

## Effects of zinc oxide and microbial phytase on digestibility of calcium and phosphorus in maize-based diets fed to growing pigs

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**ABSTRACT:** An experiment was conducted to test the hypothesis that inclusion of Zn at a pharmacological level in diets fed to pigs affects apparent total tract digestibility (ATTD) of Ca and P and standardized total tract digestibility (STTD) of Ca. The second hypothesis was that inclusion of microbial phytase increases the ATTD of Ca and P and the STTD of Ca regardless of the concentration of Zn in the diet. Fifty-six growing barrows ( $15.4 \pm 1.9$  kg average BW) were allotted to a randomized complete block design with 7 dietary treatments and 8 pigs per treatment. A maize-based basal diet was formulated with either 0 or 2,400 mg/kg Zn from ZnO and 0, 1,000, or 3,000 units of phytase (FTU) per kilogram. A Ca-free diet was used to determine basal endogenous losses of Ca. Experimental diets were fed for 13 d, and feces

were collected from the feed provided from d 6 to 11 using the marker-to-marker approach; urine was also collected from d 6 to 11. Retention of Ca, ATTD of Ca, and STTD of Ca increased ( $P < 0.01$ ) as the concentration of phytase in the diet increased and were less ( $P < 0.01$ ) if ZnO was used than if no ZnO was added to the diet. Retention of P and the ATTD of P increased ( $P < 0.0001$ ) as the concentration of phytase increased in the diet, but the increase was greater if ZnO was not added than if ZnO was added to the diet (interaction,  $P < 0.05$ ). In conclusion, pharmacological levels of Zn reduced Ca and P digestibility and retention, but this effect was partly ameliorated by the inclusion of phytase in the diets. Inclusion of microbial phytase increased the ATTD and STTD of Ca in diets and also the ATTD of P.

**Key words:** apparent digestibility, calcium, phytase, pigs, standardized digestibility, zinc

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### INTRODUCTION

Use of values for standardized total tract digestibility (STTD) of Ca may result in improved diet formulations for pigs compared with use of values for total Ca, because STTD of Ca takes the basal endogenous loss of Ca into account (González-Vega et al., 2015a,b; Merriman and Stein, 2016). Values for STTD of Ca are believed to be additive in mixed diets, which is not always the case for values for apparent total tract digestibility (ATTD). Values for ATTD or STTD of Ca may be increased by microbial phytase (Selle et al., 2009; Almeida et al., 2013; González-Vega et al., 2013), which is likely a result

of hydrolysis of phytate esters and a subsequent reduction of the ability of phytate to chelate Ca (Selle et al., 2009; González-Vega et al., 2013).

It is common industry practice to use diets with pharmacological concentrations of Zn (i.e., up to approximately 3,000 mg/kg) during the postweaning period to prevent postweaning diarrhea in pigs (Poulsen, 1995; Hill et al., 2000). However, Zn competes with Ca for absorption through channel proteins on the brush border membrane in the pig small intestine (Bertolo et al., 2001a), and it is therefore possible that elevated levels of dietary Zn interferes with absorption of Ca. In addition, Ca and Zn may bind to phytate, which may also affect absorption of Ca. However, possible interactions between Zn and phytase on the STTD of Ca have not been reported. Therefore, the objectives of this experiment were to test the hypothesis that 1) pharmacological levels of Zn affects ATTD of Ca and

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**Table 1.** Ingredient composition of experimental diets, as-fed basis

Ingredient, %	Ca free	No added ZnO			3,000 mg/kg added ZnO		
		0 FTU <sup>1</sup>	1,000 FTU	3,000 FTU	0 FTU	1,000 FTU	3,000 FTU
Corn	75.80	70.00	69.00	69.00	70.00	69.00	69.00
Cornstarch	2.22	2.08	2.27	2.27	1.78	1.97	1.97
Potato protein isolate	12.00	12.00	12.00	12.00	12.00	12.00	12.00
Soybean oil	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Calcium carbonate	–	1.75	1.75	1.75	1.75	1.75	1.75
Monosodium phosphate	0.95	0.95	0.76	0.76	0.95	0.76	0.76
L-Lys HCL	0.13	0.61	0.61	0.61	0.61	0.61	0.61
DL-Met	0.08	0.11	0.11	0.11	0.11	0.11	0.11
L-Thr	0.08	0.09	0.09	0.09	0.09	0.09	0.09
L-Trp	0.05	0.05	0.05	0.05	0.05	0.05	0.05
L-His	0.09	0.10	0.10	0.10	0.10	0.10	0.10
Lactose	5.00	8.66	8.66	8.66	8.66	8.66	8.66
Phytase premix <sup>2</sup>	–	–	1.00	1.00	–	1.00	1.00
Sodium chloride	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin–mineral premix <sup>3</sup>	0.20	0.20	0.20	0.20	0.20	0.20	0.20
ZnO	–	–	–	–	0.30	0.30	0.30
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00

<sup>1</sup>FTU = units of phytase.

