PAPER

Effects of non-phytate phosphorus levels and phytase sources on growth performance, serum biochemical and tibia parameters of broiler chickens

Xian R. Jiang,[1] Fa H. Luo,[1] Ming R. Qu,[2] Valentino Bontempo,[3] Shu G. Wu,[1] Hai J. Zhang,[1] Hong Y. Yue,[1] Guang H. Qi[1]

[1]Feed Research Institute, Chinese Academy of Agricultural Sciences, Beijing, China
[2]College of Animal Science and Technology, Jiangxi Agricultural University, Nanchang, China
[3]Dipartimento di Scienze Veterinarie per la Salute, la Produzione Animale e la Sicurezza Alimentare, Università di Milano, Italy

Abstract

A 3×3 factorial arrangement with dietary non-phytate phosphorus (NPP) levels and phytase sources (3- and 6-phytase) was conducted to evaluate the effects of NPP levels, phytase sources and their possible interactions on growth performance, serum biochemical and tibia parameters of broiler chickens from hatch to 42 days of age. A total of 540 1-day-old Arbor Acres male broiler chicks were randomly allocated into nine dietary treatments, each containing 5 replicates pens with 12 birds per pen. Interaction was statistically significant in the performance till day 21 of trial, supplementation of low NPP diet decreased body weight (BW) (P<0.001), depressed average daily gain (ADG) (P<0.001) and deteriorated average daily feed intake (ADFI) (P<0.001) over day 42. During the 8-to-21-day period, even if interaction between NPP levels and phytase sources was significant (P<0.01), BW, ADG and ADFI always increased due to dietary supplementation of phytase, with source not differing. Dietary high NPP enhanced serum calcium and P concentrations on day 21 and 42 (linear contrast, P<0.01), while decreased alkaline phosphatase (AKP) activity on day 42 (linear contrast, P<0.001), and interaction was not significant. Both dietary sources of phytase decreased serum AKP activities on day 42 (P<0.001), and urea nitrogen content on day 21 (P<0.01) and 42 (P<0.001). Both phytase improved ash percentage on day 21 and P content in tibia at 21 and 42 days of age (P<0.001). The results confirmed that dietary supplementation of phytase may enhance P availability during the 8-to-21-day period. Nevertheless, no difference between the two phytase sources was observed.

Corresponding author: Prof. Shu Geng Wu, Feed Research Institute, Chinese Academy of Agricultural Sciences, Zhongguancun Nandajie 12, 100081, Beijing, China.
Tel. +86.10.82107317 - Fax: +86.10.82106054.
E-mail: wushugeng@caas.cn

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Introduction

Phosphorus (P), which is an essential mineral in growth and development of poultry, plays an important role in energy metabolism, DNA and RNA synthesis, and many other biological processes. Phosphorus deficiency can hinder growth in birds and cause the onset of rickets, or even death, if it is severe (Scott *et al.*, 1982).

Most P contained in feed ingredients of plant origin occurs as phytic acid. The salts of phytic acid are described as phytates. In general, phytate accounts for about two thirds of the total P present in plants (Nelson, 1967). Non-ruminants, such as poultry and pigs, have virtually no phytase activity of their own. Thus, the availability of P in feedstuffs of plant origin is generally very low, ranging from 30 to 40% (Nelson *et al.*, 1968). To increase P bioavailability, the most commonly used method is supplementing high dosage of inorganic P in feed, which leads to the excretion of large amounts of P in animal manure. Consequently, the cost of feed and the environmental adverse impact are increased. Moreover, phytate limits the availability of several other essential nutrients, such as minerals, protein and amino acids (Biehl and Baker, 1996).

Many studies show that microbial phytase can be used to increase the availability of P and reduce its excretion (Simons *et al.*, 1990; Schoner *et al.*, 1991b; Yi *et al.*, 1996; Waldroup *et al.*, 2000; Paik, 2003), and improve the utilization of amino acids, energy and other nutrients in non-ruminant animals (Selle *et al.*, 2000; Cowieson *et al.*, 2006a, 2006b). Supplementation of phytase in low P diets for non-ruminant animals has received much at attention due to environmental concerns and high cost of inorganic P (Viveros *et al.*, 2002).

