Influences of Enzyme Complex Supplementation on Growth, Ileal and Apparent Fecal Digestibility and Morphology of Small Intestine in Pigs *

B. G. Kim[1], J. Z. Tian[1], J. S. Lim[1], D. Y. Kil[1], H, Y. Jeon[2], Y. K. Chung[3] and Y. Y. Kim[1,]**
School of Agricultural Biotechnology, Seoul National University, Seoul 151-742, Republic of Korea

ABSTRACT : A total of 140 weaning pigs were used to determine the effects of digestive enzyme supplementation to corn-soybean meal diets on growth performance, physiological changes of small intestine, microorganisms and pH in the gastrointestinal tract. Two kinds of enzyme complex (A, B) were used in this experiment. Pigs were allotted in a completely random design (CRD) to five replicates with four pigs per pen. Diets and water were provided for *ad libitum* consumption. Treatments included 1) Control: without enzyme supplementation, 2) Enzyme A 0.05%, 3) Enzyme A 0.10%, 4) Enzyme A 0.15%, 5) Enzyme B 0.05%, 6) Enzyme B 0.10%, 7) Enzyme B 0.15% in the diets. A total of 24 crossbred barrows 25.78±0.55 kg BW fitted with simple ileal T-cannulas were used to evaluate the effect the enzyme addition on the nutrient digstibility. Pigs were allotted 4 treatments (No enzyme, enzyme A 0.05%, enzyme A 0.1%, enzyme A 0.15%), 6 replicates according to a completely random design (CRD). Another digestibility trial was followed for enzyme complex B. Twenty pigs, average 31.92±0.37 kg BW, fitted with simple ileal T-cannulas for digestibility trial. Neither enzyme A nor enzyme B affected on fecal or ileal digestibility of dry matter, gross energy, crude protein, crude fat and crude ash ($p>0.05$). The apparent fecal digestibilities of all the nutrients were higher in total feces collection method than in indirect method. At the end of feeding trial, 21 pigs were slaughtered for examining the morphological changes of small intestine and the concentration of microorganisms in the ileum and the colon. Growth performance, intestinal morphology and pH of ileum and colon were not affected by the either enzyme complex supplementation ($p>0.05$). These results suggested that enzyme complex A and enzyme complex B were of no benefit to early-weaned pigs when corn-soybean meal based diet was provided. ***(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 12 : 1729-1735)***

Key Words : Pig, Enzyme, Digestibility, Morphology, β-Glucan

INTRODUCTION

The improvement of nutrients digestibility and growth performance has been one of the most important nutritional areas. There has been considerable interest in the use of exogenous enzymes in the animal diet over the last 10-15 years. Early studies used crude amylase and protease preparations (Jensen et al., 1957; Fry et al., 1958; Burnett, 1962). Although early works were academically interesting, those would appear to have limited practical application. Recent studies referred specific name of enzymes used in the experiments (Dritz et al., 1995; Jensen et al., 1998; Mavromichalis et al., 2000), phytase and fiber degrading enzyme product were widely used (Thacker, 2000). The effects of enzyme supplementation depended on the chemical composition, the characteristics of feed ingredient, breed and the physiological status of the animal.

The experiment of enzyme supplementation was conducted in poultry first. Earlier studies with broiler demonstrated that supplementation of a barley-based diet with β-glucanase improved growth rate and subsequently allowed a higher inclusion level of barley in the broiler diets (Hesselman and Aman, 1986). Also supplementation of a rye-based diet with pentosanase reduced sticky droppings and improved the digestibility of organic matter, crude protein and starch in the small intestine of chickens (Pettersson and Aman, 1989).

In the case of pig's diet, however, the use of exogenous enzymes in diets did not show the consistent improvements as found in studies with broilers previously (Thacker et al., 1991; Inborr et al., 1993; Thacker and Baas, 1996; Jensen et al., 1998; Mavromichalis et al., 2000; Thacker, 2001). Numerous experiments reported nutrient digestibilities were increased by supplementation of exogenous enzyme in pigs (Gdala et al., 1997; Jensen et al., 1998; Yin et al., 2001) but not others (Wubben, 1998; Thacker, 2001). The difference in extend and consistency of response to enzyme supplementation in pigs has been related to age of the animal, enzyme activity and dietary fiber level. Most of enzyme studies in pigs were performed with wheat, barley or oat diet rather than corn-soybean meal diet (Graham et al., 1989; Inborr et al., 1993; Dritz et al., 1995; Jensen et al., 1998). In Korea, however, corn and soybean meal are the main ingredients in current swine feed.