<sup>2</sup>The phytase premix was prepared by mixing 980 g ground corn and 20 g Quantum Blue 5000 G (AB Vista Feed Ingredients, Marlborough, UK) or 940 g ground corn and 60 g Quantum Blue 5G (AB Vista Feed Ingredients) to provide 1,000 or 3,000 FTU per kilogram complete diet.

<sup>3</sup>The vitamin–micromineral premix provided the following quantities of vitamins and microminerals per kilogram of complete diet: 11,136 IU vitamin A as retinyl acetate, 2,208 IU vitamin D<sub>3</sub> as cholecalciferol, 66 IU vitamin E as DL-alpha tocopheryl acetate, 1.42 mg vitamin K as menadione dimethylprimidinol bisulfite, 0.24 mg thiamin as thiamine mononitrate, 6.59 mg riboflavin, 0.24 mg pyridoxine as pyridoxine hydrochloride, 0.03 mg vitamin B<sub>12</sub>, 23.5 mg d-pantothenic acid as d-calcium pantothenate, 44.1 mg niacin, 1.59 mg folic acid, 0.44 mg biotin, 20 mg Cu as copper sulfate, 126 mg Fe as iron sulfate, 1.26 mg I as ethylenediamine dihydriodide, 60.2 mg Mn as manganous sulfate, 0.125 mg Se as sodium selenite and 0.125 mg Se as selenium yeast, and 124.9 mg Zn as zinc sulfate.

P and STTD of Ca and 2) microbial phytase increases the ATTD and STTD of Ca and the ATTD of P regardless of the concentration of Zn in the diet.

## MATERIALS AND METHODS

The Institutional Animal Care and Use Committee at the University of Illinois, Urbana, IL, reviewed and approved the protocol for the experiment. Pigs used in the experiment were the offspring of L 359 boars and C-46 females (PIC, Hendersonville, TN).

### *Animals, Experimental Design, and Diets*

Fifty-six growing barrows with an average initial BW of 15.4 ± 1.9 kg were randomly allotted to a randomized complete block design with 2 blocks of 28 pigs. Within each block, pigs were randomly allotted to 7 diets with 4 replicate pigs per diet, resulting in a total of 8 replicate pigs per diet for the 2 blocks. A diet based on maize, potato protein isolate, cornstarch, and soybean oil was formulated with either 0 or 2,400 mg/kg of added Zn and 0, 1,000, or 3,000 units of phytase (FTU) per kilogram (Quantum Blue; AB Vista Feed Ingredients, Marlborough, UK; Table 1). The 2,400 mg/kg of Zn was provided by addition of 3,000 mg/kg of ZnO (Productos Maximo, Monterrey, Nuevo Leon,

Mexico; 72% Zn) to the diets. Each diet was mixed in one 125-kg batch, and all diets were fed in a mash form. A 500-g sample of each diet was collected at the time of mixing and used for diet analysis.

All diets were formulated to contain 0.70% total Ca and 0.33% standardized total tract digestible P, and the Ca-to-standardized total tract digestible P ratio was 2.10:1. A Ca-free diet that was used to measure basal endogenous losses of Ca was also included in the experiment. Vitamins and all minerals were included in all diets except the Ca-free diet to meet or exceed the requirements for 11- to 25-kg pigs (NRC, 2012). Microminerals were included in all diets from the vitamin–mineral premix, which supplied 125 mg/kg of Zn from zinc sulfate. Therefore, the 3 diets without ZnO were calculated to contain 125 mg/kg of added Zn and the 3 diets containing 3,000 mg of ZnO were calculated to contain a total of 2,525 mg/kg of added Zn. The Zn provided by maize and potato protein isolate was not included in the calculated concentrations of Zn in the diets.

