Super dose *Buttiauxella* phytase continuously hydrolyzes phytate and improves amino acids digestibility and mineral balance in growing pigs fed phosphorous deficient diet §

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Abstract: The experiment was conducted to evaluate the efficacy of *Buttiauxella* phytase in growing pigs fed P deficient diets. Ten ileal T-cannula pigs were used in a doubly replicated 5 × 4 incomplete Latin square design (5 diets with 4 periods). The 5 diets were P deficient basal diet with 0, 500, 1,000, or 20,000 FTU/kg phytase and positive diet with adequate P. There were linear increases ($P < 0.01$) in the apparent ileal digestibility (AID) of DM, energy, CP, Ca, total P, phytate and some AA, as well as digestibility, retention and utilization of Ca, total P, Mg, Mn and Zn, with increasing phytase level. Pigs fed 20,000 FTU/kg further increased ($P < 0.05$) AID of Ca, total P, phytate and DM than pigs fed diets with 500 or 1,000 FTU/kg phytase. AID of some AA and digestibility of Na, K, Mn and Zn were improved by the super dose phytase, but not by normal dose (500 or 1,000 FTU/kg) phytase. However, hindgut fermentation of crude fiber and NDF ($P < 0.05$) were depressed by the super dose phytase supplementation. In conclusion, Super dose of phytase (20,000 FTU/kg) hydrolyzed most of the phytate and consequently further improved mineral use, protein utilization.

Keywords: amino acid digestibility, hindgut fermentation, mineral balance, phytase, pigs

INTRODUCTION

In 1991, an *Aspergillus niger*-phytase feed enzyme with the capacity to hydrolyse dietary phytate was introduced to the feed ingredient market in The Netherlands. It was developed as commercial feed additive to replace inorganic P and reduce P excretion worldwide in the following decades. New generation phytases with improved catalytic properties and stabilities keep coming to the market to meet the the global feed market demand for more efficient CP and mineral digestibility. A phytase variant from Buttiauxella sp. (Axtra Phy) was reported to have improved pepsin stability at pH 2.0 by *in vitro* studies compared to its wild type counterpart isolated from soil. In addition, it has 2 to 4 fold higher activity at pH 3.0-4.5 than *E. coli* phytase (Yu et al. 2014). Incorporation of an enzyme with such properties in animal feed may ensure early digestion of phytic acid. However, there is limited information for its efficacy *in vivo* of this novel phytase in weaned pigs since it was developed.

Supplementation with phytase has been reported to increase the digestibility of Ca, Mg, Mn, Zn, Cu, and Fe in pigs at a dosage level of 500 to 1,500 phytase FTU/kg of feed (Pallauf et al. 1992; Adeola et al. 1995). Kies et al. (2006) reported that a super dose of phytase (>10,000 FTU/kg) could further improve mineral digestibility. However, the effects of phytase doses greater than 10,000 FTU/kg on mineral retention were not reported. In addition, whether or not exogenous phytase has the capacity to enhance amino acid digestibility and protein utilization in pigs remains argued ((Adeola and Sands 2003; Liao et al. 2005; Selle and Ravindran 2008). Selle et al. (2012) argued that the responses may be due to improper digesta sample collection and evaluation (needs to be confirmed) and endogenous amino acid loss. Super dose phytase (>10,000 FTU/kg) may further hydrolyze phytate and generate larger magnitude response on apparent ileal AA digestibility. However, limited research has been conducted in this field.

In the current study the novel Buttiauxella phytase was p examined in growing pigs at 0, 500, 1000 and a super dose of 20,000 FTU/kg, on apparent ileal digestibility of various nutrients, hind gut fermentation and minerals balance in growing pigs fed corn soybean based diet.

MATERIALS AND METHODS

Preparation of phytase

The *Buttiauxella* phytase (Axtra Phy) used in this experiment was provided by Danisco Animal Nutrition (Aarhus, Denmark). Its biochemical and catalytical properties have been reported by Yu et al. (2014). The optimal pH range for this phytase is from 2.5 to 5.5. The phytase activity of this phytase was analyzed in quadruplicate according to the method of Engelen et al. (2001). The actual phytase activity was 5,108 FTU/g of product before mixed with the experiment diet components. One FTU of enzyme activity is defined as the amount of enzyme that liberates 1 μmol of inorganic P per min at 37°C and pH 5.5 (Engelen et al. 2001).

