Evaluation of the effects of pharmacological zinc oxide and phosphorus source on weaned piglet growth performance, plasma minerals and mineral digestibility

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Crossbred pigs (n = 720; average age = 28 ± 3 days and weight = 9.5 ± 0.3 kg) were used in a 20-day trial in order to determine the influence of phosphorus (P) source and various doses of pharmacological zinc (Zn) on growth performance, plasma minerals and mineral digestibility. Pigs (five intact males and five females per pen) were randomly allotted to treatments in a 3 x 3 factorial arrangement with three sources of dietary P (4.5 g/kg digestible P, 4.5 g/kg digestible P plus 2500 phytase units (FTU)/kg, or 5.5 g/kg digestible P) and three dietary levels of supplemental Zn (0, 1750 or 3500 mg/kg) from ZnO (82% Zn) with eight pens per treatment. Diets were formulated to exceed all nutrient requirements, including calcium (Ca), P and Zn from day 0 to 20. Zn supplementation increased (quadratic P < 0.05) average daily feed intake. There was a significant Zn level x P source interaction on average daily gain and feed conversion ratio (FCR). Pigs fed 4.5 g/kg digestible P without or with 2500 FTU/kg phytase gained more per day (quadratic P < 0.05) and had better FCR (quadratic P < 0.05) when they were fed 1750 mg/kg supplemental Zn. However, pigs fed 5.5 g/kg digestible P gained more per day (linear P < 0.05) and were more efficient (linear P < 0.05) when they were fed 3500 mg/kg supplemental Zn. Plasma Zn and Zn digestibility increased (linear P < 0.05) as pharmacological Zn supplementation increased from 0 to 3500 mg/kg, irrespective of P source. However, Ca, P, sodium (Na), potassium (K) and copper (Cu) digestibility were reduced (P < 0.05) as pharmacological Zn supplementation increased, and this was mitigated or exacerbated by the supplementation of 5.5 g/kg digestible P or phytase. In conclusion, increasing the dietary inclusion of pharmacological Zn may impact growth performance in young pigs through the interaction with minerals such as Ca, P, Na and K. Pharmacological Zn may reduce Na or K digestibility and indirectly reduce water secretion into the lumen, resulting in an increase in faecal dry matter as pharmacological Zn supplementation in the diet increased.

Keywords: minerals, phosphorus, phytase, pig, zinc oxide

Implications

Supplementation of young pigs’ diets with pharmacological Zn resulted in an improvement in average daily gain and feed conversion ratio, especially in the presence of phytase with 1750 mg/kg Zn from ZnO or additional digestible P with 3500 mg/kg Zn from ZnO. Increasing pharmacological Zn from 0 to 3500 mg/kg reduced apparent P, Na, K and Cu digestibility, indicating interactions between pharmacological Zn and dietary minerals, which may influence growth performance. When supplementing pharmacological Zn, the concentrations of Ca, P and other minerals supplied in the diet need to be considered. In addition, the Na and K digestibility data may indicate a possible role of pharmacological Zn in water secretion and subsequent effects on faecal dry matter or diarrhoea post-weaning.

Introduction

Zinc (Zn) in the form of zinc oxide (ZnO) is commonly fed at pharmacological doses (1500 to 3500 mg/kg) to piglets in order to mitigate diarrhoea post-weaning (Carlson et al., 1999). However, Hill et al. (2001) reported a quadratic effect of pharmacological Zn from ZnO supplementation on average daily gain (ADG) and feed efficiency (feed conversion ratio (FCR)) using nursery pigs. The authors reported an increase in ADG and improvements in FCR, as levels of Zn were increased to 2000 mg/kg, but the pigs fed 3000 mg/kg supplemental Zn were 8% lighter and 5% less efficient.
compared with the nursery pigs fed 2000 mg/kg supplemental Zn. In a different trial, pigs fed 1750 mg/kg Zn from ZnO were heavier and more efficient than pigs fed 0 or 3500 mg/kg Zn, especially in the presence of 2500 FTU/kg phytase added over the top of the diet (Walk et al., 2013). Phytase hydrolyses phytate, a known chelator of Zn, and supplementation with high levels of dietary phytase increases serum Zn to a similar level as that of 1750 mg/kg supplemental Zn from ZnO (Walk et al., 2013). Standard levels of phytase increased plasma Zn and retained Zn (mg/day) in 9-kg piglets (Revy et al., 2004), further indicating that phytase has an influence on Zn availability in weaned pigs.

