

Effects of Phytase and Carbohydrases Supplementation to Diet with a Partial Replacement of Soybean Meal with Rapeseed Meal and Cottonseed Meal on Growth Performance and Nutrient Digestibility of Growing Pigs

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ABSTRACT : An experiment was conducted to investigate the effects of microbial phytase (Natuphos[®]) supplementation in combination with carbohydrases (composed of enzymes targeted to soybean meal (SBM) dietary components such as α -galactosides and galactomannans; Endo-Power[®]) to corn-soybean meal based diet (CSD) and complex diet (CD) with a partial replacement of SBM with rape seed meal (RSM) and cotton seed meal (CSM) on growth performance and nutrient digestibility of growing pigs. A total of 168 growing pigs averaging 13.18 \pm 1.77 kg of initial body weight was arranged as a 2 \times 2 factorial design with main effects of diet types (corn-SBM based diet (CSD) and complex diets (CD; 5% of SBM was replaced with 2.5% of RSM and 2.5% of CSM in diet for phase I (0 to 3 weeks) and 6% of SBM was replaced with 3% of RSM and 3% of CSM in diet for phase II (4 to 7 weeks))) and enzyme supplementation (none and 0.1% of phytase (500 FTU/kg diet) and 0.1% of carbohydrases). The diet with enzyme application were formulated to have a 0.18% unit lower aP than diets without enzyme application. Each treatment had three replicates with 14 pigs per replicate. To determine supplementation effect of phytase and carbohydrases on ileal amino acid digestibility of SBM, RSM and CSM, a total of 18 T-cannulated pigs (initial body weight; 13.52 \pm 1.24kg) were assigned to six dietary treatments in the present study. Dietary treatments in metabolic trial included 1) SBM diet, 2) SBM diet+with enzymes (phytase (500 FTU/kg) and carbohydrases at 0.1%, respectively), 3) CSM diet, 4) CSM diet+enzymes, 5) RSM diet and 6) RSM diet+enzymes. During whole experimental period (0 to 7 wks), there was no difference in growth performance between diets (CSD and CD). However, dietary phytase and carbohydrases supplementation significantly improved gain/feed ratio (G:F) of growing pigs. During the phase II (4-7 weeks), dietary phytase and carbohydrases supplementation significantly improved all fecal nutrient digestibilities (Dry matter (DM), gross energy (GE), crude protein (CP), crude fat (CF), calcium (Ca) and phosphorus (P)). Dietary phytase and carbohydrases supplementation improved significantly overall ileal amino acid digestibilities of SBM, RSM and CSM based diets ($p < 0.05$). The simultaneous inclusion of phytase and carbohydrases in both of CSD and CD reduced feed cost per kg body weight gain (FCG). Also, results suggest that 2.5 to 3% of RSM and CSM, respectively, might be used as a protein source in growing pig diets without having an adverse effect on the growth performance and nutrient digestibility and simultaneous phytase and carbohydrases addition improves nutritional value of SBM, RSM and CSM by improving ileal amino acid digestibilities. (*Asian-Aust. J. Anim. Sci.* 2003, Vol 16, No. 9 : 1339-1347)

Key Words : Phytase, Carbohydrases, Growth Performance, Ileal Amino Acid Digestibility, Growing Pigs

INTRODUCTION

The concept of using enzyme products as animal feed additives has attracted considerable interest within the feed industry as a means for improving performance, lowering supplemental nutrient requirement, and lowering potential for environmental pollution from manure.

Microbial phytase is one of the most commonly used enzymes in monogastric animal diets. The efficacy of microbial phytase in improving overall phosphorus availability in monogastric animals is now clearly established (Coelho and Kornegay, 1996). But current evidence shows that there are additional benefits of improved amino acids and energy utilization (Ravindran et al., 1999ab).

Soybean meal (SBM) is the main protein source in

swine feeds in most parts of the world. However, soybean like other legume seeds contain anti-nutritional factors (ANFs). Of those of ANFs, α -galactosides and galactomannans, relatively highly contained in SBM, are well known as flatulence-producing factors. Those of ANFs are not digested by monogastric animals because they do not have endogenous enzyme system to degrade it. Thus, there is little doubt that the feeding value of SBM could be enhanced by the elimination or modification of ANFs (Coon et al., 1990; Leske et al., 1993).

Rape seed meal (RSM) contains high quality protein but its use in diets of monogastric animals has been limited by the relatively high level of fiber and oligosaccharides (2.5%), resulting in low energy yield and less than optimum protein utilization (Slominske and Campbell, 1990). Cotton seed meal (CSM) can also be good protein sources for swine diets but its utilization in swine diet as a protein supplementation is limited due to low lysine levels and low palatability and by the presence of ANFs such as gossypol and oligosaccharides. Nevertheless, the availability of these

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Table 1. Formula and chemical composition of experimental diets (0-3 wk) for growth trial

