Effects of Phytase and Enzyme Complex Supplementation to Diets with Different Nutrient Levels on Growth Performance and Ileal Nutrient Digestibility of Weaned Pigs

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ABSTRACT : An experiment was conducted to investigate the effect of microbial phytase (Natuphos®) supplementation in combination with enzyme complex (composed of enzymes targeted to SBM dietary components such as α-galactosides and galactomannans: Endo-Power®) to diet with low nutrient levels on growth performance and ileal nutrient digestibility of weaned pigs. A total of 210 crossbred weaned pigs (Landrace×Yorkshire×Duroc), 6.68±0.98 kg of initial body weight, were randomly allotted to five dietary treatments, based on weight and age, according to a randomized complete block design. There were three pens per treatment and 14 pigs per pen. The dietary treatments were 1) CON (Control diet with no phytase and enzyme complex (EC)), 2) LP+EC 100 (Control diet with 0.15% unit lower available phosphorus (aP) level+0.1% phytase (500 FTU/kg diet) and 0.1% enzyme complex), 3) LP+EC 80 (Control diet with 0.15% unit lower aP level+0.08% phytase (400 FTU/kg diet) and 0.08% enzyme complex, 4) LPEA+EC 100 (Control diet with 0.15% unit lower aP and 3% lower ME and amino acid levels (lysine, methionine, threonine and typtophan)+0.1% phytase (500 FTU/kg diet) and 0.1% enzyme complex), 5) LPEA+EC 80 (Control diet with 0.15% unit lower aP and 3% lower ME and amino acid levels+0.08% phytase (400 FTU/ kg diet) and 0.08% enzyme complex). For the determination of ileal nutrients digestibility, a total of 15 T-cannulated pigs (initial body weight; 7.52±1.24 kg; 3 replicates per treatment) were used in the present study. Piglets were weighted and allotted into same dietary treatments as one in growth trial and phase I experimental diets were provided for ileal digestibility study. There was no significant difference ($p>0.05$) in average daily gain (ADG) and average daily feed intake (ADFI) among dietary treatments during the whole experimental period (0 to 5 weeks). However, piglets in LP+EC 100 group had a significantly higher gain/feed ratio (G:F) than piglets had in control ($p<0.05$). Crude protein, energy and phosphorus digestibilities were significantly improved when both of phytase and enzyme complex were supplemented at the revel of 0.1%, respectively to diets with low nutrient level (aP or (and) ME and amino acids) ($p<0.05$). Piglets in LP+EC 100 and LPEA+EC 100 groups showed significantly higher phosphorus content (%) in bone than that of piglets in control group ($p<0.05$). Supplementation of both of phytase and enzyme complex at 0.1%, respectively, to diet with low nutrient levels (aP or (and) ME and amino acids) significantly improved total ileal essential amino acid and nonessential amino acid digestibilities compared to control group ($p<0.05$). In conclusion, the results from the present study suggest that the simultaneous inclusion of phytase and enzyme complex to diets at recommended level is advantageous with respect to improving growth performance and nutrient digestibility of weaned pigs and may contribute to increased economic return when added to corn-soy based weaned pig diets. ***(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 4 : 523-532)***

Key Words : Phytase, Enzyme Complex, Growth Performance, Ileal Digestibility, Weaned Pigs

INTRODUCTION

Recently, with the advancements in enzyme-producing technology as well as a better understanding of the role of enzymes in animal nutrition, the use of enzymes in pig diets is becoming more widespread and contributes to the reduction of environmental pollutants from animal 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). Besides, current evidence shows that there are the additional benefits of improved amino acids and energy utilization (Ravindran et al., 1999ab). However, not all of the data indicate that phytase affects AA and energy availability. Biehl and Baker (1997) reported no effect of phytase on true nitrogen-corrected ME in cecectomized roosters fed dehulled SBM.

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. SBM contains approximately 22% non starch polysaccharides (NSP) and of NSPs, galactosyl oligosaccharides, i.e., α-galactosides and galactomannans, known as flatulence-producing factors are relatively highly contained in SBM. Those of aniti-nutritional factors (ANF) are not digested by monogastric animals because they do not have endogenious enzyme system to degrade it. Thus, there is little doubt that the feeding value of soybean meal could be enhanced by the elimination or modification of some NSP (non starch polysaccharide) components (Coon et al., 1990; Leske et al., 1993). Therefore nowadays many

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Table 1. Explanation of enzyme treatments to diets with different nutrient levels in the present study

Treatments[1]	Phytase (g/ton)	Enzyme complex (g/ton)	Down spec levels		
			aP	ME	Amino acids (Major-limiting amino acids)[2]
Control	-	-	-	-	-
LP+EC 100	1,000	1,000	0.15%	-	-
LP+EC 80	800	800	0.15%	-	-
LPEA+EC 100	1,000	1,000	0.15%	3%	3%
LPEA+EC 80	800	800	0.15%	3%	3%

[1] CON=Control diet with no phytase (Natuphos®) and enzyme complex (EC; Endo-Power®). LP+EC 100=Control diet with 0.15% unit lower available phosphorus (aP) level+0.1% phytase (500 FTU/kg diet) and 0.1% enzyme complex. LP+EC 80=Control diet with 0.15% unit lower aP level+0.08% phytase (400 FTU/kg diet) and 0.08% enzyme complex. LPEA+EC 100=Control diet with 0.15% unit lower aP and 3% lower ME and amino acid levels+0.1% phytase (500 FTU/kg diet) and 0.1% enzyme complex. LPEA+EC 80=Control diet with 0.15% unit lower aP and 3% lower ME and amino acid levels+0.08% phytase (400 FTU/kg diet) and 0.08% enzyme complex.