The aim of this experiment was to investigate the effects of enzyme complex supplementation in growing pigs' diet on growth performance, ileal and apparent fecal

* Financial support for this project was provided by CTC Bio Ltd., Seoul, Korea.
** Corresponding Author: Y. Y. Kim. Tel: +82-2-880-4801, Fax: +82-2-878-5839, E-mail: yooykim@snu.ac.kr
[2] CTC Bio Ltd., Seoul, Korea.
[3] DangJin Livestock Cooperative Feed Mill, Korea.
Received September 9, 2003; Accepted July 28, 2004

Table 1. The specifications of enzyme complex A and B

	Enzyme complex A (units/g)	Enzyme complex B (units/g)
β-glucanase	150	1,000
xylanase	4,000	1,200
α-amylase	1,000	25
pectinase	25	-
protease	500	-

digestibilities of nutrients, morphology of small intestine, and pH in the gut of pigs.

MATERIALS AND METHODS

Animals and experimental design

A total of 140 crossbred pigs ([Landrace×Yorkshire] ×Duroc) with an average body weight of 5.36±0.11 kg were used in 11 weeks feeding trial. Pigs were grouped based on body weight, and assigned to seven treatments in a completely randomized design (CRD) in five replicates with four pigs per pen. The treatments included 1) Control: without enzyme supplementation, 2) Enzyme A 0.05%, 3) Enzyme A 0.10%, 4) Enzyme A 0.15%, 5) Enzyme B 0.05%, 6) Enzyme B 0.10%, 7) Enzyme B 0.15% in the diets. The specifications of enzyme complex A and B are presented in Table 1. Enzyme A contained both carbohydrases and proteases while enzyme B consisted of carbohydrases.

Experimental diet and vitamin-trace mineral premixes formulas

The basal diets were formulated to contain approximately 3,265 ME kcal/kg for the young period (d 0-35) and 3,275 ME kcal/kg for the growing period (d 35-77), and 1.51%, 1.34%, 1.20% and 0.97% lysine for d 0-7, d 7-21, d 21-35 and d 35-77, respectively. Other dietary nutrients met or exceeded NRC (1998) standard. The ingredient and chemical composition of experimental diets are presented in Table 2.

Feeding trials

Pigs were housed in half-slotted concrete floors pen (0.90×2.15 m^2 for four pigs) during the day 0-35, and moved in concrete-floored half-plastic woven slurry pen (1.26×2.55m^2 for four pigs) during the day 36-77, and were allowed *ad libitum* access to water and meshed-diet during entire experimental period. Body weight and feed intake were recorded at d 7, d 21, d 35 and d 77. Body weight gain was calculated by the difference between the initial body weight and final body weight. Feed conversion ratio was calculated by dividing the amount of feed consumed with the corresponded body weight gain.

Digestive trials

For the digestibility trial, twenty four pigs (PIC, 25% Meishan; 25.78±0.55 kg average initial weight) fitted with simple ileal T-cannulas were housed in an individual metabolic cage. The cannula was a rigid, light weight, yet extremely durable plastic and had an internal diameter of 11 mm. Pigs were allotted 4 treatments (No enzyme, enzyme A 0.05%, enzyme A 0.1%, enzyme A 0.15%), 6 replicates according to a completely random design (CRD). The amount of feed consumed and total excreta were recorded daily during the metabolic trial. Another experiment was followed for enzyme complex B. Twenty pigs initially weighing 31.92±0.37 kg fitted with simple ileal T-cannulas were used. Experimental design, basal diet and other procedures were the same with the enzyme A digestive trial.

Pigs were given seven days of adaptation period, and two days of feces collection and three days of ileal sample collection were followed. Collected excreta were pooled and dried in an air-forced drying oven at 60°C for 72 h and

Table 2. Percentage composition of experimental diets

Item	d 0-7	d 7-21	d 21-35	d 35-77
Ingredient, %				
Corn	33.00	48.00	61.80	68.45
SBM-44	35.20	32.33	30.70	26.20
Lactose	20.00	10.00	-	-
SDPP	6.00	4.00	2.00	-
Wheat[1]	3.00	3.00	3.00	3.00
L-lysine-HCl	-	-	-	0.05
TCP	1.15	1.05	0.90	0.70
Limestone	0.85	0.82	0.80	0.75
Salt	0.20	0.20	0.20	0.30
Vit. mixture[2]	0.20	0.20	0.20	0.25
Min. mixture[3]	0.30	0.30	0.30	0.20
Antibiotics[4]	0.10	0.10	0.10	0.10
Total	100.00	100.00	100.00	100.00
Calculated compositon[5]				
ME, kcal/kg	3,266.16	3,266.49	3,265.22	3,275.60
CP	22.81	21.19	20.02	18.07
Lysine	1.51	1.34	1.20	0.97
Ca	0.80	0.75	0.69	0.60
Total P	0.66	0.64	0.61	0.50
β-glucan content[6]	0.58	0.34	0.33	0.55

[1] Enzymes were premixed in wheat and add to the diet at the appropriate treatment level at the expense of wheat.