Diet types and enzymes	Corn-SBM based diets		Complex diets	
	-	+	-	+
Ingredients (%)				
Corn	48.82	49.45	46.54	47.98
Soybean meal	36.08	36.08	32.44	32.20
Bakery byproduct	6.00	6.00	6.00	6.00
Animal fat	3.00	3.00	4.00	3.42
Rapeseed meal	-	-	2.50	2.50
Cottonseed meal	-	-	2.50	2.50
C-molasses	2.50	2.50	2.50	2.50
Tri calcium phosphate	1.75	0.69	1.74	0.69
Limestone	0.56	0.79	0.54	0.78
Salts	0.31	0.31	0.25	0.25
Trace mineral premix ¹	0.20	0.20	0.20	0.20
L-lysine	0.19	0.19	0.21	0.22
Vitamin premix ²	0.12	0.12	0.12	0.12
Mecadox	0.10	0.10	0.10	0.10
Endopower ^{®*}	-	0.10	-	0.10
Natuphos ^{®**}	-	0.10	-	0.10
Tiamulin	0.10	0.10	0.10	0.10
CTC	0.10	0.10	0.10	0.10
Choline chloride (25%)	0.05	0.05	0.05	0.05
DL-methionine (50%)	0.12	0.12	0.10	0.10
Total	100.00	100.00	100.00	100.00
Calculated analysis (%)				
ME (kcal/kg)	3,300	3,300	3,300	3,300
Crude protein	20.50	20.50	20.50	20.50
Calcium	0.90	0.65	0.90	0.65
Total phosphorus	0.70	0.51	0.71	0.53
Available phosphorus	0.40	0.22	0.40	0.22
Lysine	1.30	1.30	1.30	1.30
Total sulfur amino acids	0.72	0.72	0.72	0.72

¹ Supplied per kg diet: 162 mg Fe, 96 mg Cu, 72 mg Zn, 47 mg Mn, 0.9 mg I, 0.9 mg Co, 0.3 mg Se.

² Supplied per kg diet: 9,600 IU vitamin A, 1,800 IU vitamin D₃, 24 mg vitamin E, 1.5 mg vitamin B₁, 12 mg vitamin B₂, 2.4 mg vitamin B₆, 0.045 mg vitamin B₁₂, 1.5 mg vitamin K₃, 24 mg pantothenic acid, 45 mg niacin, 0.09 mg D-biotin, 0.75 mg folic acid, 18 mg Ethoxyquin.

* 0.1% of phytase (Natuphos[®]; 500 FTU/kg diet) and 0.1% of carbohydrases (Endopower[®]) were supplemented.

less expensive protein sources in swine diet formulation can provide an avenue for reducing the cost of feed production.

It is expected that supplementation of simultaneous phytase and carbohydrases targeted to corn-SBM based diets will improve growth performance and nutrient utilization of pigs by degradation of ANFs such as phytate, α -galactosides, galactomannans and non starch polysaccharides (NSP). It is also expected that RSM and CSM can be partially used in growing pig diet formulation without deterioration of growth performance if phytase and carbohydrases addition affects positively on nutritional values of RSM and CSM.

Therefore, this study was conducted to investigate the effects of microbial phytase (Natuphos[®]) supplementation

Table 2. Formula and chemical composition of experimental diets (4-7 wk) for growth trial

Diet types and enzymes	Corn-SBM based diets		Complex diets	
	-	+	-	+
Ingredients (%)				
Corn	69.99	59.88	58.55	59.64
Soy bean meal	30.68	30.68	26.24	26.04
Animal fat	3.00	3.00	3.65	3.23
Rape seed meal	-	-	3.00	3.00
Cotton seed meal	-	-	3.00	3.00
C-molasses	2.50	2.50	2.50	2.50
Tri calcium phosphorus	1.51	0.63	1.50	0.62
Limestone	0.55	0.77	0.53	0.75
Salts	0.30	0.30	0.30	0.30
Trace mineral premix ¹	0.20	0.20	0.20	0.20
L-lysine	0.10	0.10	0.14	0.14
Vitamin premix ²	0.11	0.11	0.11	0.11
Olaquinox	0.10	0.10	0.10	0.10
Endopower ^{®*}	-	0.10	-	0.10
Natuphos ^{®**}	-	0.10	-	0.10
CTC	0.10	0.10	0.10	0.10
Choline chloride (25%)	0.04	0.04	0.04	0.04
DL-methionine (50%)	0.07	0.07	0.04	0.03
Total	100.00	100.00	100.00	100.00
Calculated analysis (%)				
ME (kcal/kg)	3,300	3,300	3,300	3,300
Crude protein	18.44	18.44	18.60	18.50
Calcium	0.80	0.60	0.80	0.60
Total phosphorus	0.64	0.48	0.66	0.51
Available phosphorus	0.35	0.20	0.35	0.20
Lysine	1.10	1.10	1.10	1.10
Total sulfur amino acids	0.65	0.65	0.65	0.65

¹ Supplied per kg diet: 150 mg Fe, 96 mg Cu, 72 mg Zn, 47 mg Mn, 0.9 mg I, 0.9 mg Co, 0.3 mg Se.

² Supplied per kg diet: 9,600 IU vitamin A, 1,800 IU vitamin D₃, 24 mg vitamin E, 1.5 mg vitamin B₁, 12 mg vitamin B₂, 2.4 mg vitamin B₆, 0.045 mg vitamin B₁₂, 1.5 mg vitamin K₃, 24 mg pantothenic acid, 45 mg niacin, 0.09 mg D-biotin, 0.39 mg folic acid, 7.2 mg ethoxyquin.