[2] Lysine, methionine, threonine and typtophan.

nutritionists have focused on use of enzymes targeted to SBM dietary components in diet formulations for swine to improve nutritional values of SBM.

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. Since all enzymes have a substrate selectivity, conceivably, each enzyme, by inactivating the substrates (regarded as ANFs) specifically in gastro intestinal tract, may facilitate the action of another enzyme on target substrates and improve the nutritional value of raw materials in the feed. Therefore, it is expected that supplementation of both of phytase and enzyme complex targeted to SBM dietary components to corn-soy based diets improve growth performance and nutrient utilization of pigs by degradation of ANFs such as phytate, α-galactosides and galactomannans. Despite the increasing likehood of phytase and enzyme complex being used simultaneously in practical diet formulation, published reports on their combined application are limited.

Therefore, this study was conducted to investigate the effect of microbial phytase (Natuphos®) supplementation in combination with enzyme complex (Endo-Power® which is based on α-galactosidase, galactomannanase, beta-glucanase and xylanase enzymes) to diet with a low aP or (and) ME and amino acids on growth performance and ileal nutrient digestibility of weaned pigs and to establish economic implications for the simultaneous inclusion of phytase and enzyme complex in corn-soy based diets for weaned pigs.

MATERIALS AND METHODS

Enzyme preparation

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 which is commercially recommended inclusion rate for swine diets by BASF. 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 enzyme complex preparation (Endo-Power®, EASY BIO System, Seoul, Korea) contained 7 unit/g α-galactosidase activity, 22 unit/g galactomannanase activity, 300 unit/g xylanase activity and 220 unit/g β-glucanase activity. The commercially recommended inclusion rate of this enzyme product is 0.1% of diets for weaned pigs. 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 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 per 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

An experiment was conducted to investigate the effect of microbial phytase (Natuphos®) supplementation in combination with enzyme complex (Endo-Power®) to diet with different nutrient levels on growth performance and ileal nutrient digestibility of weaned pigs.

A total of 210 crossbred weaned pigs (Landrace× Yorkshire×Duroc), 6.68±0.98 kg of initial body weight, were randomly allotted to five dietary treatment, based on weight and age, according to a randomized complete block design. There were three pens per treatment and 14 pigs per pen.

The dietary treatments were 1) CON (Control diet with no phytase (Natuphos®) and enzyme complex (EC; Endo-Power®), 2) LP+EC 100 (Control diet with 0.15% unit lower available phosphorus (aP) level+0.1% phytase (500

Table 2. Formula and chemical composition of experimental diets (Phase I, 0-2 wk)

Item	Control[1]	LP+EC100	LP+EC80	LPEA+EC100	LPEA+EC80
Ingredients (%)					
Corn	31.66	32.74	32.78	36.57	36.61
SBM (dehulled)	23.52	23.60	23.60	21.72	21.72
Whey powder	20.00	20.00	20.00	20.00	20.00
Bakery byproduct	10.00	10.00	10.00	10.00	10.00
SDPP	5.00	5.00	5.00	5.00	5.00
Soy oil	4.44	3.93	3.93	2.00	2.00
Fish meal	2.00	2.00	2.00	2.00	2.00
MCP	0.88	0.17	0.17	0.17	0.17
Limestone	0.80	0.66	0.66	0.68	0.68
ZnO	0.34	0.34	0.34	0.34	0.34
Vitamin premix[2]	0.30	0.30	0.30	0.30	0.30
Trace mineral premix[3]	0.24	0.24	0.24	0.24	0.24
Acidifier	0.15	0.15	0.15	0.15	0.15
Apramycin	0.15	0.15	0.15	0.15	0.15
L-lysine	0.11	0.11	0.11	0.10	0.10
DL-methionine (50%)	0.11	0.11	0.11	0.08	0.08
Salt	0.10	0.10	0.10	0.10	0.10
Mecadox	0.10	0.10	0.10	0.10	0.10
Choline chloride (25%)	0.10	0.10	0.10	0.10	0.10
Endo-power®	-	0.10	0.08	0.10	0.08
Natuphos®	-	0.10	0.08	0.10	0.08
Total	100.00	100.00	100.00	100.00	100.00
Calculated chemical composition (%)					
ME (kcal/kg)	3,480	3,480	3,480	3,375	3,375
Crude protein	22.85	22.98	22.98	22.36	22.36
Calcium	0.80	0.63	0.63	0.63	0.63
Total phosphorus	0.67	0.52	0.52	0.52	0.52
Available phosphorus	0.45	0.30	0.30	0.30	0.30
Lysine	1.50	1.50	1.50	1.45	1.45
Total SAA	0.82	0.82	0.82	0.80	0.80

[1] See Table 1 for abbreviation.
[2] Supplied per kg diet: 9,600 IU vitamin A, 1,800 IU vitamin D_3, 24 mg vitamin E, 1.5 mg vitamin B_1, 12 mg vitamin B_2, 2.4 mg vitamin B_6, 0.045 mg vitamin B_{12}, 1.5 mg vitamin K_3, 24 mg Pantothenic acid, 45 mg Niacin, 0.09 mg D-Biotin, 0.75 mg Folic acid, 18 mg Ethoxyquin.
[3] Supplied per kg diet: 162 mg Fe, 96 mg Cu, 72 mg Zn, 46.49 mg Mn, 0.9 mg I, 0.9 mg Co, 0.3 mg Se.

FTU/kg diet) and 0.1% enzyme complex). 3) LP+EC 80 (Control diet with 0.15% unit lower aP level+0.08% phytase (400 FTU/kg diet) and 0.08% enzyme complex. 4) LPEA+EC 100 (Control diet with 0.15% unit lower aP and 3% lower ME and amino acid levels+0.1% phytase (500 FTU/kg diet) and 0.1% enzyme complex). 5) LPEA+EC 80 (Control diet with 0.15% unit lower aP and 3% lower ME and amino acid levels+0.08% phytase (400 FTU/kg diet) and 0.08% enzyme complex). Summary table of experimental design was shown in Table 1.