The types of phytase used in animal feeds are mainly 3- (EC 3.1.3.8) and 6-phytase (EC 3.1.3.26). The former, which catalises the conversion of myo-inositol hexakisphosphate and water to 1L-myo-inositol 1,2,4,5,6-pentakisphosphate and orthophosphate, is derived from fungus and microbes (Shieh and Ware, 1968); and the latter, which catalyses the conversion of myo-inositol hexakisphosphate and water to 1L-myo-inositol 1,2,3,4,5-pentakisphosphate and orthophosphate, is derived from plants and *E. coli* (Reddy *et al.*, 1982). Previous studies have mainly focused on the utilisation of 3-phytase derived from *A. niger* (Farrell *et al.*, 1993; Panda *et al.*, 2007) and 6-phytase derived from *E. coli* (Nyannor and Adeola, 2008) as feed additives for broilers. Some differences between 3- and 6-phytase were reported *in vitro*, such as optimum pH, heat stability, resistance to proteolytic enzymes (Simon and Igbasan, 2002), and *in vivo* experiments on efficacy to improve P utilisation (Augspurger and Baker, 2004; Payne *et al.*, 2005). However, the efficacy of 3- and 6-phytase has been seldom compared. We thus conducted this study to compare the efficacy of the two phytase sources (3-phytase derived from *A. niger* and 6-phytase derived from *E. coli*) with different NPP levels in broiler chickens.

Materials and methods

Bird husbandry and dietary treatments

Five hundred and forty day-old males Arbor Acres broiler chicks were housed in thermo-

statically-controlled and electrically-heated cages and fed a nutritionally complete corn soybean meal starter diet (National Research Council, 1994) from day 1 to 7. On day 8, after overnight feed withdrawal, chicks were weighed and divided into 9 homogeneous groups. Each experimental diet was fed *ad libitum* to 5 replicates of 12 chicks and each chick with free access to water from day 8 to 42 after hatching. The feeding trial of this study was carried out in Nan Kou pilot base of the Chinese Academy of Agricultural Sciences. All the experimental procedures were approved by the Animal Care and Use Committee of the Feed Research Institute - Chinese Academy of Agricultural Sciences. The chicks were housed in cages (100×90×60 cm, length×width×height) with 12 birds each. Lights were continuously on the first day post-hatch, after which a 23L:1D lighting schedule was maintained all through the duration of the feeding trial. Temperature was maintained at 32 to 35°C during the first 3 days and gradually decreased by 3°C each consecutive week until 24°C. Feed and water were provided *ad libitum* throughout the trial.

The experiment was a 3×3 factorial arrangement of the treatments with 3 non-phytate phosphorus (NPP) levels (2.5, 3.5 and 4.5 g/kg for a 8-to-21-day starter period and 1.5, 2.5 and 3.5 g/kg for a grower period of 22 to 42 days and three phytase sources (control, 400 FTU/kg 3-phytase and 400 FTU/kg 6-phytase). The two experimental phytases, whose types and sources of extraction were different (3-phytase derived from *A. Neiger* and 6-phytase from *E. coli*), were purchased from two different companies (BASF Vitamins Co. Ltd., Shenyang, China; and VSAIN GROUP for Environmental Protection Development Co. Ltd., Hebei, China), and both with activity of 5000 FTU/g. The corn soybean meal-based starter and grower diets were formulated according to the National Research Council (1994) requirements for all nutrients, with the exception of lower NPP (Table 1).

Measurement of growth performance

Body weight (BW) and feed intake were recorded for each replicate on 21 and 42 days of age. Average daily weight gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) were calculated for starter, grower and the overall (8 to 42 days) periods. Mortality was recorded as it happened, and dead bird was weighted to adjust the FCR.

Analysis of serum

On day 21 and 42, one bird from each replicate was randomly chosen and 5 mL blood sample was collected via heart puncture. Serum was obtained by centrifugation at 1800×g for 15 min at 4°C.

The concentrations of calcium (Ca), P and urea nitrogen (UN) in serum and serum alkaline phosphatase (AKP) activity were analysed photometrically in a 722 visible spectrophotometer using the commercial kits (Nanjing Jiancheng Institute of Biological Engineering, Nanjing, China).

Tibia parameters

One bird from each replicate was randomly chosen at 21 or 42 days of age. Both tibiae were removed from the carcasses immediately after the birds had been slaughtered via exsanguination of the left jugular vein and stored at -20°C. The tibiae were stripped of muscle after defreezing. Breaking strength was determined using a three-point-bend-method, with the supports set 40 mm apart and a vertical hydraulic force applied at the midpoint of the bone shaft and the peak force required to break the bone was recorded on a tensometer, which was conducted at the Force Institute of the Chinese Academy of Sciences.