In addition to influencing Zn concentrations in the blood, Walk et al. (2013) reported a negative influence of pharmacological Zn from ZnO supplementation on plasma phosphorus (P), regardless of phytase supplementation. The authors hypothesised that Zn from ZnO precipitates with P, resulting in a reduction of P digestion and absorption. The antagonistic relationship between P and Zn is well-defined in rats (Cabell and Earle, 1965; Zemel and Bidari, 1983). For example, Cabell and Earle (1965) reported that rats fed low dietary Zn (18 mg/kg) gained 43% less weight when fed diets with 1.2% dietary calcium (Ca) and P compared with rats fed low dietary Zn with 0.30% Ca and P. However, Martinez et al. (2005) reported no effect of supplemental Zn from ZnO on plasma P, and there are no known reports evaluating the influence of pharmacological Zn on P digestibility. Therefore, the objectives of this experiment were to evaluate growth performance, faecal scores, plasma minerals and apparent total tract mineral digestibility of piglets fed graded levels of pharmacological Zn from ZnO and different sources of P (4.5 g/kg digestible P, 4.5 g/kg digestible P plus 2500 FTU/kg of phytase or 5.5 g/kg digestible P) from day 0 to 20 post-weaning.

Material and methods

The trial was conducted according to the Animals (Scientific Procedures) Act 1986, implemented by the Department for Health, Social Security and Public Safety in Northern Ireland.

Animals and husbandry

A total of 720 crossbred (PIC337) weaned pigs (28 ± 3 days post-farrowing) were sorted by BW and randomly allotted to nine dietary treatments in a 20-day experiment. Dietary treatments were replicated using eight pens (blocked over time with four replicates/block) of 10 pigs of mixed sex in each pen (five intact males and five females). The average initial BW of each pen was 9.5 ± 0.3 kg. Dietary treatments were arranged as a 3 × 3 factorial, which included three sources of supplemental P (4.5 g/kg digestible P, 4.5 g/kg digestible P plus 2500 phytase units (FTU)/kg or 5.5 g/kg digestible P) and three levels of supplemental Zn from ZnO (0, 1750 or 3500 mg/kg). The phytase used was derived from an Escherichia coli six-phytase expressed in Trichoderma reesei and contained a declared activity of 5000 FTU/g (Quantum Blue; AB Vista Feed Ingredients, Marlborough, UK). Phytase activity in the feed was analysed according to modified methods of Engelen et al. (2001), where one phytase unit is defined as the amount of enzyme required to release 1 mole of inorganic P/min from rice bran phytate at pH 4.5 and 60°C. Titanium dioxide was added (3.0 g/kg) to the diets as an indigestible marker.

Diets were fed in a pelleted form from day 0 to 20 post-weaning. The ingredient compositions, in addition to the calculated and analysed nutrient inclusion levels of the experimental diets, are presented in Tables 1 and 2. The phytase, additional P from monosodium phosphate and Zn were added over the top of the basal diet in place of corn and soyabean meal. All diets were formulated to meet or exceed National Research Council (1998) requirements for 5- to 20-kg pigs, including Ca, P and Zn. All pigs were housed in a closed-sided barn with natural lighting and at least 2 h of artificial lighting per day at a stocking density of 0.38 m²/pig. The pens were composed of concrete walls, plastic slats and plastic pen dividers and contained a stainless steel bowl drinker and multi-space plastic dry feeder to facilitate ad libitum access to water and feed.

Response variables

Pig BW and feed disappearance were measured at the beginning of the experiment (day 0) and again on day 20 to calculate average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR). Faecal consistency scores were recorded daily on a pen basis from day 1 to 10 inclusive. Scoring was based on a 4-point scale (1: firm; 2: soft, spreads slightly; 3: very soft; and 4: watery), according to methods of Wellock et al. (2006). Scores were assessed by the same stockperson who was unaware of the treatment allocation of pigs. Two scores were taken per pen and pooled to obtain an average faecal consistency score per day. Blood samples were collected from two pigs per pen via jugular venipuncture on day 20. Samples (~6 ml/tube) were collected into plastic tubes containing no anticoagulant. Blood was allowed to clot at room temperature, centrifuged at 1155 × g for 5 min and plasma was separated and stored at −20°C until further mineral analyses. Grab faecal samples were collected from the entire pen once daily and pooled per pen from day 18 to 20. The pooled faecal samples were dried for 48 h at 80°C and milled before mineral analyses. Diet dry matter (DM), CP, Ca, P, Zn and sodium (Na) and plasma and faecal Ca, magnesium (Mg), P, potassium (K), Na, copper (Cu) and Zn were determined at the Scientec Analytical Services Ltd (Cawood, North Yorkshire, UK). For determination of Ca, Mg, P, K, Na, Cu or Zn, diet and faecal samples were ashed at 510°C, and subsequently diets, faecal samples and plasma samples were digested with a mixture of nitric and hydrochloric acid. The diluted and filtered samples were then aspirated into an ICP-OES (Perkin Elmer Optima 5300DV; Perkin Elmer, Llantrisant, UK), and the optical emission was measured at the wavelength selected for that mineral using standards of known concentration. Titanium in the diets and faecal samples was analysed according to methods of Short et al. (1996). CP concentration in the diets
Table 1: Composition and chemical composition of experimental diets (as-fed basis)