The Experimental Animal Allotment Program (Kim and Lindemann, 2007) was used to randomly allot the 28 pigs in each block to the 7 diets. Pigs were individually housed in stainless steel metabolism crates that were equipped with a slatted metal floor, a stainless steel feeder, a nipple drinker, and a screen floor that allowed for total fecal collection. A urine

tray was installed below the screen floor, which allowed for total collection of urine.

### Feeding and Sample Collection

Pigs were fed experimental diets for 13 d, and the quantity of feed provided per pig daily was calculated as 3 times the daily maintenance energy requirement (i.e., 197 kcal ME/kg BW<sup>0.60</sup>; NRC, 2012) and divided into 2 equal meals that were provided at 0800 and 1700 h. Pigs had free access to water throughout the experiment. The initial 5 d were considered a period of adaptation to the diets. A color marker (indigo carmine) was added to the morning meal on d 6 and a second marker (ferric oxide) was added to the morning meal on d 11 according to the marker-to-marker approach (Adeola, 2001). Fecal collections were initiated when the first marker appeared in the feces and ceased when the second marker appeared. Fecal samples were stored at -20°C immediately after collection. Urine collection was initiated on d 6 in the morning and ceased on d 11 in the morning, and 20% of the collected urine was stored at -20°C. Orts that were collected during the collection period were dried in a forced-air oven at 65°C, and the weight was subtracted from feed allotments to calculate feed consumption.

### Sample Analysis

Fecal samples were dried in a forced-air oven at 65°C and ground in a Wiley mill (model 4; Thomas Scientific, Swedesboro, NJ) using a 1-mm screen, and subsamples were collected for analysis after all the ground materials had been mixed. Diets and fecal samples were analyzed for DM by oven drying at 135°C for 2 h (method 930.15; AOAC, 2007). Diet, fecal, and urine samples were analyzed for Ca and P by inductively coupled plasma-optical emission spectroscopy (method 985.01A, B, and D; AOAC, 2007) after wet ash sample preparation (method 975.03 B[b]; AOAC, 2007). Diets were analyzed for GE using an isoperibol bomb calorimeter (model 6300; Parr Instruments, Moline, IL), and benzoic acid was the internal standard. Diets were also analyzed for N using the combustion procedure (method 990.03; AOAC, 2007) on an Elementar Rapid N-cube protein/nitrogen apparatus (Elementar Americas, Inc., Mt. Laurel, NJ), with aspartic acid used as the internal standard, and CP was subsequently calculated as N × 6.25. Diets were also analyzed for ash (method 942.05; AOAC, 2007) and for ADF and NDF using Ankom Technology methods 12 and 13, respectively (Ankom<sup>2000</sup> Fiber Analyzer, Ankom Technology, Macedon, NY). Alfalfa meal was used as the internal standard for the ADF and NDF analyses. Diets were also analyzed for phytase activity (method 2000.12;

AOAC, 2007) and for Zn by inductively coupled plasma-optical emission spectroscopy (method 985.01A, B, and D; AOAC, 2007) after wet ash sample preparation (method 975.03 B[b]; AOAC, 2007).

### Calculations and Statistical Analysis

Values for ATTD of Ca and P were calculated for the 6 Ca-containing diets (NRC, 2012). The basal endogenous losses of Ca were determined from pigs fed the Ca-free diet according to González-Vega et al. (2015a). To obtain the STTD of Ca, ATTD values were corrected for basal endogenous losses according to González-Vega et al. (2015a). Retention of Ca was calculated as previously outlined (Almeida and Stein, 2010) using the following equation:

$$\text{Car} = \{[\text{Cai} - (\text{Caf} + \text{Cau})]/\text{Cai}\} \times 100,$$

in which Car is Ca retention (%), Cai is the intake of Ca (g), Caf is the fecal output of Ca, and Cau is the urinary output of Ca (g) over the collection period. Retention of P was also calculated using this equation.

Data were analyzed as a 2 × 3 factorial using PROC MIXED of SAS (SAS Inst. Inc., Cary, NC). The model included the fixed effects of Zn, phytase, and the interaction between Zn and phytase and the random effects of block and replicate within block. The LSMEANS procedure was used to calculate mean values, and means were separated using the PDIF option if significant differences were observed. The pig was the experimental unit and an  $\alpha$  level of 0.05 was used to assess significance among means, whereas differences were considered tendencies if the *P*-value was between 0.05 and 0.10.

