

## Research Article

# Effect of Phytase Transgenic Corn Supplemented in Feed Diets on the Utilization of Calcium and Phosphorus in Bama Boars

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## Abstract

The study aimed to evaluate the nutritional composition and the efficacy of phytase transgenic corn. Ten-month old castrated Bama boars and replicated a 6x6 Latin square design were used to evaluate the effect of phytase on utilization of calcium of phosphorus. Six-dietary treatments included: Phosphorus adequate control diet (PC), PTC0 (low-phosphorus diet), PTC500 (low-phosphorus diet with 500 FTU phytase/kg in PTC), PTC1000 (low-phosphorus diet with 1000 FTU phytase/kg in PTC), PTC5000 (low-phosphorus diet with 5000 FTU phytase/kg in PTC), microbe500 (low-phosphorus diet with 500 FTU phytase/kg from *Aspergillus Niger*). Contents of the proximate components, minerals, vitamins and amino acid of PTC did not differ from CC. The PTC has lower concentrations of phytate phosphorus than CC, the apparent digestibility of calcium and phosphorus of the PTC0 group is lower ( $p < 0.05$ ). No obvious dose-dependent relationship about the apparent digestibility of calcium and phosphorus showed among PTC500, PTC1000 and PTC5000. Therefore, the nutritional compositions of PTC had substantial equivalence to that of control corn except for phytate phosphorus concentrations, the PTC addition can improve the apparent digestibility of phosphorus and calcium and reduce the excrement of inorganic phosphorus, PTC might be an economically efficient way to decrease environment pollutions.

**Keywords:** Phytase transgenic corn; Nutritional composition; Calcium; Phosphorus; Utilization

## Abbreviations

PTC: Phytase Transgenic Corn; CC: Conventional Corn; PC: Phosphorus Adequate Control; AD: Apparent Digestibility; SD: Standard Deviation; OECD: Organization for Economic Co-operation and Development; WHO: World Health Organization; GMO: Genetically Modified Organism

## Introduction

Phosphorus and calcium are two of the essential mineral elements in animal growth, which play important role in bone development and energy metabolism [1]. Phytate is the major storage form of phosphorus in many plant feed ingredients [2-4]. However, phytate-phosphorus is poorly utilized by nonruminant animals such as pigs and poultry, and thereby inorganic phosphorus needs to be added to the feed to meet their phosphorus requirements [5]. The unavailable phytate-phosphorus will be excreted and consequently cause environmental pollution problems. In addition, phytate in plants can chelate divalent metal ions such as calcium and iron in feeding process, and result a poor utilization of these elements [6].

Some research reported that supplementation of exogenous microbial phytase in feed might be one of the most effective methods to enhance the utilization of phosphorus and reduce its excretion in nonruminant animals [7,8]. But this way is often hard to be applied because of the high cost and inconvenience of production in

developing countries [9]. With the development of biotechnology, foreign phytase gene was efficiently expressed in maize, which is extremely promising in terms of environmental health [10,11]. However, public concerns have been raised in regard to the nutritional value, the usage and their potential effects of genetically modified crops on animal and human health [12].

Up to date, several studies have been conducted to compare the feed safety values of PTC with CC in poultry, such as laying hens and broiler [13,14]. However, the nutritional values and the utilization of calcium and phosphorus have been observed inadequately between PTC and CC when pigs consuming diets formulated with these corns. Therefore, the aim of this study was: 1) To assess whether the nutritional composition (proximate components, mineral, vitamin energy and amino acids) of PTC were equivalent to CC; and 2) To evaluate the efficacy of corn-based phytase as a dietary supplement for poultry on the utilization of calcium of phosphorus.

## Materials and Methods

### Materials

Genetically modified PTC TPY002 and its CC were provided by Beijing Origin Agritech Limited, and the phytase from *Aspergillus Niger* was purchased from Beijing Challenge Group. The whole grain from PTC and CC were ground to a 0.5-mm mesh screen before analysis or inclusion into the diets.