[2] Supplied per kilogram of diet: 16,000 IU of vitamin A; 3,200 IU of vitamin D_3; 35 IU of vitamin E; 5 mg of vitamin K_3; 6 mg of riboflavin; 16 mg of calcium pantothenic acid; 32 mg of niacin; 128 μg of d-biotin; 20 μg of vitamin B_{12} (d 0-35), 12,800 IU of vitamin A; 2,560 IU of vitamin D_3; 28 IU of vitamin E; 4 mg of vitamin K_3; 5 mg of riboflavin; 13 mg of calcium pantothenic acid; 27 mg of niacin; 102 μg of d-biotin; 20 μg of vitamin B_{12} (d 35-77).

[3] Supplied per kilogram of diet: 281 mg of Cu (copper sulfate); 288 mg of Fe (ferrous sulfate); 0.3 mg of I (calcium iodate); 49 mg of Mn (manganese sulfate); 0.3 mg of Se (sodium selenite); 143 mg of Zn (zinc sulfate) (d 0-35), 187 mg of Cu (copper sulfate); 190 mg of Fe (ferrous sulfate); 0.2 mg of I (calcium iodate); 32 mg of Mn (manganese sulfate); 0.2 mg of Se (sodium selenite); 96 mg of Zn (zinc sulfate) (d 35-77).

[4] Avilamycin 20 mg per kilogram of diet.

[5] Calculated value.

[6] Analyzed value.

ground with 1 mm Wiley mill for chemical analyses. Ileal samples were collected continuously in vinyl bags between 08:00 h and 24:00 h on collection day. Ileal samples were immediately frozen and freeze dried (Ilsin Eng. Co, Korea), then ground with an 1 mm Wiley mill.

Chemical analyses

Chemical analyses of proximate nutrients in diets, feces and ileal samples were conducted according to the method of AOAC (1995). Gross energy content of diets, feces and ileal samples were analyzed by using an adiabatic bomb calorimeter (Parr Instrument, Moline, IL).

Morphology of small intestine

At the end of feeding trial, 21 pigs (3 pigs per treatment, 56.27±0.17 kg body weight) were slaughtered for examining the morphological changes of small intestine and the concentration of microorganisms in the ileum and the colon. The samples of small intestine were obtained each (10 cm in length) at proximal, middle and distal part of the small intestine from the gastric pylorus to the ileo-caecal valve. These were fixed in neutral-buffered formalin and processed by the standard paraffin method. Sections (9-10 cm) were stained with haematoxylin and eosin, and examined under a light microscope. Measurements of villus height and crypt depth were taken only from sections where the plane of section ran vertically from tip of villus to base of an adjacent crypt. From each section, a calibrated eyepiece graticule was used to measure 10 of the tallest well oriented villi from tip to crypt mouth, and 10 associated crypts from crypt mouth to base (Hampson et al., 1988).

pH in the ileum and the colon

Samples were taken from the distal part of the ileum, and the middle part of the colon. The MK-250 pH-meter (Shimazu, Japan) was used to measure pH of small intestine.

Statistical analyses

Statistical analysis was carried out by comparing means according to least significant difference (LSD) multiple range test, using the General Linear Model (GLM) procedure of SAS (1985).

RESULTS AND DISCUSSION

Growth performance

The effects of enzyme complex supplementation on the growth performance were shown in Table 3. During the day 0-35, average daily gain (ADG) was not affected by the enzyme complex supplementation ($p>0.05$). Average daily gain was higher in enzyme complex B 0.15% treatment than enzyme complex B 0.10% treatment ($p<0.05$). Average daily feed intake (ADFI) and feed conversion ratio (FCR) were not different among treatments ($p>0.05$).