<table>
<thead>
<tr>
<th>Item</th>
<th>Basal diet</th>
<th>Basal + P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingredient (g/kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wheat</td>
<td>309.9</td>
<td>306.5</td>
</tr>
<tr>
<td>Soybean meal (48%)</td>
<td>197.5</td>
<td>198.0</td>
</tr>
<tr>
<td>Whey powder</td>
<td>140.0</td>
<td>140.0</td>
</tr>
<tr>
<td>Micronised barley</td>
<td>75.0</td>
<td>75.0</td>
</tr>
<tr>
<td>Fish meal</td>
<td>72.5</td>
<td>72.5</td>
</tr>
<tr>
<td>Soya oil</td>
<td>59.1</td>
<td>60.0</td>
</tr>
<tr>
<td>Micronised wheat</td>
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<td>50.0</td>
</tr>
<tr>
<td>Micronised maize</td>
<td>25.0</td>
<td>25.0</td>
</tr>
<tr>
<td>Cooked de-hulled oats</td>
<td>25.0</td>
<td>25.0</td>
</tr>
<tr>
<td>Sugar/sucrose</td>
<td>12.5</td>
<td>12.5</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>9.3</td>
<td>9.3</td>
</tr>
<tr>
<td>Piglet starter premix†</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Limestone flour</td>
<td>4.7</td>
<td>4.7</td>
</tr>
<tr>
<td>Monosodium phosphate</td>
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<td>4.6</td>
</tr>
<tr>
<td>L-lysine HCl</td>
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</tr>
<tr>
<td>Sodium bicarbonate</td>
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<td>2.7</td>
</tr>
<tr>
<td>L-threonine</td>
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<td>2.2</td>
</tr>
<tr>
<td>α-methionine</td>
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<td>2.1</td>
</tr>
<tr>
<td>L-tryptophan</td>
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<td>0.4</td>
</tr>
<tr>
<td>L-valine</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Salt</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Choline chloride (60%)</td>
<td>0.00</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Calculated content (g/kg)

| Dry matter                  | 906.2      | 902.0     |
| Digestible lysine           | 13.6       | 13.6      |
| CP                          | 222.3      | 222.2     |
| Lactose                     | 107.1      | 107.1     |
| Calcium                     | 8.0        | 8.2       |
| Total phosphorus            | 6.9        | 7.9       |
| Digestible phosphorus       | 4.5        | 5.5       |
| Phytate phosphorus          | 1.6        | 1.6       |
| Zinc (mg/kg)                | 120.0      | 120.0     |
| Sodium                      | 3.3        | 3.3       |
| Digestible energy (MJ/kg)   | 16.0       | 16.0      |

Chemical analysis (g/kg)

| Dry matter                  | 912.8      | 913.7     |
| CP                          | 218.1      | 223.0     |
| Calcium                     | 8.9        | 9.6       |
| Total phosphorus            | 6.8        | 8.0       |
| Phytate phosphorus          | 2.2        | 2.2       |
| Zinc (mg/kg)                | 188.0      | 179.0     |
| Sodium                      | 2.8        | 3.2       |

†Five additional diets, identical to the basal diet were formulated to contain zinc from ZnO (82% Zn; PigZin; DSM Nutritional Products Ltd, Derbyshire, UK) at 1750 or 3500 mg/kg and 0 or 2500 FTU/kg of phytase at the expense of corn.

‡Two additional diets, identical to the basal + P diet were formulated to contain zinc from ZnO (82% Zn; PigZin) at 1750 or 3500 mg/kg at the expense of corn.

