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The Effects of Enzyme Complex on Performance, Intestinal Health and Nutrient Digestibility of Weaned Pigs

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ABSTRACT: Two experiments were conducted to evaluate the effect of supplementing a corn-soybean meal-based diet with an enzyme complex containing amylase, protease and xylanase on the performance, intestinal health, apparent ileal digestibility of amino acids and nutrient digestibility of weaned pigs. In Exp. 1, 108 piglets weaned at 28 d of age were fed one of three diets containing 0 (control), 100, or 150 ppm enzyme complex for 4 wks, based on a two-phase feeding program namely 1 to 7 d (phase 1) and 8 to 28 d (phase 2). At the end of the experiment, six pigs from the control group and the group supplemented with 150 ppm enzyme complex were chosen to collect digesta samples from intestine to measure viscosity and pH in the stomach, ileum, and cecum, as well as volatile fatty acid concentrations and composition of the microflora in the cecum and colon. There were linear increases ($p<0.01$) in weight gain, gain: feed ratio and digestibility of gross energy with the increasing dose rate of enzyme supplementation during the whole experiment. Supplementation with enzyme complex increased the digesta viscosity in the stomach ($p<0.05$) and significantly increased ($p<0.01$) the concentrations of acetic, propionic and butyric acid in the cecum and colon. Enzyme supplementation also significantly increased the population of *Lactobacilli* ($p<0.01$) in the cecum and decreased the population of *E. coli* ($p<0.05$) in the colon. In Exp. 2, six crossbred barrows (initial body weight: 18.26±1.21 kg), fitted with a simple T-cannula at the distal ileum, were assigned to three dietary treatments according to a replicated 3×3 Latin Square design. The experimental diets were the same as the diets used in phase 2 in Exp. 1. Apparent ileal digestibility of isoleucine ($p<0.01$), valine ($p<0.05$) and aspartic acid ($p<0.05$) linearly increased with the increasing dose rate of enzyme supplementation. In conclusion, supplementation of the diet with an enzyme complex containing amylase, protease and xylanase improved piglet performance. This is likely a result of improvement in nutrient digestibility, volatile fatty acid concentrations and bacteria ratio in the large intestine. (**Key Words:** Enzyme Complex, Weaned Pigs, Performance, Intestinal Health, Apparent Ileal Digestibility)

INTRODUCTION

Enzymes have been used in livestock production for more than 20 yrs, especially in diet containing cereals with high levels of soluble non-starch polysaccharides (NSP) such as wheat, oats, barley and rye (Yin et al., 2000a, 2000b, 2001c; Yu et al., 2007). In recent years, however, enzymes have also been accepted by the industry for corn-based diets in both poultry and pigs (Fang et al., 2007; Olukosi et al., 2007; Francesch and Geraert, 2009; Willamil et al., 2012). Corn contains 0.9% soluble and 6% insoluble NSPs (contained approximately 42 g/kg xylan), while soybean contains 6% soluble and 18 to 21% insoluble NSPs (contained approximately 17 g/kg xylan) (Bach Knudsen, 1997). The NSPs in corn mainly consist of insoluble arabinoxylans (Summers, 2001). Fang et al. (2007) reported that the supplementation of xylanase-based enzymes in corn-soybean based-diet improved the digestibility of energy and NDF and also improved average daily gain of growing pigs. This may indicate that corn-soybean based-diet can provide the substrate for xylanase to work on.

Although the use of NSP-degrading carbohydrases has been widely and successfully used in poultry fed NSP-rich diets (Francesch and Geraert, 2009), the results are not as clear in pig (Mavromichalis et al., 2000; Cadogan et al., 2003; Barrera et al., 2004; Woyengo et al., 2008). The possible causes of the differences of performance in these studies could be the differences in cereal grain types and

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quality, animal age, and the bioefficacy of enzyme used. Therefore, the specific objective of these experiments was to evaluate the effects of supplementation with an enzyme complex containing amylase, protease and xylanase on performance, nutrient digestibility and the intestinal health of weaned pigs.

MATERIALS AND METHODS

All procedures used in these experiments were approved by the China Agricultural University Institutional Animal Care and Use Committee (Beijing, China).