* 0.1% of phytase (Natuphos[®]; 500 FTU/kg diet) and 0.1% of carbohydrases (Endopower[®]) were supplemented.

in combination with carbohydrases (composed of enzymes targeted to SBM dietary components such as α -galactosides, galactomannans and NSP: Endo-Power[®]) to corn-SBM based diet (CSD) and complex diet (CD) with a partial replacement of SBM with RSM and CSM on growth performance and nutrient digestibility of growing pigs and to establish economic implications for the simultaneous inclusion of those two enzyme products in CSD and CD for growing pigs.

MATERIALS AND METHODS

Enzyme preparations

The following enzymes were used in the present study. The microbial phytase (a 10% dilution form of Natuphos[®] 5,000 granulate, BASF, Germany) contained 500 FTU/g

phytase activity. One unit phytase (FTU) is defined as the quantity of enzyme that release 1 µmol inorganic phosphorus/min from 0.00015 mol/L sodium phytase at pH 5.5 at 37°C. The carbohydrases preparation contained 7 unit/g α-galactosidase activity, 22 unit/g galactomannanase activity, 300 unit/g xylanase activity and 220 unit/g β-glucanase activity (Endo-Power[®], EASY BIO System, Inc., Seoul, Korea). One unit of α-galactosidase is defined as the amount of enzyme that liberates 0.1 µmol nitro phenol from 2 mmol of pNPG (p-nitrophenyl-alpha-d-galactoside) per minute at 30°C and pH 4.0. One unit of galactomannase is defined as the amount of enzyme that liberates 0.1 µmol total reducing sugars/min from 0.5% galactomannan at 30°C and pH 4.0. One unit of xylanase is defined as the amount of enzyme that liberates 1 mg total reducing sugar/10 min. from 0.5% xylan at 30°C and pH 4.0. One unit of β-glucanase is defined as the amount of enzyme that liberates 1 mg of total reducing sugar per 10 min. from 0.4% β-glucan at 30°C and pH 4.0.

General experimental procedure

A total of 168 growing pigs averaging 13.18±1.77 kg of

initial body weight was assigned to the feeding trial. Growing period (13.18 to 39.81 kg) was divided into two phase (phase I: 0 to 3 weeks; phase II: 4 to 7 weeks). Experiment was arranged as a 2×2 factorial design with main effects of diet types (corn-soy bean meal based diet (CSD) or complex diets (CD: 5% of SBM was replaced with 2.5% of RSM and 2.5% of CSM in diet for phase I and 6% of SBM was replaced with 3% of RSM and 3% of CSM in diet for phase II and enzyme supplementation (none or 0.1% of phytase (500 FTU/kg diet) and 0.1% of carbohydrases in the diet). Each treatment had three replicates with 14 pigs per replicate.

The formulas and chemical composition of experimental diets are presented in Tables 1, 2.

The diets were formulated to have 3,300 kcal of ME/kg and 1.3% lysine for phase I and 3,300 kcal of ME/kg and 1.1% lysine for phase II. All other nutrients met or exceeded NRC (1998) requirements. Pigs were housed in concrete floored pens, equipped with a self feeder and a nipple waterer, and allowed *ad libitum* access to feed and water throughout the experimental period.

In the present studies, it was assumed that phytase (Natuphos[®]) would increase aP content 0.18%. Therefore,

Table 3. Formula and chemical composition of experimental diets for ileal amino acid digestibility trial

Diet types and enzymes	Soybean meal		Cottonseed meal		Rapeseed meal	
	-	+	-	+	-	+
Ingredients (%)						
Soybean meal	42.84	42.64	-	-	-	-
Cottonseed meal	-	-	59.29	59.09	-	-
Rapeseed meal	-	-	-	-	58.99	58.79
Corn starch	40.49	40.49	20.45	20.45	21.42	21.42
Glucose	10.00	10.00	10.00	10.00	10.00	10.00
Animal fat	2.25	2.25	6.00	6.00	6.00	6.00
Tri calcium phosphorus	1.91	1.91	0.27	0.27	0.34	0.34
Limestone	0.52	0.52	2.00	2.00	1.26	1.26
Salt	0.30	0.30	0.30	0.30	0.30	0.30
Trace mineral premix ¹	0.24	0.24	0.24	0.24	0.24	0.24
Vitamin premix ²	0.10	0.10	0.10	0.10	0.10	0.10
Olaquinox	1.00	1.00	1.00	1.00	1.00	1.00
Endopower ^{**}	-	0.10	-	0.10	-	0.1
Natuphos ^{**}	-	0.10	-	0.10	-	0.10
Choline chloride (25%)	0.10	0.10	0.10	0.10	0.10	0.10
Cr ₂ O ₃	0.25	0.25	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00	100.00	100.00
Calculated analysis (%)						
ME (kcal/kg)	3,300	3,300	2,908	2,908	3,005	3,005
Crude protein	20.00	20.00	20.00	20.00	20.00	20.00
Calcium	0.90	0.90	0.90	0.90	0.90	0.90
Total phosphorus	0.60	0.60	0.60	0.60	0.60	0.60
Available phosphorus	0.44	0.44	0.16	0.16	0.17	0.17
Lysine	1.11	1.11	0.86	0.86	1.24	1.24
Total sulfur amino acids	0.52	0.52	0.60	0.60	0.94	0.94

¹ Supplied per kg diet: 162 mg Fe, 96 mg Cu, 72 mg Zn, 47 mg Mn, 0.9 mg I, 0.9 mg Co, 0.3 mg Se.