After breaking strength measurement, the broken tibiae were boiled in distilled water for

Table 1. Diet composition and nutrient level of experimental diets.

	8-to-21-day diet			22-to-42-day diet		
	Low	Medium	High	Low	Medium	High
Ingredients, g/kg						
Corn	530.8	527.3	523.7	629.1	625.5	622.0
Soybean meal	377.2	377.8	378.5	299.0	299.7	300.3
Soybean oil	56.2	57.4	58.6	41.3	42.5	43.7
DL-Met	2.5	2.5	2.5	1.3	1.3	1.3
Limestone	19.9	16.0	12.2	21.4	17.5	13.7
Dicalcium phosphate	6.8	12.4	17.9	1.3	6.9	12.4
NaCl	3.0	3.0	3.0	3.0	3.0	3.0
Mineral premix°	2.0	2.0	2.0	2.0	2.0	2.0
Choline chloride	1.0	1.0	1.0	1.0	1.0	1.0
Antioxidant	0.4	0.4	0.4	0.4	0.4	0.4
Vitamin premix#	0.2	0.2	0.2	0.2	0.2	0.2
Calculated composition[§]						
AME, MJ/kg	13.39	13.39	13.39	13.39	13.39	13.39
Crude protein, g/kg	230	230	230	200	200	200
Lysine, g/kg	12.4	12.2	12.2	10.5	10.3	10.2
Methionine, g/kg	5.6	5.3	5.6	4.0	4.0	4.1
Met+Cys, g/kg	9.0	9.0	9.0	7.2	7.2	7.2
Ca, g/kg	10.0	10.0	10.0	9.0	9.0	9.0
Total P, g/kg	4.8	5.8	6.8	3.7	4.7	5.7
NPP, g/kg	2.5	3.5	4.5	1.5	2.5	3.5

DL-Met, DL-methionine; AME, apparent metabolizable energy; Met+Cys, methionine+cysteine; P, phosphorus; NPP, non-phytate phosphorus. °Provided the following per kg of diet: copper, 8 mg; zinc, 75 mg; iron, 80 mg; manganese, 100 mg; selenium, 0.15 mg; iodine, 0.35 mg. #Provided the following per kg of diet: retinyl acetate, 4.3 mg; cholecalcipherol, 0.0625 mg; DL-alpha-tocopherol, 18.75 mg; menadione, 2.65 mg; cyanocobalamin, 0.025 mg, biotin, 0.0325 mg; folic acid, 1.25 mg; niacin, 50 mg, D-pantothenic acid, 12 mg; riboflavin, 6 mg; thiamin, 2 mg. §Calculated based on National Research Council (1994) feed ingredient tables.

5 min to facilitate removal of any remaining muscle and connective tissue, oven-dried at 105°C for 24 h, cooled in a dessicator, weighed, and incinerated in a muffle furnace at 550±20°C for 6 h in porcelain crucible, cooled in a desiccator, and weighed. Ash content was expressed as a percentage of dry bone weight.

Phosphorus content in the ash of tibia was determined using the Association of Official Analytical Chemists (AOAC, 2000) official method no. 984.27.

Statistical analysis

Data were analysed as a 3×3 factorial treatment arrangement by ANalysis Of Variance (ANOVA) using the MIXED procedure of SAS software® (SAS, 2008). The model included the fixed effects of NPP level and phytase source as well as the NPP level×phytase source interaction. The Tukey's significant difference *post hoc* test was used to determine which means differed. Treatment effects were considered significant at P<0.05, whereas a trend for a treatment effect was noted when P<0.10.