Diets and enzyme complex preparation

The enzyme complex used in this study contained amylase, protease and xylanase; it was supplied by Danisco Animal Nutrition, UK. The pure enzyme product provided 2,000 U/g of amylase, 40,000 U/g of protease and 20,000 U/g xylanase. One unit of xylanase is defined as the amount of enzyme which liberates 0.5 μmol of reducing sugar (expressed as xylose equivalents) from a cross-linked oat spelt xylan substrate in one minute; one unit of protease is defined as the amount of enzyme which liberates 1μmol of phenolic compound (tyrosine equivalents) from a casein substrate per minute; one unit of protease is defined as the amount of enzyme which liberates 1 μmol of glucosidic linkages from a water insoluble cross-linked starch polymer substrate per minute.

In Exp. 1, all diets were pelleted (1.5 mm) at 70°C. They were formulated to contain similar levels of nutrients and to meet or exceed the nutrient requirements for piglets (NRC, 1998). The residual enzyme activity of amylase, protease and xylanase after pelleting were 152, 3,832 and 2,107 U/kg for phase 1 and 242, 5,773 and 3,150 U/kg for phase 2. The ingredient composition and the analyzed nutrient content for phase 1 (1 to 7 d) and phase 2 (8 to 28 d) are shown in Table 1. Diets used in Exp. 2 were the same as the diets used in phase 2 in Exp. 1.

Experimental design

Exp. 1. Growth performance, intestinal health and nutrient digestibility: In Exp. 1, a total of 108 crossbred piglets (Duroc×Landrace×Large White) weighing 7.51±1.10 kg were used in a 28-d experiment. The three treatments consisted of a corn-soybean meal-based control diet and two experimental diets. The two experimental diets were the control diet supplemented with 100 or 150 ppm enzyme complex. Chromic oxide was included at 0.25% as an indigestible index for the determination of nutrient digestibility. The pigs were allocated to one of the three dietary treatments on the basis of weight and gender in a randomized complete block design with six replicates per treatment and six pigs per pen.

Table 1. Diet composition and nutrients levels of experimental diets fed to determine the effects of enzyme supplementation

Ingredient (%)	Basal diet	
	Phase 1	Phase 2
Corn	51.84	57.71
Soybean meal	12.16	25.23
Extruded full fat soybean meal	10.00	8.00
Fish meal	2.00	-
Whey powder	12.00	5.00
Sugar	1.00	-
Dried porcine soluble	3.00	-
Spray-dried plasma protein	3.60	-
Soy oil	0.66	0.74
Dicalcium phosphate	1.14	1.20
Limestone	0.79	0.84
Salt	0.20	0.30
L-Lysine-HCl (78%)	0.27	0.13
Methionine	0.10	0.05
Threonine	0.10	0.04
Tryptophan	0.04	0.01
Chromic oxide	0.25	0.25
ZnO	0.35	-
Mineral and vitamin premix[1]	0.50	0.50
Nutrient levels[2]		
Digestible energy (kcal/kg)	3,400	3,400
Dry matter	92.65	91.82
Crude protein	20.05	19.86
Calcium	0.80	0.70
Phosphorus	0.65	0.60
Available phosphorus	0.50	0.38
Lysine	1.40	1.18
Digestible lysine	1.27	1.01
Methionine	0.41	0.35
Digestible methionine	0.38	0.30
NDF	10.43	11.48
ADF	4.16	4.34

[1] Premix provided the following per kg of complete diet for growing pigs: vitamin A, 9,000 IU; vitamin D_3, 3,000 IU; vitamin E, 64 IU; vitamin K_3, 3 mg; vitamin B_{12}, 12 μg; riboflavin, 5.5 mg; pantothenic acid, 15 mg; niacin, 40 mg; choline chloride, 551 mg; folacin, 0.8 mg; vitamin B_1, 1.5 mg; vitamin B_6, 3 mg; biotin, 100 μg; Mn, 40 mg; Fe, 100 mg; Zn, 100 mg; Cu, 150 mg; I, 0.3 mg; Se, 0.3 mg.
[2] Nutrient levels were calculated values.

The pigs were housed in pens of 1.2×2 m^2 with half cement floor and half woven mesh floor. All pigs had free access to feed and water throughout the 28-d experiment. The temperature of the pig barn was controlled between 23 and 32°C. Pigs and feeds were weighed at d 0, 7 and 28 of the experiment in order to calculate weight gain, feed intake and gain:feed ratio. Fresh fecal grab samples were collected on d 26 to 28 and pooled by pen. Approximately 100 g of fresh feces were collected directly from the floor of each pen into sterile plastic bags and immediately stored at -20°C until chemical analysis.