Table 3. Growth performance of weaning and growing pigs

	Control	Enzyme complex A			Enzyme complex B			SE
		0.05%	0.10%	0.15%	0.05%	0.10%	0.15%	
Body weight								
d 0	5.36	5.36	5.37	5.34	5.37	5.36	5.36	0.11
d 7	6.88	6.72	6.49	6.34	6.53	6.59	6.50	0.12
d 21	11.53	11.46	11.05	11.10	11.35	10.93	11.05	0.24
d 35	18.88	18.24	17.62	18.65	18.47	17.65	17.73	0.28
d 77	53.69	51.48	51.47	50.54	50.96	49.35	51.63	0.60
Average daily gain								
d 0-7	217	194	161	143	166	176	163	7.30
d 7-21	332	339	326	335	345	310	325	10.35
d 21-35	525	484	469	540	508	480	477	13.00
d 35-77	829[a]	792[abc]	806[ab]	764[bc]	774[bc]	755[c]	807[ab]	7.70
Overall	628	599	599	589	592	571	601	6.69
Average daily feed intake								
d 0-7	223	203	193	172	176	191	183	5.86
d 7-21	578	620	563	576	606	549	550	14.99
d 21-35	961	923	886	1033	940	894	865	26.53
d 35-77	1,971	1,925	1,945	1,878	1,925	1,775	1,977	22.84
Overall	1,375	1,349	1,342	1,333	1,347	1,248	1,352	17.12
Feed conversion ratio								
d 0-7	1.03	1.05	1.27	1.47	1.06	1.09	1.13	0.06
d 7-21	1.75	1.85	1.74	1.74	1.77	1.78	1.74	0.03
d 21-35	1.84	1.89	1.89	1.92	1.85	1.86	1.81	0.02
d 35-77	2.38	2.43	2.41	2.46	2.48	2.35	2.45	0.02
Overall	2.20	2.25	2.24	2.26	2.27	2.18	2.25	0.01

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

Table 4. Effects of enzyme A supplementation on apparent digestibility (%) of nutrients by total feces collection method and indirect method in growing pig[1]

Item	Enzyme A level (%)				SE
	0.00	0.05	0.10	0.15	
Total feces collection method					
Dry matter	90.37	89.64	88.83	89.89	0.60
Gross energy	89.87	89.72	88.50	89.65	0.57
Crude protein	89.47	90.23	87.79	89.66	0.63
Crude fat	89.01	86.93	86.99	89.45	0.93
Indirect method					
Dry matter	64.64	77.62	70.48	63.52	3.52
Gross energy	67.86	75.48	80.22	61.68	2.95
Crude protein	60.13	75.17	66.93	61.93	3.43
Crude fat	63.53	71.75	65.71	66.31	2.91

[1] Total of 24 pigs, average initial body weight was 25.78±0.55 kg.

Table 5. Effects of enzyme B supplementation on apparent digestibility (%) of nutrients by total feces collection method and indirect method in growing pig[1]

Item	Enzyme B level (%)				SE
	0.00	0.05	0.10	0.15	
Total feces collection method					
Dry matter	91.25	88.04	88.73	90.51	0.57
Gross energy	91.39	87.95	88.90	90.55	0.56
Crude protein	90.69	87.45	88.23	90.89	0.54
Crude fat	88.35	83.27	81.29	86.80	1.22
Indirect method					
Dry matter	79.15	73.07	77.28	77.27	1.28
Gross energy	80.45	74.41	78.48	78.50	1.20
Crude protein	79.78	68.14	74.05	78.60	1.59
Crude fat	74.02	68.13	65.14	70.74	1.59

[1] Total of 20 pigs, average initial body weight was 31.92±0.37 kg.

In some experiments, enzyme supplementation to the diet improved growth performance in pigs (Bedford et al., 1992; van Lunen and Schulze, 1996). However, other feeding trials with enzyme supplementation showed no improvement in feed intake or growth performance of pigs (Thacker et al., 1991; Thacker et al., 1992; Bedford et al., 1992; Thacker and Baas, 1996; Jensen et al., 1998; Mavromichalis et al., 2000; Thacker, 2001).

Dierick and Decuypere (1994) demonstrated a total mean retention time through the stomach and small intestine of pig was about 4-5 h which was comparably longer than that of broiler. Consequently, additional digestive enzyme in diet would be less effective in pigs compared to poultry. The substrates for supplemented enzymes such as β-glucan and xylan, in the basal diets also would not be enough. And the greater bacterial proliferation in the gut of the pig could reduce the effect of exogenous enzyme.

Fecal digestibility

The effects of enzyme complex A supplementation to the corn-soybean meal diet on the nutrients digestibility by total feces collection method and indirect method was shown in Table 4. The digestibilities of dry matter (DM), gross energy (GE), crude protein (CP), crude fat (CF), crude ash (CA) and calcium (Ca) were not improved by enzyme A complex addition in both method.

