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

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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.

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., 2000b; 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 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 catalyses the conversion of myo-inositol hexakisphosphate and water to 1L-myo-inositol 1,2,3,4,5,6-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 (Nynan 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-
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. Neyer 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/kg. 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).

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

| Ingredients, g/kg | Low 22-to-42 day diet | Medium 22-to-42 day diet | High 22-to-42 day diet | Low 8- to 21-day diet | Medium 8- to 21-day diet | High 8- to 21-day diet |
|-------------------|-----------------------|--------------------------|------------------------|-----------------------|--------------------------|------------------------|
| Corn | 530.8 | 577.3 | 523.7 | 651.1 | 655.5 | 622.0 |
| Soybean meal | 377.2 | 378.5 | 378.5 | 379.0 | 379.7 | 300.3 |
| Soybean oil | 58.2 | 58.6 | 58.6 | 41.3 | 41.3 | 41.7 |
| DL-Met | 2.5 | 2.5 | 2.5 | 1.3 | 1.5 | 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 | 10.5 | 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+cystine; 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, 0.025 mg. †Provided the following per kg of diet: retinyl acetate, 4.3 mg; cholecalciferol, 0.0625 mg; DL-alpha-tocopherol, 18.75 mg; menadione, 2.85 mg; cyanocobalamin, 0.025 mg; biotin, 0.025 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.
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        | Day 21       | Day 42    | Day 8        | Day 21       | Day 42    |
| NPP level      |       |          |           |              |              |           |              |              |           |
| Low Control    | 119.2 | 649.4a   | 1778      | 27.01a      | 60.9b        | 47.4b      | 45.4b       | 120.3c      | 90.3c      | 1.690      | 1.992      | 1.919      |
| Low 3-phytase  | 118.0 | 659.3a   | 1995      | 32.0a       | 62.0b        | 49.8b      | 56.0bc      | 120.0bc     | 96.0bc     | 1.677      | 2.071      | 1.966      |
| Low 6-phytase  | 117.8 | 659.5a   | 1987      | 33.7a       | 61.8b        | 50.6b      | 56.3b       | 120.0bc     | 96.0bc     | 1.676      | 2.111      | 1.995      |
| Medium Control | 117.2 | 657.4a   | 2132      | 38.6a       | 70.2c        | 57.6c      | 62.7cd      | 145.9c      | 112.4cd    | 1.627      | 2.078      | 1.957      |
| Medium 3-phytase| 118.2 | 659.5a   | 2133      | 38.5a       | 70.3c        | 57.5c      | 60.7d       | 142.1c      | 109.6cd    | 1.589      | 2.022      | 1.905      |
| Medium 6-phytase| 117.9 | 670.5b   | 2125      | 39.4a       | 68.3c        | 57.4c      | 62.4cd      | 141.2c      | 109.7cd    | 1.585      | 2.035      | 1.911      |
| High Control   | 117.4 | 689.7a   | 2322      | 40.8a       | 77.8c        | 63.0c      | 64.7c       | 152.9c      | 117.6c     | 1.592      | 1.970      | 1.872      |
| High 3-phytase | 119.4 | 655.3a   | 2294      | 38.3a       | 78.0b        | 62.1b      | 61.0cd      | 151.7c      | 115.5d     | 1.596      | 1.954      | 1.864      |
| High 6-phytase | 118.5 | 667.5a   | 2237      | 39.2a       | 74.7c        | 60.5c      | 61.4cd      | 145.9c      | 112.4cd    | 1.572      | 1.962      | 1.858      |
| SEM            | 1.12  | 15.9a    | 61.5      | 1.12        | 2.52         | 1.75      | 1.31        | 4.23        | 2.72       | 0.0520     | 0.0558     | 0.0478     |

Main effects

| NPP | Day 8  | Day 21 | Day 42 | Day 8  | Day 21 | Day 42 | Day 8  | Day 21 | Day 42 | Day 8  | Day 21 | Day 42 |
|-----|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| Low | 118.34 | 560.5c | 1853c  | 31.54  | 61.8b  | 49.8b  | 52.88c | 126.3c | 96.9c  | 1.681  | 2.058  | 1.960  |
| Medium | 118.11 | 662.1b | 2130b | 38.83  | 69.9b  | 57.5b  | 61.93b | 143.1b | 110.6b | 1.601  | 2.045  | 1.924  |
| High | 118.44 | 670.5a | 2294a | 39.49  | 76.8a  | 61.9a  | 62.38b | 150.2b | 115.0b | 1.567  | 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 | 0.0222 | 0.0276 |