At the end of the experiment, six pigs close to average group body weight were chosen from the control group and the group supplemented with 150 ppm enzyme complex. These 12 pigs were fasted for 24 h. Pigs were subsequently killed after they had free access to feed for two hours. The abdomen was immediately opened and the entire gastrointestinal tract was tied to collect digesta in the different parts of the tract (stomach, small intestine, cecum and colon). The pH of the digesta in the stomach, ileum and cecum was measured with a Temperature-Corrected pH-Meter (HI8424 pH meter, Hanna Instruments, China). The fresh digesta taken from the stomach, ileum and cecum was immediately refrigerated on ice and then centrifuged at 4,000×g for 10 min at 10°C. The supernatant was then used to determine viscosity using a Physica MCR 301 (H-PTD200, Anton Paar, Graz).

Exp. 2. Apparent ileal digestibility: In Exp. 2, six crossbred barrows (Duroc×Landrace×Large White) with an average body weight of 18.06±1.2 kg were surgically fitted with a simple T-cannula at the distal ileum according to the methods described by Stein et al. (1998). After the surgery, the pigs were housed in adjustable, 1.2×0.7×0.96 m^3 metabolic crates with perforated floors that allowed for freedom of movement. The pigs were fed increasing amounts of commercial diet twice daily and had unlimited access to water. After a 12-d recovery period, pigs were randomly assigned to one of three treatments according to a replicated 3×3 Latin square design.

Each experimental period lasted 7 d, consisting of 5 d for acclimation to the experimental diets and 2 d collection of ileal digesta. The daily feed allowance was divided into two equal portions and was fed at 08:00 and 16:30 h every day. Ileal digesta were collected continuously for 12 h from 08:00 to 20:00 h on d 6 and 7 (Stein et al., 1998). A plastic bag was attached to the cannula using a cable tie to collect the digesta flowing into the bag. Bags were changed every 20 min. Ileal digesta were stored on ice during the 12 h collection period. After each day's collection, digesta were stored at -20°C to prevent bacterial degradation of the amino acids in the digesta. Digesta were lyophilized and a sub-sample was collected for analysis.

Chemical analysis

Chemical analysis of feed, feces and digesta: Feed samples were collected at the beginning of each experiment. Fecal samples in Exp. 1 were thawed, heat dried (65°C, 72 h) in an oven and ground to pass through a 1-mm sieve. Digesta samples in Exp. 2 were freeze dried using a Virtis Genesis Wizzard 2.0 Freeze Dryer (SP industries, Warminster).

In Exp. 1, feed and fecal samples were analyzed for dry matter (AOAC method 930.15, 2000), crude protein (AOAC method 988.05, 2000) and calcium (AOAC method 965.17, 2000). Phosphorus content was analyzed using an UV-visible spectrophotometer (Hitachi, U-1,000 Tokyo, Japan). Gross energy was determined by an Automatic Adiabatic Oxygen Bomb Calorimeter (Parr 1281 Automatic Energy Analyzer, Moline, IL). NDF and ADF were determined using fibre bags and Fibre Analyzer (Ankom Technology, Macedon, NY) following an adaptation of the procedure described by Van Soest et al. (1991). The concentration of NDF was analyzed using heat stable α-amylase and sodium sulphite without correction for insoluble ash. The ADF fraction was analyzed in a separate sample. The content of chromium was determined by an Automatic Absorption Spectrophotometer (Hitachi Z-5000 Automatic Absorption Spectrophotometer, Tokyo, Japan) according to Williams et al. (1962). Total starch was determined according to the method described by Thivend et al. (1972).

In Exp. 2, The amino acid content of the feed samples and digesta was assayed using ion-exchange chromatography with an Automatic Amino Acid Analyzer (L-8800 Hitachi Automatic Amino Acid Analyzer, Tokyo, Japan) after hydrolyzing with 6 N HCl at 110°C for 24 h. Cystine was determined as cysteic acid and methionine as methionine sulfone after preoxidation with performic acid and pre-column derivation using phenylisothiocyanate (L-8800 Hitachi Automatic Amino Acid Analyzer, Tokyo, Japan). Tryptophan was determined after hydrolyzing with 4 M NaOH at 110°C for 22 h using phenylisothiocyanate (Model 76337, Agilent Technologics, Waldbronn, Germany).