Pettersson and Aman (1989) found significant improvement of organic matter, crude protein and starch digestibility when β-glucanase/pentosanase enzyme complex was added in poultry diet. And the supplementation with xylanase increased ileal xylose digestibility (Gdala et al., 1997). However, Wubben (1998) demonstrated enzyme (cellulase and hemicellulase) supplementation did not affect the digestibility of dry matter, crude protein, organic matter and amino acids. Thacker (2001) also dbserved enzyme (protease, cellulase, xylanase, α-galactosidase and amylase) supplementation had no effect on nutrient digestibility.

In present experiment, enzyme complex (β-glucanase, xylanase, α-amylase, pectinase, protease) was supplemented and this enzyme complex was expected to enhance the nutritional value of vegetable protein sources through increased utilization of their non-starch polysaccharides. Not enough substrate and/or low activity of the enzyme in the gastrointestinal tract could be the reason for the lack of beneficial response.

The effects of enzyme complex B addition on the nutrients digestibility by total feces collection method and indirect method were shown in Table 5. The digestibilities of dry matter, gross energy, crude protein, crude fat, were not improved by enzyme B addition, either.

The supplementation of β-glucanase increased the digestibility of mixed linked β-glucan (Li et al., 1996b; Jensen et al., 1998). In their experiment, however, the digestibility of starch and nitrogen was not changed, same as present experiments. On the other hand, in a recent experiment, enzyme (β-glucanase and xylanase) treatment increased ileal digestibility of energy and some amino acids (Yin et al., 2001). These inconsistent results could be explained by the difference of basal diet. Li et al. (1996a) said that β-glucanase was effective enzyme on the nutrient digestibility in the barley-soybean meal based diet but not in the wheat-, corn- or rye-soybean meal diet.

The comparison of two different digestibility trials in the present study was interesting. The apparent fecal digestibilities of all the nutrients were higher in total feces collection method than in indirect method. Considering chromium (Cr) level of feces and ileal samples as air dry base were 0.48 and 0.64% respectively, Cr might be accumulated or absorbed in the large intestine. Gross energy and crude protein content was 7% and 50% higher in feces than in the ileal samples.

Table 6. Effects of enzyme A and emzyme B supplementation on ileal digestibility (%) of nutrients in growing pig

Item	Enzyme level (%)				SE
	0.00	0.05	0.10	0.15	
Enzyme complex A[1]					
Dry matter	76.72	75.96	75.97	76.04	0.95
Gross energy	77.70	76.30	76.59	76.26	0.91
Crude protein	83.64	82.29	83.14	82.71	0.59
Crude fat	91.63	90.20	90.22	87.89	0.61
Enzyme complex B[2]					
Dry matter	77.12	76.48	76.19	79.47	1.07
Gross energy	79.65	79.67	79.37	81.45	0.96
Crude protein	84.61	84.76	81.41	83.54	1.06
Crude fat	83.87	81.90	82.49	85.35	0.96
Crude ash	52.40	52.11	58.14	52.29	1.56

[1] Total of 24 pigs, average initial body weight was 25.78±0.55 kg.
[2] Total of 20 pigs, average initial body weight was 31.92±0.37 kg.

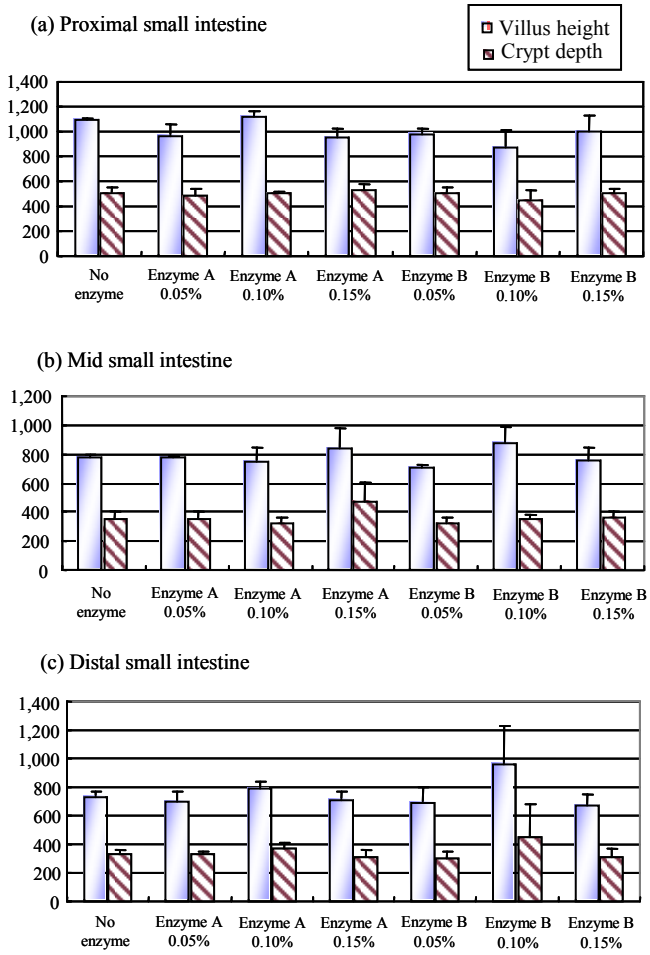

Figure 1. Villus height (μm) and crypt depth (μm) of proximal (a), mid (b) and distal (c) small intestine.