Results and discussion

Growth performance

Body weight, ADG, ADFI and FCR of broiler chickens are summarised in Table 2. Compared to low NPP diet, medium and high NPP diets increased BW by 18 and 20% on day 21 and by 15 and 23% on day 42 (P<0.001), and increased ADFI and ADG from day 8 to 21, 22 to 42 and 8 to 42 (P<0.001). Feed conversion ratio of birds fed high NPP diets had tendency to be lower than low NPP diets during starter, grower and whole periods (P=0.069; P=0.086; P=0.060). Previous studies have reported that broilers cannot utilise phytic acid and clearly showed slower growth and feed intake when animals were fed low NPP diet, being P supply provided by corn and soybean meal (Schoner *et al.*, 1991a; Manangi and Coon, 2008). No significant differences were detected in BW, ADG, ADFI and FCR among the control, 3- and 6-phytase treatments, but both diets with the different phytase sources tended to improve BW on day 21 (P=0.107), ADG from day 8 to 21 (P=0.098) and ADFI from day 8 to 21 (P=0.062). Kornegay *et al.* (1996) reported that phytase was very effective in improving P availability. Also, the improvement in BW and ADG via supplementing phytase may be due to the improvement in the availability and absorption of nutrients through increasing the digestibility of the ingested diets (Abudabos, 2012; Attia *et al.*, 2012). The improved performance of chickens fed low NPP diet with phytase compared to control during the first period suggests that 2.5 g/kg NPP diet is in fact deficient in P during this period. This finding is in agreement with previous studies (Simons *et al.*, 1990; Kornegay *et al.*, 1996; Panda *et al.*, 2007) which noted that adding phytase made a positive effect on broilers in lower NPP level condition. There were significant interactions between NPP levels and phytase sources affecting BW at 21 day of age (P<0.01), ADG (P<0.01) and ADFI (P<0.001) from day 8 to 21, and ADFI from day 8 to 42 (P=0.040). However, the results showed that the increase (BW, ADG

Table 2. Effects of non-phytate phosphorus levels and phytase sources on growth performance of 540 broiler chickens (5 replicates/treatment).

	Phytase source	BW, g			ADG, g/d			ADFI, g/d			FCR		
		Day 8	Day 21	Day 42	Day 8 to 21	Day 22 to 42	Day 8 to 42	Day 8 to 21	Day 22 to 42	Day 8 to 42	Day 8 to 21	Day 22 to 42	Day 8 to 42
NPP level													
Low	Control	119.2	499^{a}	1778	27.0^{a}	60.9	47.4	45.4^{a}	120.3	90.3^{a}	1.690	1.992	1.919
Low	3-phytase	118.0	593^{b}	1895	33.9^{b}	62.0	50.8	56.9^{bc}	128.3	99.7^{ab}	1.677	2.071	1.966
Low	6-phytase	117.8	589^{b}	1887	33.7^{b}	61.8	50.6	56.3^{b}	130.3	100.7^{abc}	1.676	2.111	1.995
Medium	Control	117.2	657^{bc}	2132	38.6^{bc}	70.2	57.6	62.7^{cd}	145.9	112.6^{cd}	1.627	2.078	1.957
Medium	3-phytase	119.2	659^{bc}	2133	38.5^{bc}	70.3	57.5	60.7^{bcd}	142.1	109.6^{bcd}	1.589	2.022	1.905
Medium	6-phytase	117.9	670^{c}	2125	39.4^{c}	69.3	57.4	62.4^{bcd}	141.2	109.7^{bcd}	1.585	2.035	1.911
High	Control	117.4	689^{c}	2322	40.8^{c}	77.8	63.0	64.7^{d}	152.8	117.6^{d}	1.592	1.970	1.872
High	3-phytase	119.4	655^{bc}	2294	38.3^{bc}	78.0	62.1	61.0^{bcd}	151.7	115.5^{d}	1.596	1.954	1.864
High	6-phytase	118.5	667^{c}	2237	39.2^{c}	74.7	60.5	61.4^{bcd}	145.9	112.1^{bcd}	1.572	1.962	1.858
SEM		1.12	15.9	61.5	1.12	2.52	1.75	1.31	4.23	2.72	0.0520	0.0558	0.0478
Main effects													
NPP													
Low		118.34	560.5^{a}	1853^{a}	31.54^{a}	61.6^{a}	49.6^{a}	52.88^{a}	126.3^{a}	96.9^{a}	1.681	2.058	1.960
Medium		118.11	662.1^{b}	2130^{b}	38.83^{b}	69.9^{b}	57.5^{b}	61.93b	143.1^{b}	110.6^{b}	1.601	2.045	1.924
High		118.44	670.5^{b}	2284^{c}	39.43^{b}	76.8^{c}	61.9^{c}	62.38b	150.2^{b}	115.0^{b}	1.587	1.962	1.865
SEM		0.644	9.18	35.5	0.646	1.45	1.01	0.758	2.44	1.57	0.0301	0.0322	0.0276
Phytase													
Control		117.95	615.1	2077	35.47	69.6	56.0	57.57	139.7	106.8	1.636	2.013	1.916
3-phytase		118.89	636.0	2107	36.91	70.1	56.8	59.55	140.7	108.2	1.621	2.016	1.911
6-phytase		118.05	642.1	2083	37.43	68.6	56.1	60.06	139.1	107.5	1.611	2.036	1.921
SEM		0.644	9.18	35.5	0.646	1.45	1.01	0.758	2.44	1.57	0.0301	0.0322	0.0276
P-value													
Level		ns	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.069	0.086	0.060
Source		ns	0.107	ns	0.098	ns	ns	0.062	ns	ns	ns	ns	ns
Interaction		ns	0.003	ns	0.001	ns	ns	<0.001	ns	0.040	ns	ns	ns