## Composition analysis

The concentrations of proximate components, mineral, vitamin and amino acids of PTC and CC were analyzed according to standard methods for protein (GB 87 5009.5-2010, China, 2010), total fat (GB/T 5009.6-2003), carbohydrate (GB/Z 88 21922-2008), fiber (GB/T 5009.88-2008), calcium (GB/T 5009.92-2003), copper 89 (GB/T 5009.13-2003), iron, magnesium and manganese (GB/T 5009.90-2003), 90 potassium (GB/T 5009.92-2003), zinc (GB/T 5009.14-2003), selenium (GB 5009.93-2010), phosphorus (GB/T 5009.87-2003), sodium (GB/T 5009.91-2003), thiamine (GB/T 5009.84-2003), riboflavin (GB/T 5009.85-2003), folic acid (GB/T 5009.211-2008), niacin (GB/T 5009.89-2003), free biotin (GB 5413.19-2010), Amino acid content (GB/T 5009.124-2003). Each nutrient was detected triple.

## Determination of phytase activity

Phytase activity in corns was determined according to the standard method (GB/T 18634-2009, China, 2009). One phytase activity unit (FTU) is defined as the quantity of enzyme that releases 1 $\mu$ mol of inorganic phosphorus per min from 5.5mmol/L sodium phytate at pH 5.5 at 37°C.

## Experimental scheme and design

**Animals:** Ten-month old castrated Bama boars were bought from Beijing Tonghe Shengtai Institute of Comparative Medicine (License: SCXK (Beijing), 2015-0004). They were individually housed in stainless steel cages with the laboratory temperature 20°C-25°C and humidity 40-70%. All pigs were surgically fitted with post-valve T-intestinal cannulas after 36-hour fasting control. Six swines, which were well recovered from the surgery and with good appetite, were selected. The protocol was approved by the Experimental Animal Welfare and Ethics Committee of National Institute for Nutrition and Health, Chinese Center for Diseases Control and Prevention.

**Dietary treatments:** According to the references [15,16], six-dietary treatment in the present study included: (1) Phosphorus adequate control diet (PC): daily feed with adequate phosphorus to meet its requirements of the pigs; (2) PTC0: low-phosphorus diet in which phosphorus content was reduced by 1/3 on the basis of PC diet but the ratio of calcium to phosphorus was unchanged; (3) PTC500:

low-phosphorus diet added PTC with 1000 FTU phytase/kg; (4) PTC1000: low-phosphorus diet added PTC with 1000 FTU phytase/kg; (5) PTC5000: low-phosphorus diet added PTC with 5000 FTU phytase/kg; (6)microbe 500: low-phosphorus diet added 500 FTU phytase/kg from *Aspergillus Niger*.

Feed formula was prepared to meet the nutritional requirements of this small type pigs [17]. Soybean meal and vegetable oil was non-genetically modified. The detailed formula was shown in Table 1.

**Experimental procedure:** A replicated 6 $\times$ 6 Latin square design was applied for PTC with different level of phytase activity and CC comparison. Each diet was supplied for 6 days. All swines were fed twice per day with equal amounts of each meal at 12-hour intervals (from 8:00 to 20:00). On the seventh and eighth day, faeces were collected by self-designed honeycomb ducts with once per hour from 8:00 to 20:00 and frozen immediately at -20°C. After two days faeces collection, pigs were given a new diet. All the faeces were freeze-dried, ground through a 0.250-mm mesh screen and then determined the concentration of calcium, phosphorus and chromium.

## Analytical methods

The digestibility of calcium and phosphorus was calculated by Apparent Digestibility (AD). Parameters of digestibility were calculated using the following equation [18,19]:

$$AD\% = 100 - (Cr_{feed} / Cr_{feces}) * (A_{feces} / A_{feed}) * 100$$

Where  $Cr_{feed}$  is the content of chromium in diet (mg/kg),  $Cr_{feces}$  is the content of chromium in feces (mg/kg),  $A_{feces}$  is the content of nutrients in digesta (g/kg), and  $A_{feed}$  is the content of nutrients in diet (g/kg).

The data were analyzed with the SPSS software (version 19.0; SPSS, Inc., Chicago, IL, USA). Descriptive data were presented as mean  $\pm$  standard deviation (SD). Reference values from Organization for Economic Co-operation and Development (OECD) was used to compare the difference in nutrients level between PTC and CC. Differences among comparisons of digestibility values obtained from pigs were analyzed by the variance analysis. A two-tailed p-value < 0.05 was considered statistically significant.

