Phytase inclusion in pig diets improves zinc status but its effect on copper availability is inconsistent


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ABSTRACT: Complexation of dietary phytate with cations is a major cause of reduced bioavailability of Zn and possibly Cu in pig diets. We conducted 2 studies with 2 treatments in young growing pigs (8 to 40 kg) to estimate potential contributions of phytase to availability and supply of Zn and Cu, respectively. Each treatment comprised 10 pens with 8 pigs each as experimental units. In Exp. 1, 500 phytase units (FTU)/kg of microbial phytase (Natuphos 5000G; BASF) was added to a diet containing 15 mg Zn from ZnSO₄ and 160 mg/kg Cu from CuSO₄ in addition to Cu and Zn from feed ingredients. In Exp. 2, 500 FTU/kg was added to a diet containing 45 mg Zn from ZnSO₄ without added CuSO₄. Feces were collected to determine nutrient digestibility, blood was collected, and pigs were killed to determine Cu and Zn in the liver. In both experiments, phytase inclusion increased (P < 0.001) Zn digestibility by on average 10% units, serum Zn level (P < 0.001) by 0.4 mg/L, and liver Zn content (P < 0.001) by 129 mg/kg DM. In Exp. 1 phytase increased (P = 0.03) Cu digestibility by 6% units but reduced (P = 0.04) liver Cu content by 35 mg/kg DM. In Exp. 2 phytase reduced (P < 0.001) Cu digestibility by 16% units without affecting liver Cu content. Results indicate that the effect of phytase on Cu availability depends on dietary Cu and Zn content and the response variable studied. In conclusion, the consistent effects of phytase on indices of Zn status allow a reduction of Zn inclusion in phytase-supplemented diets.

Key words: availability, copper, phytase, pigs, zinc

INTRODUCTION

Copper and Zn are essential trace elements in diets of growing pigs. Deficiency may impair growth and immune responses (NRC, 2005). Adequate amounts of Cu and Zn are generally added to pig diets via the premix. However, the majority of the dietary Cu and Zn is excreted in the manure, thus contributing to the accumulation of the elements in the soil and surface water. Complexation of dietary phytate with cations is a major cause of reduced bioavailability of Zn and possibly Cu in pig diets. Inclusion of phytase may improve their availability thus allowing a reduction in the dietary inclusion of these elements (e.g., Adeola et al., 1995). Practical implementation requires quantitative insight in the contribution of phytase to the Cu and Zn supply of the pigs. Because of the antagonism between Cu and Zn, the influence of phytase may be affected by the high, growth-promoting Cu content generally included in nursery diets. Thus, the objective of 2 studies was to determine the effect of the inclusion of phytase in pig diets with high and low Cu content on Cu and Zn availability in young growing pigs.

MATERIAL AND METHODS

Experimental protocols were approved by an ethical committee of Wageningen University and Research Centre.

Animals, Housing, and Design

In each of 2 studies, 1 dietary treatment was included with and without 500 phytase units (FTU) of microbial phytase (Natuphos 5000G, BASF) per kilogram, a commonly included dosage in practical diets. One phytase unit is defined as the amount of enzyme required to release 1 μmol of inorganic P/min from sodium phytate at 37°C. Each treatment comprised 10 pens with 4 female pigs.
and 4 castrated male hybrid pigs [Large White × (Dutch Landrace × Great Yorkshire)] each as experimental unit. At the day of weaning, pigs (26 d of age; 8 kg initial BW) were blocked on the basis of BW and allocated to pens and treatments in a randomized block design. In the 56-d experiment, pigs were housed in climate-controlled rooms with 8 pens of 4.8 m² with plastic coated mesh floors, plastic fences, and stainless steel feeders.

**Diets and Feeding**

In Exp. 1, all pigs received the same prestarter diet with 10 mg Zn as ZnSO₄·H₂O and without added phytase during a 2-wk depletion period. From days 15 to 56, pigs received a starter diet with 15 mg Zn with or without added phytase according to the experimental treatment. All diets contained 160 mg Cu as CuSO₄·5H₂O. In Exp. 2, pigs received prestarter and starter diets without added Cu, with 45 mg Zn from ZnSO₄·H₂O and with or without added phytase according to the experimental treatment.

The analyzed phytase content, corrected for (intrinsic) phytase in the basal diets, was 800 and 595 FTU/kg in Exp. 1 and 2, respectively. In both experiments, diets were based on barley (*Hordeum vulgare*), wheat (*Triticum aestivum*), corn (*Zea mays*), soybean (*Glycine max*) meal, rape seed meal, potato (*Solanum tuberosum*) protein, corn gluten meal, whey powder, oil, synthetic AA, vitamins, and minerals. These ingredients contributed on average 35 mg Zn and 7 mg Cu per kg of diet. In all diets, Cr₂O₃ was included as indigestible marker. Diets were freely available in stainless steel feeders and water from nipple drinkers.

**Observations and Chemical Analysis**

Body weight and feed intake were determined in 2-wk periods and growth rate and feed efficiency were calculated. Nutrient digestibility was determined by grab sampling of feces of all pigs in 6 out of the 10 replicates on days 34 (Exp. 1) or 27 (Exp. 2) and day 55 (Exp. 1 and 2) after weaning. Samples of diets and freeze-dried feces were ground through a 0.75-mm mesh sieve and analyzed for moisture by oven drying (ISO 6496) and for trace elements by atomic absorption spectrometry after combustion of a 5 g sample at 550°C and dissolving the residue in HCl (ISO 6869). Blood samples were collected by puncture of the vena jugularis on days 35 (Exp. 1) or 28 (Exp. 2) and 56 (Exp. 1 and 2) from 2 piglets (female and castrate) per pen in all replicates. Serum levels of Cu and Zn were determined after dilution of 0.5 mL with 4.5 mL diluent (0.1% Triton X-100 in 0.05% HNO₃) and measured against standards with inductively coupled plasma atomic emission spectroscopy (ICP-AES; Perkin Elmer 3300DV).

