Effect of Feeding Organic Acid With or Without Enzyme on Intestinal Microflora, Intestinal Enzyme Activity and Performance of Weaned Pigs

Defa Li*, S. D. Liu, S. Y. Qiao, G. F. Yi, C. Liang[1] and P. Thacker[2]
Ministry of Agriculture Feed Industry Center, China Agricultural University, Beijing, China

ABSTRACT : Ninety-six, 35 day old, crossbred pigs, were fed either a basal diet based on corn, soybean meal, fish meal and whey or one of three similar diets supplemented with either 0.5% organic acid or enzyme both alone and in combination. Neither organic acid nor enzyme produced any significant ($p<0.05$) improvements in daily gain or feed conversion. Organic acid, both alone and in combination with enzyme, significantly ($p=0.04$) decreased the pH in the lower colon. None of the treatments produced any effects on *E. Coli* or *Lactobacillus* numbers in any part of the gastrointestinal tract. Feeding enzyme increased trypsin ($p=0.01$), chymotrypsin ($p=0.03$) and amylase ($p=0.08$) levels in the jejunum. Chymotrypsin levels were higher ($p=0.04$) in the ileum of pigs fed enzyme. Serum glucose levels were lower ($p=0.01$) on day 14 in pigs fed enzyme either alone or in combination with acid. Under the conditions of this experiment (10% dietary whey, pigs weaned at 35 days of age), neither organic acid nor enzyme were effective in improving starter pig performance. Therefore, there would appear to be little justification for the routine inclusion of these products in diets fed to pigs weaned at 35 days or later. *(Asian-Aus. J. Anim. Sci. 1999. Vol. 12, No. 3 : 411-416)*

Key Words : Pigs, Performance, Enzymes, Organic Acids, Bacteria, pH

INTRODUCTION

A post-weaning growth check, characterized by slow growth, general unthriftiness and scouring is often observed in piglets during the first week after weaning (Barnett et al., 1989). Manners (1976) stated that this post-weaning lag may be related to the inability of the weaned pig to secrete a sufficient quantity of hydrochloric acid to lower pH in the stomach.

There are several consequences of this reduced acid-secreting ability. Firstly, hydrochloric acid is involved in the activation of pepsinogens (Mayes, 1990). Therefore, piglets with an elevated gastric pH are likely to experience a reduction in the efficiency of protein digestion (Kirchgessner and Roth, 1982; Easter, 1988). In addition, since gastric pH plays a role in preventing the movement of viable bacteria from the environment into the upper small intestine (Mayes, 1990), the limited acid-secreting ability may result in an increase in scouring. Another explanation for the post-weaning growth check is the observation that levels of several pancreatic enzymes are depressed at weaning (Owsley et al., 1986; Lindemann et al., 1986; Makkink et al., 1994). Jensen et al. (1997) reported significant reductions in the levels of trypsin, chymotrypsin and amylase in recently weaned 28 day old pigs compared with suckling pigs.

Since both acid-secreting ability and digestive enzyme secretion are low in the weaned pig, supplementation with either an exogenous source of enzyme or an organic acid may help to improve starter pig performance at weaning. Therefore, the present study was conducted to evaluate the effects of feeding an organic acid or an enzyme additive, both alone and in combination, on the performance of weaned pigs as well as to monitor the effects of these additives on pH, microflora populations and enzyme activity in the gastrointestinal tract.

* Corresponding Author: Defa Li.
[1] Kemin Industries (Asia) Pte Ltd., 12 Senko Drive, Singapore
[2] Department of Animal Science, University of Saskatchewan, Saskatoon, Canada.
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MATERIALS AND METHODS

Animals and diets

Ninety-six, 35 day old, crossbred (Duroc × Landrace × Yorkshire) pigs, weighing an average of 10.4 ± 0.6 kg, were divided into four groups on the basis of sex, weight and litter. They were fed a basal diet based on corn, soybean meal, fish meal and whey or one of three similar diets supplemented with either 0.5% organic acid (Acid Lac, Kemin Industries Ltd., Singapore) or 700 mg/kg enzyme (Kemzyme, Kemin Industries Ltd., Singapore) both alone and in combination. All diets were provided in mash form and were formulated to provide 3.28 Mcal/kg DE, 18.9% crude protein and contained sufficient vitamins and minerals to meet or exceed NRC (1988) requirements (table 1).

