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,2 Shu G. Wu,1 Hai J. Zhang,1 Hong Y. Yue,1 Guang H. Qi1

1Feed Research Institute, Chinese Academy of Agricultural Sciences, Beijing, China
2College of Animal Science and Technology, Jiangxi Agricultural University, Nanchang, China
3Dipartimento 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 chicks 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., 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 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 hexakisphosphosphate 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 (Augsburger 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-
satisfactorily 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-phosphate 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).

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

<table>
<thead>
<tr>
<th>Ingredients, g/kg</th>
<th>Low</th>
<th>8-to-21-day diet</th>
<th>Medium</th>
<th>High</th>
<th>Low</th>
<th>22-to-42-day diet</th>
<th>Medium</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>530.8</td>
<td>527.3</td>
<td>523.7</td>
<td>629.1</td>
<td>625.5</td>
<td>622.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soybean meal</td>
<td>377.2</td>
<td>375.4</td>
<td>378.5</td>
<td>299.0</td>
<td>297.9</td>
<td>300.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soybean oil</td>
<td>56.2</td>
<td>57.4</td>
<td>58.6</td>
<td>41.3</td>
<td>42.5</td>
<td>43.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DL-Met</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>1.3</td>
<td>1.3</td>
<td>1.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Limestone</td>
<td>19.9</td>
<td>16.0</td>
<td>12.2</td>
<td>21.4</td>
<td>17.5</td>
<td>13.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>6.8</td>
<td>12.4</td>
<td>17.9</td>
<td>1.3</td>
<td>6.9</td>
<td>12.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NaCl</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mineral premix°</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Choline chloride</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antioxidant</td>
<td>0.4</td>
<td>0.4</td>
<td>0.4</td>
<td>0.4</td>
<td>0.4</td>
<td>0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin premix‡</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### 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 18000 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.**

<table>
<thead>
<tr>
<th>Ingredients, g/kg</th>
<th>Low</th>
<th>8-to-21-day diet</th>
<th>Medium</th>
<th>High</th>
<th>Low</th>
<th>22-to-42-day diet</th>
<th>Medium</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>530.8</td>
<td>527.3</td>
<td>523.7</td>
<td>629.1</td>
<td>625.5</td>
<td>622.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soybean meal</td>
<td>377.2</td>
<td>375.4</td>
<td>378.5</td>
<td>299.0</td>
<td>297.9</td>
<td>300.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soybean oil</td>
<td>56.2</td>
<td>57.4</td>
<td>58.6</td>
<td>41.3</td>
<td>42.5</td>
<td>43.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DL-Met</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>1.3</td>
<td>1.3</td>
<td>1.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Limestone</td>
<td>19.9</td>
<td>16.0</td>
<td>12.2</td>
<td>21.4</td>
<td>17.5</td>
<td>13.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>6.8</td>
<td>12.4</td>
<td>17.9</td>
<td>1.3</td>
<td>6.9</td>
<td>12.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NaCl</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mineral premix°</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Choline chloride</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antioxidant</td>
<td>0.4</td>
<td>0.4</td>
<td>0.4</td>
<td>0.4</td>
<td>0.4</td>
<td>0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin premix‡</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Calculated composition**: 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, 8.35 mg. °Provided the following per kg of diet: retinol acetate, 4.3 mcg; cholecalciferol, 0.0625 mg; DL-alpha tocopheryl, 18.75 mg; menadione, 2.85 mcg; cyanocobalamin, 0.0625 mcg; biotin, 0.0025 mcg; folic acid, 1.25 mcg; niacin, 50 mcg; D-pantothenic acid, 12 mcg; riboflavin, 0.6 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).