BW, body weight; ADG, average daily gain; g/d, gram per day; ADFI, average daily feed intake; FCR, feed conversion ratio; NPP, non-phytate phosphorus; ns, not significant.. $^{a-d}$Values within the same column with different superscripts are significantly different (P<0.05).

and ADFI) in the low level of NPP by phytase was not repeated in medium and high levels in younger birds and when the birds became older the effect of phytase disappeared. This observation may be due to the fact that younger birds are more sensitive to phytase than older ones when NPP level is low. During the 22-to-42-day period, neither type of phytase improved BW, ADG and ADFI. The results indicate that broilers' growth performance was not affected by dietary phytase along with age, which may be the reason why broiler chickens undergoing compensatory growth exhibited a feed intake relative to BW and some associated digestive adaptation greater than normal (Zubair and Leeson, 1996).

Serum biochemical parameters

Table 3 shows the effect of NPP levels and phytase sources on serum biochemical parameters. Compared to low NPP diet, high NPP diet enhanced serum Ca on day 21 (linear contrast, P<0.01) and 42 (linear contrast, P<0.001) and P content on day 21 (linear contrast, P<0.001) and 42 (linear contrast, P<0.001), but decreased AKP activity on day 42 (linear contrast, P<0.001); medium NPP diet increased serum P content on day 21 (linear contrast, P<0.01) and 42 (linear contrast, P=0.016) and serum Ca content at 42 days of age (linear contrast, P<0.01). However, serum UN was not affected by dietary NPP levels. Serum P concentrations showed an increased tendency with the rise of dietary NPP levels, which is in agreement with the results observed by Sebastian *et al.* (1996) and Viveros *et al*. (2002). In this study, enhancing NPP levels in diet also increased serum Ca concentration. In contrast, Sebastian *et al.* (1996) and Fernandes *et al.* (1999) showed that plasma Ca levels were reduced by increase levels of P supplementation. Alkaline phosphatase is crucial in osteogenesis, which is influenced by serum P concentration and sensitive to Ca and P metabolism. A low serum P concentration can induce the release of AKP, and finally increase the deposition of Ca^{2+} and PO_4^{3-} into bone tissues (Przytulski *et al.*, 1982). The AKP activities were higher in low NPP treatments than medium and high NPP treatments, which is in agreement with Fernandes *et al.* (1999) and Viveros *et al*. (2002) who suggested that the diets were deficient in P. At 42 days of age, the AKP activity decreased dramatically compared to 21 days of age, which shows that the availability of P increases in broiler chickens as they grow.

Dietary 6-phytase increased serum P content on day 21 (linear contrast, P<0.01) and serum Ca content on day 42 compared to the control (linear contrast, P<0.01). Serum P concentration increased with the supplementation of phytase strongly suggesting that phytase can increase P availability, these results being supported by data obtained by Sebastian *et al.* (1996) and Viveros *et al*. (2002). Dietary phytase enhanced serum Ca retention at 42 days of age, which is consistent with the results achieved by Viveros *et al*. (2002). Supplementation of both sources of phytase decreased serum AKP activities on day 42 (P<0.001), and urea nitrogen content on day 21 (P<0.01) and 42 (P<0.001). No significant difference was found between the sources of phytase on serum biochemical parameters of chickens at 21 and 42 days of age. There was no interaction between NPP levels and phytase sources having effect on serum biochemical parameters. The decrease of AKP activities

Table 3. Effects of non-phytate phosphorus levels and phytase sources on serum biochemical parameters of 540 broiler chickens (5 replicates/treatment).