The formulas and chemical composition of experimental diets are presented in Table 2, 3. To allow an ideal balance of major limiting amino acids (Chung and Baker, 1992), the control diets were supplemented with a small quantity of synthetic amino acids. 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 0.15% and enzyme complex (Endo-Power®) would increase ME and amino acids content 3% by enzymatic improvement of nutrient availability based on the result from an enzyme study (Kim et al., 2001a), therefore, dietary requirements for aP, ME and animo acids were reduced 0.1, 3 and 3%, respectively. Calcium to total phosphorus ratios of approximately 1.2:1.0 were specified for diets containing both of phytase and carbohydrase enzymes. As a result, calcium levels in experimental diet formulations with enzymes is reduced by approximately 20% compared to control diets without enzymes.

During the growth trial, on d 10 and 20, 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 (6 pigs) in one pen per dietary treatment on d 13 and 23 and frozen for later analyses. Six fecal samples from each pen were pooled and pooled samples were used as

Table 3. Formula and chemical composition of experimental diets (Phase II 3-5 wk)

Item	Control[1]	LP+EC100	LP+EC 80	LPEA+EC100	LPEA+EC 80
Ingredients (%)					
Corn	54.14	55.25	55.29	57.47	57.51
Soy bean meal	36.44	36.24	36.24	36.00	36.00
Animal fat	4.29	3.84	3.84	2.00	2.00
Fish meal	2.00	2.00	2.00	2.00	2.00
Dicalcium phosphate	0.94	0.65	0.65	0.64	0.64
Limestone	1.01	0.63	0.63	0.63	0.63
Vitamin premix[2]	0.12	0.12	0.12	0.12	0.12
Trace mineral premix[3]	0.24	0.24	0.24	0.24	0.24
Acidifier	0.10	0.10	0.10	0.10	0.10
L-Lysine	0.10	0.11	0.11	0.05	0.05
DL-Methionine (50%)	0.07	0.07	0.07	-	-
Salt	0.30	0.30	0.30	0.30	0.30
Mecadox	0.10	0.10	0.10	0.10	0.10
Choline Chloride (25%)	0.05	0.05	0.05	0.05	0.05
Endo-power®	-	0.10	0.08	0.10	0.08
Natuphos®	-	0.10	0.08	0.10	0.08
CTC	0.10	0.10	0.10	0.10	0.10
Total	100.00	100.00	100.00	100.00	100.00
Calculated chemical composition (%)					
ME (kcal/kg)	3,400	3,400	3,400	3,300	3,300
Crude protein	21.50	21.51	21.51	21.48	21.48
Calcium	0.80	0.60	0.60	0.60	0.60
Total phosphorus	0.60	0.45	0.45	0.45	0.45
Available phosphorus	0.30	0.15	0.15	0.15	0.15
Lysine	1.30	1.30	1.30	1.26	1.26
Total SAA	0.74	0.74	0.74	0.71	0.71

[1] See Table 1 for abbreviation.
[2] Supplied per kg diet: 9,600 IU vitamin A, 1,800 IU vitamin D_3, 24 mg vitamin E, 1.5 mg vitamin B_1, 12 mg vitamin B_2, 2.4 mg vitamin B_6, 0.045 mg vitamin B_{12}, 1.5 mg vitamin K_3, 24 mg Pantothenic acid, 45 mg Niacin, 0.09 mg D-Biotin, 0.39 mg Folic acid, 7.2 mg Ethoxyquin.
[3] Supplied per kg diet: 150 mg Fe, 96 mg Cu, 72 mg Zn, 46.49 mg Mn, 0.9 mg I, 0.9 mg Co, 0.3 mg Se.

replication unit. Three samples per treatment were used for determination of digestibilities. Total Feed and lyophilized feces (three samples per treatment) were analyzed for gross energy by bomb calorimetry (Parr 1261 Isoperibol Calorimeter, Parr Instruments, Moline, USA).

For the determination of ileal nutrient digestibility, a total of 15 T-cannulated pigs (initial body weight; 7.52±1.24 kg; 3 replicates per treatment) were used in the present study. Piglets were weighted and allotted into same dietary treatments as one in growth trial and phase I experimental diets were provided for ileal digestibility study. 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 (Fisher Schentific Pittsburgh PA) were used to collect digesta from canulated pigs. After removing a cap from the cannula, a plastic bag was attached to cannula barrel. Collected samples (three samples per treatment) 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.

At the end of experiment, six piglets with similar body weight per dietary treatment (6 replicates per treatment) were randomly selected and killed and metatarsals was removed and frozen for later analysis of phosphorus (P) and calcium (Ca) in the bone. Ca was analyzed by atomic absorption spectrophotometry. Total P was quantified colorimetrically according to AOAC (1984) procedures.

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 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. Individual piglets were used in experimental unit for digestibility trial.

Table 4. The effect of phytase and enzyme complex supplementation in combination to diet with a low aP or (and) ME and amino acids on growth performance of weaning pigs

Items	Control[1]	LP+EC 100	LP+EC 80	LPEA+EC 100	LPEA+EC 80	SEM[2]
Starter (0-2 wk):						
ADG (g)	407	432	411	416	404	6.21
ADFI (g)	624	578	594	586	603	11.21
G:F	0.65^{a}	0.75^{b}	0.69^{ab}	0.71^{ab}	0.67^{ab}	0.02
Grower (3-5 wk):						
ADG (g)	495	527	504	514	488	10.08
ADFI (g)	796	739	764	755	760	16.15
G:F	0.62	0.71	0.66	0.68	0.64	0.03
Overall (0-5 wk):						
ADG (g)	459	489	467	475	454	7.84
ADFI (g)	727	675	696	687	697	12.86
G:F	0.63^{a}	0.72^{b}	0.67^{ab}	0.69^{ab}	0.65^{a}	0.02

^{a,b} Values with different superscripts of the same row are significantly differ ($p<0.05$). [1] See Table 1 for abbreviation. [2] Standard error of the mean.