During the 28 day growth trial, all pigs were housed in groups of eight in an environmentally controlled building containing 1.8 × 2.4 m wired-floored pens equipped with self feeders. Three pens, containing four gilts and four castrates, were assigned to each treatment. Pigs were permitted *ad libitum* access to feed and water throughout the experiment. Weight gain and feed intake were recorded and used to calculate feed conversion at the completion of the trial.

The enzyme additive used in this experiment contained a blend of starch, protein and fiber-digesting enzymes of fungal (*Trichoderma reesei*) and bacterial origin (*Bacillus licheniformis, Bacillus subtilis, Bacillus acidophilus and Aspergillus niger*). According to the manufacturers specifications, the enzyme additive provided a-amylase (20,000 AU/kg), β-amylase (20,000 AU/kg), β-glucanase (330 BGU/kg), branched chain

amylase (69 AU/kg), pectinase (27,500 PCU/kg), exoproteinase (100,000 PCU), endoproteinase (100,000 PU/kg) and cellulase (3,750 CU). The organic acid mixture consisted primarily of lactic acid, fumaric acid, propionic acid, formic acid and ortho-phosphoric acid.

Table 1. Composition and nutrient levels of diets fed to determine the effects of enzyme and/or organic acids on starter pig performance (35 to 63 days)

	Control	Organic Acid	Enzyme	Combi-nation
Ingredient (% as fed)				
Corn	56.64	56.14	56.64	56.14
Soybean meal	22.00	22.00	22.00	22.00
Fish meal (Peruvian)	5.00	5.00	5.00	5.00
Whey (dried)	10.00	10.00	10.00	10.00
Soybean oil	2.00	2.00	2.00	2.00
Calcium carbonate	0.50	0.50	0.50	0.50
Dicalcium carbonate	2.00	2.00	2.00	2.00
Salt	0.10	0.10	0.10	0.10
Premix[1]	1.00	1.00	1.00	1.00
Amino acids[2]	0.76	0.76	0.76	0.76
Organic acids[3]	-	0.50	-	0.50
Enzyme[4]	-	-	+	+
Analyzed Nutrient Level (% as fed)				
Crude protein	18.90	18.90	18.90	18.90
Calcium	0.85	0.85	0.85	0.85
Phosphorus	0.70	0.70	0.70	0.70
Lysine	1.30	1.30	1.30	1.30
Threonine	0.92	0.92	0.92	0.92
Diet pH	5.70	5.15	5.52	5.14

[1] Vitamin-mineral premix provided the following per kg of complete diet: 5,512 IU vitamin A, 3,551 IU vitamin D, 66.1 IU vitamin E, 27.6 mg vitamin B_{12}, 2.2 mg vitamin K, 5.5 mg riboflavin, 13.8 mg d-pantothenate, 30.3 mg niacin, 551 mg choline chloride, 100 mg Mn, 100 mg Fe, 100 mg Zn, 250 mg Cu, 0.3 mg I, 1 mg Co, 0.3 mg Se.

[2] All diets were supplemented with 0.36% lysine hydrochloride, 0.2% methionine, 0.1% threonine and 0.1% tryptophan.

[3] Acid Lac (Kemin Industries Ltd., Singapore) consisted primarily of lactic acid, fumaric acid, propionic acid, formic acid and ortho-phosphoric acid.

[4] Kemzyme (Kemin Industries Ltd., Singapore) provided α-amylase (20,000 AU/kg), β-amylase (20,000 AU/kg), β-glucanase (330 BGU/kg), branced chain amylase (69 AU/kg), pectinase (27,500 PCU/kg), exoproteinase (100,000 PCU), endoproteinase (100,000 PU/kg) and cellulase (3,750 CU).

Blood sampling

On day 3, five pigs per treatment were selected and blood samples obtained by anterior vena cava puncture using uncoated vacutainer tubes. On day 14, seven pigs per treatment were bled. The blood samples were centrifuged for 5 min at 1000 rpm and the serum was stored at -20℃ until needed for analysis.

Digesta sampling

Seven days after weaning, 12 non-fasted pigs (3 pigs per treatment) were killed by electrocution followed by exsanguination. The viscera were immediately exposed via a midline incision and the stomach, duodenum, jejunum, ileum, cecum and colon were aseptically isolated, doubly ligated, and removed. A 5-10 cm section of the duodenum, jejunum and ileum, taken from a site as close as possible to the middle of the tract, was tied off, cut and placed in an ice chest for immediate transportation to the laboratory for microbiological assay. In addition, the pylorus was opened and samples of chyme were placed in 5 ml sealed conical tubes and also placed on ice.