## RESULTS

Pigs readily consumed their assigned diets and remained healthy throughout the experiment. Values for analyzed concentrations of Zn in diets without ZnO were between 57.8 and 138.0 mg/kg, and diets with added ZnO all were analyzed to contain between 2,525 and 2,670 mg/kg of Zn (Table 2).

There were no differences in feed intake among dietary treatments, but daily Ca intake increased ( $P < 0.05$ ) as the concentration of phytase increased (Table 3). Fecal Ca output increased ( $P < 0.05$ ) if ZnO was added to the diets, and the output of Ca in feces tended to decrease ( $P = 0.058$ ) as the concentration of phytase increased, regardless of concentration of ZnO in the diets. Urine Ca output was not affected by addition of ZnO to the diet. However, the output of Ca in urine was reduced if phytase was added to diets

**Table 2.** Analyzed composition of experimental diets, as-fed basis

Item	Ca free	No added ZnO			3,000 mg/kg added ZnO		
		0 FTU <sup>1</sup>	1,000 FTU	3,000 FTU	0 FTU	1,000 FTU	3,000 FTU
DM, %	87.20	87.69	87.89	88.07	87.97	88.11	88.07
Ash, %	2.18	3.86	3.84	3.57	3.67	4.12	4.23
GE, kcal/g	4,147	4,059	4,005	4,038	3,981	4,020	4,037
CP, %	14.39	16.30	16.20	16.40	15.66	15.44	16.53
ADF, %	2.59	2.33	2.51	2.29	2.54	2.27	2.32
NDF, %	8.66	8.09	8.21	7.96	8.45	7.83	7.84
Ca, %	0.03	0.63	0.77	0.69	0.65	0.70	0.66
P, %	0.39	0.43	0.40	0.40	0.39	0.41	0.36
Zn, mg/kg	110	58	93	138	2,640	2,520	2,670
Phytase, FTU/kg	<70	<70	1,100	3,700	<70	1,300	3,200

<sup>1</sup>FTU = units of phytase.

without ZnO, but that was not the case if phytase was added to the diets with ZnO (interaction,  $P < 0.05$ ).

Total Ca excretion was reduced ( $P < 0.0001$ ) as the concentration of phytase increased and was greater if ZnO was used ( $P = 0.001$ ) than if no ZnO was added to the diet. An interaction between phytase and ZnO was observed for Ca excretion because pigs fed diets without ZnO and 3,000 FTU had the least excretion of Ca, but that was not the case if ZnO was added to the diet ( $P < 0.05$ ). The ATTD and STTD of Ca increased ( $P = 0.001$ ) as the concentration of phytase increased and were less ( $P < 0.01$ ) if ZnO was used than if no ZnO was added to the diet. However, for Ca retention, an interaction ( $P < 0.05$ ) between phytase and ZnO was observed because pigs fed the diet without ZnO and 3,000 FTU had greater retention of Ca than pigs fed the diet without ZnO and 1,000 FTU, but if ZnO was added to the diet, no difference between the diets with 1,000 and 3,000 FTU was observed for Ca retention.

Intake of P was not affected by inclusion of phytase or ZnO in the diets (Table 4). Excretion of P was reduced as diet phytase concentration increased, but the reduction was less in diets that contained ZnO than in diets that contained no ZnO (interaction,  $P < 0.01$ ). Urine P output was not affected by addition of phytase or ZnO to the diets. Total P excretion decreased ( $P < 0.0001$ ) as the concentration of phytase increased in the diet, but the excretion was greater if ZnO was added than if ZnO was not added to the diet (interaction,  $P < 0.05$ ). Likewise, the percentage of P retention and ATTD of P increased ( $P < 0.0001$ ) as the concentration of phytase increased in the diet, but the increase was greater if ZnO was not added than if ZnO was added to the diet (interaction,  $P < 0.05$ ).

## DISCUSSION

Zinc is an essential micronutrient for all living organisms, with different roles as a structural com-

ponent of proteins, an enzymatic cofactor, and transcriptional regulator in cellular and biochemical processes (Solomons, 2013). Requirements in pigs linearly increase by age in parallel with increased BW. Requirements in pigs expressed as milligrams per kilogram diet are reduced as pig BW increases (NRC, 2012). For pigs between 11 and 25 kg, the dietary requirement is 80 mg/kg (NRC, 2012), but pharmacological concentrations of Zn (2,000 to 3,000 mg/kg) may enhance growth performance and reduce the prevalence of diarrhea (Hahn and Baker, 1993; Case and Carlson, 2002; Hill et al., 2000; Carlson et al., 2006; Hu et al., 2012).