		Serum Ca, mmol/L		Serum P, mmol/L		AKP, K unit/100 mL		UN, mmol/L	
	Phytase source	Day 21	Day 42	Day 21	Day 42	Day 21	Day 42	Day 21	Day 42
NPP level									
Low	Control	3.59	2.770	2.87	2.00	462	45.7	38.5	11.12
Low	3-phytase	3.82	2.849	3.22	1.98	443	35.6	25.4	3.39
Low	6-phytase	3.80	2.881	3.39	2.09	484	33.5	25.9	4.50
Medium	Control	3.65	2.965	3.58	2.28	385	41.8	31.9	10.55
Medium	3-phytase	3.96	2.998	3.62	2.31	463	24.2	24.7	2.92
Medium	6-phytase	3.89	3.146	3.63	2.43	483	30.9	25.8	4.69
High	Control	4.27	3.003	3.89	2.35	294	31.6	26.4	9.70
High	3-phytase	4.56	3.215	4.09	2.50	343	23.9	23.5	2.76
High	6-phytase	4.37	3.260	4.53	2.65	358	24.2	23.5	4.39
SEM		0.232	0.0704	0.151	0.133	78.5	3.53	3.21	0.567
Main effects									
NPP									
Low		3.73^{a}	2.833^{a}	3.159^{a}	2.024^{a}	463	38.3^{a}	29.9	6.50
Medium		3.84a	3.037^{b}	3.610^{b}	2.341^{b}	444	32.3^{ab}	27.5	6.06
High		4.40b	3.159^{b}	4.168^{c}	2.500^{b}	332	26.5^{b}	24.5	5.62
SEM		0.134	0.0407	0.0872	0.0766	45.3	2.04	1.85	0.327
Phytase									
Control		3.84	2.913^{a}	3.444^{a}	2.211	381	39.7^{a}	32.3^{a}	10.45^{a}
3-phytase		4.11	3.021^{ab}	3.641^{ab}	2.265	416	27.9^{b}	24.5^{b}	3.02^{c}
6-phytase		4.02	3.096^{b}	3.852^{b}	2.389	442	29.5^{b}	25.1^{b}	4.69^{b}
SEM		0.134	0.0407	0.0872	0.0766	45.3	2.04	1.85	0.327
P-value									
Level		0.003	<0.001	<0.001	<0.001	0.093	0.001	ns	ns
Source		ns	0.010	0.009	ns	ns	<0.001	0.009	<0.001
Interaction		ns	ns	ns	ns	ns	ns	ns	ns

Ca, calcium; P, phosphorus; AKP, alkaline phosphatase; UN, urea nitrogen; NPP, non-phytate phosphorus; ns, not significant. ^{a,b,c}Values within the same column with different superscripts are significantly different (P<0.05).

Table 4. Effects of non-phytate phosphorus levels and phytase sources on tibial parameters of 540 broiler chickens (5 replicates/treatment).

		Ash, %		Ash P content, %		Breaking strength, N	
	Phytase source	Day 21	Day 42	Day 21	Day 42	Day 21	Day 42
NPP level							
Low	Control	30.76	31.61^{abc}	14.72	14.88	68.6	116
Low	3-phytase	32.58	29.26^{a}	15.79	16.56	83.3	87
Low	6-phytase	34.78	30.37^{ab}	17.00	16.14	84.7	97
Medium	Control	35.12	30.39^{ab}	15.81	16.18	112.3	114
Medium	3-phytase	37.47	32.39^{bcd}	17.34	16.98	116.0	112
Medium	6-phytase	38.90	33.12^{bcd}	17.17	16.87	119.7	153
High	Control	37.81	32.68^{bcd}	16.59	16.46	139.9	132
High	3-phytase	39.35	33.74^{cd}	17.39	16.91	121.7	162
High	6-phytase	39.72	35.14^{d}	18.15	18.06	122.1	180
SEM		0.817	0.625	0.310	0.372	9.11	12.8
Main effects							
NPP							
Low		32.70^{a}	30.41^{a}	15.84^{a}	15.86^{a}	78.8^{a}	99.9^{a}
Medium		37.16^{b}	31.97^{b}	16.78^{b}	16.68^{b}	116.0^{b}	126.5^{b}
High		38.96^{c}	33.85^{c}	17.38^{b}	17.14^{b}	127.9^{b}	158.1^{c}
SEM		0.472	0.361	0.179	0.215	5.26	7.42
Phytase							
Control		34.56^{a}	31.56^{a}	15.71^{a}	15.84^{a}	106.9	120.6
3-phytase		36.46^{b}	31.79^{ab}	16.84^{b}	16.82^{b}	107.0	120.6
6-phytase		37.80^{b}	32.87^{b}	17.44^{b}	17.03^{b}	108.8	143.2
SEM		0.472	0.361	0.179	0.215	5.26	7.42
P value							
Level		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Source		<0.001	0.030	<0.001	<0.001	ns	0.054
Interaction		ns	0.005	ns	ns	ns	ns

P, phosphorus; NPP, non-phytate phosphorus; ns, not significant. ^{a,b,c}Values within the same column with different superscripts are significantly different (P<0.05).

with the supplementation of phytase indicated that it could increase P availability. Corzo *et al.* (2005) found there was a negative relation between amino acids consumption and serum uric acid concentration: the result of the experiment showed that the serum urea nitrogen concentration decreased by supplementing both sources of phytase at the end of the starter and grower periods, which may be the reason why dietary phytase improves the utilisation of amino acids in broilers (Cowieson *et al.*, 2006b).

