Effects of Dietary Thermostable Phytase Supplementation on Growth Performance and Ca and P Metabolism of Broilers.

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Abstract

A study was conducted to study the effects of thermostable phytase supplementation on the growth performance and Ca and P metabolism of broilers for 21 d. 1-day-old Arbor Acres chicks with similar initial body weight were randomly divided into 3 treatments consisting 8 pens of 10 chicks per pen. The control group was given maize-soybean meal basal diet, and the other two groups were fed the basal diet including either 500 U ordinary phytase/kg diet or 500 U thermostable phytase/kg diet. The results indicated that broilers given diets supplemented with phytase have enhanced body weight and weight gain when compared with these fed basal diet (P<0.05), however, growth performance of broilers was similar between two phytase-supplemented groups (P>0.05). Dietary phytase supplementation significantly decreased fecal P excretion (P<0.05) and therefore increased P retention (P<0.05), and this effect was more pronounced in the thermostable phytase-supplemented group (P<0.05). However, the tibia ash content and Ca and P content of tibia ash were all unaffected by diet phytase supplementation (P>0.05). The results indicated that dietary phytase supplementation can improve growth performance and metabolic status of P, and these effects appeared to be more pronounced in the thermostable phytase-supplemented group.

Key words: Thermostable phytase, growth, Ca, P, metabolism, broilers.
Introduction

Phytate, the major form of phosphorus in plant-based feeds, is poorly available to poultry since it appears to lack a meaningful ability to digest this compound, even though intrinsic phytase have been isolated from the small intestines of broilers and laying hens (Maenz et al., 1997; Maenz and Classen, 1998). Consequently, much of the phytate-bound P in the diets is extracted in animal waste and can cause deleterious pollution to environment due to excessive P excretion, besides, phytate can also exert negative influence on the availability of Ca and trace minerals since phytate can chelate these minerals and then form less digestible complexity. Inorganic phosphates mainly in the form of dicalcium phosphate and calcium phosphate are routinely added to supply animals’ requirements and ensure high growth performance. However, additional inorganic phosphorus supplementation can increase feed cost since P is usually listed as the third most expensive nutrient in poultry diets after energy and protein (Biehl et al., 1998).

One way to overcome these problems is the introduction of microbial phytase as feed additive. Phytase feed enzymes with the capacity to release phytate-bound P, reduce P load on the environment and improve growth performance of domestic animals have been well documented (Simons et al., 1990; Cabahug et al., 1999; Ravindran et al., 2000; Paik, 2003; Selle and Ravindran, 2007). The efficacy of phytase enzyme preparation depends not only on type used, inclusion rate, and the level of activity present, but also on the ability of enzyme to maintain its activity in different conditions in vivo and vitro, especially the conditions used for the pre-treatment of a diet as pelleting. The stability of added enzyme to pelleting process is a major concern of feed manufactures (Silversides and Bedford, 1999). Ordinary phytase feed enzyme can not withstand high pelleting temperature up to 80 °C and then the residual activity of exogenous phytase present in the finished pellets might become low and may not play the expected full role. However, phytase feed enzyme can be included in animal rations in stable form to avoid thermostability problems at high pelleting temperatures. Therefore, the present study was conducted to study the effect of thermostable phytase supplementation on the growth performance and Ca and P metabolism of broilers in comparison with the ordinary phytase.

Materials and Methods

Experimental Design, Animals, and Diets

The animal care and use protocol were approved by Nanjing Agricultural University Institutional Animal Care and Use Committee. The study was conducted to study the effects of thermostable phytase supplementation on growth performance and Ca and P metabolism of broilers for 21 d. 240 1-day-old Arbor Acres chicks with similar initial body weight (46.22±0.09 g) were randomly divided into 3 treatments consisting 8 replicates of 10 chicks per replicate (1 replicate per cage). The control group (CO group) was given maize-soybean meal basal diet (Table 1), the other two groups were fed basal diets including either 500 U ordinary phytase /kg diet (OP group) or 500 U thermostable phytase U/kg diet (TP group). The two phytase-supplemented diets were formulated by adding 100 g ordinary phytase/kg of diet (provided by Weifang Bio-Tech Co., Ltd., Weifang, China, Trade name is KINGPHOS®) and 100 g thermostable phytase/kg of diet (provided by same company mentioned above, Trade name is KINGPHOS HS®), respectively. The declared activity was both 5000 U/g of product for the two phytase enzymes. One unit of enzyme is defined as the amount of enzyme that liberates 1 µmol inorganic orthophosphate per min from 5 mmol/L sodium phytate at pH 5.5 and a temperature of 37 °C. Diets were given in the pellet form. Birds were housed in an environmentally controlled room. Feed and fresh water were available ad libitum at all time.