**Table 1:** Feed formula in dietary treatment for Bama boars (g/kg).

Composition	PC	PTC0	PTC500	PTC1000	PTC5000	Microbe500
Ordinary corn	766.68	766.68	724.38	682.18	343.68	754.38
Soybean meal (43%)	150	164.09	164.09	164.09	164.09	164.09
Fish meal (65%)	20	20	20	20	20	20
<i>Aspergillus Niger</i> phytase	0	0	0	0	0	12.3
PTC	0	0	42.3	84.5	423	0
Soybean oil	10	10	10	10	10	10
Salt	5	5	5	5	5	5
Chromium trioxide	5	5	5	5	5	5
Premix (1%)	10	10	10	10	10	10
Calcium hydrogen phosphate*	25	12.5.00	12.5.00	12.5	12.5.00	12.5.00
Stone powder*	8.32	6.73	6.73	6.73	6.73	6.73
Total phosphorus (%)	0.64	0.44	0.42	0.45	0.45	0.43

**Note:** Calcium hydrogen phosphate including calcium 20.35%, phosphorus 17.62%, stone powder including calcium 35%.

**Table 2:** Comparison of nutrients' content between PTC and CC.

Nutrients	PTC	CC	Reference values*
<b>Proximate components</b>			
Protein (g/100g)	7.93 ± 0.83	7.29 ± 0.16	6.00-12.70
Total fat (g/100g)	4.35 ± 0.07	4.40 ± 0.14	3.1-5.8
Carbohydrate (g/100g)	70.22 ± 8.31	64.36 ± 1.17	-
Fiber (g/100g)	11.53 ± 2.22	10.76 ± 1.32	8.30-11.90
<b>Minerals</b>			
phosphorus (g/100g)	0.26 ± 0.00	0.22 ± 0.01	0.23-0.72
potassium(mg/100g)	190.21 ± 121.03	195.99 ± 114.74	320-720
magnesium (mg/100g)	115.60 ± 8.57	96.71 ± 0.25	82-1000
calcium (mg/100g)	5.04 ± 0.28	6.43 ± 1.65	3-100
iron (mg/100g)	2.54 ± 0.05	2.05 ± 0.45	0.1-10
zinc (mg/100g)	18.50 ± 0.71	23.50 ± 4.95	1.2-3.0
sodium (mg/100g)	0.83 ± 0.49	0.64 ± 0.23	0-150
<b>Vitamins</b>			
Vitamin A (µg/100g)	7.67 ± 0.01	5.74 ± 0.01	-
Vitamin E (mg/100g)	4.41 ± 1.87	4.33 ± 0.30	-
Thiamine (mg/100g)	0.17 ± 0.08	0.17 ± 0.05	0.23-0.86
Riboflavin (mg/100g)	0.03 ± 0.00	0.03 ± 0.01	0.025-0.56
Vitamin B <sub>6</sub> (mg/100g)	0.32 ± 0.06	0.36 ± 0.01	-
Vitamin B <sub>12</sub> (µg/100g)	0.27 ± 0.07	0.30 ± 0.06	-
<b>Phytate</b>			
phytate phosphorus (g/100g)	0.02 ± 0.01	0.12 ± 0.01	-
Ratio of phytate phosphorus to phosphorus (%)	7.70%	54.50%	

**Note:** \*Data from OECD (2002); - indicated data not given.

## Results

### The content of nutritional composition in PTC and CC

Analyzed nutrient contents of the corns were presented in Table 2. Contents of the proximate components, minerals and vitamins of PTC did not differ greatly from CC. All vales of both corns were in the range of reference values recommended by OECD. The content of phytate phosphorus, the ratio of phosphorus to phytate phosphorus in PTC was obviously lower than that in CC (7.7% and 54.5%).

The content of amino acids in PTC and CC were presented in Table 3. Although there was a little difference between the two corns, all vales were in the range of reference values recommended by OECD.

### The phytase activity of PTC

The genetical modified phytase maize was cultivated by the gene gun mediated transformation with *Aspergillus-Niger* phyA2 gene. The phytase in the PTC and *Aspergillus Niger* were both acid phytase. The phytase activity was 11832 FTU/kg for PTC, and 40691FTU/kg for *Aspergillus Niger*.

### The apparent digestibility of calcium and phosphorus

All pigs were normal in diet intake and water drinking, and were in good condition throughout the whole experiment. The apparent digestibility of calcium and phosphorus was presented in Table 4.

