The supplementation of low-P diets with microbial 6-phytase expressed in *Aspergillus oryzae* increases P and Ca digestibility in growing pigs

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**ABSTRACT:** A trial was conducted to evaluate the dose response of a novel microbial 6-phytase expressed in *Aspergillus oryzae* (Ronozyme HiPhos; DSM Nutritional Products, Basel, Switzerland) in pigs. Forty-eight individually housed pigs (Landrace × Pietrain; 52 kg BW; 24 males and 24 females) were distributed among 6 experimental treatments consisting of a low-P diet (3.5 g P/kg; 1.1 g digestible P/kg), which was supplemented with 500, 1000, 2000, or 4000 units of phytase activity/kg, and a standard-P diet (4.5 g P/kg; 1.8 g digestible P/kg) that was supplemented with CaHPO₄. After 17 d, fresh feces were sampled from all pigs and the apparent total tract digestibility of DM, OM, ash, P, and Ca was measured using TiO₂ as indigestible marker. Blood samples were also obtained from each pig and serum was analyzed for P and Ca concentrations. The nonsupplemented low-P diet increased Ca and reduced P blood serum concentrations (*P* < 0.05) relative to the standard-P diet (10.8 vs. 10.2 and 6.7 vs. 7.7 mg/dL, respectively). Phytase supplementation of the low-P diet reduced Ca (from 10.8 to 9.9 mg/dL; linear, *P* < 0.001) and increased P concentrations (from 6.7 to 8.0 mg/dL; linear and quadratic, *P* < 0.001) in serum and reduced P concentration in feces (from 13.7 to 7.6 g/kg DM; linear and quadratic, *P* < 0.001). Phytase improved the total tract digestibility of P (from 29.0 to 62.3%; linear and quadratic, *P* < 0.001 and *P* < 0.05), Ca (from 54.0 to 75.7%; quadratic, *P* < 0.01), and ash (from 46.2 to 57.7%; quadratic, *P* < 0.01). In conclusion, the microbial 6-phytase tested improves the apparent total tract digestibility of P in growing pigs and reduces P excretion in feces in a dose-dependent manner.

**Key words:** digestibility, phosphorous, phytase, pigs


**INTRODUCTION**

Phytate-bound P from plant-derived feed ingredients accounts for most of the P in practical pig diets (Reddy et al., 1982). Phytate is the mixed salt of phytic acid and its P is poorly available to pigs because the endogenous phytase activity (*FYT*) present in the small intestine cannot efficiently dephosphorylate it. As a result, pigs need to be supplemented with highly available inorganic P sources [e.g., CaHPO₄ (dicalcium phosphate; DCP)] to satisfy P requirements. However, the large proportion of unavailable P is still excreted in feces and causes environmental concern. Microbial phytases facilitate the use of phytate-P by pigs, alleviate P pollution, and reduce the need for supplementation with inorganic P sources. The aim of this study was to test the efficacy of a novel microbial 6-phytase added at different dosages to a low P diet on growth performance, Ca and P blood serum concentrations, and apparent total tract digestibility of DM, OM, ash, P, and Ca in growing pigs.

**MATERIALS AND METHODS**

This experiment was conducted at the Experimental Farm of the Institut de Recerca i Tecnologia Agroalimentària (IRTA) following approval by IRTA’s Ethical Committee on Animal Experimentation. Forty-eight pigs (Landrace × Pietrain; 24 males and 24 females; 52 ± 5.3 kg initial BW) from IRTA’s farm herd were used. Pigs were housed individually in 2 identical rooms of 24 pens each provided with forced ventilation for thermal regulation. Each pen had 1 feeder and 1 water nipple to allow for ad libitum access to feed and water.

At the start of the experiment, pigs were blocked by BW and sex and randomly assigned to the 6 experimental treatments, providing a total of 8 replicates.

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The pigs were fed the experimental diets for 17 d. Individual BW and feed intake were recorded at the start and at the end of the trial. At the end of the experiment, a blood sample was obtained from all the pigs and serum was analyzed for Ca and P concentration. Fresh feces were also obtained at that time from each pig and were stored at –20°C until analysis. Feed and feces were analyzed for DM, ash, Ca, P, and TiO₂. Digestibility values were previously calculated by the index method as described previously (Willamil et al., 2012).

The effects of dietary treatment were analyzed using the GLM procedure (SAS Institute Inc., Cary, NC) with pig as the experimental unit. For the performance variables, the model included the effect of sex and initial BW was used as the covariate. Linear and quadratic polynomial contrasts were used to determine the response to phytase supplementation of the low-P diets. Results are presented as least squares means and multiple comparisons were performed when the effect of dietary treatment was significant. The level of significance was set at P < 0.05.

**RESULTS AND DISCUSSION**

The presence of the enzyme preparation in the low-P supplemented diets was confirmed (671, 1529, 2659, and 4448 FYT/kg, respectively). Basal phytase activities were also measured in the nonsupplemented low-P and standard-P diets (150 and 114 FYT/kg, respectively). These can be explained by the native phytases present in the used feed ingredients (Viveros et al., 2000). Analyzed Ca concentrations were similar among diets and ranged between 5.9 and 6.4 g/kg whereas P concentrations ranged between 2.9 and 3.3 g/kg in the low-P diets and was 4.2 g/kg in the standard-P diet.