Tibia parameters

Effects of dietary NPP levels and phytase sources on tibia parameters are presented in Table 4. Compared to low NPP diet, medium and high NPP diets increased ash percentage, P content and breaking strength on day 21 and 42 (P<0.001). Supplementation of both sources of phytase significantly improved the ash percentage on day 21 and P content of tibia at 21 and 42 days of age (P<0.001). Dietary 6-phytase enhanced ash percentage (linear contrast, P=0.039) and tended to increase breaking strength (linear contrast, P=0.094) in tibia of chickens at 42 days of age compared to control diet. There was a significant interaction between NPP levels and phytase sources on ash percentage at 42 days of age (P<0.01). The ash percentage and P contents in ashes of bone are the main parameters for mineral deposition in animal bones. In fact, the ash content is closely related to P concentration. Enhancement of ash percentage and P content of tibia with application of either source of phytase suggests that phytase can increase mineral deposition in P deficient diet, which is in agreement with the results described by Sebastian *et al.* (1996) and Viveros *et al.* (2002). Breaking strength reflects the rigidity of bones as a whole. In the present study, the low breaking strength values in medium NPP treatment on day 42 meant that the tibia was more fragile, thus likely indicating the diet was deficient in P.

Conclusions

The results showed that phytase supplementation in every NPP level diet and especially in low NPP diets can improve growth performance along with serum biochemical and tibia parameters of chickens. Dietary supplementation of phytase may enhance P availability during the 8-to-21-day period. There seems to be no difference between 3- and 6-phytase on the above-mentioned aspects.

References

Abudabos, M.A., 2012. Phytate phosphorus utilization and intestinal phytase activity in laying hens. Ital. J. Anim. Sci. 11:e8.

AOAC, 2000. Official methods of analysis. 17th ed., Association of Official Analytical Chemists, Arlington, VA, USA.

Attia, Y.A., El-Tahawy, W.S., Abd El-Hamid, A.E., Hassan, S.S., Nizza, A., El-Kelaway, M.I., 2012. Effect of phytase with or without multienzyme supplementation on performance and nutrient digestibility of young broiler chicks fed mash or crumble diets. Ital. J. Anim. Sci. 11:e56.

Augspurger, N.R., Baker, D.H., 2004. High dietary phytase levels maximize phytate-phosphorus utilization but do not affect

protein utilization in chicks fed phosphorus- or amino acid-deficient diets. J. Anim. Sci. 82:1100-1107.

Biehl, R.R., Baker, D.H., 1996. Efficacy of supplemental 1 alpha-hydroxycholecalciferol and microbial phytase for young pigs fed phosphorus- or amino acid-deficient corn-soybean meal diets. J. Anim. Sci. 74:2960-2966.

Corzo, A., Fritts, C.A., Kidd, M.T., Kerr, B.J., 2005. Response of broiler chicks to essential and non-essential amino acid supplementation of low crude protein diets. Anim. Feed Sci. Tech. 118:319-327.

Cowieson, A.J., Acamovic, T., Bedford, M.R., 2006a. Phytic acid and phytase: implications for protein utilization by poultry. Poultry Sci. 85:878-885.

Cowieson, A.J., Acamovic, T., Bedford, M.R., 2006b. Supplementation of corn-soy-based diets with high concentrations of an Escherichia coli-derived phytase: effects on broiler chick performance and digestibility of amino acids, minerals and energy. Poultry Sci. 85:1389-1397.

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

Fernandes, J.I.M., Lima, F.R., Mendonca, C.X., Mabe I.Jr., Albuquerque, R., Leal, P.M., 1999. Relative bioavailability of phosphorus in feed and agricultural phosphates for poultry. Poultry Sci. 78:1729-1736.

Kornegay, E.T., Denbow, D.M., Yi, Z., Ravindran, V., 1996. Response of broilers to graded levels of microbial phytase added to corn-soybean meal based diets containing three levels of non-phytate phosphorus. Brit. J. Nutr. 75:839-852.

