^b	80.10 ^a	0.98	0.034
Val	71.96 ^{ab}	70.87 ^b	71.10 ^b	77.70 ^{ab}	78.80 ^a	1.15	0.044
IAA	78.28 ^b	78.04 ^b	78.05 ^b	83.09 ^{ab}	83.86 ^a	0.87	0.042
Ala	67.42	63.84	66.57	72.95	75.01	1.44	0.064
Asp	80.45	80.57	79.65	84.84	85.26	0.82	0.057
Cys	53.93 ^b	65.75 ^{ab}	56.89 ^{ab}	70.48 ^a	69.79 ^a	2.26	0.045
Glu	82.65 ^c	82.56 ^c	83.35 ^{bc}	87.36 ^{ab}	88.35 ^a	0.76	0.014
Gly	69.29	68.55	68.55	72.49	73.16	0.96	0.385
Pro	78.97 ^c	79.46 ^{bc}	80.21 ^{bc}	84.15 ^{ab}	85.37 ^a	0.83	0.028
Ser	79.67 ^{ab}	79.83 ^{ab}	78.44 ^b	84.12 ^a	84.27 ^a	0.80	0.038
Tyr	79.06	78.04	76.16	82.94	82.01	0.96	0.139
DAA	73.93 ^b	74.83 ^{ab}	73.73 ^b	79.92 ^a	80.40 ^a	0.99	0.044

PC, positive control; SEM, standard error of the mean; IAA, indispensable amino acids; DAA, dispensable amino acids.

¹⁾ Negative control, similar to PC except available P.

^{abc} Values within a row with different superscript letters differ significantly at $p < 0.05$.

than low-P without phytase, respectively. For His, IAA, Glu, Pro, and DAA, the apparent ileal AA digestibility of low-P group supplemented with phytase 1,000 U/kg were higher ($p < 0.05$) than PC; and the same effects to Glu, Pro, and DAA in low-P supplemented with phytase 750 U/kg group. Among supplemented phytase groups, the apparent ileal AA digestibility of His, Thr, Val, IAA, Glu, Pro, Ser, and DAA in 1,000 U/kg were higher ($p < 0.05$) than that of 500 U/kg; whereas 750 U/kg were higher ($p < 0.05$) than 500 U/kg of Thr, Ser, and DAA.

In the grower period, on day of 63, the results of the apparent ileal AA digestibility are summarized in Table 6. There was a significant difference ($p < 0.05$) between PC and low-P without phytase group for the AID of the AAs except for Arg, Lys, Ala, and Gly. However, there was no significant difference ($p > 0.05$) between the PC and the low-P diet with supplement phytase 750 or 1,000 U/kg groups except for Met. The AID of most AA in low-P with 500 U/kg phytase group was lower ($p < 0.05$) than PC apart from Arg, Lys, Ala, Cys, and Gly. It was observed that in the low-P with supplement phytase 750 U/kg group the AID of most AAs was higher than other treatment groups, compared with low-P without phytase group, His, Thr, Asp, Cys, Pro, and Ser was 3.3%, 9.3%, 6.2%, 21.5%, 6.0%, and 7.1% higher ($p < 0.05$); while, IAA and DAA was 5.7% and 8.9% higher ($p > 0.05$), respectively.

Nutrients absorption and retention

Data of two trial periods on the apparent retentions of DM, CP,

Table 6. Effect of phytase on apparent ileal digestibility of amino acid in 63-d old Youxian Sheldrake