**Table 3:** Comparison of amino acids' content between PTC and CC (g/100g).

Nutrients	PTC	CC	Reference values*
Aspartic Acid	0.55	0.48	0.48-0.85
Threonine	0.24	0.23	0.27-0.58
Serine	0.38	0.33	0.35-0.91
Glutamic	1.56	1.44	1.25-2.58
Glycine	0.23	0.22	0.26-0.49
Alanine	0.67	0.6	0.56-1.04
Valine	0.33	0.32	0.21-0.85
Methionine	0.12	0.13	0.10-0.46
Isoleucine	0.17	0.15	0.22-0.50
Leucine	1	0.93	0.79-2.41
Tyrosine	0.19	0.17	0.12-0.79
Phenylalanine	0.37	0.34	0.32-0.64
Lysine	0.24	0.22	0.05-0.55
Hlstdine	0.19	0.2	0.15-0.38
Argnine	0.33	0.28	0.22-0.64
Proline	0.55	0.59	0.63-1.36
Tryptophane	0.06	0.06	-
Cystine	0.1	0.1	-

**Note:** \*Data from OECD (2002); - indicated data not given.

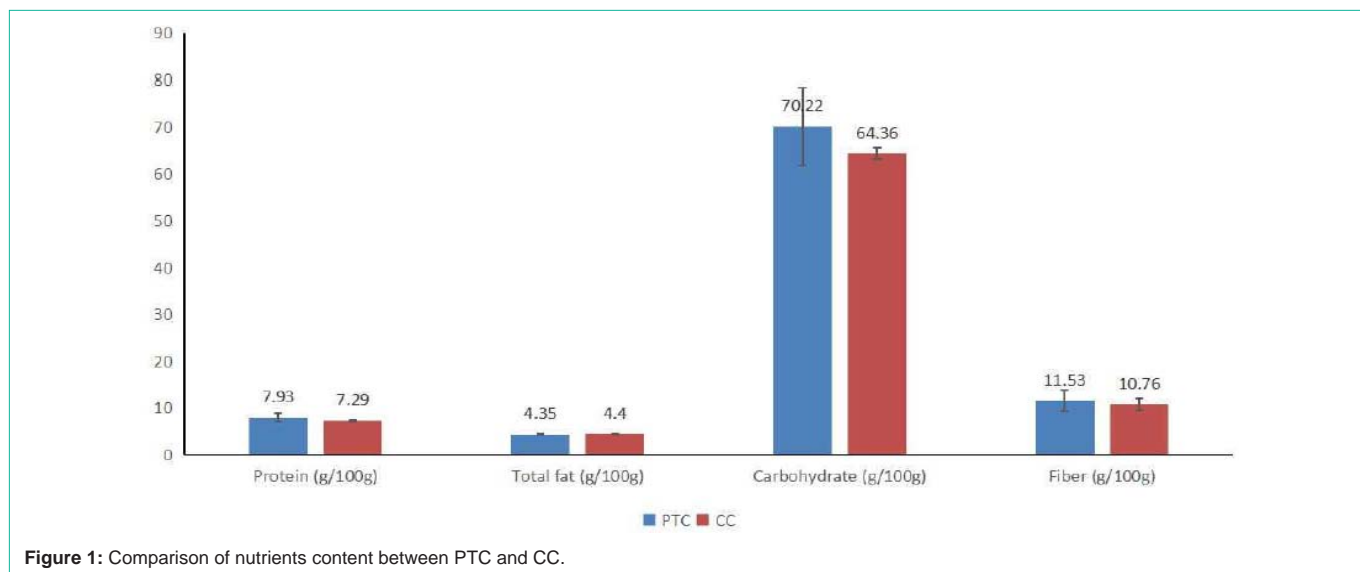


Figure 1: Comparison of nutrients content between PTC and CC.