Animals and experiment diets

Ten crossbred [Duroc × (Landrace × Large White)] pigs were obtained from a local commercial pig farm (Beijing Beilangzhong pig farm) and fed a corn-soybean meal based diet containing 0.9% Ca and 0.8% total P for three weeks before surgery. The pigs were housed in adjustable stainless steel metabolism crates (1.4 × 0.70 × 0.6 m^3). Pigs (19.26 ± 1.06 kg) were surgically fitted with a simple T-cannula at the distal ileum. After surgery, pigs were returned to their metabolic crates and allowed a 14-day recovery period. During this period, the pigs were fed increasing amounts of a commercial grower diet twice daily and had unlimited access to water.

After the recovery period, the pigs were assigned to one of five treatments according to a

doubly replicated 5 × 4 incomplete Latin square design (5 diets with 4 periods, n = 8). Each period consisted of a five day adjustment period followed by a three day total collection of feces and urine and then a two day collection of ileal digesta. Pigs were fed their diets at 2.1 times their maintenance energy requirement as recommended by the Feeding Standard of Swine (2004) based on their body weight at the beginning of each period. The daily feed allowance was offered in two equal portions at 0800 and 1700 h.

The five treatments consisted of a low- P diet (0.43% Ca, 0.38% total P and 0.21% non-phytate P) supplemented with 0 (negative control, NC), 500, 1,000 or 20,000 FTU/kg *Buttiauxella* phytase, respectively, as well as a positive control (PC) formulated to be adequate in all nutrients (0.64% Ca, 0.52% total P and 0.42% non-phytate P). All diets were fed in mash form with 0.25% chromic oxide inclusion as an indigestible marker. Table 1 shows the ingredient and chemical composition of the PC diet and NC diets.

Total urine and fecal samples were collected twice per day. Feces were collected by placing a plastic bag over the anus of each pig. The collected feces were placed in plastic bags and stored at -20°C. Urine was collected in buckets from drop pans under each pen. A 25% HCl solution was added to each bucket to maintain the urinary pH below 3. Each day, the total urine volume was measured and a 10% aliquot was filtered through gauze. Fifty mL of the mixed urine sample was transferred into a screw-capped tube and immediately stored at -20°C until analysis. At the end of the collection period, urine samples from the three day collection were pooled for each pig and strained through glass wool before analysis. Feces were thawed, pooled for each pig within the given period, homogenized, sub-sampled, dried at 65°C for 72 h and ground through a 1-mm screen. The procedures for the collection were conducted according to the methods described by Song et al. (2003).

Ileal digesta was collected continuously for 12 h from 0800 to 2000 h on days 9 and 10 according to procedures described by Stein et al. (1998). In brief, a 200 mL plastic bag was

attached to the open cannula using a cable tie. Bags were removed whenever they were filled with digesta or at least every 30 min and stored at -20°. At the end of each two day collection period, ileal samples were thawed and lyophilized in a vacuum-freeze dryer (Tofflon Freezing Drying Systems, Shanghai, China), ground through a 1-mm screen, and thoroughly mixed. A representative sub-sample was then taken for chemical analysis. Table 2 shows the analyzed nutrient composition of the experimental diets (as-fed basis).

Analytical procedures

Analysis of DM (AOAC method 930.15), CP (AOAC method 984.13), crude fiber (AOAC method 978.10), Ca (AOAC method 927.02) and P (AOAC method 984.27) were conducted according to the methods of AOAC (2007). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined using fiber bags and Fibre Analyzer, (Ankom Technology, Macedon, NY, USA) following an adaptation procedure described by van Soest et al. (1991). GE (gross energy) was determined by Automatic Energy Analyzer (Parr 1281, Moline, IL, USA). P content was analyzed using a UV-visible Spectrophotometer (Hitachi, U-1000, Tokyo, Japan). Amino acids in diets and digesta were assayed using Ion-Exchange Chromatography according to Wang et al. (2011). Mg, Na, K, Cu, Fe, Mn, and Zn were analyzed with Flame Atomic Absorption Spectrophotometry (AA-6300, Shimadzu Corp., Tokyo, Japan). Phytate (phytic acid) was analyzed on an Ion Chromatograph System (Dionex DX-500, CA, USA) with a Dionex CarboPac PA-100 column (4 × 250 mm) (Tran et al. 2011). The determination of chromium content and AID of nutrients followed the method of Wang et al. (2011).

Statistical analysis

The data obtained were analyzed using the GLM procedure of SAS (SAS Institute, Cary, NC). Individual pigs served as the experimental unit. The model included pig, period, and treatment. Contrast statement in GLM procedure of SAS was applied to conduct polynomial

orthogonal contrasts for linear and quadratic responses. Coefficients for unequally spaced contrasts were generated by PROC IML of SAS. The alpha level used in the determination of significance for all analysis was 0.05 and trends (alpha < 0.10) are also reported.