² Supplied per kg premix: 9,600 IU vitamin A, 1,800 IU vitamin D3, 24 mg vitamin E, 1.5 mg vitamin B1, 12 mg vitamin B2, 2.4 mg vitamin B6, 0.045 mg vitamin B12, 1.5 mg vitamin K3, 24 mg pantothenic acid, 45 mg niacin, 0.09 mg D-biotin, 0.75 mg folic acid, 18 mg ethoxyquin.

* 0.1% of phytase (Natuphos[®]; 500 FTU/kg diet) and 0.1% of carbohydrases (Endopower[®]) were supplemented.

Table 4. Chemical composition and amino acid composition of SBM, RSM and CSM used for ileal amino acid digestibility trial

Items	Soybean meal	Rapeseed meal	Cottonseed meal
Chemical composition (As fed basis)			
Gross energy (kcal/kg)	4,434	4,470	4,404
Crude protein (%)	46.65	33.90	33.73
Crude fat (%)	1.04	0.81	1.05
Crude ash (%)	6.07	7.92	6.89
Calcium (%)	0.55	0.36	0.34
Total phosphorus (%)	0.64	1.11	0.89
Amino acid composition			
Essential amino acids (%)			
Arginine	2.35	1.93	0.98
Histidine	1.51	0.66	0.88
Isoleucine	2.42	1.23	1.09
Leucine	3.08	2.51	2.03
Methionine	0.49	0.44	0.57
Lysine	2.25	1.52	1.61
Phenylalanine	2.63	1.57	1.83
Threonine	1.23	1.27	1.07
Valine	2.19	1.53	1.48
Sub-total (%)	18.77	12.76	12.12
Non-essential amino acids (%)			
Cystine	0.62	0.10	0.59
Alanine	1.57	1.47	1.46
Aspartic acid	2.92	2.34	1.53
Glutamic acid	6.94	4.34	6.63
Glycine	1.66	1.66	1.45
Proline	1.61	2.07	1.40
Serine	1.74	2.15	1.58
Tyrosine	1.72	0.97	1.11
Sub-total (%)	18.15	15.00	15.17
Total amino acids (%)	36.92	27.76	27.29

dietary requirements for aP were reduced 0.18%. Calcium to total phosphorus ratios of approximately 1.3:1.0 were specified for all experimental diets. As a result, calcium and aP levels in experimental diet formulations with phytase and carbohydrases was reduced by approximately 27% compared to diets without enzymes supplementation.

During the growth trial, on d 15 and 30, chromium oxide (Cr_2O_3) was added at 0.25% of the diet to serve as an indigestible marker. Fresh fecal samples were taken from each pig (14 pigs) in one pen per dietary treatment on d 18 and 33 and frozen for later analyses. Feed and lyophilized feces were analyzed for gross energy by bomb calorimetry (Parr 1261 Isoperibol Calorimeter, Parr Instruments, Moline, USA).

Ileal digestibility trial

To determine phytase and carbohydrases addition effect on ileal amino acid digestibility of SBM, RSM and CSM, a total of 18 T-cannulated pigs (initial body weight: 13.52 ± 1.24 kg) were assigned to six dietary treatments in the present study. Dietary treatments in metabolic trial included 1) SBM diet (SBM based diet), 2) SBM diet+with

enzymes (phytase and carbohydrases at 0.1%, respectively), 3) CSM diet (CSM based diet) 4) CSM diet+enzymes, 5) RSM diet (RSM based diet) and 6) RSM diet+enzymes. Each basal diets were semipurified diets and contained only SBM or RSM or CSM as the protein source (Table 3). Corn starch, glucose and animal fat were used as energy sources. Amino acid composition of SBM, RSM and CSM used in the present study is presented in Table 4.

There were 5 d adjustment period and 3 d collection period. All surgical procedure were previously described by Giesting and Easter (1991). Digesta were collected from 08:00 to 20:00 during collection period. Sterile sampling bags (Fisger Scientific Pittsburgh PA) were used to collect digesta from cannulated pigs. After removing a cap from the cannula, a plastic bag was attached to cannula barrel. Collected samples were place on plastic containers and stored at -10°C for chemical analysis. All samples were freeze-dried. Freeze-dried samples were ground using 1 mm Wiley mill and used for measuring nutrient composition.

Analysis of the experimental diets and excreta was done according to the methods of the AOAC (1990). The amino acid content of the experimental feed and excreta was determined following acid hydrolysis with 6 N HCl at 110°C for 24 h, using an amino acid analyzer (Biochrom 20, Pharmacia Biotech, England). Sulfur-containing amino acids were analyzed after cold performic acid oxidation (Moore, 1963) overnight with subsequent hydrolysis.

Statistical analysis of the data

Statistical analysis was carried out by comparing means according to Duncan's Multiple Range Test (Duncan, 1955), using the General Linear Model (GLM) procedure of SAS (1985). Data were analyzed as a randomized complete block design. Pigs were blocked by initial weight with pen as the experimental unit. Factorial analysis method was used to locate the interactive effect between diet types (CSD and CD) and enzyme treatments (none and phytase and carbohydrases addition).