Experiment Procedures

Body weight and feed intake on a cage basis were recorded from d 1 to 21 for the calculation of the average body weight gain, feed intake and Feed/Gain ratio. For the determination of P retention at 17 d of age, clean stainless steel collection trays were placed under each cage (six per treatment) and feces from birds were collected for 72 h. Feces were mixed thoroughly and stored under -20 °C. Prior to chemical analysis, subsamples of feces were collected, weighed, and dried for 48 h in an oven at 65 °C. The dried excreta were
allowed to equilibrate to atmospheric conditions for 24 h before being weighed. Feed and excreta samples were then ground through a 0.45-mm screen and analyzed (AOAC, 1990) for P (965.17). At d 21, 24 poult-sample from three treatments (1 bird per cage) was selected randomly and killed by cervical dislocation, the right tibiae was removed, cleaned from adhering tissue. For bone ash determination, tibiae were dried 110 °C for 12 h, extracted with ether, dried again, and weighed. The dry fat-free bones were ashed in a muffle furnace at 550 °C, and the bone ash were analyzed (AOAC, 1990) for P (965.17) and Ca (968.08).

Statistical Analysis

All data were analyzed as a completely randomized design using one-way ANOVA (SPSS, 2008). The differences were considered to be significant at P<0.05.

Result

Growth Performance

Broilers given diets supplemented with phytase (Table 2), either ordinary one or the thermostable one, have increased body weight (P<0.05) than these fed the basal diet, which appeared to result from enhanced body weight gain (P<0.05) and feed intake (P<0.05) rather than improved feed conversion efficiency (P>0.05). However, there was no significant difference in the growth performance of broilers between the two different phytase-supplemented groups (P>0.05), though growth parameters were numerically higher in thermostable phytase-supplemented group.

Ca and P Metabolism

As indicated in table 3, tibia ash content was uninfluenced by phytase supplementation (P>0.05), and the same effects were also observed for Ca and P content of tibia ash (P>0.05), though which were numerically higher in the phytase-supplemented groups when compared with the control. As expected, dietary phytase supplementation significantly decreased P excretion in feces (P<0.05) and therefore enhanced P retention of broilers (P<0.05). Besides, it is also worth noting that the effect of thermostable phytase on P retention of broilers was more pronounced than the ordinary one (P<0.05).

Discussion

The results obtained from present study confirmed the beneficial influence of dietary phytase supplementation on the growth performance of broilers in terms of body weight, weight gain, and feed intake. The released mineral and trace mineral elements chelated by phytic acid along with possible increased starch digestibility (Knuckles and Betschrsr, 1987) and improved availability of protein, amino acids and other nutrients (Selle et al., 2000; Selle and Ravindran, 2007) may attributed to the enhanced growth performance. It is generally thought that responses to exogenous phytase supplementation in feed intake and body weight gain are more robust and consistent than feed efficiency responses (Selle and Ravindran, 2007). In our study, feed conversion ratio was uninfluenced by phytase supplementation, which was in accordance with the previous reports by (Ahmad et al., 2000) and (Viveros et al., 2002), and this effect is probably as result of simultaneous increases in weight gain and feed consumption or declining feed efficiency responses to phytase with time, which may attributes to concurrent improvements in broiler strains, feeds and management techniques (Rosen, 2003).

Traditionally, estimates of P and Ca needs for growing broilers have been based primarily upon tibia ash or toe ash values, and Ca and P contents in tibia ash are used as the primary criterion of P and Ca adequacy. Reports regarding the effects of dietary phytase supplementation on mineral tibia of broilers are conflicting (Qian et al., 1996; Sebastian et al., 1996; Ahmad et al., 2000; Viveros et al., 2002), which may due to the age of animals, dietary mineral elements content especially Ca, diet compositions, phytase inclusion rate, the level of activity present, and the stability of the enzyme to maintain its activity in different conditions, both in vivo or vitro. In our study, there was no significant difference in the tibia ash content and Ca and P content in tibia between treatments, which is supported by reports by (Ahmad et al., 2000) and may result from the adequate Ca and P level in the basal diet that may satisfy broilers’ needs.
Table 1: Ingredients and nutrient composition of basal diet (g/kg, as-fed basis unless otherwise stated).