P-value

| Level | ns     | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Source | ns | 0.107  | ns     | 0.008  | ns     | 0.062  | ns     | 0.040  | ns     |
| Interaction | ns | 0.003  | ns     | 0.001  | ns     | 0.062  | ns     | 0.040  | 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. *Values within the same column with different superscripts are significantly different (P<0.05).
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 Ca2+ and PO43− into bone tissues (Przytulska 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).

| NPP level | Phytase source | Serum Ca, mmol/L | Serum P, mmol/L | AKP, K unit/100 mL | UN, mmol/L |
|-----------|----------------|------------------|-----------------|-------------------|------------|
|           |                | Day 21 | Day 42 | Day 21 | Day 42 | Day 21 | Day 42 | Day 21 | Day 42 | Day 21 | Day 42 |
| Low       | Control        | 3.59   | 2.770  | 2.87   | 2.00   | 462    | 45.7   | 38.5   | 11.12  |
| Low       | 3-phytase      | 3.82   | 2.849  | 2.22   | 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   |

Main effects

| NPP     | Phytase | Serum Ca, mmol/L Day 21 | Serum P, mmol/L Day 21 | AKP, K unit/100 mL Day 21 | UN, mmol/L Day 21 |
|---------|---------|-------------------------|------------------------|--------------------------|------------------|
| Low     | Control | 3.73a                   | 2.833a                 | 2.150a                   | 2.024a            |
| Medium  | 3-phytase| 3.84a                   | 3.037a                 | 3.610a                   | 2.341a            |
| High    | 6-phytase| 4.40b                   | 3.159b                 | 4.168b                   | 2.500b            |
| SEM     |         | 0.154                   | 0.0407                 | 0.0872                   | 0.0760            |

P-value

| Level   | Source | P-value | Interaction | ns | ns | ns | ns | ns | ns |
|---------|--------|---------|-------------|----|----|----|----|----|----|
| 0.003   | ns     | <0.001  | 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,cValues 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.

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

| NPP level | Phytase source | Ash, % Day 21 | Ash, % Day 42 | Ash P content, % Day 21 | Ash P content, % Day 42 | Breaking strength, N Day 21 | Breaking strength, N Day 42 |
|-----------|----------------|---------------|---------------|------------------------|------------------------|-----------------------------|-----------------------------|
| Low       | Control        | 30.76         | 31.61c        | 14.72                  | 14.88                  | 68.6                        | 116                         |
| Low       | 3-phytase      | 32.58         | 29.26a        | 15.79                  | 16.56                  | 83.3                        | 87                          |
| Low       | 6-phytase      | 34.78         | 30.37b        | 17.00                  | 16.14                  | 84.7                        | 97                          |
| Medium    | Control        | 35.12         | 30.39b        | 15.81                  | 16.18                  | 112.3                       | 114                         |
| Medium    | 3-phytase      | 37.47         | 32.39c        | 17.34                  | 16.98                  | 116.0                       | 112                         |
| Medium    | 6-phytase      | 38.90         | 33.12c        | 17.17                  | 16.87                  | 119.7                       | 153                         |
| High      | Control        | 37.81         | 32.68c        | 16.59                  | 16.46                  | 139.9                       | 132                         |
| High      | 3-phytase      | 39.35         | 33.74c        | 17.39                  | 16.91                  | 121.7                       | 162                         |
| High      | 6-phytase      | 39.72         | 35.14c        | 18.15                  | 18.06                  | 122.1                       | 180                         |
| SEM       |                | 0.817         | 0.625         | 0.310                  | 0.372                  | 9.11                        | 12.8                        |

P: phosphorus; NPP: non-phytate phosphorus; ns, not significant. a-cValues within the same column with different superscripts are significantly different (P<0.05).
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