Phytase supplementation of the low-P diet improved the ADG and G:F of the pigs (quadratic, P < 0.05; Table 1). This is most likely due to the release of phytate-bound P that contributes to the P requirement of the pigs. Because low-P diets were used, this may be the main explanation for the increased performance. However,

**Table 1.** Effect of microbial 6-phytase supplementation to low-P diets on performance, Ca, and P blood serum concentration and apparent total tract digestibility of nutrients in growing pigs

<table>
<thead>
<tr>
<th>Item</th>
<th>Low P FYT/kg phytase&lt;sup&gt;2&lt;/sup&gt;</th>
<th>500 FYT/kg phytase</th>
<th>1000 FYT/kg phytase</th>
<th>2000 FYT/kg phytase</th>
<th>4000 FYT/kg phytase</th>
<th>Standard P FYT/kg phytase</th>
<th>Root MSE&lt;sup&gt;3&lt;/sup&gt;</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADG, kg</td>
<td>0.79</td>
<td>0.89</td>
<td>0.88</td>
<td>0.91</td>
<td>0.80</td>
<td>0.88</td>
<td>0.128</td>
<td>0.045</td>
</tr>
<tr>
<td>ADFI, kg</td>
<td>2.49</td>
<td>2.32</td>
<td>2.03</td>
<td>2.19</td>
<td>2.13</td>
<td>2.22</td>
<td>0.455</td>
<td>0.243</td>
</tr>
<tr>
<td>G:F</td>
<td>0.33</td>
<td>0.39</td>
<td>0.44</td>
<td>0.42</td>
<td>0.39</td>
<td>0.41</td>
<td>0.083</td>
<td>0.032</td>
</tr>
<tr>
<td>Ca in blood serum, mg/dL</td>
<td>10.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.46&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>P in blood serum, mg/dL</td>
<td>6.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.49&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>&lt;0.001&lt;sup&gt;abc&lt;/sup&gt;</td>
</tr>
<tr>
<td>DM digestibility, %</td>
<td>81.5</td>
<td>81.4</td>
<td>81.9</td>
<td>81.9</td>
<td>79.3</td>
<td>80.7</td>
<td>2.46&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>0.054</td>
</tr>
<tr>
<td>OM digestibility, %</td>
<td>83.4</td>
<td>83.1</td>
<td>83.5</td>
<td>83.3</td>
<td>80.9</td>
<td>82.5</td>
<td>2.35&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>0.025</td>
</tr>
<tr>
<td>Ash digestibility, %</td>
<td>46.2</td>
<td>50.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>52.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>57.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>51.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>48.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.87&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>0.042</td>
</tr>
<tr>
<td>P digestibility, %</td>
<td>29.0</td>
<td>36.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>42.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>55.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>62.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>37.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.10&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>&lt;0.001&lt;sup&gt;abc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ca digestibility, %</td>
<td>54.0</td>
<td>65.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>68.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>75.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>60.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>58.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.41&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>0.004&lt;sup&gt;abc&lt;/sup&gt;</td>
</tr>
<tr>
<td>P in feces, g/kg DM</td>
<td>13.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.69&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>&lt;0.001&lt;sup&gt;abc&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Within a row, means without a common superscript differ (P < 0.05).
<sup>1</sup>FYT = phytase activity.
<sup>2</sup>Ronozyme HiPhos (DSM Nutritional Products, Basel, Switzerland).
<sup>3</sup>MSE = mean squared error.
<sup>4</sup>Linear effect of phytase supplementation of the negative control diet.
<sup>5</sup>Quadratic effect of phytase supplementation of the negative control diet.
other extraphosphoric effects of phytases (e.g., increased CP and AA digestibility) have been reported in P-adequate diets and these may also be responsible for part of the performance improvements (Selle and Ravindran, 2008).

Without phytase supplementation, reducing the P content of the diet by removing DCP and replacing the corresponding amount of Ca with CaCO₃ resulted in higher Ca and lower P concentrations in the blood serum of the pigs ($P < 0.05$). This is indicative that the low-P diet was deficient in P (Pointillart, 1991). Phytase contributed to counteract this deficiency as indicated by the increased P and reduced Ca serum concentrations ($P < 0.001$) in response to increasing doses of the enzyme. As expected from digestibility values of P in feedstuffs, the low-P diet (without DCP) presented a lower total tract digestibility of P than the standard-P diet (29.0 vs. 37.7%; $P < 0.05$). Differences were not observed for total tract digestibility of Ca, ash, DM, and OM or the P concentration in feces. Phytase supplementation of the low-P diet improved the total tract digestibility of P (from 29.0 to 36.3, 42.3, 55.9, and 62.3%; linear and quadratic, $P < 0.001$ and $P < 0.05$, respectively), Ca (from 54.0 to 65.1, 68.9, 75.7, and 60.4%; quadratic, $P < 0.01$), and ash (from 46.2 to 50.4, 52.6, 57.7, and 51.2%; quadratic, $P < 0.01$) and reduced P concentration in feces (from 13.7 to 11.3, 10.4, 8.0, and 7.6 g/kg DM; linear and quadratic, $P < 0.001$). These increases in P digestibility are in agreement with other studies (Almeida and Stein, 2012). Together with the performance data, these data confirm that inorganic P could be fully replaced by a microbial 6-phytase in grower–finisher pig diets, which is in agreement with observations by Augspurger et al. (2009).

In conclusion, the microbial 6-phytase tested improves the apparent total tract digestibility of P in growing pigs and reduces P excretion in feces in a dose-dependent manner.

LITERATURE CITED