RESULTS AND DISCUSSION

Growth performance

Table 4 showed the effect of microbial phytase supplementation in combination with enzyme complex to diet with different nutrient levels on growth performance of weaned pigs. During the whole experimental period (0 to 35 days), there was no significant difference ($p>0.05$) in average daily gain (ADG) and average daily feed intake (ADFI) among dietary treatments. However, piglets in LP+EC100 group had a significantly higher gain: feed ratio (G:F) than piglets had in control ($p<0.05$). Regardless of dietary aP, ME and amino acid levels, piglets fed diets with phytase in combination with enzyme complex at the recommended level (0.1%, respectively) tended to have greater G:F than piglets fed control diet. During the entire experimental period, best G:F was found in LP+EC100 treatment.

In the present study, supplemental phytase and enzyme complex at recommended levels (0.1%, respectively) improved growth performance of weaned piglets fed a low aP diet ($p<0.05$) and had a tendency to improve growth performance of piglet fed diet with low aP, ME and amino acids. Growth performance of piglets fed a reduced aP, ME and amino acid diet with two enzyme products at 0.08% (20% lower dosage level than recommended level), respectively, performed similarly to control piglets. These results indicate that simultaneous supplementation of phytase and enzyme complex had a positive effects on growth performance and nutrient availability of piglets and economic application of phytase and enzyme complex is possible in practical condition.

There have been many studies to show positive effect of phytase (Jongbloed, 1987; Cromwell et al., 1995; Kornegay et al., 1998) or enzyme complex supplementation (Kim et al., 2001a,b; Pettey et al., 2002), individually on growth performance of pigs.

Recently, Kim et al. (2001a) reported that enzyme complex (Endo-Power®) supplementation (0.1%) to corn-soy based diets for weaned piglets improved ADG and G:F by 3 and 7%, respectively. In continued study, Kim et al. (2001b) found that there was no difference in growth performance between growing pigs fed a control diet and those fed a diet with 5% lower ME levels and with enzyme complex (Endo-Power®) at 0.05% level. Other unpublished research (EASY Bio System, Inc.) in large-scale commercial swine operations tends to support the improvement in feed efficiency observed in the present study.

The enzyme complex used in this study contained enzymes targeted to SBM dietary components which are mainly α-galactosides (mainly raffinose (1.0%) and stachyose (4.6%) and β-mannans (1.2%) and known as ANFs for poultry and swine diets. These ANFs are not digestible by pigs and other monogastric animals because they do not have endogenous enzymes to degrade those of ANFs. Consequently, 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 protein digestibility and growth in swine (Veldman et al., 1993; Gdala et al., 1997). The presence of β-mannans in the diet has also shown to diminish growth performance and inhibit nutrient absorption in poultry (Vorha and Kratzer, 1964; Verman and McNab, 1982) and swine (Blackburn and Jonson, 1981; Rainbird et al., 1984; Edwards et al., 1988).

It is well known that phytate is one of the most important ANFs in monogastric animals (Jongbloed, 1987). Phytate in vegetable feedstuffs can make insoluble complex with minerals (Chan, 1988), starch (Thompson, 1986), proteins (Ravindran et al., 1999a,b) and digestive enzymes (Nair et al., 1991; Caldwell, 1992) in the gastro intestinal tract. Therefore, if microbial phytase can effectively remove phytate in diets, it is acceptable that more nutrients will be available for host animals and this will contribute to improve growth performance of pigs.

Table 5. The effect of phytase and enzyme complex supplementation in combination to diet with a low aP or (and) ME and amino acids on nutrient digestibilities of weaned pigs

Item	Control[1]	LP+EC 100	LP+EC 80	LPEA+EC 100	LPEA+EC 80	SEM[2]
Phase I (0-2 wk):						
Dry matter	83.13^{b}	84.84^{a}	83.65^{b}	83.53^{b}	83.38^{b}	0.20
Gross energy	83.87^{b}	85.12^{a}	84.10^{ab}	84.07^{ab}	83.66^{b}	0.19
Crude protein	78.35^{c}	81.28^{a}	79.15^{b}	79.11^{b}	78.26^{c}	0.29
Ether extract	70.42	73.89	70.96	71.57	70.28	0.54
Calcium	40.48	46.75	45.68	46.58	44.71	1.12
Phosphorus	36.50^{c}	47.44^{a}	44.01^{ab}	45.44^{ab}	42.85^{b}	1.10
Phase II (3-5 wk):						
Dry matter	75.03^{c}	79.14^{a}	75.63^{bc}	76.07^{b}	75.52^{bc}	0.40
Gross energy	76.24^{c}	79.32^{a}	76.34^{c}	77.15^{b}	75.53^{d}	0.35
Crude protein	76.01^{c}	78.47^{a}	77.12^{b}	77.29^{b}	75.23^{d}	0.30
Ether extract	58.35^{ab}	62.86^{a}	58.72^{ab}	60.39^{ab}	55.95^{b}	0.79
Calcium	56.19	61.71	58.82	60.52	57.03	0.89
Phosphorus	36.52^{d}	54.24^{a}	46.05^{bc}	48.77^{b}	43.05^{c}	1.66

^{a,b,c,d} Values with different superscripts of the same row are significantly differ ($p<0.05$). [1] See Table 1 for abbreviation. [2] Standard error of the mean.