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Basal diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize</td>
<td>570</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>310</td>
</tr>
<tr>
<td>Corn gluten meal</td>
<td>39</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>31</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>18</td>
</tr>
<tr>
<td>Limestone</td>
<td>13</td>
</tr>
<tr>
<td>L-Lysine</td>
<td>1.5</td>
</tr>
<tr>
<td>DL-Methionine (99%)</td>
<td>1.3</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>3.0</td>
</tr>
<tr>
<td>Zeolite</td>
<td>3.2</td>
</tr>
<tr>
<td>Premixa</td>
<td>10</td>
</tr>
</tbody>
</table>

Calculated composition:

| ME (MJ/kg)      | 12.5 |
| CP              | 209  |
| Calcium         | 9.8  |
| Available phosphorus | 4.2 |
| Lysine          | 10.5 |
| Methionine      | 4.9  |
| Methionine + cystine | 8.4 |

*Premix provided per kilogram of diet: transretinyl acetate, 24 mg; cholecalciferol, 6 mg; all-rac-α-tocopherol acetate, 20 mg; menadione, 1.3 mg; thiamin, 2.2 mg; riboflavin, 8 mg; nicotinamide, 40 mg; choline chloride, 400 mg; calcium pantothenate, 10 mg; pyridoxine-HCl, 4 mg; biotin, 0.04 mg; folic acid, 1 mg; vitamin B<sub>12</sub>(cobalamin), 0.013 mg; Fe (from ferrous sulfate), 80 mg; Cu (from copper sulphate), 7.5 mg; Mn (from manganese sulphate), 110 mg; Zn (from zinc oxide), 65 mg; I (from calcium iodate), 1.1 mg; Se (from sodium selenite), 0.3 mg; bacitracin zinc, 30 mg.

Table 2: Effects of thermostable phytase supplementation on growth performance of broilers.

<table>
<thead>
<tr>
<th>Item</th>
<th>Body weight, g</th>
<th>Body weight gain, g/d</th>
<th>Feed intake, g/d</th>
<th>Feed/Gain ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO</td>
<td>683&lt;sup&gt;b&lt;/sup&gt;</td>
<td>29.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>42.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.43</td>
</tr>
<tr>
<td>OP</td>
<td>722&lt;sup&gt;a&lt;/sup&gt;</td>
<td>32.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>46.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.44</td>
</tr>
<tr>
<td>TP</td>
<td>731&lt;sup&gt;a&lt;/sup&gt;</td>
<td>32.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>46.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.40</td>
</tr>
<tr>
<td>SEM</td>
<td>5</td>
<td>0.3</td>
<td>0.6</td>
<td>0.02</td>
</tr>
</tbody>
</table>

<sup>SEM = Standard error of means (n=8).<br>a-cMeans within a column with no common superscript differ significantly (P<0.05).</sup>

Table 3: Effects of thermostable phytase supplementation on Ca and P metabolism of broilers.

<table>
<thead>
<tr>
<th>Item</th>
<th>Tibia ash (%)</th>
<th>Bone mineral</th>
<th>P excretion (g/kg DM intake)</th>
<th>P retention (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ca (%)</td>
<td>P (%)</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>38.1</td>
<td>37.2</td>
<td>18.1</td>
<td>3.7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>OP</td>
<td>37.2</td>
<td>38.6</td>
<td>20.1</td>
<td>2.9&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>TP</td>
<td>37.9</td>
<td>38.1</td>
<td>19.3</td>
<td>2.6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>SEM</td>
<td>2.4</td>
<td>1.4</td>
<td>0.9</td>
<td>0.2</td>
</tr>
</tbody>
</table>

<sup>SEM = Standard error of means (n=6).<br>a-cMeans within a column with no common superscript differ significantly (P<0.05).</sup>

It is well known that exogenous phytase in the feeds enhance phytate degradation through releasing phytate-bound P, and therefore reduce P excretion and improve P retention (Simon et al., 1990; Paik, 2003), which is once more demonstrated by the results of the present study. In
our study, thermostable phytase is more effective in improving the metabolic status of P than the ordinary phytase. Most of the added feed enzymes inactivation takes place during conditioning, when the feed is heated with steam, especially the pelleting process. By adding the thermostable enzymes to the formulation partially overcome this problems, and the better efficiency by thermostable phytase supplementation obtained in present study may because of the high residual activity of the thermostable phytase in the finished pellets after high temperature pelleting and refining process.

**Conclusion**

Dietary phytase supplementation, either the ordinary one or thermostable one, can enhance the growth performance of broilers in term of body weight, weight gain, and improve the P metabolic status evidenced by lower P excretion and therefore higher P retention, and these effects appeared to be more pronounced in the thermostable phytase supplemented group.

**References**