RESULTS

Apparent ileal digestibility of AA, Ca, P and phytate

Pigs fed PC diet had greater ($P < 0.05$) apparent ileal digestibility (AID) of Ca and total P than pigs fed the NC diet (Table 5). There were linear increases ($P < 0.01$) in AID of Ca, P and phytate and linear decrease ($P < 0.01$) in phytate concentration in freeze-dried digesta with increasing dose of *Buttiauxella* phytase (Figure 1). Phytase supplementation increased ($P < 0.05$) AID of Ca, P and phytate compared with pigs fed NC diet. Furthermore, pigs fed 20,000 FTU/kg phytase had greater ($P < 0.05$) AID of Ca, P and phytate and lower ($P < 0.05$) phytate concentration in freeze dried digesta than pigs fed 500 or 1000 FTU/kg phytase.

There were linear increases ($P < 0.05$) in the AID of phenylalanine, threonine, valine, alanine, aspartic acid, glutamic acid, serine and tyrosine and linear tendency increases in AID of isoleucine, lysine and cysteine with increasing dose of *Buttiauxella* phytase. All of AA AID were numerically increased by phytase supplementation, and significant improvement for leucine, lysine, threonine, aspartic acid and serine were observed between pigs fed super dose of phytase (20,000 FTU/kg) and diets without phytase. However, no significant difference was observed among pigs fed NC diet or diets with 500 or 1000 FTU/kg phytase.

DM, energy, CP and fiber digestibility and hindgut fermentation

There were linear increases ($P < 0.05$) in AID of DM, energy and CP and apparent total tract digestibility (ATTD) of CP with increasing dose of *Buttiauxella* phytase (Table 4). Pigs fed 20,000 FTU/kg phytase had greater ($P < 0.05$) AID of DM than pigs fed diets with 500 or 1000 FTU/kg phytase and greater ($P < 0.05$) AID of energy than pigs fed diet having 1000

FTU/kg phytase. Super dose of phytase (20,000 FTU/kg) supplementation improved (P < 0.05) AID and ATTD of CP compared to pigs fed NC diet while no difference was observed among pigs fed diets without phytase or with 500 or 1000 FTU/kg phytase.

Phytase supplementation linearly decreased (P < 0.05) ATTD of NDF and hindgut digestibility of DM, energy, crude fiber, NDF and ADF. Pigs fed 20,000 FTU/kg phytase had lower (P < 0.05) ATTD of NDF and hindgut digestibility of crude fiber and NDF compared to pigs fed diets without phytase. Hindgut fermentation of crude protein was not affected by treatment.

Minerals balance

There were linear increases (P < 0.05) in the digestibility, retention and utilization of Ca, P and Mg with increasing dose of *Buttiauxella* phytase, as well as in the digestibility of Na and K (Tables 5). Pigs fed PC diet had greater (P < 0.05) digestibility, retention and utilization of Ca and P than pigs fed the NC diet. Pigs fed 20,000 FTU/kg phytase had greater (P < 0.05) digestibility and utilization of Ca and P, but lower (P < 0.05) P retention than pigs fed PC diet. Super dose of phytase (20,000 FTU/kg) supplementation further improved digestibility, retention and utilization of Ca and P compared to pigs fed diets with 500 or 1000 FTU/kg phytase. Pigs fed 20,000 FTU/kg phytase had greater digestibility of Na, K and Mg and retention and utilization of Mg than pigs fed diets without phytase. However, digestibility of Na and K were not significant improved by 500 or 1000 FTU/kg phytase supplementation.

Phytase supplementation linearly increased (P < 0.05) digestibility, retention and utilization of Mn and Zn. Pigs fed 20,000 FTU/kg phytase had greater digestibility of Mn, Zn, Mg and retention and utilization of Zn than pigs fed diets without phytase. However, these measurements were not significant improved by 500 or 1000 FTU/kg phytase supplementation. The balance of Cu and Fe were not affected by the dietary inclusion of phytase.

DISCUSSION

If phytase completely degrades phytate, it would theoretically liberate 2.82 g/kg P and 3.04 g/kg Ca, which implies that supplementation with reduced dietary Ca in phytase supplemented diets is feasible. Considering that high dietary Ca levels may disrupt phytate degradation, Selle suggested dietary Ca levels should be kept to a minimum in phytase supplemented pig and poultry diets without compromising skeletal integrity or growth performance (Selle et al. 2009). Therefore, in the present experiment, the Ca level in phytase supplemented diets was reduced to maximize the phytase activity, and no lameness pigs were observed in the current experiment.