RESULTS AND DISCUSSION

Growth performance

Table 5 showed the effects of simultaneous phytase and carbohydrases supplementation to corn-SBM based diet (CSD) and complex diet (CD) with a partial replacement of SBM with RSM and CSM on growth performance of growing pigs

During the phase I (0 to 3 weeks), there was no significant difference in any criteria of growth performance among dietary treatments.

During the phase II (4 to 7 weeks), G:F (gain/feed ratio) of pigs fed CSD was higher ($p < 0.05$) than that of pig fed CD and simultaneous phytase and carbohydrases

Table 5. Effects of simultaneous phytase and carbohydrases supplementation to corn-SBM based diet (CSD) and complex diet (CD) on growth performance of growing pigs

Diet types and enzymes ³	CSD*		CD		SE ²	Statistical significance ¹		
	-	+	-	+		Diets	Enzymes	D×E
Phase I (0-3 wk)								
ADG (g)	473	506	461	500	55.39	NS	NS	NS
ADFI (g)	831	838	842	841	90.22	NS	NS	NS
F/G	1.76	1.66	1.84	1.69	0.17	NS	NS	NS
Phase II (4-7 wk)								
ADG (g)	572	611	557	602	62.93	NS	NS	NS
ADFI (g)	1,230	1,236	1,250	1,276	128.23	NS	NS	NS
F/G	2.15 ^{ab}	2.02 ^c	2.24 ^a	2.12 ^{bc}	0.09	0.0164	0.0028	NS
Overall (0-7 wk)								
ADG (g)	529	566	516	559	58.53	NS	NS	NS
ADFI (g)	1,059	1,065	1,075	1,090	98.79	NS	NS	NS
F/G	1.98 ^{ab}	1.87 ^b	2.07 ^a	1.93 ^{ab}	0.11	NS	0.0427	NS

* CSD: Corn-soybean meal based diet; CD: complex diet. ^{a,b,c} Values with different superscripts of the same row are significantly differ (p<0.05).

¹ NS: Not statistically significant. p>0.05. ² Pooled standard error. ³ 0.1% of phytase (Natuphos[®]; 500 FTU/kg diet) and 0.1% of carbohydrases (Endopower[®]) were supplemented.

supplementation increased G:F of growing pigs (p<0.01). There was no interaction between diet types and enzyme treatments.

During the whole experimental period (0-7 weeks), average daily gain (ADG) was not significantly influenced by simultaneous phytase and carbohydrases supplementation but G:F of pigs was significantly increased by enzyme supplementation (p<0.05). There was no difference in growth performance between CSD and CD.

Simultaneous phytase and carbohydrases improved G:F of growing pigs fed diet with a 0.18% unit lower aP (p<0.05). This result is in good agreement with that in our previous study. Shim et al. (2003) reported that simultaneous phytase and carbohydrases supplementation (0.1%, respectively) increased G:F of piglets fed corn-SBM based diet with a 0.15% unit lower aP than control diet.

There have been many studies to show positive effect of phytase (Jongbloed, 1987; Cromwell et al., 1995; Kornegay et al., 1998) or carbohydrases supplementation (Kim et al., 2001a,b; Pettey et al., 2002), on growth performance of pigs fed corn-SBM based diets.

In the present study, it is not clear that positive effects on growth performance was mainly driven by phytase or in combination with carbohydrases because two enzymes were supplemented simultaneously to diets. The response of pigs to the addition of phytase can be markedly influenced by the level of supply. In the present study, 500 FTU/kg of phytase can be the lower dose of supply for the improvement of growing performance. In fact, the major result of the influence of the addition of phytase is an improvement in P digestibility. However, in such cases, it is clear that any process which will reduce ANF concentration by enzyme application will enhance performance and relax some of the constraint on feed formulation. The possibility exists that supplementation of swine diets with a

combination of phytase and carbohydrases will enhance nutrient utilization further and as a result, improve growth performance of pigs. Conceivably, one enzyme, by inactivating the anti-nutritional factors in gastro intestinal tract, may facilitate the action of another enzyme on target substrates and the absorption of liberated nutrients. This idea is supported by the results from a broiler study. Ravindran et al. (1999b) reported that the improvements in nitrogen and energy digestibilities in diets with the combination were much greater than those observed with the individual enzymes (phytase and xylanase). They suggested that these two feed enzymes could facilitate each other's activity by providing greater substrates access and thereby further reducing the antinutritive properties of phytates and non starch polysaccharides (NSPs). More recently, Selle et al. (2003) confirmed beneficial effects of simultaneous inclusion of phytase and xylanase in wheat-based broiler diets. They reported that the most pronounced improvements in growth performance were associated with the combined supplementation of phytase and xylanase in all three broiler experiments.

Based on the fact mentioned above, it appears that there might be some additive effects of combination of two enzymes on improvement of growth performance of pigs in the present study. It is also noteworthy that the experimental diets with enzyme combination were formulated to contain 0.18% unit lower aP compared to control diet in the present study.