Ileal digestibility

The influence of enzyme complex A or B supplementation to the corn-soybean meal diet on the ileal digestibility was shown in Table 6. As discussed previously, the effects of enzyme supplementation on the nutrient digestibility were varied. In this experiment, the digestibilities of dry matter, gross energy, crude protein, crude fat, were not affected by enzyme complex addition.

In addition to the previous discussion, the age of the animal could influence on the effect of enzyme supplementation. In a recent experiment, however, enzyme (β-glucanase and xylanase) treatment increased ileal digestibility of energy and some amino acids in 31 days old pigs (Yin et al., 2001).

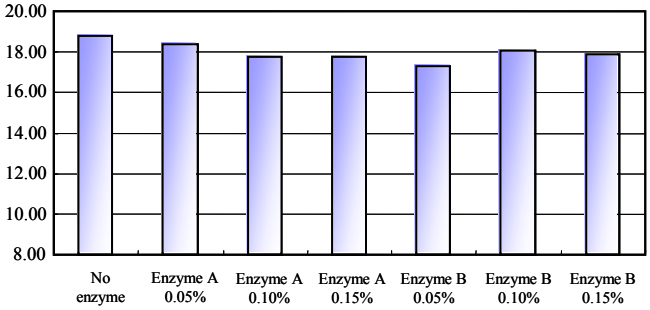

Figure 2. Length (m) of small intestine.

Morphology of small intestine

The effects of enzyme complex supplementation on the villus height and crypt depth were shown in Figure 1. There was no improvement in the villus height and crypt depth of the proximal, mid and distal part of small intestine by the supplementation of enzyme complex ($p>0.05$). The anti-nutritional factors have been shown to incite enzyme inhibition through cell surface-legume agglutinin interaction and/or cause villus atrophy (Makinde et al., 1997). In the case of early weaning pigs, weaning and exposure of the neonatal intestine to feed antigens have been implicated in delaying maturation of the intestine (Arvola et al., 1992). In this experiment, the age of pig was relatively older therefore, the negative effect of weaning on the villus height and crypt depth would be compensated and additional improvement by exogenous enzyme would not be found. Hall and Byrne (1989) observed the evidence of intestinal repair, as indicated by an increased crypt cell production rate three weeks after weaning.

The effect of enzyme complex supplementation on the length of small intestine was shown in Figure 2. The length of small intestine was not different among treatments ($p>0.05$). Contrary toexpected hypothesis, small intestine length was numerically the highest in no enzyme supplemented treatment.

Kelly et al. (1991) reported that the marked differences in live weight contributed to the difference in the weight of digestive tract of pigs weaned at 14 d age during the 5 d post-weaning. In this experiment, however, the slaughter weight was almost the same among treatment (21 pigs; 56.27±0.17 kg body weight).

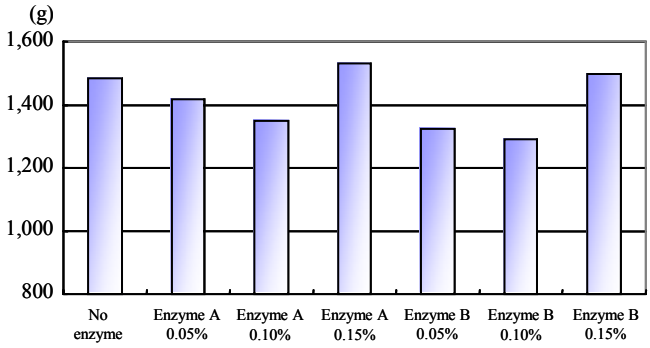

Figure 2. Weight (g) of small intestine.

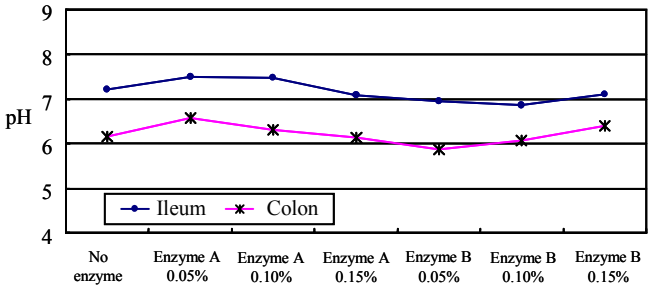

Figure 3. pH of the ileum and colon.