Manangi, M.K., Coon, C.N., 2008. Phytate phosphorus hydrolysis in broilers in response to dietary phytase, calcium, and phosphorus concentrations. Poultry Sci. 87:1577-1586.

National Research Council, 1994. Nutrient requirements of poultry. 9th rev. ed. National Academy Press, Washington, DC, USA.

Nelson, T.S., 1967. The utilization of phytate phosphorus by the chick: a review. Poultry Sci. 46:862-871.

Nelson, T.S., Sheih, T.R., Wodzinski, R.J., Ware, J.H., 1968. The availability of phytate phosphorus in soybean meal before and after treatment with a mold phytase. Poultry Sci. 47:1842-1848.

Nyannor, E.K.D., Adeola, O., 2008. Corn expressing an Escherichia coli-derived phytase gene: comparative evaluation study in broiler chicks. Poultry Sci. 87:2015-2022.

Paik, I.K., 2003. Application of phytase, microbial or plant origin, to reduce phosphorus excretion in poultry production. Asian Austral. J. Anim. 16:124-135.

Panda, A.K., Rao, S.V.R., Raju, M.V.L.N., Gajula, S.S., Bhanja, S.K., 2007. Performance of broiler chickens fed low non phytate phosphorus diets supplemented with microbial phytase. J. Poultry Sci. 44:258-264.

Payne, R.L., Lavergne, T.K., Southern, L.L., 2005. A comparison of two sources of phytase in liquid and dry forms in broilers. Poultry Sci. 84:265-272.

Przytulski, T., Ko win-Podsiadly, M., Klemke, A., 1982. Relationship between serum alkaline phosphatase genetic polymorphism and activity of the enzyme in Large White pigs. Ann. Genet. Sel. Anim. 14:43-48.

Reddy, N.R., Sathe, S.K., Salunkhe, D.K., 1982. Phytates in legumes and cereals. Adv. Food Res. 28:1-92.

SAS, 2008. SAS/STAT user's guide: version 9.2. SAS Inst. Inc., Cary, NC, USA.

Schoner, F.J., Hoppe, P.P., Schwarz, G., 1991a. Comparative effects of microbial phytase and inorganic phosphorus on performance and on retention of phosphorus, calcium and crude ash in broilers. J. Anim. Physiol. An. N. 66:248-255.

Schoner, F.J., Hoppe, P.P., Schwarz, G., 1991b. Reduction of phosphorus excretion in broiler production by supplementing microbial phytase. pp 481-486 in Proc. 103rd Nat. Congr. of Association of German Agricultural Investigation and Research Institutes, Ulm, Germany.

Scott, M.L., Nesheim, M.C., Young, R.J., 1982. Nutrition of the chicken. 3rd ed. Scott M.L. & Associates Publ., Ithaca, NY, USA.

Sebastian, S., Touchburn, S.P., Chavez, E.R., Lague, P.C., 1996. The effects of supplemental microbial phytase on the performance and utilization of dietary calcium, phosphorus, copper, and zinc in broiler chickens fed corn-soybean diets. Poultry Sci. 75:729-736.

Selle, P.H., Ravindran, V., Caldwell, R.A., Bryden, W.L., 2000. Phytate and phytase: consequences for protein utilization. Nutr. Res. Rev. 13:255-278.

Shieh, T.R., Ware, J.H., 1968. Survey of microorganisms for the production of extracellular phytase. J. Appl. Microbiol. 16:1348-1351.

Simon, O., Igbasan, F., 2002. In vitro properties of phytases from various microbial origins. Int. J. Food Sci. Tech. 37:813-822.

Simons, P.C.M., Versteegh, H.A.J., Jongbloed, A.W., Kemme, P.A., Slump, P., Bos, K.D., Wolters, M.G.E., Beudeker, R.F., Verschoor, G.J., 1990. Improvement of phosphorus availability by microbial phytase in broilers and pigs. Brit. J. Nutr. 64:525-540.

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

Waldroup, P.W., Kersey, J.H., Saleh, E.A., Fritts, C.A., Yan, F., Stilborn, H.L., Crum, R.C.Jr., Raboy, V., 2000. Nonphytate phosphorus requirement and phosphorus excretion of broiler chicks fed diets composed of normal or high available phosphate corn with and without microbial phytase. Poultry Sci. 79:1451-1459.

Yi, Z., Kornegay, E.T., Ravindran, V., Denbow, D.M., 1996. Improving phytate phosphorus availability in corn and soybean meal for broilers using microbial phytase and calculation of phosphorus equivalency values for phytase. Poultry Sci. 75:240-249.

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