In the present study, phytase supplementation at levels of 500, 1,000 and 20,000 FTU/kg hydrolyzed 62.8, 70.6 and 90.5 % of the phytate in the ileum respectively, which resulted in 2.25, 2.90 and 9.66 times lower phytate at the ileum than for pigs fed the NC diet. The extremely lower digesta phytate content for pigs fed 20,000 FTU/kg phytase may contribute the improvement of energy, AA and mineral digestibility. Yu et al. (2012) have recently reported that pepsin activity reduction is relieved by the hydrolysis of phytate (IP6) and the aggregating capabilities of IP esters (IP_{1-6}) on soy protein and β-casein decrease dramatically from IP_6 to IP_5 and become negligible with IP_{1-4}. These may be one of the mechanisms that phytase benefit CP and AA digestibility

The efficacy of phytase in improving the AA availability is still a matter of debate. The magnitude of improvement generated by phytase ranges from no effect to fairly substantial effects (Liao et al. 2005; Nitrayova et al. 2006; Kiarie et al. 2010; Zeng et al. 2011). The interaction between phytase and AA digestion might thus be multifaceted. In the current study, phytase supplementation numerically improved the AID of CP and all of AA in a corn-SBM diet fed to growing pigs compared to diets without phytase. The magnitude of improvement

was larger for pigs fed diets with the super dose (20,000 FTU/kg) phytase than with normal dose phytase (500 or 1,000 FTU/kg). Furthermore, the super dose phytase supplementation significantly improved AID of leucine, lysine, threonine, aspartic acid and serine while no difference was observed among pigs fed NC diet or diets with 500 or 1000 FTU/kg phytase. This aggressive unambiguous response may attribute to the extremely higher degradation of phytate in ileum for pigs fed the super dose phytase, which remove, almost completely, the anti-nutritive effects of phytate in the gut.

Super dose of phytase (20,000 FTU/kg) supplementation improved ($P < 0.05$) AID of DM and energy compared to pigs fed NC diet while no difference was observed among pigs fed diets without phytase or with 500 or 1000 FTU/kg phytase. This may partly be explained by the very low digesta phytate content for pigs fed 20,000 FTU/kg phytase. However, the ATTD of energy was not affected by phytase supplementation which may be explained by reduction of crude fiber and NDF fermentation in hindgut. The NC diet only contains 0.38% total P, and approximate 80% P was absorbed in the upper intestine for pigs fed diet with 20,000 FTU/kg phytase. This only allowed small amount of P flow in to hindgut, which may depresses microbial fermentation in the lower intestine due to a possible deficiency of P and Ca (Metzler et al. 2008).

The results of current study agreed with previous results that supplementation of microbial phytase to low-P diets improved P digestibility Zeng et al. (2011). The amount of digestible P generated by 500, 1,000 and 20,000 FTU/kg is estimated at 0.73, 0.79 and 1.56 g/kg, respectively. For P digestibility, our results were in agreement with the results of reports that used super doses of microbial phytase (Augspurger and Baker 2004; Kies et al. 2006). However, the mechanism of the continuing improvement in P digestibility as a result of inclusion of super phytase doses remains unclear. Kies et al. (2006) provided two possible explanations: one is that phytate may be degraded faster or to a greater extent at a super dose

(> 10,000 FTU/kg); the other is that a large part of the active phytase escapes the stomach to continue working in the small intestine. In order to investigate which site of digestive tracts is responsible for the additional effect on mineral digestibility at the high phytase dose, data on phytate degradation in the jejunum will be needed.

Cowieson et al. (2004) reported that phytate increased the excretion of endogenous Na in broilers, and Woyengo et al. (2009, 2010) subsequently reported that phytic acid increases Na secretion in the jejunum and reduces the AID of Na in piglets. These reports indicate that phytate may interfere with intestinal uptakes of AA (and glucose) via Na-dependent transport systems (Woyengo et al. 2011). Liao et al. (2005) reported that a high phytic acid diet reduced the AID of energy and AA compared with a low phytic acid diet. However, in the current studies phytase supplementation attenuated the negative nutritional effect of phytic acid. Gagne et al. (2002) found that phytase increased post-prandial plasma concentrations of α-amino nitrogen in growing pigs and suggested this was indicative of phytase enhancing AA absorption. Kies et al. (2005, 2006) subsequently reported that phytase supplementation improved the digestibility of Na and K coupled with increased post-prandial plasma concentrations of glucose. Our results presented in this study agree with these findings of improved digestibility of Na and K, and enhancement of GE and AA utilization with phytase supplementation.