Digesta was removed from the remaining parts of the digestive tract by gently massaging the intestine and collecting the digesta in 50 ml Biomex beakers. The pH of the different parts of the gastrointestinal tract were determined using a Cole Farmer bench top pH meter. Five 111 samples of digesta for enzyme assay were taken from the jejunum and ileum, placed in self-sealing conical tubes and immediately frozen in liquid nitrogen.

Chemical analysis of diets

The crude protein, calcium and phosphorus content of the diets were determined using the methods of the AOAC (1990). Crude protein was analyzed using the Kjeldahl method (AOAC method 988.05), calcium by titration with 0.1 N KMnO, (AOAC method 927.02) and total phosphorus was determined colorimetrically using a molybodovanadate reagent (AOAC method 965.05).

Samples of the diets were hydrolyzed with 6 M HCl at 110 ℃ for 24 h and analyzed for lysine and threonine using high-performance liquid chromatography (Shimadzu LC 10 Liquid Chromatograph). Dietary pH was determined in triplicate by weighing 20 g of diet into a 250 ml beaker and adding 100 ml of de-ionized water (Radecki et al., 1988). The pH was read using a Cole Panner pH meter.

Analysis of serum samples

Serum samples were analyzed for alkaline amino transferase, blood urea nitrogen, cholesterol, creatinine kinase and glucose using a Technicon RA-1000 autoanalyzer and commercial kits supplied by the Zhongsheng High-Tech Bioengineering Company (Beijing, China).

Microbiological analysis of digesta

About 0.5 g of digesta from the stomach, duodenum, jejunum and ileum were aseptically placed into sterile tubes containing 9.5 ml of sterile diluent for microbial determination. The diluent contained 4.5 g KH_2PO_4, 6 g NaH_2PO_4, 0.5 g L-cysteine, 1 g glucose, 0.5 g Tween 80 and 1000 ml of distilled water. *Lactobacilli* were enumerated on Man-Rogosa-Sharpe agar plates after being incubated for 48 h in a 5% CO incubator (Heraeus Model BE-5060 Incubator). *Escherichia coli* were enumerated on MacConkey agar plates after incubation for 24 h. All plates were incubated at 37℃.

Analysis of enzyme activity in digesta

Frozen digesta samples were slowly thawed at 0-4℃ and then centrifuged (Heraeus Biofuge 22R Centrifuge)

Table 2. Effect of feeding organic acid and/or enzyme on the performance of weaned pigs (35 to 63 days)[1]

	Control	Organic acid	Enzyme	Combination	SEM[2]	p Value
Daily gain (g)	380	399	412	401	9.66	0.22
Feed intake (g)	677	685	669	668	25.34	0.96
Feed conversion	1.78	1.72	1.62	1.67	0.06	0.33

[1] No means significantly different ($p<0.05$). [2] Standard Error of the Mean.

Table 3. Effect of organic acid and/or enzyme additive on the pH in various sections of the gastrointestinal tract[1]

	Control	Organic acid	Enzyme	Combination	SEM[2]	p Value
Pylorus	4.2	3.2	3.8	2.5	0.46	0.13
Fundus	3.5^{a}	2.8^{ab}	2.8^{ab}	1.8^{b}	0.40	0.09
Jejunum	5.7^{ab}	5.3^{b}	6.4^{a}	5.6^{ab}	0.22	0.05
Ileum	6.4	6.2	6.8	5.9	0.32	0.30
Cecum	5.9^{a}	5.5^{a}	5.5^{a}	4.9^{b}	0.17	0.02
Lower colon	6.6^{a}	5.5^{b}	6.5^{a}	5.6^{b}	0.28	0.04

[1] Means with different subcripts are significantly different ($p<0.05$). [2] Standard Error of the Mean.