Items	PC	Phytase (U/kg)				SEM	p-value
		0 ¹⁾	500	750	1,000		
Arg	89.18	84.75	85.75	87.80	86.89	0.53	0.057
His	87.87 ^a	83.04 ^b	84.98 ^{bc}	85.82 ^{bc}	85.24 ^{abc}	0.47	0.014
Ile	81.17 ^a	73.07 ^b	73.81 ^b	78.07 ^{ab}	76.63 ^{ab}	0.97	0.037
Leu	85.57 ^a	79.11 ^b	80.22 ^b	83.49 ^{ab}	82.38 ^{ab}	0.76	0.039
Lys	81.36	74.94	76.04	78.38	76.83	0.78	0.069
Met	74.08 ^a	60.35 ^b	58.29 ^b	63.62 ^b	63.64 ^b	1.75	0.031
Phe	85.60 ^a	79.12 ^b	80.16 ^b	83.55 ^{ab}	82.15 ^{ab}	0.75	0.032
Thr	79.08 ^a	69.38 ^b	72.67 ^{bc}	75.86 ^{bc}	74.92 ^{ac}	0.96	0.009
Val	79.12 ^a	69.35 ^b	70.38 ^b	74.93 ^{ab}	73.63 ^{ab}	1.14	0.036
IAA	82.56 ^a	74.79 ^b	75.81 ^b	79.06 ^{ab}	78.03 ^{ab}	0.86	0.023
Ala	78.58	66.81	66.29	74.55	73.26	1.62	0.062
Asp	83.22 ^a	76.24 ^b	77.80 ^{bc}	80.93 ^{bc}	79.70 ^{abc}	0.76	0.023
Cys	80.59 ^a	61.00 ^b	71.66 ^a	74.10 ^a	72.40 ^a	1.93	0.015
Glu	87.54 ^a	81.50 ^b	82.79 ^b	85.42 ^{ab}	84.62 ^{ab}	0.67	0.025
Gly	71.71	61.16	64.57	67.50	66.65	1.19	0.059
Pro	84.80 ^a	77.06 ^b	79.64 ^{bc}	81.71 ^{bc}	80.94 ^{abc}	0.79	0.019
Ser	83.79 ^a	76.08 ^b	79.21 ^{bc}	81.45 ^{bc}	80.68 ^{abc}	0.77	0.012
Tyr	82.35 ^a	74.34 ^b	76.23 ^b	79.38 ^{ab}	78.09 ^{ab}	0.89	0.034
DAA	81.57 ^a	71.77 ^c	74.77 ^{bc}	78.13 ^{ab}	77.04 ^{abc}	1.01	0.017

PC, positive control; SEM, standard error of the mean; IAA, indispensable amino acids; DAA, dispensable amino acids.

¹⁾ Negative control, similar to PC except available P.

^{abc} Values within a row with different superscript letters differ significantly at $p < 0.05$.

P, Ca, and apparent metabolizable energy (AME) are presented in Table 7. At the end of the starter period, there was no significant difference ($p > 0.05$) observed among groups supplemented with phytase on the apparent retention of DM, CP, and P. The supplementation of phytase increased ($p < 0.05$) the apparent retention of Ca and AME. Compared with low-P without phytase group, apparent retention of Ca was 17.3% and 23.9% higher ($p < 0.001$)

Table 7. Effect of phytase on apparent availability (%) in Youxian Sheldrake

Items	PC	Phytase (U/kg)				SEM	p-value
		0 ¹⁾	500	750	1,000		
28 d							
DM	65.94	61.68	64.47	69.06	70.07	1.03	0.051
CP	48.30	47.86	51.42	56.16	60.65	1.87	0.137
AME	70.33 ^{bc}	67.55 ^c	70.33 ^{bc}	74.27 ^{ab}	76.02 ^a	0.91	0.010
Phosphorus	48.71	46.08	48.60	53.24	53.55	0.99	0.059
Calcium	37.06 ^b	35.32 ^b	35.72 ^b	41.43 ^a	43.79 ^a	0.86	<0.001
63 d							
DM	65.89 ^a	62.05 ^b	67.59 ^a	67.52 ^a	68.67 ^a	0.70	0.012
CP	57.25 ^a	49.40 ^b	55.10 ^a	54.44 ^a	59.10 ^a	0.90	0.002
AME	71.58 ^{ab}	68.55 ^b	73.05 ^a	72.42 ^a	73.77 ^a	0.58	0.030
Phosphorus	43.43 ^{bc}	41.49 ^c	43.46 ^{bc}	48.63 ^a	46.72 ^{ab}	0.84	0.037
Calcium	42.36 ^a	38.89 ^b	42.54 ^a	45.04 ^a	44.68 ^a	0.59	0.002

PC, positive control; SEM, standard error of the mean; DM, dry matter; CP, crude protein; AME, apparent metabolizable energy.