In the present study, even though G:F of pigs fed CSD was higher than that of pig fed CD during the phase II (4 to 7 weeks), overall (0 to 7 weeks), no significant difference in growth performance was found between pigs fed CSD and those fed CD. One possible explanation for this growth response to diet types is the inclusion rate of RSM and CSM in diets (2.5 to 3%, respectively) might be too low to

Table 6. Effects of simultaneous phytase and carbohydrases supplementation to corn-SBM based diet (CSD) and complex diet (CD) on fecal nutrient digestibility and nutrient excretion of growing pigs

Diets and enzymes ³	CSD*		CD		SE ²	Statistical significance ¹		
	-	+	-	+		Diets	Enzyme	D×E
Fecal Nutrient digestibilities								
Phase I (0-3 wk)								
Dry matter	79.00	81.08	78.90	80.17	1.51	NS	NS	NS
Gross energy	79.85	80.43	78.55	80.12	1.13	NS	NS	NS
Crude protein	76.88 ^b	79.86 ^a	76.51 ^b	78.78 ^{ab}	1.90	NS	0.0146	NS
Ether extract	63.87	68.50	62.22	65.41	4.18	NS	NS	NS
Calcium	57.09	64.00	56.81	58.96	6.48	NS	NS	NS
Phosphorus	39.42 ^b	54.99 ^a	37.84 ^b	49.49 ^{ab}	9.96	NS	0.0164	NS
Phase II (4-7 wk)								
Dry matter	80.50 ^b	81.60 ^a	79.53 ^c	81.45 ^a	0.92	0.0241	0.0001	NS
Gross energy	80.86	81.87	80.44	81.66	0.92	NS	0.0439	NS
Crude protein	77.29 ^b	78.91 ^a	76.59 ^b	78.71 ^a	1.07	NS	0.0001	NS
Ether extract	79.73 ^{ab}	81.12 ^a	77.94 ^b	81.00 ^a	1.86	NS	0.0340	NS
Calcium	55.39 ^{ab}	61.16 ^a	49.53 ^b	59.47 ^{ab}	6.60	NS	0.0371	NS
Phosphorus	45.35 ^{ab}	50.66 ^a	39.92 ^b	48.15 ^{ab}	5.56	NS	0.0262	NS
Nutrient excretion (kg/head/49d)**								
Dry matter	9.17 ^b	8.56 ^c	9.66 ^a	8.96 ^b	0.46	0.0096	0.0010	NS
Nitrogen	0.44 ^a	0.40 ^b	0.45 ^a	0.42 ^a	0.02	NS	0.0017	NS
Calcium	0.28 ^a	0.18 ^b	0.30 ^a	0.19 ^b	0.06	NS	0.0001	NS
Phosphorus	0.24 ^a	0.17 ^b	0.26 ^a	0.20 ^b	0.04	0.0430	0.0001	NS

* See Table 5 for abbreviation. ** Nutrient excretion was calculated based on fecal nutrient digestibilities during phase I and II. ^{a,b,c} Values with different superscripts of the same row are significantly differ ($p < 0.05$). ¹ NS: Not statistically significant, $p > 0.05$. ² Pooled standard error. ³ 0.1% of phytase (Natuphos[®]; 500 FTU/kg diet) and 0.1% of carbohydrases (Endopower[®]) were supplemented.

negatively affect the growth response and nutrient digestibility of piglets. Because of the high fiber content and ANFs of CSM and RSM and limited information on its effect on growth performance, the use of CSM and RSM in pig diets has been limited. In the literature various recommendations for inclusion levels of RSM and CSM in diets for piglets are given. These recommended levels are variable depending on the varieties used (e.g. high- or low-ANFs, such as glucosinolate and gossypol, cultivars, cultivated countries). Henkel and Mosenthin (1989) reported that the recommended levels of RSM for piglets diet should be less than 5% and Bell (1984) concluded that RSM can be used by 10% in piglet diets. Aherne and Kennelly (1985) reported that diets for piglets may contain up to 15% of rape seed meal, whereas Thacker (1990) recommended an inclusion rate of up to 5% in starter diets and 10% in grower-finisher and sow diets. Various reviews about the use of CSM for feeding pigs are also available (Buitrago et al., 1977; Aherne and Kennelly, 1983; Tanksley, 1990), one of the most practical suggestions is to include less than 10% of CSM in diets for young pigs, increasing this amount in older animals and breeding stock (Buitrago et al., 1977).

Fecal nutrient digestibility and nutrient excretion

During the phase I (0 to 3 weeks), there was no difference in overall fecal nutrient digestibilities between CSD and CD ($p > 0.05$). However, simultaneous phytase and

carbohydrases supplementation significantly increased crude protein (CP) and phosphorus (P) digestibilities of growing pigs ($p < 0.05$).

During the phase II (4 to 7 weeks), dry matter (DM) digestibility of pigs fed CSD was higher than that of pig fed CD ($p < 0.05$) but there was no difference in other nutrient digestibilities between CSD and CD ($p > 0.05$). However, simultaneous phytase and carbohydrases supplementation significantly increased all nutrient digestibilities (DM, gross energy (GE), CP, crude fat (CF), calcium (Ca) and P) of pigs ($p > 0.05$).

These results are in good agreement with those of our previous study (Shim et al., 2003). In a previous study, simultaneous phytase and carbohydrases supplementation to corn-SBM based diet with a 0.15% lower aP than control diet increased apparent DM, GE, CP and P digestibilities of weaned pigs (Table 6).