The results of the present studies also support the study of Adeola et al. (1995), which indicated that the growth-promoting effect of phytase may be due to an overall increase in the availability of minerals. Phytase supplementation of diets has been reported to improve Zn, Cu and Mg absorption in weanling pigs (Pallauf et al. 1992; Kies et al. 2006). The improvement in Mg absorption and retention observed in the present experiments further suggests that a super dose of phytase (>10,000 FTU/kg) may continue to hydrolyze soluble phytate in the upper small intestine as the solubility of Ca-phytate decreases rapidly at a pH

above approximately 5, but the Mg salt precipitates at a higher pH.

In conclusion, the *Buttiauxella* phytase used in this study was efficacious at concentrations up to 20,000 FTU/kg for pigs, based on the response criteria of the total tract digestibility of P, Ca, Na and Mg and the AID of phytate, GE and CP. This super phytase dose of phytase further improved phytate degradation in the ileum and enhanced CP and AA utilization as well as increased the availability of minerals.

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Table 1. Ingredient and chemical composition of the experimental diets (as-fed basis)[£]

	Positive Control	Negative Control
Ingredient (%)		
Corn	70.52	70.52
Soybean meal	24.62	24.62
Corn starch	-	1.67
Ground wheat	0.40	0.40
Soybean oil	1.09	0.44
Limestone	0.72	0.70
Dicalcium phosphate	1.30	0.30
Salt	0.30	0.30
L-Lysine- HCl (78 %)	0.29	0.29
DL-Methionine	0.01	0.01
Chromic oxide	0.25	0.25
Vitamin and mineral premix[1]	0.50	0.50
Total	100.00	100.00

[£]Vitamin and mineral premix provided the following per kilogram of diet: vitamin A, 12,000 IU as vitamin A acetate; vitamin D, 2,500 IU as vitamin D_3; vitamin E, 30 IU as DL-α-tocopheryl acetate; 12 µg of vitamin B_{12}; vitamin K, 3 mg as menadione sodium bisulfate; D-pantothenic acid, 15 mg as calcium pantothenate; 40 mg of nicotinic acid; choline, 400 mg choline as choline chloride; Mn, 30 mg as manganese oxide; Zn, 80 mg as zinc oxide; Fe, 90 mg as iron sulfate; Cu, 10 mg as copper sulfate; I, 0.35 mg as ethylenediamine dihydroiodide; and Se, 0.3 mg as sodium selenite.

Table 2. Analyzed nutrient composition of experimental diets (as-fed basis)

	Positive Control	Phytase level (FTU/kg)			
		0	500	1,000	20,000
Phytase activity (FTU/kg)	31	34	546	1018	19026
DM (%)	87.35	87.79	87.17	87.93	87.78
CP (%)	17.29	17.80	17.24	17.44	17.18
Ca (%)	0.64	0.41	0.44	0.43	0.45
P (%)	0.52	0.38	0.37	0.38	0.38
Phytate-P (%)	0.22	0.23	0.23	0.23	0.21
Sodium, Na (g/kg)	1.27	1.29	1.28	1.29	1.30
Potassium, K (g/kg)	0.64	0.66	0.65	0.64	0.65
Iron, Fe (mg/kg)	111	104	114	111	116
Copper, Cu (mg/kg)	14.99	15.85	14.99	16.68	15.52
Manganese, Mn (mg/kg)	29.14	29.70	30.98	30.02	30.50
Zinc, Zn (mg/kg)	88.80	72.03	78	72	72
Magnesium, Mg (g/kg)	1.96	1.93	1.92	2.00	1.94
Essential AA (%)					
Arginine	1.09	1.16	1.09	1.08	1.08
Histidine	0.48	0.48	0.46	0.46	0.45
Isoleucine	0.67	0.70	0.70	0.68	0.66
Leucine	1.46	1.49	1.50	1.49	1.45
Lysine	0.91	0.95	0.92	0.91	0.89
Methionine	0.30	0.30	0.29	0.30	0.29
Phenylalanine	0.89	0.90	0.84	0.84	0.85
Threonine	0.59	0.64	0.67	0.67	0.64
Tryptophan	0.18	0.17	0.19	0.18	0.18
Valine	0.82	0.81	0.83	0.84	0.84
Nonessential AA (%)					
Alanine	0.91	0.96	0.95	0.93	0.91
Aspartic acid	1.63	1.62	1.70	1.70	1.62
Cystine	0.35	0.34	0.34	0.33	0.33
Glutamic acid	3.47	3.50	3.38	3.39	3.29
Glycine	0.67	0.68	0.70	0.69	0.67
Proline	1.08	1.09	1.04	1.11	1.06
Serine	0.81	0.88	0.86	0.88	0.83
Tyrosine	0.62	0.56	0.54	0.55	0.56

Table 3. Effects of phytase on apparent ileal digestibilities (AID) (%) of amino acids, Ca, P and phytate in growing pigs