Enzyme activity: Sterile cell scrapers were used to obtain the jejunal and ileal mucosa (1 to 2 g and stored in capped tubes), which were placed in liquid N and stored at -80°C until needed for analysis of sucrase and maltase activities. Determination of sucrase and maltase activities of jejunal and ileal mucosa was performed using the corresponding reagent kits (Jiancheng Bioengineering Institute, Nanjing, China). One sucrase unit is defined as the quantity of enzyme required to hydrolyze 1 nmol of sucrose per mg protein minute, at pH 5.5 and 37°C; Maltase was similarly defined using maltose.

Volatile fatty acid analysis: Samples of digesta from the cecum and colon of individual pigs were taken for the analysis of volatile fatty acids. Volatile fatty acid concentrations in the digesta were determined using a modified method of Porter and Murray (2001). A 1 g sample was diluted with 2 ml of 0.1% HCl solution and put on ice for 30 min and then centrifuged at 12,000×g at 0°C for 15 min. Exactly 1 ml of the supernatant was passed through a 0.22 μm Nylon Membrane Filter (Millipore, Bedford, OH) and then 5 μl of the solution was injected into a Gas Chromatographic System (Agilent HP 6890 Series, Santa Clara, CA).

Microbiology analysis: The cecal and colonic contents

Table 2. Effect of enzyme supplementation on performance of weaned pigs[1]

	Enzyme[2]			SEM[3]	p-value		
	0	100	150		Treatment	Linear	Quadratic
Weight gain (g/d)							
d 1 to 7	188[a]	208[ab]	219[b]	9.28	0.09	0.03	0.66
d 8 to 28	416[a]	466[b]	479[b]	10.63	<0.01	<0.01	0.18
d 1 to 28	359[a]	401[b]	414[b]	9.13	<0.01	<0.01	0.20
Feed intake (g/d)							
d 1 to 7	260	262	274	8.81	0.50	0.29	0.62
d 8 to 28	716	737	730	15.90	0.64	0.93	0.81
d 1 to 28	602	618	616	12.93	0.65	0.52	0.62
Gain:feed ratio							
d 1 to 7	0.72	0.79	0.80	0.03	0.08	0.04	0.34
d 8 to 28	0.57[b]	0.63[a]	0.66[a]	0.01	<0.01	<0.01	0.07
d 1 to 28	0.60[b]	0.65[a]	0.67[a]	0.01	<0.01	<0.01	0.24

[ab] Means in the same row with different superscripts differ (p<0.05).
[1] Data represents mean of six pens with six pigs per pen. [2] Enzyme contained 2,000 U/g of amylase, 40,000 U/g of protease and 20,000 U/g xylanase.
[3] SEM = Standard error of the means.

were immediately placed in sterile 50 ml capped tubes and stored on ice until laboratory analysis was conducted within 12 h. A modification of the method as described by Orban et al. (1997) was used to determine the populations of *Lactobacilli* and *E. coli*. Cecal and colonic contents (1 g) were serially diluted 1:9 in sterile resazurin solution (KH_2PO_4 0.3 mol/L, NaOH 0.22 mol/L and resazurin 4.3 mmol/L). Appropriate serial dilutions were used to enumerate the two bacteria (10^{-5}, 10^{-6}, 10^{-7} for *Lactobacilli* and 10^{-2}, 10^{-3}, 10^{-4} for *E. coli*). *E. coli* (Mac Conkey agar) was incubated aerobically at 37°C for 12 to 18 h. *Lactobacilli* (MRS agar) was incubated anaerobically at 37°C for 12 to 18 h. The microbial enumerations of digesta are expressed as $\log_{10}$ Colony-Forming Units per gram.

Statistical analysis

All data were processed using SAS (SAS Institute, Cary, NC) and statistically analyzed by ANOVA as a complete block design with pen in Exp. 1 and individual pig as a block in Exp. 2. Control statement in GLM model was applied to determine linear and quadratic effects of enzyme concentrations. Statistical differences among treatments were determined by the Student-Newman-Keuls Multiple Range Test. Significance was taken at p<0.05 with a trend being p<0.10.