Based on the facts mentioned above and the results of growth trial in this study, it appears that the positive growth performances response to the enzyme combination (phytase and enzyme complex) were largely driven by enzymatic inactivation of ANFs in diets and improved nutrient digestibilities as found in the digestibility trial in this study. It is also noteworthy that the experimental diets with enzyme combination were formulated to contain 0.15% unit lower aP or (and) 3% lower ME and amino acid levels.

In most experiments on phytase, the efficacy of microbial phytase has been compared with inorganic P sources. From many studies on phytase (Natuphos®), it was concluded that, for pigs, a dietary phytase dose of 500 FTU/kg is equivalent to the amount of P provided by 1 g monocalcium phosphate (MCP)/kg diet. It is acceptable that 1 g of MCP is equivalent to 0.1% aP in the diet. However, in this study, all treatment diets were formulated to contain 0.15% lower aP than that of control diet. Nevertheless, in the preset study, pigs fed on LP+EC 100 group showed better growth performance than pigs in control group ($p<0.05$). It is not clear that this improvement of growth response is driven by complementary action between phytase and enzyme complex or aP level in treatment diets was not limiting growth performance of weaned pigs.

In the present study, since two enzymes were supplemented simultaneously to all treatment diets, it is also not clear that positive effects on growth performance was mainly driven by phytase or in combination with enzyme complex. 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 enzyme complex will enhance nutrient utilization further. 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. (1999a) 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.

The main aim of the present study was not to examine whether there is synergetic effects of phytase and enzyme complex on growth performance and nutrient utilization or not. Therefore, further study will be needed to investigate whether phytase and enzyme complex have synergic effects with respect to improving growth performance and nutrient availability and to validate possible mechanism(s) contributing to the observed complementary action between phytase and enzymes in enzyme complex in corn-soy basis diets.

Total tract digestibility and ileal amino acid digestibility

The effect of phytase and enzyme complex supplementation in combination to diets with a low aP or (and) ME and amino acids on fecal nutrient digestibility (%) in weaned pigs were present in Table 5. During the phase I (0-2 weeks), there was no difference in calcium and crude fat digestibilities among dietary treatments. However, total tract crude protein, energy and phosphorus

Table 6. The effect of phytase and enzyme complex supplementation in combination to diet with a low aP or (and) ME and amino acids on ileal amino acid digestibility of weaned pigs

Item	Control	LP+EC 100	LP+EC 80	LPEA+EC 100	LPEA+EC 80	SEM[2]
Essential amino acids						
Arginine	83.60^{c}	86.11^{a}	84.61^{b}	84.82^{b}	81.62^{d}	0.41
Histidine	74.87	77.49	75.72	76.04	74.65	0.45
Isoleucine	82.86	85.41	83.29	84.61	80.47	0.75
Leucine	81.52	84.31	82.52	83.55	80.85	0.90
Lysine	81.04	83.30	81.19	82.77	81.03	0.76
Phenylalanine	77.70^{b}	81.24^{a}	80.16^{a}	80.42^{a}	77.35^{b}	0.50
Threonine	79.92	82.62	79.74	80.50	79.10	0.77
Valine	78.66^{ab}	81.86^{a}	79.32^{ab}	81.51^{ab}	78.23^{b}	0.54
Methionine	80.60^{b}	86.16^{a}	83.45^{ab}	85.91^{a}	79.63^{b}	0.89
Sub-mean	80.08^{c}	83.17^{a}	81.11^{bc}	82.24^{ab}	79.21^{c}	0.44
Non-essential amino acids						
Alanine	80.50^{ab}	82.34^{a}	80.43^{ab}	81.86^{a}	79.49^{b}	0.35
Aspartic acid	78.55^{b}	81.12^{a}	80.78^{a}	79.51^{ab}	77.96^{b}	0.38
Glutamic acid	78.86	80.49	79.29	79.90	78.70	0.57
Glycine	73.81^{ab}	76.69^{a}	73.15^{ab}	73.92^{ab}	72.34^{b}	0.56
Proline	86.24^{c}	90.72^{a}	88.09^{b}	89.93^{a}	85.64^{c}	0.54
Serine	75.48^{b}	79.61^{a}	77.31^{ab}	78.77^{a}	75.17^{b}	0.58
Tyrosine	78.20^{cd}	82.92^{a}	79.36^{bc}	81.02^{ab}	76.57^{d}	0.64
Sub-mean	78.80^{cd}	81.98^{a}	79.77^{bc}	80.70^{b}	77.98^{d}	0.40
Total mean	79.52^{cd}	82.64^{a}	80.52^{bc}	81.55^{ab}	78.67^{d}	0.40

[a,b,c,d] Values with different superscripts of the same row are significantly differ ($p<0.05$). [1] See Table 1 for abbreviation. [2] Standard error of the mean.

digestibilities were significantly improved when both of phytase and enzyme complex were supplemented at the revel of 0.1%, respectively to diets with low nutrient levels (aP or (and) ME and amino acids) ($p<0.05$). Piglets fed on LP+EC 100, LP+EC 80 and LPEA+EC 80 groups had significantly higher P digestibility than those fed on control group ($p<0.05$).

During the phase II (3-5 weeks), there was similar trend in overall nutrient digestibilities with that observed during the phase I. The apparent DM, gross energy, crude protein and P digestibilities were significantly higher in LP+EC 100 and LPEA+EC 100 groups than in control group ($p<0.05$). During the whole experimental period, P digestibility was significantly improved in LP+EC 80 and LPEA+EC 80 groups than in control group.