§Supplied per kilogram of diet: vitamin A (retinyl acetate), 13 180 IU; vitamin D (cholecalciferol), 3000 IU; vitamin E (α-tocopheryl acetate), 180 IU; vitamin K (menadione dimethylpyrimidinol bisulphate), 3.0 mg; folic acid, 1.0 mg; niacin, 25.0 mg; calcium pantothenate, 12.3 mg; riboflavin, 8.0 mg; vitamin B12, 0.02 mg; thiamine, 3.0 mg; pyridoxine, 4.0 mg; biotin, 0.1 mg; selenium (sodium selenite, 4.4%), 0.2 mg; iodine (calcium iodate anhydrous), 2.0 mg; iron (iron sulphate monohydrate), 150 mg; manganese (manganese oxide), 45 mg; copper (copper sulphate pentahydrate), 160 mg; and zinc (zinc sulphate monohydrate), 110 mg.

was determined using the DUMAS method and a Leco FP-528 N analyser (LECO Corporation, St. Joseph, MI, USA), and the DM content was determined by gravimetry. Plasma alkaline phosphatase activity was determined by Central Analytical Services (Midlothian, Scotland, UK) using a United Kingdom Accreditation Service-approved method and spectrophotometry (RX Imola Analyser; Randox Laboratories; Dungloe, Ireland). Apparent mineral digestibility was calculated using the following equation: apparent digestibility = 100 - [(markerfaeces × (mineralfaeces / mineraldiet))]/100.

Statistical analysis

All data were analysed as a completely random design using the fit model platform (JMP 10.0; SAS Institute Inc., Cary, NC, USA). The statistical model included a 3 × 3 factorial arrangement of treatments to determine the influence of pharmacological Zn, P source and the interaction. When differences were significant, interactive or main effect means were separated using orthogonal contrast statements. Significant interactions were separated using linear and quadratic contrasts between Zn dose (0, 1750 or 3500 mg/kg Zn from ZnO) within each P source (4.5 g/kg digestible P, 4.5 g/kg digestible P plus 2500 FTU/kg of phytase or 5.5 g/kg of digestible P). Significant main effects of Zn from ZnO were separated using equally spaced linear and quadratic contrast statements. Significant main effects of P source were separated using orthogonal contrast statements to determine differences between 4.5 and 5.5 g/kg of digestible P or the effect of phytase supplementation (2500 FTU/kg) compared with diets containing 4.5 and 5.5 g/kg of digestible P. Pen served as the experimental unit and significance was accepted at P<0.05. Initial BW appeared to be different for pigs fed the different pharmacological Zn levels (P=0.04). Therefore, initial BW was used as a covariate when evaluating treatment differences in ADG, ADFI and FCR. Replicate pen was also included in the model to account for differences between replicating the treatments over time.

Results

The analysed chemical composition and phytase recoveries of the diets were similar to formulated values and within acceptable ranges when sampling and assay variations were considered (Tables 1 and 2). Mortality was >1% and was not influenced by pharmacological Zn or P source or the interaction (data not shown). Initial BW appeared to be influenced by pharmacological Zn supplementation. However, this effect was likely the result of replication over time, and therefore initial BW was used as a covariate in the model to determine treatment differences for ADG, ADFI and FCR.

Growth performance

From weaning to 20 days post-weaning, ADFI increased (quadratic P<0.05), irrespective of P source, from 0.395, 0.454 and 0.431 kg/day for pigs fed 0, 1750 and 3500 mg/kg of Zn from ZnO, respectively (Table 3). There was no main
effect of P source or Zn level × P source interaction on ADFI. There was a significant Zn level × P source interaction on ADG and FCR from weaning to 20 days post-weaning (Table 3). Pigs fed 4.5 g/kg digestible P or 4.5 g/kg digestible P plus 2500 FTU/kg phytase gained more per day (quadratic
\[ P < 0.05 \]) and had better FCR (quadratic \[ P < 0.05 \]) when they were fed 1750 mg/kg supplemental Zn. However, pigs fed 5.5 g/kg digestible P gained more per day (linear \[ P < 0.05 \]) and were more efficient (linear \[ P < 0.05 \]) when they were fed 3500 mg/kg supplemental Zn.