RESULTS

Performance and nutrient digestibility

The performance of the pigs in Exp. 1 is presented in Table 2. During the first phase, pigs fed diets supplemented with enzyme complex at 150 ppm tended to increase weight gain (p = 0.09) and gain:feed ratio (p = 0.08) compared with pigs fed the control diet. There were linear increases (p<0.01) in weight gain and gain:feed ratio with increasing dose rate of enzyme supplementation during phase 2 and the whole experimental period. There were no differences for feed intake (p>0.05) between the treatments.

There was a linear increase in digestibility of gross energy (p<0.01) with the increasing dose rate of enzyme supplementation. Pigs fed diets supplemented with enzyme complex tended to have increased digestibility of crude protein (p = 0.07) and dry matter (p = 0.09) (Table 3). However, the digestibility of starch (p>0.05) was not affected by enzyme supplementation.

Volatile fatty acid

Table 4 shows the effects of enzyme supplementation on

Table 3. Effect of enzyme supplementation on nutrient digestibility (%) of weaned pigs[1]

	Enzyme[2] (ppm)			SEM[3]	p-value		
	0	100	150		Treatment	Linear	Quadratic
Dry matter	85.82	86.92	87.44	0.38	0.09	0.01	0.54
Energy	84.45[a]	85.89[b]	86.26[b]	0.40	0.05	<0.01	0.29
Crude protein	79.51	81.93	82.30	0.74	0.07	0.02	0.27
Starch	99.49	99.51	99.56	0.06	0.62	0.42	0.79

[ab] Means in the same row with different superscripts differ (p<0.05).
[1] Data represents mean of six pens with six pigs per pen. [2] Enzyme contained 2,000 U/g of amylase, 40,000 U/g of protease and 20,000 U/g xylanase.
[3] SEM = Standard error of the means.

Table 4. Effect of enzyme supplementation on volatile fatty acid concentrations (mg/g of digesta) in the cecum and colon of weaned pigs[1]

	Enzyme[2] (ppm)		SEM[3]	p-value
	0	150		
Cecum				
Acetic acid	4.21	6.81	0.17	<0.01
Propionic acid	2.11	2.87	0.12	0.01
Butyric acid	0.85	1.35	0.08	0.01
Colon				
Acetic acid	3.20	5.48	0.20	<0.01
Propionic acid	1.36	2.34	0.06	<0.01
Butyric acid	0.74	1.20	0.05	<0.01

[ab] Means in the same row with different superscripts differ (p<0.05).
[1] Data represents mean of six pens with six pigs per treatment.
[2] Enzyme contained 2,000 U/g of amylase, 40,000 U/g of protease and 20,000 U/g xylanase.
[3] SEM =Standard error of the means.

cecal and colonic volatile fatty acid concentrations. Supplementation with enzyme complex significantly increased (p<0.01) the concentrations of acetic, propionic and butyric acids in both the cecum and colon compared with pigs fed the control diet.

Microbial counts in large intestine

The effect of enzyme supplementation on cecal and colonic microbiota is presented in Table 5. Enzyme supplementation significantly increased the population of *Lactobacilli* (p<0.01) in the cecum. In the colon, the population of *E. coli* (p<0.05) was lower for pigs fed the diets supplemented with enzyme than those without enzyme supplementation.

Table 5. Effect of enzyme supplementation on large intestinal microbiota in the cecum and colon of weaned pigs[1, 2]

	Enzyme[3] (ppm)		SEM[4]	p-value
	0	150		
Cecum				
E. coli	7.06	6.51	0.17	0.18
Lactobacilli	7.86	8.33	0.05	<0.01
Colon				
E. coli	8.05	7.25	0.13	0.02
Lactobacilli	8.33	8.84	0.11	0.06

[ab] Means in the same row with different superscripts differ (p<0.05).
[1] Data represents mean of six pens with six pigs per treatment.
[2] Bacterial numbers are expressed as $\log_{10}$ colony forming units per gram.
[3] Enzyme contained 2,000 U/g of amylase, 40,000 U/g of protease and 20,000 U/g xylanase.
[4] SEM = Standard error of the means.

Digesta viscosity, pH and mucosa enzymatic activity

Viscosities of digestive contents in the stomach (p<0.05) were greater for pigs fed the diet supplemented with enzyme but no differences (p>0.05) were observed in the ileam and cecum. Supplementing the diet with the enzyme tended to decrease the pH in the cecum (p<0.05), but there was no differences (p>0.05) in the pH of the stomach or ileam (Table 6).