	Positive Control	Phytase level (FTU/kg)				SEM	*p*-value		
		0	500	1,000	20,000		Treat	Linear	Quadratic
Ca	61.1[b]	44.0[c]	56.9[b]	59.1[b]	68.9[a]	2.29	<0.01	<0.01	<0.01
P	56.1[bc]	38.3[d]	54.3[c]	59.5[b]	79.5[a]	2.10	<0.01	<0.01	<0.01
Phytate	19.4[c]	11.1[c]	62.8[b]	70.6[b]	90.5[a]	4.25	<0.01	<0.01	<0.01
Essential AA									
Arginine	87.9	89.3	89.2	88.8	89.9	0.59	0.21	0.27	0.60
Histidine	81.3	80.6	84.2	83.1	85.0	1.69	0.33	0.24	0.36
Isoleucine	82.4	83.4	85.1	84.2	85.9	0.88	0.07	0.10	0.68
Leucine	82.7[c]	83.6[bc]	85.7[ab]	85.1[abc]	86.4[a]	0.86	0.03	0.11	0.28
Lysine	79.2[b]	79.4[b]	81.9[ab]	81.2[ab]	82.5[a]	0.87	0.03	0.10	0.16
Methionine	88.4	87.9	87.2	87.0	88.3	0.79	0.62	0.45	0.46
Phenylalanine	82.6	82.9	83.3	83.0	85.1	0.82	0.23	0.04	0.97
Threonine	65.8[c]	68.8[bc]	71.7[ab]	71.8[ab]	74.4[a]	1.17	<0.01	0.01	0.11
Tryptophan	68.6	68.2	72.9	71.8	74.1	1.85	0.12	0.18	0.25
Valine	76.0[b]	77.1[ab]	79.7[a]	79.1[ab]	80.7[a]	1.18	0.05	0.13	0.32
Nonessential AA									
Alanine	74.5[b]	76.7[ab]	78.4[a]	77.1[ab]	79.7[a]	1.02	0.01	0.05	0.94
Aspartic acid	75.5[b]	76.2[b]	78.8[ab]	78.9[ab]	80.8[a]	1.10	0.01	0.03	0.15
Cystine	62.3	60.9	62.4	63.0	66.7	2.09	0.38	0.09	0.54
Glutamic acid	84.5	83.9	84.7	83.7	86.6	0.78	0.10	0.01	0.79
Glycine	60.8	61.1	64.7	63.1	67.9	1.84	0.06	0.03	0.41
Proline	73.7	73.9	69.8	70.4	73.8	2.48	0.59	0.53	0.38
Serine	75.5[c]	77.5[bc]	79.5[ab]	79.6[ab]	81.7[a]	1.09	<0.01	0.04	0.24
Tyrosine	81.1	78.4	79.3	78.5	83.0	1.47	0.15	0.02	0.85

[a-c]Means in the same row with different superscripts differ ($p < 0.05$).

Table 4. Effects of phytase on apparent ileal digestibility (AID), total tract apparent digestibility (ATTD) and hindgut fermentation of nutrient in growing pigs (Exp. 2)

Items	Positive Control	Phytase level (FTU/kg)				SEM	*p*-value		
		0	500	1,000	20,000		Treat	Linear	Quadratic
AID									
Dry matter	72.3[b]	71.6[b]	72.9[b]	72.8[b]	74.9[a]	0.58	<0.01	<0.01	0.27
Gross energy	74.4[b]	74.0[b]	75.4[ab]	74.6[b]	76.5[a]	0.58	0.03	0.01	0.61
Crude protein	74.1[b]	74.0[b]	75.4[ab]	75.9[ab]	78.0[a]	0.96	0.03	0.01	0.21
Crude fiber	22.1	23.6	23.0	22.8	23.2	0.80	0.75	0.97	0.56
NDF	27.4	27.0	27.5	26.1	27.6	1.02	0.82	0.61	0.58
ADF	22.4	22.2	23.2	22.5	22.8	0.80	0.91	0.79	0.83
ATTD									
Dry matter	90.8	89.9	90.8	90.2	90.7	0.40	0.44	0.38	0.65
Gross energy	90.5	90.1	90.7	89.9	90.3	0.39	0.71	0.78	0.81
Crude protein	88.2[ab]	87.1[b]	88.8[ab]	88.4[ab]	89.7[a]	0.50	0.01	<0.01	0.07
Crude fiber	63.7	64.6	62.4	62.6	59.4	1.95	0.42	0.07	0.59
NDF	64.6[a]	64.8[a]	62.5[ab]	60.8[ab]	58.6[b]	1.47	0.02	0.02	0.07
ADF	51.7	49.0	50.6	48.8	46.7	1.69	0.40	0.18	1.00
Hindgut fermentation									
Dry matter	18.6	18.3	17.9	17.4	15.8	0.71	0.07	0.02	0.48
Gross energy	16.1	16.0	15.3	15.3	13.8	0.70	0.16	0.04	0.55
Crude protein	14.1	13.1	13.4	13.2	12.4	1.05	0.87	0.52	0.96
Crude fiber	41.6[a]	41.0[a]	39.4[a]	39.8[a]	36.3[b]	2.15	<0.01	<0.01	0.47
NDF	37.2[a]	37.8[a]	34.9[ab]	34.8[ab]	31.0[b]	1.79	<0.01	<0.01	0.06
ADF	29.3	26.8	27.4	26.3	23.9	1.82	0.15	0.03	0.94