There have been many researchers who found improvement in P, Ca, energy and amino acid digestibilities when pigs were fed a corn-SBM based diet supplemented with microbial phytase or carbohydrases, individually. In a previous study using same enzyme product with one used in the present study, Kim et al. (2001a) found that supplementing carbohydrases improved ileal gross energy and amino acids digestibility by 7% and 3%, respectively in weaned piglets.

A large number of data evaluating the use of microbial phytase reported that improved bioavailability of P, calcium,

Table 7. Effects of simultaneous phytase and carbohydrases supplementation to SBM, RSM and CSD based diets on apparent ileal amino acid digestibility of growing pigs

Diet types and enzymes ²	SBM		RSM		CSM		SE ¹
	-	+	-	+	-	+	
Essential amino acids							
Arg	84.23 ^b	87.60 ^a	73.37 ^d	77.31 ^c	71.79 ^d	76.31 ^c	5.97
His	80.56 ^{ab}	84.53 ^a	73.56 ^c	77.30 ^{bc}	74.36 ^c	76.50 ^{bc}	4.52
Ile	79.75 ^a	80.96 ^a	67.14 ^b	71.65 ^b	65.82 ^b	68.82 ^b	6.84
Leu	74.91 ^{ab}	79.03 ^a	67.71 ^{bc}	70.73 ^{abc}	65.70 ^c	71.09 ^{abc}	5.87
Lys	78.26 ^{ab}	80.25 ^a	66.19 ^c	73.76 ^{abc}	67.33 ^{bc}	72.76 ^{abc}	7.32
Phe	79.07 ^a	83.35 ^a	66.79 ^b	69.42 ^b	67.47 ^b	70.67 ^b	6.94
Thr	74.64 ^{ab}	80.19 ^a	65.04 ^b	69.11 ^{ab}	67.42 ^b	73.91 ^{ab}	7.28
Val	79.59 ^a	83.89 ^a	67.61 ^b	70.26 ^b	69.48 ^b	71.73 ^b	6.97
Met	78.49 ^{ab}	82.49 ^a	68.33 ^{bc}	74.48 ^{abc}	67.89 ^c	73.44 ^{abc}	6.98
Sub-mean	78.83 ^b	82.48 ^a	68.42 ^d	72.67 ^c	68.58 ^d	72.80 ^c	6.52
Non-essential amino acids							
Ala	78.17 ^a	83.70 ^a	68.86 ^{ab}	70.92 ^{ab}	62.68 ^b	69.86 ^{ab}	9.50
Asp	73.58 ^{ab}	78.85 ^a	61.70 ^d	65.38 ^{cd}	65.16 ^{cd}	68.87 ^{bc}	6.61
Glu	78.02 ^b	82.57 ^a	71.54 ^c	74.72 ^{bc}	73.14 ^c	75.37 ^{bc}	4.17
Gly	69.04 ^{ab}	73.98 ^a	61.60 ^b	66.50 ^{ab}	63.36 ^b	67.54 ^{ab}	5.79
Pro	77.34 ^{ab}	82.78 ^a	66.94 ^c	70.17 ^c	72.13 ^{bc}	76.77 ^{ab}	6.02
Ser	79.55 ^{ab}	84.18 ^a	68.60 ^c	73.23 ^{bc}	73.16 ^{bc}	78.33 ^{ab}	5.96
Tyr	78.15 ^{ab}	82.33 ^a	69.52 ^b	73.33 ^b	72.69 ^b	73.21 ^b	5.80
Sub-mean	76.27 ^b	81.20 ^a	66.97 ^c	70.61 ^{cd}	68.90 ^{de}	72.85 ^c	6.26
Total-mean	77.69 ^b	81.91 ^a	67.77 ^d	71.75 ^c	68.73 ^d	72.82 ^c	5.24

* See Table 5 for abbreviation. ^{a, b, c, d, e} Values with different superscripts of the same row are significantly differ (p<0.05). ¹ Pooled standard error. ² 0.1% of phytase (Natuphos[®]; 500 FTU/kg diet) and 0.1% of carbohydrases (Endopower[®]) were supplemented.

zinc, protein/amino acids and energy were found when adequate amounts of phytase (500 FTU/kg feed) was supplemented to pig diets (Kornegay and Qian, 1996; Yi et al., 1996; Kornegay et al., 1998). It has been known that supplementation of pig diets with microbial phytase increase the availability of dietary phosphorus in the order of 26.5% to 44.2% (Mroz et al., 1994; Mroz and Jongbloed, 1998). The similar result also occurred in this digestibility study. Dietary phytase and carbohydrases supplementation increased phosphorus digestibility in order of 16% to 35%. This increase comes from the effect of phytase on phytate phosphorus, which has a typical digestibility value of 30% in young pigs (Jongbloed and Kemme, 1990).

In the present study, nutrient excretion was calculated based on results of fecal nutrient digestibility. Dietary phytase and carbohydrases supplementation reduced significantly total DM, nitrogen, Ca and P excretion per pig during the whole experimental period (p<0.05%). These nutrient excretion responses are basically related to improvement in nutrient digestibility by supplementation of phytase and carbohydrases.