The bioavailability of several minerals including Zn is affected by phytate (*myo*-inositol hexaphosphate; Lopez et al., 2002). Phytate is the main storage form for P in plants (Selle et al., 2009) but is also considered an antinutritional factor for humans and animals, as it has the capacity to chelate nutritionally important cations such as  $\text{Cu}^{+2}$ ,  $\text{Zn}^{+2}$ ,  $\text{Mg}^{+2}$ ,  $\text{Mn}^{+2}$ ,  $\text{Fe}^{+2}$ , and  $\text{Ca}^{+2}$  (Persson et al., 1998; Maenz et al., 1999; Selle et al., 2009). Phytase (*myo*-inositol hexaphosphate phosphohydrolase) hydrolyzes the phospho-ester bond between phytate and P, which will release phytate P, and phytase may also enhance macro- and micromineral availabilities by releasing the cations that are bound to phytate and possibly also increase the utilization of energy and AA (Selle and Ravindran, 2008).

The observation that microbial phytase supplementation increased the ATTD of Ca and P is in agreement with results from previous experiments (Igbasan et al., 2001; Guggenbuhl et al., 2007; Almeida and Stein, 2010; Poulsen et al., 2010; González-Vega et al., 2013, 2015a,b). Correction of ATTD values of a nutrient for basal endogenous losses results in calculation of values for STTD (NRC, 2012). The basal endogenous loss of Ca obtained from pigs fed the Ca-free diet was 0.43 g/kg DMI, which is in agreement with the value (0.40 g/kg DMI) reported by González-Vega et al. (2015b), who also used a diet based on maize and potato protein iso-

**Table 3.** Calcium balance and apparent total tract digestibility (ATTD) and standardized total tract digestibility (STTD) of Ca for pigs fed diets containing different levels of microbial phytase (0, 1,000, or 3,000 units of phytase [FTU]) without or with ZnO addition<sup>1</sup>

Item	No added ZnO			3,000 mg/kg added ZnO			SEM	P-value		
	0 FTU	1,000 FTU	3,000 FTU	0 FTU	1,000 FTU	3,000 FTU		Phytase	Zn	Phytase × Zn
Feed intake, g/d	724	725	806	750	781	774	140	0.723	0.914	0.966
Ca intake, g/d	4.65	5.34	5.52	4.85	5.45	5.14	0.25	0.023	0.907	0.426
Fecal Ca output, g/d	1.51	1.40	1.12	1.69	1.62	1.47	0.13	0.058	0.021	0.804
Urine Ca output, mg/d	618 <sup>ab</sup>	887 <sup>a</sup>	463 <sup>b</sup>	856 <sup>a</sup>	613 <sup>ab</sup>	517 <sup>ab</sup>	90	0.037	0.552	0.010
Ca excretion, % of intake	44 <sup>ab</sup>	41 <sup>b</sup>	28 <sup>c</sup>	52 <sup>a</sup>	41 <sup>b</sup>	41 <sup>b</sup>	2.4	<0.0001	0.001	0.024
ATTD of Ca, %	68.1	74.5	79.7	65.4	69.7	72.0	2.3	0.001	0.008	0.555
STTD of Ca, <sup>2</sup> %	70.0	76.1	81.3	67.2	71.3	73.7	2.3	0.001	0.008	0.578
Ca retention, % of intake	55 <sup>bc</sup>	58 <sup>b</sup>	71 <sup>a</sup>	47 <sup>c</sup>	58 <sup>b</sup>	58 <sup>b</sup>	2.4	<0.0001	0.001	0.024

<sup>a-d</sup>Values within a column without a common superscript are different ( $P < 0.05$ ).

<sup>1</sup>Data are means of 8 observations per treatment, except for the 3,000 ZnO and 0 phytase diet, which had only 7 observations.

<sup>2</sup>Values for STTD were calculated by correcting ATTD values for basal endogenous losses. Basal endogenous losses were determined from pigs fed the Ca-free diet as  $0.430 \pm 0.18$  g/kg of DMI.

late. The increase in STTD of Ca that was observed as phytase was added to the diets also is in agreement with previous data (González-Vega et al., 2015a,b). These results confirm that phytate may chelate dietary Ca, but if phytate is hydrolyzed by phytase, the chelated Ca will be released and absorbed, which will increase retention of both Ca and P (Sauer et al., 2003; Poulsen et al., 2010). However, González-Vega et al. (2013) did not observe an increase in P retention as phytase was added to the diet, as was the case in this experiment, but the diets used by González-Vega et al. (2013) were very low in Ca, which may have prevented P from being retained because for P to be retained in bone tissue, both Ca and P need to be available (Crenshaw, 2001; Stein et al., 2006).