<table>
<thead>
<tr>
<th>NPP level</th>
<th>Phytase source</th>
<th>BW, g</th>
<th>ADG, g/d</th>
<th>ADFI, g/d</th>
<th>FCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Day 8</td>
<td>Day 21</td>
<td>Day 42</td>
<td>Day 8</td>
<td>Day 21</td>
</tr>
<tr>
<td>Low</td>
<td>Phytase</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-phytase</td>
<td>119.2</td>
<td>499a</td>
<td>1778b</td>
<td>27.0a</td>
<td>60.9a</td>
</tr>
<tr>
<td>3-phytase</td>
<td>118.0</td>
<td>598a</td>
<td>1895b</td>
<td>33.9a</td>
<td>62.0a</td>
</tr>
<tr>
<td>Medium</td>
<td>Control</td>
<td>117.8</td>
<td>589a</td>
<td>1887b</td>
<td>33.7a</td>
</tr>
<tr>
<td>3-phytase</td>
<td>117.2</td>
<td>657a</td>
<td>2312b</td>
<td>38.6a</td>
<td>70.2a</td>
</tr>
<tr>
<td>6-phytase</td>
<td>118.8</td>
<td>590a</td>
<td>2133b</td>
<td>38.5a</td>
<td>70.3b</td>
</tr>
<tr>
<td>High</td>
<td>Control</td>
<td>117.4</td>
<td>689a</td>
<td>2322b</td>
<td>40.8a</td>
</tr>
<tr>
<td>3-phytase</td>
<td>119.4</td>
<td>655a</td>
<td>2294a</td>
<td>38.3a</td>
<td>78.0a</td>
</tr>
<tr>
<td>6-phytase</td>
<td>118.5</td>
<td>667a</td>
<td>2237a</td>
<td>39.2a</td>
<td>74.7b</td>
</tr>
<tr>
<td>SEM</td>
<td>1.12</td>
<td>15.9a</td>
<td>61.5a</td>
<td>1.12</td>
<td>2.52</td>
</tr>
</tbody>
</table>

Main effects

NPP

<table>
<thead>
<tr>
<th>NPP</th>
<th>Phytase</th>
<th>Control</th>
<th>6-phytase</th>
<th>3-phytase</th>
<th>6-phytase</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>118.34</td>
<td>560.5a</td>
<td>1853a</td>
<td>31.5a</td>
<td>61.8a</td>
<td>49.8a</td>
</tr>
<tr>
<td>Medium</td>
<td>118.11</td>
<td>602.1a</td>
<td>2130a</td>
<td>38.83</td>
<td>69.9a</td>
<td>57.5a</td>
</tr>
<tr>
<td>High</td>
<td>118.44</td>
<td>670.5a</td>
<td>2284a</td>
<td>39.43</td>
<td>76.8a</td>
<td>61.9a</td>
</tr>
<tr>
<td>SEM</td>
<td>0.644</td>
<td>9.18</td>
<td>35.5</td>
<td>0.646</td>
<td>1.45</td>
<td>1.01</td>
</tr>
</tbody>
</table>

P-value

Level | ns | <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 | 0.088 | ns | ns | 0.062 | ns | ns | ns | ns |
Interaction | ns | 0.007 | ns | 0.008 | ns | ns | 0.062 | ns | 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. *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<0.01); 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...
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).

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

<table>
<thead>
<tr>
<th>Phytase source</th>
<th>Ash, %</th>
<th>Ash P content, %</th>
<th>Breaking strength, N</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 21</td>
<td>Day 42</td>
<td>Day 21</td>
</tr>
<tr>
<td>NPP level</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>30.76</td>
<td>31.61&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>14.72</td>
</tr>
<tr>
<td>3-phytase</td>
<td>32.58</td>
<td>29.26&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.79</td>
</tr>
<tr>
<td>6-phytase</td>
<td>34.78</td>
<td>30.37&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>17.00</td>
</tr>
<tr>
<td>Medium</td>
<td>35.12</td>
<td>30.39&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>15.81</td>
</tr>
<tr>
<td>Control</td>
<td>37.47</td>
<td>32.39&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17.34</td>
</tr>
<tr>
<td>3-phytase</td>
<td>38.90</td>
<td>33.12&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>17.17</td>
</tr>
<tr>
<td>6-phytase</td>
<td>37.81</td>
<td>32.68&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>16.59</td>
</tr>
<tr>
<td>High</td>
<td>39.55</td>
<td>33.74&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>17.39</td>
</tr>
<tr>
<td>3-phytase</td>
<td>39.72</td>
<td>35.14&lt;sup&gt;d&lt;/sup&gt;</td>
<td>18.15</td>
</tr>
<tr>
<td>6-phytase</td>
<td>0.817</td>
<td>0.625</td>
<td>0.310</td>
</tr>
</tbody>
</table>