Ileal amino acid digestibilities

Table 7 showed the effect of simultaneous phytase and carbohydrases supplementation to SBM, RSM and CSM based diets on ileal amino acid digestibilities of growing pigs. Simultaneous phytase and carbohydrases supplementation improved significantly ileal essential

amino acid (EAA) as well as nonessential amino acid (NEAA) digestibilities in SBM, RSM and CSM based diets (p<0.05). It was observed that ileal EAA and NEAA digestibilities of SBM based diet was much higher than those of RSM and CSM based diet. RSM based diet had a similar ileal EAA and NEAA digestibilities with CSM based diet.

This result is in good agreement with those from other studies. Sauer and Thacker (1986) reported that amino acid digestibilities of RSM are lower than SBM. Imbeah and Sauer (1991) reported that averaged ileal EAA digestibilities in growing pigs fed SBM and RSM based diets was 82.5% and 75.4% respectively. Prawirodigdo et al. (1997) reported that the availability of ileal digestible nitrogen from CSM is low compared with SBM.

Many explanations for the lower digestibility of RSM and CSM compared to SBM have been proposed, centering on fiber, unpalatable tannins, lignins, pectins, cellulose (de Lange et al., 1990) and glucosinolates and gossypol. Fiber alone induces a faster passage rate which reduces the opportunity for digestion to some degree (Imbeah and Sauer 1991). Lignin occurring mostly in the hulls may decrease protein digestibility by hydrophobic binding of amino acids and increasing feed passage. RSM (rapeseed meal) hulls contain significant non-lignin non-extractable polyphenyls (tannin type) that may also interfere with digestion. Pectins, by forming a gel matrix, may restrict proteolytic access. Fiber may also increase endogenous N loss through

Table 8. Economic analysis for the simultaneous inclusion of phytase and carbohydrases in CSD and CD diets with a low aP for growing pigs (0 to 7 weeks)*

Diet types and enzymes ²	CSD**		CD		SE ¹
	-	+	-	+	
TWG ³ (kg/pig)	25.94	27.73	25.29	27.38	2.87
TFC ⁴ (USD/pig)	10.02	10.37	10.11	10.48	0.96
FCG (USD /pig)	0.386	0.373	0.399	0.382	0.017

* Not significant. ** See table 5 for abbreviation.

¹ Pooled standard error. ² 0.1% of phytase (Natuphos[®]; 500 FTU/kg diet) and 0.1% of carbohydrases (Endopower[®]) were supplemented.

³ Abbreviations: TWG=Total weight gain per pig, TFC=Total feed cost per pig, FCG=Feed cost/kg body weight gain.

⁴ Averaged feed production cost for each treatment diet (phase I and phase II) were 193.25 USD/ton for CSD without enzymes, 198.58 USD/ton for CSD with enzymes, 192 USD/ton for CD without enzymes and 196.25 USD/ton for CD with enzymes, respectively.

abrasive effects or binding endogenous protein (Mitaru et al., 1984; Sauer and Thacker 1986; de Lange et al., 1990; Imbeah and Sauer, 1991). Grala et al. (1997) found low apparent and true ileal digestibilities resulted from a high flow of both endogenous N (nitrogen) and undigested dietary N in young pigs (12-25 kg) fed RSM.

In the present study, it appears that the positive amino acid digestibility response to the enzyme combination (phytase and carbohydrases) were largely driven by enzymatic inactivation of ANFs such as phytate, α -galactosides, galactomannans and non starch polysaccharides (NSP) in SBM, RSM and CSM. It is known that undigested α -galactosides and β -mannans are used by microflora at the lower intestines and finally produced extra gases (Calloway et al., 1966) and negatively related to energy and amino acid digestibility in swine (Veldman et al., 1993; Gdala et al., 1997). It is well known that phytic acid can also reduce amino acid digestion and absorption through phytate-protein/amino acid or phytate-mineral-protein/amino acid complexes and can interfere with intestinal pepsin and trypsin enzyme activity (Nair et al., 1991; Caldwell, 1992; Ravindran et al., 1999a,b).

Economic analysis

Table 8 summarized economic analysis data for the simultaneous inclusion of phytase and carbohydrases in CSD and CD with a low aP for growing pigs. As shown in the table, the simultaneous inclusion of phytase and carbohydrases in both of CSD and CD reduced feed cost per kg body weight gain (FCG). Pigs fed CDS had a tendency to have lower FCG than those fed CD. This result suggests that dietary enzymatic enhancement with phytase and carbohydrases may contribute to increase economic benefits when added to corn-SBM based diet or complex diet containing partially RSM and CSM as protein sources for growing pigs.

In conclusion, the data from this study indicates that the simultaneous inclusion of phytase and carbohydrases to

diets at 0.1% level, respectively, is advantageous with respect to improving growth performance and nutrient digestibility of growing pigs and may contribute to increased economic return when added to corn-SBM based diet or complex diet for growing pigs. Our data clearly suggest that application of both of phytase and carbohydrases in growing pig diets can be a good way to reduce negative impact of pig production on environment via a decrease in the excretion of N and P. Also, our results suggest that 2.5 to 3% of RSM and CSM, respectively might be used as a protein source in growing pig diets without having an adverse effect on the growth performance and nutrient digestibility and simultaneous phytase and carbohydrases addition improves nutritional value of SBM, RSM and CSM by improving ileal amino acid digestibilities.

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