Table 2
<table>
<thead>
<tr>
<th>Diet</th>
<th>Formulated phytase (FTU/kg)</th>
<th>Analysed phytase (FTU/kg)</th>
<th>Formulated Zn (mg/kg)</th>
<th>Analysed Zn (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>0</td>
<td>75</td>
<td>120</td>
<td>193</td>
</tr>
<tr>
<td>Basal</td>
<td>0</td>
<td>50</td>
<td>1750</td>
<td>1782</td>
</tr>
<tr>
<td>Basal</td>
<td>0</td>
<td>144</td>
<td>3500</td>
<td>4123</td>
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<tr>
<td>Basal + phytase</td>
<td>2500</td>
<td>2505</td>
<td>120</td>
<td>183</td>
</tr>
<tr>
<td>Basal + phytase</td>
<td>2500</td>
<td>2655</td>
<td>1750</td>
<td>1554</td>
</tr>
<tr>
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<td>2065</td>
<td>3500</td>
<td>4223</td>
</tr>
<tr>
<td>Basal + dP</td>
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<td>71</td>
<td>120</td>
<td>179</td>
</tr>
<tr>
<td>Basal + dP</td>
<td>0</td>
<td>184</td>
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<td>2146</td>
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<tr>
<td>Basal + dP</td>
<td>0</td>
<td>214</td>
<td>3500</td>
<td>4456</td>
</tr>
</tbody>
</table>

1 Diets were analysed in duplicate for Zn using ICP-OES (Perkin Elmer Optima 5300DV; Perkin Elmer, Llantrisant, UK) and phytase recoveries according to methods of Engelen et al. (2001).
2 Digestible phosphorus.

Table 3
<table>
<thead>
<tr>
<th>Pharmacological ZnO (mg/kg)</th>
<th>Supplemental phosphorus</th>
<th>ADFI (kg/day)</th>
<th>ADG (kg/day)</th>
<th>FCR</th>
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<tr>
<td>0</td>
<td>4.5 g/kg dP</td>
<td>0.382</td>
<td>0.290</td>
<td>1.282</td>
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<td>1750</td>
<td>4.5 g/kg dP</td>
<td>0.444</td>
<td>0.408</td>
<td>1.085</td>
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<td>3500</td>
<td>4.5 g/kg dP</td>
<td>0.425</td>
<td>0.368</td>
<td>1.161</td>
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<tr>
<td>0</td>
<td>2500 FTU/kg</td>
<td>0.414</td>
<td>0.363</td>
<td>1.149</td>
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<tr>
<td>1750</td>
<td>2500 FTU/kg</td>
<td>0.482</td>
<td>0.447</td>
<td>1.082</td>
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<tr>
<td>3500</td>
<td>2500 FTU/kg</td>
<td>0.424</td>
<td>0.376</td>
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<tr>
<td>0</td>
<td>5.5 g/kg dP</td>
<td>0.388</td>
<td>0.334</td>
<td>1.210</td>
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<tr>
<td>1750</td>
<td>5.5 g/kg dP</td>
<td>0.436</td>
<td>0.395</td>
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<td>3500</td>
<td>5.5 g/kg dP</td>
<td>0.443</td>
<td>0.414</td>
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<tr>
<td>0</td>
<td>0.395</td>
<td>0.329</td>
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<td>1.214</td>
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<tr>
<td>1750</td>
<td>0.454</td>
<td>0.417</td>
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<td>1.092</td>
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<td>3500</td>
<td>0.431</td>
<td>0.386</td>
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<td>0.010</td>
<td>0.014</td>
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<tr>
<td>4.5 g/kg dP</td>
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<td>0.417</td>
<td>0.355</td>
<td>1.176</td>
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<tr>
<td>2500 FTU/kg</td>
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<td>0.440</td>
<td>0.395</td>
<td>1.121</td>
</tr>
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<td>5.5 g/kg dP</td>
<td></td>
<td>0.423</td>
<td>0.381</td>
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<td>0.010</td>
<td>0.014</td>
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<tr>
<td>Pharmacological ZnO</td>
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<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
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<tr>
<td>Linear ZnO</td>
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<td>0.0002</td>
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<td>&lt;0.0001</td>
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<tr>
<td>Quadratic ZnO</td>
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<td>&lt;0.0001</td>
<td></td>
<td>&lt;0.0001</td>
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<tr>
<td>Phosphorus source</td>
<td>ns²</td>
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<td>0.017</td>
<td></td>
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<tr>
<td>4.5 g/kg vs 5.5 g/kg dP</td>
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<td>ns</td>
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<td>dP vs phytase</td>
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<td>Pharmacological ZnO × P source</td>
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</tbody>
</table>

ADF = average daily feed intake; ADG = average daily gain; FCR = feed conversion ratio; dP = digestible phosphorus.