[a-c]Means in the same row with different superscripts differ ($p < 0.05$).

Table 5. Effects of phytase on macro-minerals balance in growing pigs

	Positive Control	Phytase level (FTU/kg)				SEM	*p*-value		
		0	500	1,000	20,000		Treat	Linear	Quadratic
Calcium, Ca									
Intake (g/d)	6.87	4.43	4.77	4.58	4.82				
Digestibility (%)	66.5^{b}	47.0^{c}	63.8^{b}	66.8^{b}	75.4^{a}	1.90	<0.01	<0.01	<0.01
Retention (g/d)	4.19^{a}	1.81^{d}	2.86^{c}	2.80^{c}	3.47^{b}	0.13	<0.01	<0.01	<0.01
Utilization (%)	60.8^{b}	40.8^{c}	59.6^{b}	61.2^{b}	72.0^{a}	2.31	<0.01	<0.01	<0.01
Phosphorus, P									
Intake (g/d)	5.59	4.09	3.99	4.12	4.03				
Digestibility (%)	58.7^{b}	39.5^{c}	60.8^{b}	62.4^{b}	83.7^{a}	1.51	<0.01	<0.01	<0.01
Retention (g/d)	3.23^{a}	1.59^{c}	2.41^{b}	2.54^{b}	3.31^{a}	0.08	<0.01	<0.01	<0.01
Utilization (%)	57.5^{b}	39.1^{c}	60.3^{b}	61.8^{b}	82.1	1.64	<0.01	<0.01	<0.01
Sodium, Na									
Intake (g/d)	1.36	1.39	1.38	1.38	1.39				
Digestibility (%)	89.2^{b}	88.5^{b}	91.1^{ab}	89.7^{b}	92.8^{a}	0.91	0.02	<0.01	0.37
Retention (g/d)	0.72	0.73	0.77	0.75	0.71	0.08	0.99	0.67	0.86
Utilization (%)	53.6	52.7	56.0	54.9	50.4	5.53	0.96	0.51	0.74
Potassium, K									
Intake (g/d)	0.69	0.71	0.70	0.69	0.70				
Digestibility (%)	80.7^{ab}	76.8^{b}	80.7^{ab}	79.4^{b}	84.9^{a}	1.77	0.05	<0.01	0.31
Retention (g/d)	0.06	0.11	0.10	0.12	0.13	0.03	0.34	0.38	0.85
Utilization (%)	8.6	15.0	14.7	18.6	18.5	3.54	0.28	0.35	0.74
Magnesium, Mg									
Intake (g/d)	2.11	2.07	2.07	2.15	2.08				
Digestibility (%)	26.1^{c}	29.0^{bc}	33.1^{ab}	35.7^{a}	38.2^{a}	2.17	<0.01	0.03	0.09
Retention (g/d)	0.42^{c}	0.42^{c}	0.52^{bc}	0.61^{ab}	0.68^{a}	0.05	<0.01	<0.01	0.04
Utilization (%)	20.1^{c}	20.1^{c}	24.5^{bc}	28.3^{ab}	32.7^{a}	2.38	<0.01	<0.01	0.06

$^{a-d}$Means in the same row with different superscripts differ ($p < 0.05$).