Table 6 showed the effect of phytase supplementation in combination with enzyme complex to diet with low nutrient levels on ileal amino acid digestibilities of weaned pigs. Averaged ileal disgestibility of essential animo acids was significantly improved ($p<0.05$) when two enzyme products were supplemented together to diet with low aP or (and) ME and amino acids at the 0.1% level. However, supplementing two enzyme products at the 0.08% level to diet with low aP or (and) ME and amino acids did not improve overall essential amino acid digestibility ($p>0.05$). This result suggest that 0.08% of phytase and enzyme complex supplementation, respectively was not enough to improve significantly overall ileal amino acid digestibility.

Overall, similar trend was observed in nonessential amino acid digestibility. Averaged ileal nonessential amino acid digestibilities were significantly improved when two enzymes were supplemented together to diet with low aP or (and) ME and amino acids at the 0.1% level.

The results of this digestibility study agree with those reported by many researchers who found improvement in P, energy and amino acids digestiblities when pigs were fed a corn-soy basis diet supplemented with microbial phytase or enzyme complex, individually. A large number of data evaluating the use of microbial phytase reported that improved bioavailability of P, calcium, 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 same result also occurred in this digestibility study. Dietary phytase and enzyme complex supplementation increased phosphorus digestibility in order of 24 to 48%. 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 previous studies using same enzyme product with one used in the present study, Kim et al. (2001a) found that supplementing enzyme complex improved ileal gross energy and amino acids digestibility by 7 and 3%, respectively in weaned piglets. In another study, Kim et al (2001b) showed that enzyme complex addition increased apparent ileal true digestibility of amino acids by 7% when

Table 7. The effect of phytase and enzyme complex supplementation in combination to diet with a low aP or (and) ME and amino acids on nutrient excretion and bone mineral composition of weaned pigs

Item	Control	LP+EC 100	LP+EC 80	LPEA+EC 100	LPEA+EC 80	SEM[2]
Excretion (kg/head/35d)						
Dry matter	4.59[a]	3.68[c]	4.33[b]	4.27[b]	4.28[b]	0.082
Nitrogen	0.24[a]	0.20[b]	0.24[a]	0.22[a]	0.24[a]	0.004
Calcium	0.12	0.10	0.11	0.14	0.12	0.006
Phosphorus	0.12[a]	0.08[c]	0.09[b]	0.10[b]	0.10[b]	0.004
Bone mineral composition (%)						
Calcium	38.10	43.65	41.19	43.63	43.33	0.57
Phosphorus	23.39[b]	25.69[a]	23.28[b]	25.42[a]	23.02[b]	0.24

[a,b,c] Values with different superscripts of the same row are significantly differ ($p<0.05$). [1] See Table 1 for abbreviation. [2] Standard error of the mean.

Table 8. Economic analysis for the simultaneous inclusion of phytase and enzyme complex in corn-soy based diets with a low aP or (and) ME and amino acids for weaned pigs

Item	Control[1]	LP+EC 100	LP+EC 80	LPEA+EC 100	LPEA+EC 80	SEM[2]
TWG[3] (kg/pig)	16.08	17.12	16.33	16.62	15.90	0.27
TFC[4] (USD/pig)	14.14	13.23	13.60	13.32	12.9	0.25
FCG (USD /pig)	0.879[a]	0.773[b]	0.833[ab]	0.801[b]	0.811[ab]	0.01

[a,b] Values with different superscripts of the same row are significantly differ ($p<0.05$). [1] See Table 1 for abbreviation. [2] Standard error of the mean.

[3] Abbreviations: TWG=total weight gain per pig, TFC=toal feed cost per pig, FCG=feed cost/kg body weight gain.

[4] Feed production cost for each diets were 555.7 USD/ton for control diet. 560.2 USD/ton for LP−EC 100 diet, 558.1 USD/ton for LP+EC 80 diet. 553.8 USD/ton for LPEA−EC 100 diet CP, 530.3 USD/ton for LPEA−EC 80 diet. respectively.

included in a corn-SBM diet at 0.05%. At the present study, addition of phytase and enzyme complex at 0.1%, respectively, to diet with a low aP increased ileal total amino acid digestibility by 4%.

As shown in the present digestibility study, the mechanisms directing the growth responses observed in the growth trial appear to be closely related to improvements in overall nutrient digestibility. However, the increase in overall nutrient digestibility was observed only when two enzyme products were supplemented to diet at recommended levels (0.1%). The question still remains concerning how growth performance of piglets fed on LPEA+EC 80 group performed similarly to that of the control piglets, even though digestibility of overall nutrients except for phosphorus was not affected by the presence of the two enzyme products at lower level in this study. This growth response with the addition of phytase and enzyme complex in LPEA+EC 80 treatment group might be due to the fact that dietary aP level rather than energy and amino acids levels was main limiting factor for growth of piglets in this study.

Fecal nutrients excretion and bone mineral composition

Table 7 shows the response of nutrient excretion and bone mineral composition to dietary treatments. In the present study, nutrient excretion was calculated based on results of fecal nutrient digestibility.

Total DM and phosphorus excretion per pig for during the whole experimental were significantly reduced by dietary phytase and enzyme complex supplementation ($p<0.05$%). There was no significant difference in calcium excretion among dietary treatments ($p>0.05$). Nitrogen excretion was significantly lower in LP+EC 100 group than in any other dietary treatments ($p<0.05$).

The calcium content in bone was not affected by dietary treatments. However, P content in bone was significantly higher in LP+EC 100 and LPEA+EC100 groups than in other dietary treatments ($p<0.05$).