1 Data are means of eight replicates of 10 pigs per replicate pen.
2 Not significant (P > 0.05).
Zinc oxide, phytase and phosphorus in weaned pigs

Table 4 Influence of pharmacological zinc oxide and phosphorus source (phytase or additional phosphorus from monosodium phosphate) on faecal dry matter and apparent faecal mineral digestibility of 17-kg pigs1

<table>
<thead>
<tr>
<th>Pharmacological ZnO (mg/kg)</th>
<th>Supplemental phosphorus</th>
<th>Faecal DM (%)</th>
<th>Ca (%)</th>
<th>Mg (%)</th>
<th>P (%)</th>
<th>K (%)</th>
<th>Na (%)</th>
<th>Cu (%)</th>
<th>Zn (%)</th>
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DM = dry matter; dP = digestible phosphorus.

1Data are means of eight replicates of 10 pigs per replicate pen.

2Not significant (P > 0.05).

Faecal consistency scores

Faecal consistency scores were <2.0 for all the days measured and not significantly influenced by Zn level, P source or the interaction (data not shown). Faecal DM was influenced (quadratic P < 0.05) by supplemental Zn level, whereas DM was 23.36, 24.98 or 23.95% in pigs fed 0, 1750 or 3500 mg/kg supplemental Zn from ZnO, respectively (Table 4). There was no main effect of P source or Zn level × P source on faecal DM.

Apparent mineral digestibility

Apparent faecal digestibility of Ca, Mg, P, K and Cu were influenced by a significant Zn level × P source interaction (Table 4). Supplemental Zn had no influence on apparent faecal Ca digestibility, except in the presence of 4.5 g/kg digestible P plus 2500 FTU/kg of phytase, which resulted in a linear (P < 0.05) reduction in Ca digestibility as supplemental Zn in the diet increased from 0 to 3500 mg/kg. When pigs were fed diets supplemented with 4.5 g/kg digestible P without phytase, apparent faecal Cu (linear P < 0.05), Mg (quadratic P < 0.05), P (linear P < 0.05) and K (linear P < 0.05) digestibilities were reduced as supplemental Zn from ZnO increased from 0 to 3500 mg/kg. Similarly, supplementation of pig diets with 4.5 g/kg digestible P plus 2500 FTU/kg of phytase reduced (linear P < 0.05) apparent faecal Mg, P, K and Cu digestibilities as supplemental Zn from ZnO increased from 0 to 3500 mg/kg. However, when pigs were fed 5.5 g/kg digestible P, there was no influence of supplemental Zn on apparent faecal digestibility of P or K and a quadratic (P < 0.05) influence of supplemental Zn on the apparent faecal digestibility of Mg and Cu. Apparent faecal digestibility of Na was reduced (linear P < 0.05) as Zn supplementation in the diet increased from 0 to 3500 mg/kg, irrespective of P source. In addition, irrespective of P source, apparent faecal digestibility of Zn increased (linear P < 0.05) from −17.1 to −8.82 or 6.68% as supplemental Zn in the diets increased from 0 to 1750 or 3500 mg/kg, respectively.
Plasma minerals
There was no effect of supplemental Zn or P source on any mineral evaluated in the plasma (Table 5). Supplemental Zn or P source had no effect on the concentrations of Ca, Mg, K or Na in the plasma. However, there was a significant main effect of supplemental Zn on plasma Cu, Zn and alkaline phosphatase concentrations (Table 5). Cu concentrations in the plasma decreased linearly ($P < 0.05$) from 2.13 to 1.92 and 1.86 mg/kg as supplemental Zn increased in the diet from 0 to 1750 and 3500 mg/kg, respectively. Plasma Zn concentrations increased (quadratic $P < 0.05$) from 0.83 to 2.02 or 4.81 mg/kg as dietary Zn supplementation increased from 0 to 1750 or 3500 mg/kg, respectively. Plasma alkaline phosphatase increased (linear $P < 0.05$) from 17.5 to 23.3 or 26.9 U/l as supplemental Zn increased from 0 to 1750 or 3500 mg/kg, respectively. In addition, there was a significant main effect of P source on plasma P and Cu (Table 5). Pigs fed 5.5 g/kg digestible P had a higher ($P < 0.05$) concentration of P in their plasma than pigs fed diets containing 4.5 g/kg digestible P without phytase, but this was not different from the plasma P concentration in pigs fed diets with 4.5 g/kg digestible P plus phytase. Pigs fed 4.5 g/kg digestible P plus phytase had reduced ($P < 0.05$) plasma Cu concentrations compared with pigs fed diets supplemented with 4.5 or 5.5 g/kg digestible P without phytase.