¹⁾ Negative control, similar to PC except available P.

^{abc} Values within a row with different superscript letters differ significantly at $p < 0.05$.

in supplementation with 750 and 1,000 U/kg phytase group, AME was also increased 9.9% and 12.6% ($p < 0.05$). At the end of the grower period, there were remarkable effects ($p < 0.05$) of supplement phytase on the apparent retention of DM, CP, P, Ca, and AME compared with NC group. For DM, CP, Ca, and AME, there was no difference ($p > 0.05$) among supplemented with phytase groups. For P, supplement phytase with 750 U/kg have shown the best effect and increased by 17.2% ($p < 0.05$) compared with low-P without phytase.

DISCUSSION

The presence of PA in diets for nonruminants has a general negative effect on the growth performance because of resultant lower availability of P in diet. Diets of nonruminants are supplemented with phytase for the purpose of improving the utilization of phytate P and reducing the amount of P flowing into the environment. Phytase hydrolyzes phytate to release inorganic P, which is used by the animals to meet their requirement. This study have shown that in the addition of 1,000 U/kg phytase to low-P diets increases ADG by 7.5% and 8.2%, and decreases F/G by 4.1% and 5.4% (starter and grower, respectively). Likewise, the entire period, phytase addition (750 and 1,000 U/kg) to low-P diets decreases F/G by 4.7% and 5.0%, respectively. Since the report of Simons et al [14] that phytase addition (1,500 formazan turbidity units [FTU]/kg) to diets containing 4.5 g/kg total P increased weight gain (733 g vs 338 g) and feed efficiency (1.50 vs 1.85) of broilers from 0 to 24 days of age. Predictably, the addition of phytase to P inadequate diets has been consistently shown to enhance growth performance. For instance in ducks [15] and broiler chickens [16], researchers found similar improvements in growth performance when low-P diets was supplemented with phytase or inorganic P. When supplemental phytase was added to P-deficient diets, improvements in growth performance might have been due to increased availability of P.

Tibia ash content usually reflects the absorption of Ca, P, and other minerals, which are also the main indicators of bone status. For P deficiency diets, tibia ash content is more sensitive than growth performance as a standard to evaluate P requirements [6]. Our data show that phytase supplementation of low-P diets had no significant effect on tibia ash at 28 or 63 days of age, but it shows an increasing trend, which is in agreement with the findings of Onyango et al [17], Walk et al [18], and Li et al [19]. For example, Li et al [19] reported phytase fed at 500 or 1,000 FTU/kg increased tibia ash weight and ash percentage compared to that of birds fed 0.20% non-phytate phosphorus (NPP) diet without phytase at 21 d age. Walk et al [18] reported tibia ash was reduced in broilers fed with NC compared to broilers fed with other diets; and, phytase supplementation improved tibia ash comparable with the PC. Huff et al [20] did not find any improvements in tibia ash content when phytase was added to low-P diets. A possible reason for the varied results could be the different ages and also

the sources of Phytase used in the various studies. In this study, results have shown an effect on d 28 and 63 tibia in Ca and P content. However, in some studies [21,22] it was reported that Ca and P in the tibia ash were relatively constant and were not greatly affected by phytase supplementation.

Plasma concentrations of Ca and P are the main indicators of poultry nutritional status of Ca and P. In a low-P diet, the regulatory mechanism mobilizes the bone Ca and P to maintain normal Ca and P homeostasis. It has been reported in various studies about the effect of supplementing phytase to low-P diets on serum concentrations of Ca and P. Viveros et al [21] have reported the increased levels of serum P but decreased Ca when microbial phytase was added to low-P diets in chickens. Onyango et al [17] found that supplementation of three different phytase to a low-P diet resulted in no different serum Ca concentrations but higher serum P levels than those fed the low-P diet. Yang et al [22] have reported a reduction of the dietary NPP content from 0.45% to 0.25% did not negatively reduce the bone or plasma concentrations of Ca and P. In this study, supplementation of different levels of phytase to a low-P diet increased the serum concentrations of Ca and P, especially at the end of grower period.