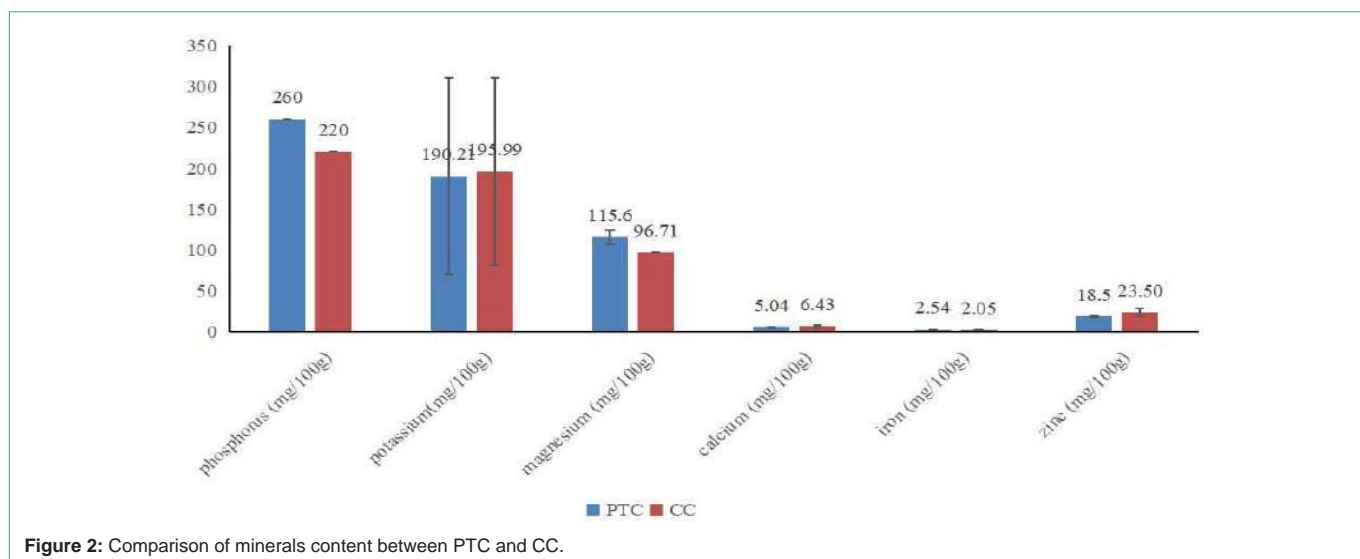


Figure 2: Comparison of minerals content between PTC and CC.

Compared with the PTC0 group, the apparent digestibility of calcium and phosphorus were significantly higher in groups of CC, PTC500, PTC1000, PTC5000 and microbe500 (calcium: 59.31%; 47.87; 61.15%; 61.88%; 60.27%; 60.57%; phosphorus: 42.96%; 30.72%; 46.13%; 47.44%; 49.02; 45.49 all  $p < 0.05$ ). The apparent digestibility of calcium and phosphorus in PTC500, PTC1000, PTC5000 group was similar as groups of microbe500 (all  $p > 0.05$ ). However, no obviously dose-dependent manner between the apparent digestibility of calcium and phosphorus and different level of phytase activity was showed among groups of PTC500, PTC1000 and PTC5000.

The result displayed that the addition of phytase corn with 500 FTU/kg and above enzyme activity could reduce the amount of calcium hydrogen phosphate by 1/3 when preparing feed for pigs. In addition, phytase activity in PTC was effective as that from *Aspergillus Niger*.

## Discussion

The PTC is an innovative way of adding exogenous microbial

phytase to corns for monogastric animals feeding. According to the guidance of World Health Organization (WHO) workshop, nutritional components analysis between PTC and its control production is one of important aspects for safety evaluation of foods or food components from plants derived by modern biotechnology [20]. To date, the principle of “substantial equivalence” has been used internationally for safety assessing of Genetically Modified Organism (GMO). There may be some change in key nutrients’ content between GMO and their control production, which could be related to natural variation, meteorological conditions, soil conditions, etc. Therefore, it can be regarded as there is no difference between the GMO and control production as long as the values were in the range of variation [21].

Several studies showed that microbial phytase supplementation can significantly improve phosphorus utilization in nonruminant animal diets [22,23] However, it cost highly and could not use extensively in the feed production. The PTC derived by exogenous microbial phytase and supplemented into diet, could be an