The reduction in ATTD and STTD of Ca that was observed as ZnO was added to the diet is in agreement with data indicating that pharmacological levels of ZnO reduced Ca and P absorption in 7.3-kg pigs fed diets containing 0.78% total P and 0.95% Ca (Meyer et al., 2002). However, Walk et al. (2015) reported that ATTD of Ca was not influenced by dietary Zn in the absence of phytase, but if phytase was added to the diet, a linear reduction in Ca digestibility was observed as dietary Zn increased from 0 to 3,500 mg/kg. In the present experiment, a reduction in retention of Ca was also observed in pigs fed diets containing ZnO compared with pigs fed diets without ZnO, confirming that pharmacological levels of Zn has a negative effect on absorption and retention of Ca and that the positive effect of phytase on digestibility of Ca is reduced if ZnO is added to the diets. Recently, it was reported that addition of pharmacological concentrations of ZnO and 1,000 FTU to diets fed to weanling pigs reduced growth performance during the nursery phase compared with pigs fed diets containing phytase but no added ZnO (Blavi et al., 2016). A lack of response to phytase on growth performance of 7.2-kg pigs was also

reported if pharmacological concentrations of ZnO were included in diets (Martínez et al., 2005).

High dietary Ca accentuates the negative effect of phytate on Zn bioavailability in broiler chickens (Bafundo et al., 1984), rats (Forbes et al., 1984), and fish (Gatlin and Phillips, 1989), although the mechanism by which Ca reduces Zn availability is different from the effects of phytate. High dietary Ca also reduces blood and bone concentrations of Zn in post-weaning pigs (Hsu et al., 1975) but does not affect the apparent absorption of Zn (Whiting and Bezeau, 1958). It is therefore possible that if addition of microbial phytase to diets increases the digestibility of Ca, as was demonstrated in this experiment and in previous experiments (González-Vega et al., 2015a,b), the additional absorbed Ca may have a negative effect on the Zn status of pigs. However, Ca may improve Zn absorption from phytate-containing foods in humans (Lönnerdal et al., 1984; Petterson et al., 1994).

The approximate pH of the intestine where absorption of metal ions takes place coincides with the pH at which these complexes precipitate (Champagne, 1988). The order of mineral potency as inhibitors of phytate hydrolysis at a neutral pH is  $Zn^{+2} \gg Fe^{+2} > Mn^{+2} > Fe^{+3} > Ca^{+2} > Mg^{+2}$  (Maenz et al., 1999). Multiple mineral-phytate complexes such as Ca-Zn-phytate are more stable than single mineral complexes such as Ca-phytate or Zn-phytate (Maenz et al., 1999) and if 2 cations, such as  $Ca^{+2}$  and  $Zn^{+2}$ , are simultaneously presented, they act together to increase phytate precipitation (Simpson and Wise, 1990). The formation of Zn-Ca-phytate complexes in the small intestine may be a major mechanism by which phytate reduces dietary Zn availability (Fordyce et al., 1987). Therefore, it may be hypothesized that high dietary Zn increases the negative effect of phytate on Ca digestibility, which may be

**Table 4.** Phosphorus balance and apparent total tract digestibility (ATTD) of P for pigs fed diets containing different levels of microbial phytase (0, 1,000, or 3,000 units of phytase [FTU]) without or with ZnO addition<sup>1</sup>