The weight of small intestine was not affected by the enzyme supplementation (Figure 3). The effect of enzyme supplementation on the weight of small intestine was not significant ($p>0.05$). The weight of small intestine was numerically higher (14% and 16% respectively) as exogenous enzyme supplementation level was increased both in enzyme A and B complex. This difference might be explained by the increase the efficiency of feed particles, degradation in gastrointestinal tract due to enzyme addition. The present study also suggested that the enzyme complex supplementation did not affect the length of small intestine.

pH in the ileum and the colon

The effect of enzyme supplementation on the acidity in ileum and colon was shown in the Figure 4. The pH in ileum was 0.95 higher than that of colon. The lower pH in the colon was caused by the volatile fatty acids produced by the microorganisms which would be more abundant in colon. However, enzyme supplementation did not significantly affected on the acidity in the ileum or in the middle colon ($p>0.05$).

The results of this experiment could show the effect of two enzyme complexes supplementation to the pig diet. However, our results could not represent all the enzymes nor all the diets because many factors influenced on the effect of enzyme, such as the age of animal, basal diet and feed processing. In the case of β-glucan, for instance, the solubility increased from 45 to 62% when it was pelleted (Graham et al., 1989). Thus, when we determine enzyme supplementation level, we have to consider the solubility changes by the feed processing. Most of experiments related to carbohydrase reported that their destination was improving digestibility by degrading cell wall content. By the way, some cell wall content itself could positively affect the animal health (Dritz et al., 1995).

In conclusion, results of the present study demonstrated that enzyme complex A and enzyme complex B had no influence on the growth performance, nutrients digestibilities, intestinal morphology and pH in the gastrointestinal tract in early-weaned and growing pigs fed diets based on corn-soybean meal.

REFERENCES

AOAC. 1995. Official Methods of Analysis (16th ed). Association of Official Analytical Chemists. Washington, DC.

Arvola, T., I. Rantala, A. Martinen and E. Isolauri. 1992. Early dietary antigens delay the development of gut mucosal barrier in preweaning rat. Pediatr. Res. 32:301-305.

Bedford, M. R., J. F. Phatience, H. L. Classen and J. Inborr. 1992. the effect of dietary enzyme supplementation of rye- and barly-based diets on digestion and subsequent performance in weanling pigs. Can. J. Anim. Sci. 72:97-105.

Burnett, G. S. 1962. The effect of damaged starch, amylolyitc enzymes, and proteolytic enzymes on the utilization of cereals by chickens. Br. Poult. Sci. 3:89-103.

Dierick, N. A. and J. A. Decuypere. 1994. Enzyme and growth in pigs. In: Principles of pig science (Ed. D. J. A. Cole, J. Wiseman and M. A. Varley). Nottingham University Press. Nottingham, UK. pp. 169-195.

Dritz, S. S., J. Shi, T. L. Kielian, R. D. Goodband, J. L. Nelssen, M. D. Tokach, M. M. Chengappa, J. E. Smith and F. Blecha. 1995. Influence of dietary β-glucan on growth performance, nonspecific immunity, and resistance to streprococcus suis infection in weanling pigs. J. Anim. Sci. 73:3341-3350.

Fry, R. E., J. B. Allred, L. S. Jensen and J. McGinnis. 1958. Influence of enzyme supplementation and water treatment on the nutritional value of different grains for poults. Poult. Sci. 37:372-375.

Gdala, J., H. N. Johansen, K. E. B. Knudsen, I. H. Knap, P. Wagner and O. B. Jorgensen. 1997. The digestibility of carbohydrates, protein and fat in the small and large intestine of piglets fed non-supplemented and enzyme supplemented diets. Anim. Feed Sci. Technol. 65:15-33.

Graham, H., J. G. Fadel, C. W. Newman and R. K. Newman. 1989. Effect of pelleting and β-glucanase supplementation on the ileal and fecal digestibility of a barley-based diet in the pig. J. Anim. Sci. 67:1293-1298.

Hall, G. A. and T. F. Byrne. 1989. Effects of age and diet on small intestinal structure and function in gnobiotic piglets. Res. Vet. Sci. 47:387-392.

Hampson, D. J., Z. F. Fu and W. C. Smith. 1988. Preweaning supplementary feed and porcine post-weaning diarrhoea. Res. Veter. Sci. 44:309.