In the present study, it appears that nutrient excretion response is basically related to improvement in nutrient digestibility by supplementation of phytase and enzyme complex. P excretion was reduced by 33 to 50% when two enzyme products were supplemented to treatment diets. This P excretion reduction comes from the effect of phytase on degradation of phytate phosphorus in the diets and lower total P content in treatment diets than one in control diet. This result is in good agreement with many previous studies to show that phytase supplementation to swine diets reduced P excretion by 30 to 40% (Jongbloed et al., 1992; Pierce et al., 1994; Cromwell et al., 1993;1995; Kornegay et al., 1998).

Bone ash content has been widely used in accessing efficacy of phytase on P bioavailability and bone mineralization (Hoppe et al., 1991). It is well known that microbial phytase supplementation of low-phosphorus pig diets improves bone mineralization and increase P content in bone (Yong et al., 1993; Yi et al., 1996). Surprisingly, in the present study, supplementation of both phytase and enzyme complex to a diet with low aP or (and) ME and amino acids at 0.1%, respectively, significantly increased P content in bone ($p<0.05$) in comparison to control group. This improvement suggests that the combination of phytase and enzyme complex had a strong positive effect on P availability of weaned pigs.

Economic analysis

Table 8 summarized economic analysis data for the simultaneous inclusion of phytase and enzyme complex in corn-soy based diets with a low aP or (and) ME and amino acids for weaned pigs. As shown in the table. feed cost per kg weight gain was highest in control group and LP+EC 100 group and LPEA+EC 100 group had a significantly lower feed cost per kg weight gain than control group had ($p<0.05$). Regardless of dosage levels of two enzyme products. dietary phytase and enzyme complex addition to diet with low aP or (and) ME and amino acid levels reduced feed cost per kg weight gain compared to control group.

In the present study. economic analysis data clearly suggest that phytase and enzyme complex addition can reduce supplemental P and ME and amino acid requirements of weaned pigs and contribute to reduction of pig production cost.

In conclusion. the results from the present study suggest that the simultaneous inclusion of phytase and enzyme complex to diets at recommended level is advantageous with respect to improving growth performance and nutrient digestibility of weaned pigs and may contribute to increased economic return when added to corn-soy based weaned pig diets.

REFERENCES

AOAC. 1984. Official Methods of Analysis (14th Ed.) Association of Official Analytical Chemists, Washington, DC.

AOAC. 1990. Official Method of Analysis 15th ed., Association of Official Analytical Chemists. Washington, DC.

Biehl, R. R. and D. H. Baker. 1997. Microbial phytase improves amino acid utilization in young chicks fed diets based on soybean meal but not diets based on peanut meal. Poult. Sci. 76:355-360.

Blackburn, N. A. and I. T. Johnson. 1981. The effect of guar gum on the viscosity of gastrointestinal contents and on glucose uptake from the perfushed jejunum in the rat. Br. J. Nutr. 46:239-249.

Cahn, T. T., A. J. A. Aarnink, J. B. Schutte, A. Sutton, D. J. Langhout and M. W. A. Verstegen. 1998. Dietary protein affects nitrogen excretion and ammonia emission from slurry of growing-finishing pigs. Livest. Prod Sci. (56):181-191.

Caldwell, R. A. 1992. Effect of calcium and phytic acid on the activation of trypsinogen and the stability of trypsin. J. Agric. Food Chem. 40:43-46.

Calloway, D. H., D. J. Colasito and R. D. Mathews. 1966. Gases produced by human intestinal flora. Nature. 212:1238.

Chan, H. C. 1988. Phytate and cation binding activity. M.S. Thesis, Texas Tech University, Lubbock, TX.

Chung, T. K. and D. H. Baker. 1992. Ideal amino acid pattern for 10 kilogram pigs. J. Anim. Sci. 70:3102-3111.

Coelho, M. B. and E. T. Kornegay. 1996. Phytase in animal nutrition and wate management. BASF Corp., Mount Olive, NJ.

Coon, C. N., K. L. Leske, O. Akavanichan and T. K. Cheng. 1990. Effect of oligosaccharide-free soybean meal on true metabolizable energy and fiber digestion in adult roosters. Poult. Sci. 69:787-793.

Cromwell, G. L., R. D. Coffey, G. R. Parker, H. J. Monegue and J. H. Randolph. 1995. Efficacy of a recombinant-derived phytase in improving the bioavailability of phosphorus in corn-soybean meal diets for pigs. J. Anim. Sci. 73:2000-2008.

Cromwell, G. L., T. S. Stahly, R. D. Coffey, H. J. Monegue and J. H. Randolph. 1993. Efficacy of phytase in improving the bioavailability of phosphorus in soybean meal and corn-soybean meal diets for pigs. J. Anim. Sci. 71:1831-1840.

Duncan, D. B. 1955. Multiple range and multiple F test. Biometrics 11:1-42.

Edwards, C. A., I. T. Johnson and N. W. Read. 1988. Do viscous polysaccharides slow absorption by inhibiting diffusion or convection? Eur. J. Cli. Nutr. 42:307-312.

Gdala, G. A., J. M. Jansman, L. Buraczewska, J. Huisman and P. van Leeuwen. 1997. The influence of -galactosidase supplementation on the ileal digestibility of lupin seed carbohydrates and dietary protein in young pigs. Anim. Feed Sci. Technol. (67):115-125.

Giesting, D. W. and R. A. Easter. 1991. Effect of protein source and fumaric acid supplementation on apparent ileal digestibility of nutrients by young pigs. J. Anim. Sci. 69:2497-2503.

Hoppe, P. P., F. J. Schoner, H. Wiesche, G. Shwarz and A. Safer. 1991. Phosphorus equivalency of Aspergillus-niger-phytase for piglets fed a grain-soybean-meal diet. J. Anim. Physiol. Anim. Nutr. 69:225.