Item	No added ZnO			3,000 mg/kg added ZnO			SEM	P-value		
	0 FTU	1,000 FTU	3,000 FTU	0 FTU	1,000 FTU	3,000 FTU		Phytase	Zn	Phytase × Zn
P intake, g/d	3.15	2.88	3.23	2.89	3.18	2.80	0.16	0.996	0.286	0.042
Fecal P output, g/d	1.17 <sup>a</sup>	0.85 <sup>b</sup>	0.62 <sup>c</sup>	1.23 <sup>a</sup>	1.21 <sup>a</sup>	0.96 <sup>b</sup>	0.05	<0.0001	<0.0001	0.008
Urine P output, mg/d	62	56	57	49	58	49	9	0.962	0.497	0.619
P excretion, % of intake	40.5 <sup>ab</sup>	31.9 <sup>c</sup>	21.1 <sup>d</sup>	46.1 <sup>a</sup>	35.1 <sup>bc</sup>	34.3 <sup>bc</sup>	1.8	<0.0001	<0.0001	0.015
ATTD of P, %	61.5 <sup>cd</sup>	70.0 <sup>b</sup>	80.6 <sup>a</sup>	55.6 <sup>d</sup>	63.2 <sup>c</sup>	67.5 <sup>bc</sup>	1.5	<0.0001	<0.0001	0.040
P retention, % of intake	59.5 <sup>cd</sup>	68.2 <sup>b</sup>	78.9 <sup>a</sup>	53.9 <sup>d</sup>	64.9 <sup>bc</sup>	65.7 <sup>bc</sup>	1.8	<0.0001	<0.0001	0.015

<sup>a-d</sup>Values within a column without a common superscript are different ( $P < 0.05$ ).

<sup>1</sup>Data are means of 8 observations per treatment, except for the 3,000 ZnO and 0 phytase diet, which had only 7 observations.

the reason for the reduction in STTD of Ca that was observed as ZnO was added to the diets.

Another possible explanation for the negative effects of pharmacological levels of Zn on STTD of Ca is that  $\text{Ca}^{+2}$  and  $\text{Zn}^{+2}$  compete for a common transport pathway on the brush border membrane, and this transporter has greater affinity for  $\text{Zn}^{+2}$  than for  $\text{Ca}^{+2}$  (Bertolo et al., 2001b). High dietary ZnO may, therefore, produce more ionic  $\text{Zn}^{+2}$  ready for absorption in the stomach and proximal parts of the small intestine, and as a consequence, transport capacity for  $\text{Ca}^{+2}$  is reduced, which results in a reduced absorption and digestibility of Ca. However, additional research is needed to confirm this hypothesis. The observation that there was no interaction between addition of ZnO and phytase on ATTD and STTD of Ca indicates that the increased digestibility of Ca that is caused by phytase is independent of the concentration of Zn in the diet.

The reduction in P retention that was observed as ZnO was added to the diets is in agreement with data indicating that pharmacological concentrations of ZnO decreases plasma P, regardless of phytase supplementation (Walk et al., 2013). Digestibility of P in pigs fed 4.5 g/kg digestible P was also reduced as supplemental Zn was added at pharmacological levels to the diet, and this effect was greater in pigs fed diets without phytase than if phytase was included in the diet (Walk et al., 2015). However, there were no effects of ZnO addition on P digestibility by pigs fed 5.5 g/kg digestible P (Walk et al., 2015), indicating that the negative effects of ZnO on ATTD of P may be overcome by addition of excess P in the diet. The antagonistic relationship between Zn and P has also been demonstrated in rats, and rats fed low Zn (18 mg/kg) with 1.20% Ca and 1.20% P had reduced weight gain compared with rats fed diets containing 42 mg/kg of Zn. However, if both Ca and P in the diet were reduced to 0.30%, no benefit of increasing dietary Zn from 18 to 42 mg/kg was observed (Cabell and Earle, 1965). Therefore, several mechanisms may be involved in reducing Zn availability, and effects of phytase are likely influenced by dietary concentrations of Ca and P.

In conclusion, results of this experiment indicate that pharmacological levels of Zn in diets for pigs may reduce Ca and P digestibility, but addition of microbial phytase to these diets may partly ameliorate this effect. As a consequence, if pigs need pharmacological levels of Zn, dietary concentrations of Ca and P in diets for pigs that are around 15 kg may need to be increased by 4 and 9.5%, respectively, or diets need to be supplemented with microbial phytase to prevent reduced absorption of Ca and P. Results of the experiment also indicate that interactions among Zn, Ca, P, and phytate may take place in the intestinal tract of pigs, which is the likely reason that supplemental Zn from ZnO reduced Ca and P digestibility and retention. The reason for the reduced absorption of Ca in diets containing pharmacological levels of Zn may be that Zn and Ca compete for the same calcium channels to be absorbed into the enterocytes. Inclusion of microbial phytase increased the ATTD and STTD of Ca and also the ATTD of P, confirming that dietary phytate interferes with Ca and P digestibility.

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