Discussion
Pigs were healthy throughout the trial, with a cull or mortality rate <1% and faecal scores <2. The basal diets were formulated to contain adequate or excess nutrients, including Ca, P and Zn, although some of the diets were supplemented with phytase. Regardless of the nutrient adequacy of the diets, the high health status of the animals, as well as the occurrence of minimal diarrhoea, ADG and FCR were influenced by supplemental Zn and P source. More specifically, ADG and FCR linearly improved in pigs fed 5.5 g/kg digestible P as supplemental Zn increased from 0 to 3500 mg/kg. However, when pigs were fed 4.5 g/kg digestible P without or with phytase, there was a quadratic effect of supplemental Zn on ADG and FCR, with pigs fed 1750 mg/kg supplemental Zn having the highest ADG and the best FCR. Improvements in growth performance associated with pharmacological Zn from ZnO have been previously reported (Carlson et al., 1999; Hill et al., 2001). Similar to the results of the present study, Hill et al. (2001) reported a quadratic effect of pharmacological Zn supplementation on ADG and feed efficiency; and nursery pigs fed 3000 mg/kg supplemental Zn from ZnO were 8% lighter and 5% less efficient compared with nursery pigs fed 2000 mg/kg supplemental Zn from ZnO. Walk et al.
(2013) fed 1750 or 3500 mg/kg of supplemental Zn from ZnO and reported no effect on ADG of 7- to 11-kg pigs and a linear reduction in ADG of 11- to 21-kg pigs. Martinez et al. (2005) fed 1000 or 4000 mg/kg Zn from ZnO to 7-kg pigs for 21 days and reported no effect of dietary Zn on growth performance. The inconsistencies in the performance benefits of pharmacological Zn from ZnO may be associated with numerous factors such as the age and health status of the pigs, the level of Zn from ZnO supplemented in the diet, the length of time the pigs are fed pharmacological Zn, the environment in which the pigs are housed, the presence of dietary phytase and the level of dietary P.

In the present trial, phytase supplementation in diets containing 4.5 g/kg digestible P improved ADG and FCR, but the magnitude of the performance response was associated with the level of supplemental Zn in the diet. Previous authors have also reported improvements in ADG or ADFI of pigs fed Ca- and P-adequate diets supplemented with phytase (Adeola et al., 1995) or phytase and pharmacological Zn from ZnO (Walk et al., 2013). However, Martinez et al. (2005) reported no effect of 500 FTU/kg of phytase on growth performance of 7-kg pigs when supplemented in diets containing various levels of pharmacological Zn from ZnO. Augspurger et al. (2004) reported a 30% reduction in microbial phytase efficacy when supplemental ZnO or ZnCl was included at 1500 mg/kg in diets fed to 7-kg pigs. Zn has an affinity to phytate; however, the binding of Zn to phytate appears to depend on the molar concentrations of Zn and Ca relative to the molar concentration of phytate and the pH of the solution (Champagne and Phillippy, 1989; Champagne et al., 1990; Gifford and Clydesdale, 1990; Simpson and Wise, 1990). In the present trial, the Zn-to-phytate molar ratio was calculated to be 2 : 1 or 4 : 1 in the diets of pigs fed 1750 or 3500 mg/kg supplemental Zn, respectively. Therefore, similar to previously published in vitro trials (Champagne et al., 1990; Simpson and Wise, 1990), Zn and phytate precipitation may have occurred more readily in pigs fed 3500 mg/kg supplemental Zn than those fed 1750 mg/kg supplemental Zn. This precipitation would result in reduced phytate hydrolysis and decreased mineral digestibility, specifically of Ca and P.

Apparent faecal Ca digestibility was not influenced by dietary Zn or by 4.5 or 5.5 g/kg digestible P in the absence of phytase. However, supplementing phytase resulted in a linear decrease in apparent faecal Ca digestibility, as dietary Zn increased from 0 to 3500 mg/kg. Similarly, the dietary inclusion of supplemental Zn from 0 to 3500 mg/kg resulted in a linear reduction in apparent faecal P digestibility, especially in pigs fed 4.5 g/kg digestible P without phytase, whereas there was no effect of supplemental Zn on apparent faecal P digestibility in pigs fed 5.5 g/kg digestible P. Meyer et al. (2002) reported pharmacological Zn from ZnO reduced Ca and P absorption in piglets fed diets containing 0.78% total P and 0.95% Ca. The authors concluded that, when considering pharmacological Zn supplementation in pig diets, the lowest possible level should be evaluated from an environmental and nutritional perspective.