Apparent ileal digestibility of amino acids

The pigs recovered well from the surgery and remained healthy during the entire experiment. The apparent ileal digestibility of amino acids is presented in Table 7. Enzyme supplementation linearly increased the apparent ileal

Table 6. Effects of enzyme supplementation on digesa viscosity, pH and jejunal and ileal mucosa enzymatic activity of weaned pigs[1]

	Enzyme[2] (ppm)		SEM[3]	p-value
	0	150		
Digesta Ph				
Stomach	3.46	3.27	0.75	0.43
Ileum	6.13	5.45	0.65	0.37
Cecum	5.76	5.35	0.10	0.08
Digesta viscosity (mPa s)				
Stomach	0.92	1.34	0.08	0.03
Ileum	1.06	1.12	0.03	0.18
Cecum	2.82	3.21	0.13	0.06
Enzyme activity, jejunal mucosa (U/mg of protein)				
Sucrase	18.05	19.49	1.97	0.84
Maltase	122.84	161.5	22.73	0.51
Enzyme activity, ileal mucosa (U/mg of protein)				
Sucrase	34.18	38.19	3.65	0.65
Maltase	175.24	217.32	36.95	0.84

[ab] Means in the same row with different superscripts differ (p<0.05).
[1] Data represents mean of six pens with six pigs per treatment.
[2] Enzyme contained 2,000 U/g of amylase, 40,000 U/g of protease and 20,000 U/g xylanase. [3] SEM = Standard error of the means.

Table 7. Effects of enzyme supplementation on apparent ileal digestibility of amino acids of weaned pigs[1]

	Enzyme[2]			SEM[3]	p-value		
	0	100	150		Treatment	Linear	Quadratic
Crude protein	74.15	80.57	81.95	3.25	0.08	0.04	0.33
Indispensable amino acids							
Arginine	83.27	86.53	88.59	1.82	0.06	0.02	0.66
Phenylalanine	81.82	84.30	85.66	2.10	0.21	0.09	0.73
Histidine	81.01	83.33	84.72	1.90	0.17	0.07	0.75
Isoleucine	80.21[a]	86.09[b]	86.19[b]	1.59	0.02	<0.01	0.06
Leucine	82.54	83.25	86.53	1.56	0.07	0.04	0.31
Lysine	82.61	88.99	89.22	2.82	0.08	0.05	0.20
Methionine	83.97	85.56	86.48	3.01	0.35	0.37	0.88
Threonine	78.69	81.85	83.08	2.82	0.26	0.13	0.65
Valine	79.79[a]	82.90[b]	82.99b	0.86	0.02	0.01	0.07
Tryptophan	78.23	82.52	85.42	2.99	0.10	0.04	0.76
Dispensable amino acids							
Alanine	76.62	79.94	81.60	3.75	0.35	0.18	0.77
Aspartic acid	79.22[a]	82.59[b]	82.67[b]	1.16	0.04	0.02	0.12
Glutamic acid	89.07	89.79	90.28	1.33	0.58	0.33	0.91
Cystine	68.67	71.82	73.10	3.19	0.32	0.16	0.70
Glycine	69.35	73.36	74.76	2.87	0.17	0.08	0.56
Proline	83.41	85.46	86.20	3.19	0.58	0.34	0.79
Serine	78.48	81.81	84.11	3.59	0.18	0.13	0.85
Tyrosine	87.68	88.51	89.45	3.24	0.45	0.54	0.98

[ab] Means in the same row with different superscripts differ (p<0.05).
[1] Value represents mean of six pigs. [2] Enzyme contained 2,000 U/g of amylase, 40,000 U/g of protease and 20,000 U/g xylanase.
[3] SEM = Standard error of the means.

digestibility of isoleucine (p<0.01), valine (p<0.05), aspartic acid (p<0.05), and tended to increase the apparent ileal digestibility of crude protein (p = 0.08), arginine (p = 0.06), leucine (p = 0.07), and lysine (p = 0.08). The digestibility of most amino acids was numerically increased by enzyme supplementation.