Hesselman, K. and P. Aman. 1986. The effect of β-glucanase on the utilization of starch and nitrogen by broiler chickens fed on

barley of low- or high-viscosity. Anim. Feed Sci. Technol. 15:83-93.

Inborr, J., M. Schmitz and F. Ahrens. 1993. Effect of adding fibre and starch degrading enzymes to a barley/wheat based diet on performance and nutrient digestibility in different segments of the small intestine of early weaned pigs. Anim. Feed Sci. Technol. 44:113-127.

Jensen, L. S., R. E. Fry, J. B. Allred and J. McGinnis. 1957. Improvement in the nutritional value of barley for chicks by enzyme supplementation. Poult. Sci. 36:919-921.

Jensen, M. S., K. E. B. Knudsen, J. Inborr and K. Jakobsen. 1998. Effect of β-glucanase supplementation on pancreatic enzyme activity and nutrient digestibility in piglets fed diets based on hulled and hulless barley varieties. Anim. Feed Sci. Technol. 72:329-345.

Kelly, D., J. A. Smyth and K. J. McCracken. 1991. Digestive development in the early-weaned pig. II. Effect of level of food intake on digestive enzyme activity during the immediate post-weaning. Br. J. Nutr. 65:181-188.

Li, S., W. C. Sauer, R. Mosenthin and B. Kerr. 1996a. Effect of β-glucanase supplementation of cereal-based diets for starter pigs on the apparent digestibilities of dry matter, crude protein and energy. Anim. Feed Sci. Technol. 59:223-231.

Li, S., W. C. Sauer, S. X. Huang and V. M. Gabert. 1996b. Effect of β-glucanase supplementation to hulless barley- or wheat-soybean meal diets on the digestibilities of energy, protein, β-glucans, and amino acids in young pigs. J. Anim. Sci. 74:1649-1656.

Makinde, M. O., E. Umapathy, B. T. Akingbemi, K. T. Mandisodz and E. Skadhauge. 1997. Differential response of legumes and creep feeding on gut morphology and faecal composition in weanling pigs. Comp. Biochem. Physiol. 118A:349-354.

Mavromichalis, I., J. D. Hancock, B. W. Senne, T. L. Gugle, G. A. Kennedy, R. H. Hines and C. L. Wyatt. 2000. Enzyme supplementation and particle size of wheat in diets for nursery and finishing pigs. J. Anim. Sci. 78:3086-3095.

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

Pettersson, D. and P. Aman. 1989. Enzyme supplementation of a poultry diet containing rye and wheat. Br. J. Nutr. 62:139-149.

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

Thacker, P. A. 2000. Recent advances in the use of enzymes with special reference to β-glucanases and pentosanases in swine rations. Asian-Aust. J. Anim. Sci. 13:376-385.

Thacker, P. A. 2001. Effect of enzyme supplementation on the performance of growing-finishing pigs fed barley-based diets supplemented with soybean meal or canola meal. Asian-Aust. J. Anim. Sci. 14:1008-1013.

Thacker, P. A. and T. C. Bass. 1996. Effects of gastric pH on the activity of exogenous pentosanase supplementation of the diet on the performance of growing-finishing pigs. Anim. Feed Sci. Technol. 63:187-200.

Thacker, P. A., G. L. Campbell and J. GrootWassink. 1991. The effect of enzyme supplementation on the nutritive value of rye-based diets for swine. Can. J. Anim. Sci. 71:489-496.

Thacker, P. A., G. L. Campbell and J. GrootWassink. 1992. The effect of organic acids and enzyme supplementation on the performance of pigs fed barley-based diets. Can. J. Anim. Sci. 72:395-402.

van Lunen, T. A. and H. Schulze. 1996. Influence of Trichoderma longibrachiatium xylanase supplementation of wheat and corn based diets on growth performance of pigs. Can. J. Anim. Sci. 76:271-273.

Wubben, J. E., D. M. Albin, M. R. Smiricky and V. M. Gabert. 1998. Ileal digestibilities of amino acids in growing pigs fed soybean meal and corn starch-based diets supplemented with fiber-degrading enzymes and steeping. http://porknet.outreach.uiuc.edu/pdf/IlealDigestibilitiesOf.PDF.

Yin, Y. -L., S. K. Baidoo, H. Schulze and P. H. Simmins. 2001. Effects of supplementing diets containing hulless barley variety having different levels of non-starch polysaccharides with β-glucanase and xylanase on the physiological status of the gastrointestinal tract and nutrient digestibility of weaned pigs. Livest. Prod. Sci. 71:97-107.