These previous authors reported a 20-fold increase in Zn excretion and a reduction in Zn absorption as ZnO increased from 0 to 3000 mg/kg Zn (Meyer et al., 2002). This is in contrast with the apparent faecal Zn digestibility of the present trial, which was negative and increased as ZnO supplementation increased to 3500 mg/kg. However, Buff et al. (2005) reported a 53% increase in Zn absorption and a 60% increase in Zn retention when pigs were fed 2000 mg/kg Zn from ZnO compared with a basal diet containing 162 mg/kg Zn. The discrepancies in the data may be due to collection methods, the time and age of the pigs at sampling, ADFI – and thus average daily Zn intake – and the bioavailability of the source of Zn used.

Although increasing supplemental Zn from ZnO increased Zn digestibility, it actually reduced Cu, Mg, K and Na digestibility, and this is in agreement with Meyers et al. (2002). The extent of the effect of pharmacological ZnO on Cu, Mg, K and Na digestibility varied with dietary ZnO concentration or P source, and in some cases (Mg and Cu) no real trends or explanations can be determined. However, in the case of Na and K, it is interesting to note that pharmacological ZnO may have an impact on the osmotic gradient and the flux of water through the intestinal tract. For example, Zn from ZnCl has been reported to block the K-channels that regulate enterocyte Na and Cl flux (Hoque et al., 2005), and Cl secretion is the principle determinant of luminal hydration (Berkes et al., 2003). Under normal conditions of Cl secretion, Na follows, resulting in an accumulation of NaCl and an osmotic gradient for water diffusion into the intestinal lumen (Berkes et al., 2003). Therefore, in the present trial, Zn from ZnO may have blocked both Na and K absorption from the intestinal lumen, which resulted in a linear reduction in Na and K digestibility. This decrease in luminal uptake of Na and K may have reduced water secretion into the intestinal lumen, which resulted in an increase in faecal DM with pharmacological ZnO supplementation.

The concentration of Zn or alkaline phosphatase activity in the plasma increased as supplemental Zn from ZnO increased in the diet, and this is in agreement with previous authors (Hill et al., 2001; Martinez et al., 2005; Shelton et al., 2011). Hahn and Baker (1993) plotted a quadratic effect of plasma Zn on ADG, whereas ADG increased by 18% as plasma Zn concentration increased from 0.85 to 1.99 mg/l and then decreased by 10% as plasma Zn concentration increased to 3.27 mg/l. This is in agreement with the plasma Zn concentrations in the present trial, except when supplemental P was included in the diets. For example, pigs fed 3500 mg/kg Zn from ZnO were 7% lighter and 3% less efficient than pigs fed 1750 mg/kg supplemental Zn, and this corresponds to an increase in plasma Zn from 2.02 to 4.81 mg/kg.

There was no effect of supplemental Zn on plasma P and this is in agreement with Martinez et al. (2005) but contradictory to Walk et al. (2013) who reported pharmacological ZnO reduced serum P concentration. Shelton et al. (2011) reported pharmacological ZnO reduced plasma P, but only in the presence of 150 mg/kg Cu from tri-basic CuCl. In the present trial, Cu concentration in the plasma was reduced as
supplemental Zn increased, and this has been previously reported by Hill et al. (2001). However, in other studies, pharmacological Zn from ZnO had no effect on plasma Cu concentration (Buff et al., 2005; Shelton et al., 2011) or actually increased plasma Cu concentration, except when both Zn and Cu were combined in the diet (Shelton et al., 2011). Differences in the results between the present trial and the previously reported trials may be associated with the level of Cu or Zn fed or the source of Cu supplied in the diets.

In conclusion, the performance, plasma and digestibility data from the present trial indicate a complex interaction between supplemental Zn and minerals, particularly Ca, P, Cu, Na and K. Supplemental Zn from ZnO improved growth performance, especially at 1750 mg/kg Zn with phytase. Increasing the concentration of supplemental Zn in the diet from 0, 1750 to 3500 mg/kg reduced Ca, P, Na, K, Mg and Cu digestibility, which in some cases was mitigated or exacerbated by additional P from monosodium phosphate or phytase. Understanding the factors that influence the binding of these minerals to phytate or precipitation with each other will facilitate more accurate use of these minerals in feed formulations and an improvement in animal performance.

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References

Walk, Wilcock and Magowan