Main effects

NPP

<table>
<thead>
<tr>
<th>Level</th>
<th>Ash, %</th>
<th>Ash P content, %</th>
<th>Breaking strength, N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>32.70&lt;sup&gt;a&lt;/sup&gt;</td>
<td>30.41&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.84&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Medium</td>
<td>37.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>31.97&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.78&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>High</td>
<td>38.96&lt;sup&gt;c&lt;/sup&gt;</td>
<td>33.85&lt;sup&gt;c&lt;/sup&gt;</td>
<td>17.38&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>SEM</td>
<td>0.472</td>
<td>0.361</td>
<td>0.179</td>
</tr>
</tbody>
</table>

Phytase

<table>
<thead>
<tr>
<th>Source</th>
<th>Ash, %</th>
<th>Ash P content, %</th>
<th>Breaking strength, N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>34.56&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31.58&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.71&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>3-phytase</td>
<td>36.46&lt;sup&gt;b&lt;/sup&gt;</td>
<td>31.79&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.54&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>6-phytase</td>
<td>37.80&lt;sup&gt;c&lt;/sup&gt;</td>
<td>32.87&lt;sup&gt;c&lt;/sup&gt;</td>
<td>17.44&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>SEM</td>
<td>0.472</td>
<td>0.361</td>
<td>0.179</td>
</tr>
</tbody>
</table>

P value

| Interaction | Particle | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
|            | Source   | 0.005  | ns     | ns     | ns     | ns     | ns     |

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

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


Augspurger, N.R., Baker, D.H., 2004. High dietary phytase levels maximize phytate-phosphorus utilization but do not affect...
protein utilization in chicks fed phospha-
rus- or amino acid-deficient diets. J. Anim.
Sci. 82:1100-1107.
Biehl, R.R., Baker, D.H., 1996. Efficacy of sup-
plemental 1 alpha-hydroxycholcalciferol
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 essen-
tial and non-essential amino acid supple-
mentation of low crude protein diets. Anim.
Cowieson, A.J., Acamovic, T., Bedford, M.R.,
2006a. Phytic acid and phytase: implica-
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
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.
Fernandes, J.J.M., Lima, F.R., Mendonca, C.X.,
Mabe Jr., Albuquerque, R., Leal, P.M.,
1999. Relative bioavailability of phospho-
rus in feed and agricultural phosphates for
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 contain-
ing three levels of non-phytate phospho-
Manangi, M.K., Coon, C.N., 2008. Phytate phos-
phorus hydrolysis in broilers in response
to dietary phytase, calcium, and phospho-
rus concentrations. Poultry Sci. 87:1577-
1586.
requirements of poultry. 9th rev. ed.
National Academy Press, Washington, DC,
USA.
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 phos-
phorus in soybean meal before and after
treatment with a mold phytase. Poultry Sci.
47:1842-1848.
expressing an Escherichia coli-derived
phytase gene: comparative evaluation
study in broiler chicks. Poultry Sci.
Paik, I.K., 2003. Application of phytase, micro-
bial or plant origin, to reduce phosphorus
excretion in poultry production. Asian
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 phos-
phorus diets supplemented with microbial
Payne, R.L., Lavergne, T.K., Southern, L.L.,
2005. A comparison of two sources of phy-
tase in liquid and dry forms in broilers.
Poultry Sci. 84:265-272.
Przytulski, T., Ko win-Podsiadly, M., Klemke, A.,
1982. Relationship between serum alka-
line phosphatase genetic polymorphism
and activity of the enzyme in Large White
Phytates in legumes and cereals. Adv. Food
Res. 28:1-92.
SAS Inst. Inc., Cary, NC, USA.
Comparative effects of microbial phytase
and inorganic phosphorus on performance
and on retention of phosphorus, calcium
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 supple-
mental microbial phytase on the perform-
ance 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:
Shieh, T.R., Ware, J.H., 1968. Survey of
microorganisms for the production of extracellular
16:1348-1351.
of phytases from various microbial origins.
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., 1999. Improvement of phosphorus
availability by microbial phytase in broil-
Viveros, A., Brenes, A., Arija, I., Centeno, C.,
2002. Effects of microbial phytase supple-
mentation on mineral utilization and
serum enzyme activities in broiler chicks
dfed 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.,
requirement and phosphorus excretion of
broiler chicks fed diets composed of nor-
mal 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 cal-
culation of phosphorus equivalency values
for phytase. Poultry Sci. 75:240-249.