Table 6. Effects of phytase on trace minerals balance in growing pigs

Item	Positive Control	Phytase level (FTU/kg)				SEM	*p*-value		
		0	500	1,000	20,000		Treat	Linear	Quadratic
Copper, Cu									
Intake (mg/d)	16.1	17.0	16.1	17.9	16.7				
Digestibility (%)	17.8	18.5	20.4	19.2	22.7	1.91	0.41	0.13	0.86
Retention (mg/d)	2.26	2.54	2.68	2.88	2.77	0.30	0.62	0.69	0.47
Utilization (%)	13.8	14.7	16.4	16.4	16.6	1.87	0.78	0.60	0.58
Iron, Fe									
Intake (mg/d)	119.3	111.4	122.9	119.4	125.2				
Digestibility (%)	34.0	33.3	37.7	35.2	35.6	3.31	0.90	0.83	0.80
Retention (mg/d)	39.6	36.2	45.5	40.5	42.7	4.05	0.57	0.55	0.58
Utilization (%)	32.8	32.2	36.8	34.3	34.7	3.32	0.88	0.80	0.79
Manganese, Mn									
Intake (mg/d)	31.3	31.9	33.3	32.3	32.8				
Digestibility (%)	30.6^{b}	29.7^{b}	32.4^{b}	38.5^{ab}	41.7^{a}	3.01	0.03	0.01	0.09
Retention (mg/d)	9.49	9.25	11.24	11.77	13.31	1.08	0.07	0.02	0.19
Utilization (%)	30.0	29.1	32.9	36.5	40.9	3.33	0.09	0.01	0.20
Zinc, Zn									
Intake (mg/d)	95.5	77.4	83.5	77.7	77.5				
Digestibility (%)	22.7^{b}	24.6^{b}	25.4^{b}	25.5^{b}	29.9^{a}	1.19	<0.01	<0.01	0.66
Retention (mg/d)	19.8^{ab}	17.2^{b}	19.7^{ab}	17.7^{b}	21.8^{a}	1.20	0.07	0.02	0.76
Utilization (%)	20.8^{b}	22.2^{b}	23.5^{b}	22.9^{b}	28.3^{a}	1.52	0.02	<0.01	0.81

[a-b]Means in the same row with different superscripts differ ($p < 0.05$).

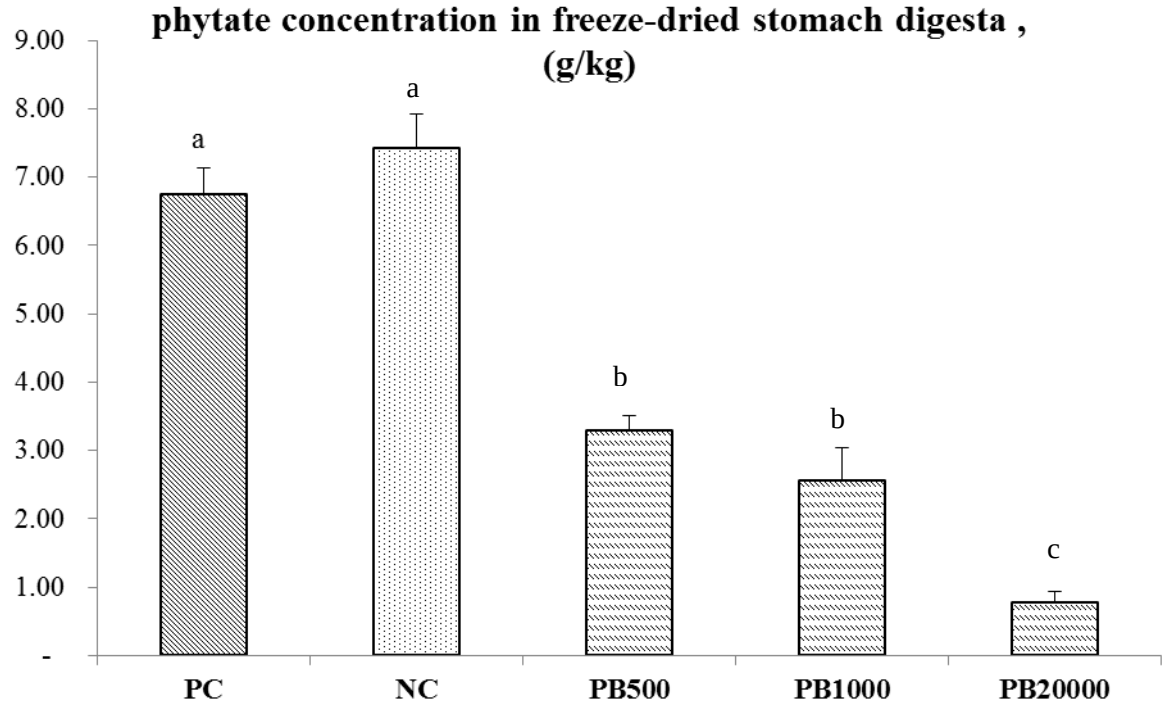

Figure 1. Effects of phytase on phytate concentration in freeze-dried stomach digesta of growing pigs. PC= positive control, NC= basal diet without phytase. PB500, PB1000 or PB20000 means diets with 500, 1,000 or 20,000 FTU/kg phytase.