DISCUSSION

Adding the enzyme blend to piglet diet improved body weight gain and gain:feed ratio over 8 to 12% in the current experiment. This result is consistent with previous reports (Pettey et al., 2002; Fang et al., 2007; Ji et al., 2008). Kim et al. (2003) reported that supplementing carbohydrase in corn-soybean based-diets to nursery pigs improved gain:feed ratio by 7 to 9%. The greater improvement (8 to 12%) in gain:feed ratio obtained in this study may be due to the different enzyme profiles used. In this study, enzyme complex contained xylanase, amylase and protease with targeted substrates including NSP, indigestible starch and protein. While in the experiment of Kim et al. (2003), the enzyme used contained β-1,4-mannanase, β-1,4mannosidase and α-1,6-galactosidase, which only targeting on NSPs.

Enzyme supplementation improved the digestibility of gross energy, crude protein, and dry matter by 2, 4 and 2%, respectively in Exp. 1. This result is consistent with previous findings (Caine et al., 1998; Café et al., 2002; Olukosi et al., 2007). For example, Caine et al. (1998) reported that supplementation of protease can increase the solubility of soybean protein and decrease the effect of trypsin inhibitors. The use of carbohydrases in corn-soybean based-diets improved ileal energy digestibility for grower-finisher pigs (Kim et al., 2006). The improvement in nutrient digestibility in our study indicates that the enzyme complex may have exerted its beneficial effects on nutrient digestibility, and thus performance, through first breaking down the plant cell wall structure and then releasing the nutrients contained in the cell wall for use by the pig (Bedford and Schulze, 1998).

Sucrase and maltase are intestinal brush border glycoside hydrolases and are responsible for the final steps of carbohydrate digestion. Hedemann et al. (2006) reported that pigs fed high insoluble fiber diets had increased mucosal enzyme activity. This suggests that the activity of these enzymes at the ileal brush border may be modulated by the amount of fermentable material reaching the hindgut. However, in the current study, no differences in activity of these enzymes were found among treatments on the jejunal and ileal brush border enzyme activities. Similarly, there was no difference on the jejunal and ileal brush border enzyme activities for pigs fed diets supplemented with or

without enzymes (Li et al., 2004; Willamil et al., 2012). Brush border enzyme activities may depend on several factors such as the section of small intestine (Hedemann et al., 2006) and pig age (Fan et al., 2002). These factors might help to explain the differences among studies.

In our study, the viscosity of the digesta in the stomach was higher for pigs fed the diet supplemented with enzyme complex than for pigs fed the diet without enzyme complex. The reason for the increase in digesta viscosity in stomach may be that enzyme supplementation degraded the insoluble NSPs fraction into short-chain polymers with increased solubility and water holding capacity of the digesta. An increase in digesta viscosity for a diet supplemented with enzyme was also observed by Li et al. (2004) and Willamil et al. (2012).

Bach Knudsen et al. (1991), Teitelbaum and Walker (2002), and Wong et al. (2006) demonstrated that soluble NSPs contained in the diet helped to stimulate the growth of commensal gut flora and thus increased the production of short-chain fatty acids, reduced pH in the large intestine and inhibited potentially harmful bacteria. In our study, the cecal and colonic volatile fatty acid concentrations were significantly higher for pigs fed the diet supplemented with enzyme complex than for those without enzymes. Högberg and Lindberg (2004) also showed similar results, where enzyme supplementation markedly increased volatile fatty acid concentrations and lowered the pH in the cecum. The increase of volatile fatty acids in the cecum and colon may have some beneficial effects. Wong et al. (2006) reported that butyrate is of great interest for it is a preferred energy source for colonocytes (Roediger, 1982); it also stimulates colon epithelial cells thus increasing the absorptive capacity of the epithelium (Topping and Clifton, 2001). This may further explain the better performance observed for pigs fed diets supplemented with enzyme complex in our study.

This study further demonstrated that adding enzymes to the piglet diets can improve apparent ileal digestibility of amino acid. Barrera et al. (2004) also reported that supplementing xylanase improved ileal digestibility of some amino acids in wheat-based diet of growing pigs. Yin et al. (2001a) and Yin et al. (2001b) reported that the inclusion of xylanase in diets containing hulless barley increased apparent ileal digestibility of some amino acids. Dierick and Decuypere (1994) suggested that xylanase supplementation could break down cell wall, eliminating the encapsulating effects of the cell wall and thus increased the digestion of protein at the proximal of the small intestine.

IMPLICATIONS

The results of the present study clearly demonstrate that the supplementation of an enzyme complex containing amylase, protease and xylanase improved performance in young pigs. This improvement was likely mediated through changes in nutrient digestibility, volatile fatty acid concentrations and bacteria in the large intestine.

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