In this study, the obtained data indicated that serum ALP activity can be reduced by supplementation of phytase in low-P diet. Similarly to the present findings, Huff et al [20] reported that diet supplemented with phytase alone significantly decreases the serum ALP activity in broiler chickens. Atia et al [23] also found the similar results in growing turkeys. When available P is deficient in poultry diets, absorption into the blood P concentration is reduced; resulting in an increased osteoclast activity to mobilize bone of P in order to maintain the blood P concentrations; and in the process of osteoblasts will produce large amounts of ALP, and raise the level of ALP in the plasma. In this study, supplementation of phytase reduced the levels of UA and BUN. Phytate hydrolyzed by phytase released the bound of protein and AA, reduced to some enzyme inhibition, the utilization of protein and AAs were improved, serum UA, serum urea nitrogen concentration was decreased [6].

Phytase addition improved the digestibility of most measured AA. At the end of starter, supplementation with phytase 1,000 U/kg improved the apparent ileal AA digestibility of His, Thr, Val, IAA, Glu, and Pro; however, at the end of grower, apparent ileal AA digestibility of His, Thr, Asp, Cys, Pro, and Ser were higher in the group supplemented with phytase 1,000 U/kg. This is in agreement with findings of Ravindran et al [3], who have found that the digestibility of Asp, Val, and Thr in particular was improved by phytase. Coweison et al [24], found that phytase improved the digestibility of Val, Thr, and Ile. Furthermore, Kiarie et al [7] reported that 1,000 FTU phytase improved the digestibility of His, Ile, Leu, Thr, and Val. These published results of the positive effect of phytase on AA digestibilities vary [25,26], however, it is clear that when phytase influences the digestibility of AA, it does not affect all AAs to the same extent. This may be associated with

differential interactions between AA groups and phytate or it may be linked to the ability of phytate to increase the loss of endogenous compounds. At present, the mechanisms underlying the AA associated responses to added phytase are primarily speculative. It has been recommended that the *de novo* formation of binary protein-phytate complexes in the acidic regions of the gastrointestinal tract, which are refractory to pepsin activity, may be the key mechanism whereby phytate depresses the digestibility of dietary AA [25]. The other possible mode of action is that phytate may induce increased endogenous AA flows [6,7,25]. Moreover, Woyengo et al [27] and Kiarie et al [7] suggested that the capacity of phytate to drag Na into the small intestinal lumen, which is ameliorated by phytase, may mean that phytate could compromise intestinal uptakes of dietary and endogenous AA by impeding Na⁺-dependent transport systems and Na⁺, K⁺ATPase activity.

Supplementation of low-P diets with phytase significantly improved apparent digestibility of DM and CP at the end of starter period, and improved AME for the entire period. Phytases were reported to decrease significantly the N and DM excretion and to increase digestibility of protein, AME and mineral availability of the diet regardless of diet type, P concentration and age of broiler chicks [28,29]. Alternatively, Liebert et al [30] have reported that phytase supplementation of maize-soy diets did not enhance AME in layers. Onyango et al [17] have reported that energy retention or the AME in the chicks was not improved by addition of any of the 3 phytase preparations, but improved apparent digestibility of DM. Similar results were demonstrated by Adeola research in Pekin ducks [15]. It was reported to be influenced by moisture, temperature, Ca and P content of the feed, age of poultry and dietary composition, methionine or lysine and metabolizable energy [29]. This study has shown that supplementation of phytase was able to improve availability of Ca and P. This was in line with Rutherfurd et al [2] and Adeola [15]. Paiva et al [16] reported that Phytase supplementation significantly increased Ca digestibility regardless of Ca and P levels of the diets. In addition, diets containing 0.6% Ca and 1,000 FTU/kg of Phytase resulted in a significant increase in P digestibility. Kiarie et al [7], demonstrated that phytase response in NC birds showing the greatest improvement on apparent retention of Ca (38%) and P (51%).

In conclusion, the obtained results in the present study indicate that supplementation with microbial phytase at 1,000 U/kg in the low-P diets resulted in better effects on growth performance, nutrient utilization, and tibia mineralization in Youxian sheldrake. Moreover, based on AA, phytase at 1,000 U/kg was shown to have the highest AID at the starter period; whereas, during the grower period this occurred at 750 U/kg.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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