Jongbloed, A. W. and P. A. Kemme. 1990. Apparent digestible phosphorus in the feeding of pigs in relations to availability, requirementsand environment 1. Digestible phosphorus in feedstuffs from plant and animal origin. Netherlands Journal of Agricultural Science 38:567-574.

Jongbloed, A. W. 1987. Phosphorus in the feeding of pigs. Ph. D. dissertation, Wageningen, Agricultural University, The Netherlands.

Jongbloed, A. W., Z. Mroz and P. A. Kemme. 1992. The effect of supplementary Aspergillus niger phytase in diets for pigs on concentration and apparent digestibility of dry matter, total phosphorus and phytic acid in different sections of the alimentary tract. J. Anim. Sci. 70:1159-1168.

Kephart, K. B. and G. W. Sherritt. 1990. Performance and nutrient balance in growing swine.

Kim, S. W., I. Mavromichalis and R. A. Easter. 2001a. Supplementation of alpha-1galactosidase and beta-1,4-mannanase to improve soybean meal utilization by nursery pig. J. Anim. Sci. 79 (Suppl. 1):106 (Abstr.).

Kim, S. W., J. H. Zhang, K. T. Soltwedel and R. A. Easter. 2001b. Supplementation of -1,6-galactosidase and 1,4-mannanase to improve soybean meal utilization by growing-finishing pigs. J. Anim. Sci. 79 (Suppl. 2):84 (Abstr.).

Kornegay, E. T. and H. Qian. 1996. Replacement of inorganic phosphorus by microbial phytase for young pigs fed on a maize soyabean meal diet. Br. J. Nutro. 76:563-578.

Kornegay, E. T., J. S. Radcliffe and Z. Zhang. 1998. Influence of phytase and diet composition on phosphorus and amino acid digestibility and phosphorus and nitrogen excretion in swine.

In: BASF Tech. Symp., Carolina Swine Nutr. Conf., Durham, NC. pp. 125-155.

Leske, K. L., C. J. Jevne and C. N. Coon. 1993. Effect of oligosaccharide additions on nitrogen corrected true metabolizable energy of soy protein concentrate. Poult. Sci. 72:664.

Moore, S. 1963. On the determination of cystine as cysteric acid. J. Biol. Sci. 238:235-237.

Mroz, Z. and A. W. Jongbloed. 1998. The influence of phytase on the availability of protein and energy in swine. In: BASF Tech. Symp., Carolina Swine Nutr. Conf., Durham, NC. pp. 65-88.

Mroz, Z., A. W. Jongbloed and P. A. Kemme. 1994. Apparent digestibility and retention of nutrients bound to phytase complexes as influenced by microbial phytase and feeding regimen in pigs. J. Anim. Sci, 72:126-132.

Nair, V. C., J. Laflamme and Z. Duvnjak. 1991. Production of phytase by Aspergillus ficcum and reduction pf phytic acid content in canola meal. J. Sci. Food Agric. 54:355.

NRC. 1998. Nutrient Requirements of Swine (10th Ed.). National Academy Press, Washington DC.

Pettey, L. A., S. D. Carter, B. W. Senne and J. A. Shriver. 2002. Effects of -mannanase addition to corn-soybean meal diets on growth performance, carcass traits and nutrient digestibility of weanling and growing-finishing pigs.

Pierce, J. L., K. L. Enright, G. L. Cromwell, L. W. Turner and T. C. Bridges. 1994. Dietary manipulation to reduce the N and P excretion by finishing pigs. J. Anim. Sci. 72(Suppl. 1):331(Abstr.).

Rainbird, A. L., A. G. Low and T. Zebrowska. 1984. Effect of guar gum on glucose and water absorption from isolated loops of jejunum in conscious growing pig. Br. J. Nutr. 52:489-498.

Ravindran, V., P. H. Selle and W. L. Bryden. 1999a. Influence of microbial phytase on apparent ileal amino acid digestibility in feedstuffs for broilers. Poult. Sci. 78:699-706.

Ravindran, V., P. H. Selle and W. L. Bryden. 1999b. Effects of phytase supplementation, individually and in combination, with glucanase on the nutritive value of wheat and barley. Poult. Sci. 78:1588-1595.

SAS. 1985. SAS User's Guide: Statistics, SAS Inst. Inc., Cary, NC.

Selle, P. H., V. Ravindran, G. Ravindran, P. H. Pittolo and W. L. Bryden. 2003. Influence of phytase and xylanase supplementation on growth performance and nutrient utilization of broilers offered wheat-based diets. Asian-Aust. J. Anim. Sci. Vol 16, 3:394-402.

Thompson, L. U. 1986. Phytic acid: a factor influencing starch digestibility and blood glucose response. In: Phytic Acid: chemistry and applications (Ed. E. Graf). Pilatus Press, Minneapolis, p. 173-194.

Veldman, A. V., W. A. G. Veen, D. Barug and P. A. van Paridon. 1993. Effect of -galactosides in feed on ileal piglet digestibility physiology. J. Anim. Nutr. (69):67-65.

Verma, S. V. S. and J. M. Mcnab. 1982. Guar meal in diets for broiler chickens. Br. Poult. Sci. 23:95-105.

Vohra, P. and F. H. Kratzer. 1964. Growth inhibitory effect of certainpolysaccharides for chickens. Poult. Sci. 43:1164-1170.

Yi, Z. E. T. Kornegay, V. Ravindran, M. D. Kindemann and J. H. Wilson. 1996. Effectiveness of Natuphos phytase in improving the bioavailabilities ofphosphorus and other nutrient in soybean meal-based semifurified diets for young pig. J. Anim. Aci. 1996. 74:1601-1611.

Young, L. G., M. Leunissen and J. L. Atkinson. 1993. Addiiton of microbial phytase to diets of young pigs. J. Anim. Sci. 1993. 71:2147-2150.