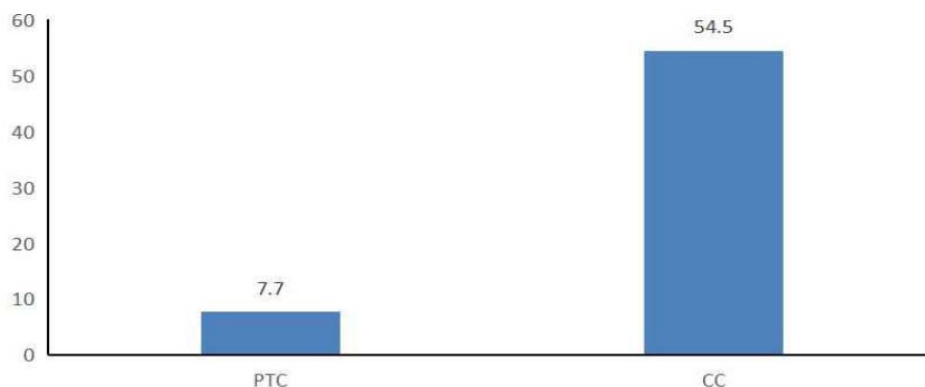


Figure 3: Ratio of phytate phosphorus to phosphorus (%).

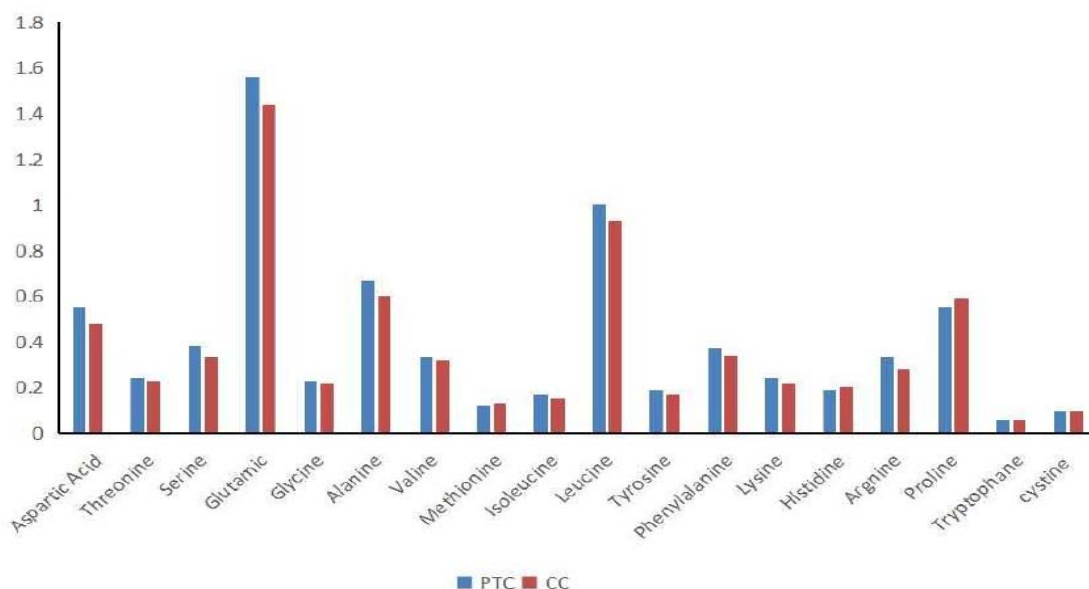


Figure 4: Comparison of amino acids content between PTC and CC (g/100g).

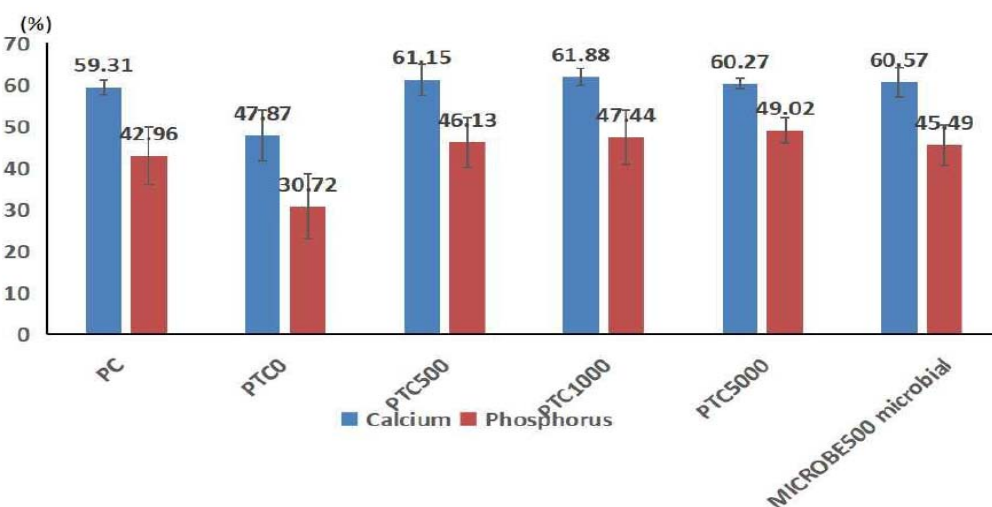


Figure 5: Comparison of the apparent digestibility of calcium and phosphorus in different diets.