In each of 4 replicates, 2 representative piglets per pen were killed at the end of the experiment. The liver was collected, freshly ground, and subsampled for further analysis. A 5-g subsample was dried at 103°C for 4 h and moisture content was calculated. Copper and Zn were determined with ICP-AES (Perkin Elmer Optima 7300 DV) in the fresh sample after treatment with a mixture of HClO₄ and H₂SO₄ at 300°C.

**Statistical Analyses**

Data were analyzed by ANOVA as randomized block design with pen as experimental unit and phytase inclusion as explanatory treatment using Genstat statistical software (VSN, Hemel Hempstead, UK). Differences were considered significant at \( P \leq 0.05 \).

### RESULTS

The inclusions of phytase did not influence growth performance (data not shown). Effects of phytase on serum levels and digestibility of Cu and Zn were similar on days 35 and 56 (Exp. 1) and on days 28 and 56 (Exp. 2); hence, pooled data are reported. Phytase drastically increased \( P < 0.001 \) serum Zn levels, liver Zn content, and Zn digestibility in both experiments (Table 1). In both experiments phytase did not affect serum Cu levels. In Exp. 1, phytase inclusion increased \( P = 0.026 \) Cu digestibility and \( P = 0.036 \) liver Cu content. In Exp.

### Table 1. Influence of the inclusion of microbial phytase in diets of weaned piglets during 6 (Exp. 1) or 8 (Exp. 2) wk on Cu and Zn levels in blood serum and liver and on total tract digestibility of Cu and Zn

<table>
<thead>
<tr>
<th>Item</th>
<th>Exp. 1</th>
<th>Exp. 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Phytase</td>
</tr>
<tr>
<td>Serum level, μmol/L¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu</td>
<td>28.9</td>
<td>29.6</td>
</tr>
<tr>
<td>Zn</td>
<td>6.7</td>
<td>13.9</td>
</tr>
<tr>
<td>Liver content, mg/kg DM</td>
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<td></td>
</tr>
<tr>
<td>Cu</td>
<td>109</td>
<td>74</td>
</tr>
<tr>
<td>Zn</td>
<td>105</td>
<td>297</td>
</tr>
<tr>
<td>Digestibility, %²</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu</td>
<td>−9.2</td>
<td>−3.7</td>
</tr>
<tr>
<td>Zn</td>
<td>0.86</td>
<td>10.1</td>
</tr>
</tbody>
</table>

¹Serum levels on days 35 and 56 (Exp. 1) and days 28 and 56 (Exp. 2) were pooled per animal prior to statistical analysis.

²Digestibility coefficients on days 34 and 55 (Exp. 1) and days 27 and 55 (Exp. 2) were pooled per pen prior to statistical analysis.
Phytase affects copper and zinc availability

2 phytase reduced \( P < 0.001 \) Cu digestibility without affecting liver Cu content.

DISCUSSION

Despite the large effect of phytase inclusion on Zn digestibility and serum Zn content, phytase inclusion did not influence growth performance in the 2 studies. These results are similar to other studies (e.g., Jondreville et al., 2005) showing that the dietary Zn supply for maximum body gain is less than for maximum plasma or serum Zn levels.

Mean Zn digestibility seems slightly underestimated, but the effects of phytase inclusion on Zn digestibility and serum and liver Zn content were consistent and very substantial, indicating that the degradation of dietary phytate released a substantial amount of Zn and may reduce complexation of endogenous Zn (Oberleas, 1996). Serum Zn levels in pigs fed control diets were lower in Exp. 1 than Exp. 2, reflecting differences between added Zn content of 15 and 45 mg/kg from ZnSO\(_4\) in Exp. 1 and 2, respectively.

In Exp. 1 the mean liver Cu content was higher and Cu digestibility was lower than in Exp. 2. This difference reflects the higher dietary Cu level of 165 mg/kg in Exp. 1 vs. 7 mg/kg in Exp. 2. Effects of phytase on Cu variables were inconsistent and differed between selected variables and between Exp. 1 and 2. The reduction in Cu digestibility by phytase inclusion, especially in Exp. 2, was remarkable and might be mediated by an interaction with additionally released Zn in the digestive tract. The antagonism between Cu and Zn is well described and may be mediated by intestinal metallothionein (MT). Synthesis of MT in the enterocytes is more responsive to Zn than Cu whereas binding affinity of MT is higher for Cu than Zn (Cousins, 1985). The increase in intestinal MT provides a temporary storage of Cu and Zn, which is eventually sloughed off and excreted. Blalock et al. (1988) found a Zn-induced increase in intestinal MT in rats fed low but not high Cu diets. This interaction may have contributed to the phytase-induced reduction in Cu digestibility in Exp. 2 with a low dietary Cu and relatively high Zn content. Indeed in other studies (e.g., Adeola et al., 1995), Cu absorption was reduced when the Zn level in the diet was increased, and an interaction between phytase and Zn level occurred. Adeola (1995) found a significant decrease in serum Cu level after phytase inclusion. Despite the decrease in Cu digestibility in Exp. 2, phytase inclusion did not reduce serum and liver Cu levels in our studies. Thus, the relevance of the reduction in Cu digestibility remains to be established.

In conclusion, the inclusion of phytase in the diet of growing pigs consistently improved the digestibility, serum, and tissue levels of Zn. This allows a reduction of Zn inclusion in phytase-supplemented diets, irrespective of the dietary Cu content. The effects on the same indices of Cu status were inconsistent and may depend on the Cu and Zn contents in the diet.

LITERATURE CITED