**Table 4:** Comparison of the apparent digestibility of calcium and phosphorus in different diets (%).

Nutrients	PC	PTC0	PTC500	PTC1000	PTC5000	MICROBE500 microbial
Calcium	59.31±1.70 <sup>a</sup>	47.87±6.12 <sup>b</sup>	61.15±3.72 <sup>a</sup>	61.88±1.97 <sup>a</sup>	60.27±1.24 <sup>a</sup>	60.57±3.67 <sup>a</sup>
Phosphorus	42.96±6.93 <sup>a</sup>	30.72±7.88 <sup>b</sup>	46.13±5.97 <sup>a</sup>	47.44±6.58 <sup>a</sup>	49.02±3.09 <sup>a</sup>	45.49±4.81 <sup>a</sup>

**Note:** Groups with same letters indicated that there was no difference among groups, while with different letters indicated there was significant difference among groups.

economically efficient way to enhance the utilization of phytate phosphorus in nonruminant animal diets [10,24]. Some studies presented that plant phytase had obvious efficacies and could reduce the excrement of phosphorus and improve the utilization of phosphorus and/or calcium in broiler chicks, brown roosters, growing pigs [16,25,26]. Furthermore, it was reported that low-phosphorus diets were more beneficial for increasing phytase activity and enhancing the utilization of phosphorus [15,27,28].

The present study used a common daily diet with adequate phosphorus and low-phosphorus diets to evaluate the utilization of calcium and phosphorus in PTC. The results showed that the effect of phytase supplementation in corn was quite pronounced. The apparent digestibility of calcium and phosphorus were significantly higher in PTC groups with phytase activity 500 FTU and above than that in PTC0 group, which was a low-phosphorus diets without phytase. In addition, the efficiency of phytase in PTC groups was similar as that from common diet and microbe. The result in our study was accordance with previous studies [13,16,25,26].

In the present study, the apparent digestibility of phosphorus and calcium in growing pigs feeding with different level of phytase activity did not increased in a dose-dependent manner, which was in accordance with Zhang et al.'s study [29]. While Torrallardona et al.'s study [15] observed the improved apparent total tract digestibility of phosphorus in growing pigs and reduced phosphorus excretion in feces in a dose-dependent manner. One of the reasons could be explained by the different source of phytase. The microbial phytase used in Torrallardona et al.'s study [15] was supplemented directly into the diet and could be released completely to digest phosphorus, while these used in our's and Zhang's study [29] were expressed in genetically modified corns. In addition, the content of inorganic phosphorus in the low phosphorus diet in this study was just reduced by a third. Calcium and phosphorus could be completely released by the 500KTU/kg phytase and could absolutely meet animal's nutritional requirement. Most of the phytase were expressed in the endosperm of maize [10], which led to the excess phytase stay in this layer and then could not be released when it was unnecessary.

## Conclusion

Based on these results, it was concluded that except phytate phosphorus concentrations, the nutritional compositions of PTC had substantial equivalence to that of control corn. The efficacy of *Aspergillus Niger*-derived phytase expressed in transgenic modified corn was comparable to that expressed in microbe. In addition, the apparent digestibility of phosphorus and calcium in diet with PTC was enhanced obviously in pigs. As a consequence, PTC added to monogastric animal diets might be an economically efficient way to reduce the excrement of inorganic phosphorus and then decrease environment pollutions.

## Declaration

**Credit authorship statement:** Conceptualization: X.Y and L.Y; Methodology: M.L and L.L; Experiments: L.L, D.M; Data Curation: L.L and J.W; Writing-Original Draft Preparation: L.L and J.W; Writing-Review & Editing: L.Y; Supervision: X.Y and L.Y; Project Administration: L.Y and X.Y.

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