Table 4. Effect of organic acid and/or enzyme addition on gastrointestinal *E. coli* and *Lactobacilllus* counts (log colony forming units)[1]

	Control	Organic acid	Enzyme	Combination	SEM[2]	p Value
E. Coli						
Stomach	5.2^{ab}	4.5^{ab}	5.8^{a}	3.9^{b}	0.43	0.08
Duodenum	5.1	4.7	5.4	4.4	0.54	0.56
Jejunum	5.4	5.1	5.5	5.2	0.73	0.97
Ileum	7.0	6.3	6.1	6.8	0.46	0.55
Lactobacillus						
Stomach	6.6	6.9	6.1	7.2	0.56	0.60
Duodenum	5.5	6.2	6.1	5.9	0.46	0.75
Jejunum	5.9	6.6	6.1	6.1	0.23	0.26
Ileum	6.8	7.3	6.6	7.7	0.46	0.36

[1] Means followed by same or no letter do not differ ($p<0.05$). [2] Standard Error of the Mean.

under refrigeration (4℃) at 13,000 rpm for 10 min. The supernatant was stored at -70℃ until determination of enzyme activity. Trypsin and chymotrypsin activity were determined using N-benzoyl-DL-arginine-p-nitroanilide (Sigma, B4875) and N-glutaryl-L-phenylalanine-p-nitroanilide (Sigma, G2505) as the substrates, respectively (Owsley et al., 1986). Standard curves for determining trypsin and chymotrypsin activity in intestinal contents were generated using P-nitroaniline (Sigma, N-2128). Amylase activity was tested by using the Amylase-PNP enzymatic Colour Test (Centronic, Lye-buffer, DK0155-1). Protein content in the supernatant was tested by the Lowry method (Lowry, 1951).

Statistical analysis

For pig performance data, a completely randomized design was used with pen as the experiment unit. For the statistical analysis of serum parameters, microbiological data and enzyme activity, the individual pig was the experimental unit. Data were subjected to an analysis of variance using the GLM procedures of SAS (1989) and Duncan's Multiple Range test was used to separate means when appropriate (SAS, 1989)

RESULTS

The effects of feeding organic acid and enzyme, both alone and in combination, on the performance of the pigs during the 28 day growth trial are summarized in table 2. Neither organic acid nor enzyme produced any significant improvements in daily gain or feed conversion. Organic acid, both alone and in combination with the enzyme, significantly ($p=0.04$) decreased pH in the lower colon and produced numerical reductions in pH in all other sections of the gastrointestinal tract (table 3). Pigs fed enzyme alone had a significantly ($p=0.05$) higher pH in the jejunum compared with pigs fed the organic acid diet. In the remaining sections of the tract, enzyme alone had no consistent effect on pH producing slight increases in some sections (ileum) and slight reductions in others (pylorus, fundus, cecum).

The effects of feeding organic acid or the enzyme additive on microflora in the gastrointestinal tract are shown in table 4. None of the treatments produced any statistically significant changes in either *E. Coli* or *Lactobacillus* numbers in any part of the gastrointestinal tract. Supplementing organic acid, both alone and in

Table 5. Effect of feeding organic acid and/or enzyme on trypsin, chymotrypsin and amylase activity in the jejunum and ileum (IU/mg protein)[1]

	Control	Organic acid	Enzyme	Combination	SEM[2]	p Value
Jejumum						
Trypsin	548[b]	398[b]	2,376[a]	2,504[a]	404	0.01
Chymotrypsin	903[b]	1,881[b]	3,909[a]	4,379[a]	752	0.03
Amylase	33,208[ab]	19,288[ab]	104,462[a]	76,566[ab]	7,045	0.08
Ileumbacillus						
Trypsin	666	653	460	577	167	0.81
Chymotrypsin	1,911[b]	1,566[b]	6,168[a]	2,150[b]	1,003	0.04
Amylase	6,077	6,749	45,372	21,867	11,774	0.14

[1] Means with different subscripts are significantly different ($p<0.05$). [2] Standard Error of the Mean.

Table 6. Effect of feeding organic acid and/or enzyme on blood biochemical criteria

	Control	Organic acid	Enzyme	Combination	SEM[2]	p Value
3 Days After Weaning						
Blood urea nitrogen (mmol/l)	4.0	3.2	5.2	5.2	0.55	0.18
Alanine amino transferase (u/l)	60	64	42	60	14.66	0.79
Glucose (mg/dl)	85	93	96	89	6.48	0.73
Creatinine kinase (u/l)	806	1,042	324	942	195	0.24
Cholesterol (mg/dl)	86	77	76	76	7.48	0.43
14 Days After Weaning						
Blood urea nitrogen (mmol/l)	5.0	4.0	3.7	3.7	0.46	0.11
Alanine amino transferase (u/l)	63	54	64	50	10.02	0.72
Glucose (mg/dl)	103[a]	100[a]	83[b]	83[b]	4.27	0.01
Creatine kinase (u/l)	688	737	563	585	147	0.82
Cholesterol (mg/dl)	69	61	56	51	5.57	0.16

[1] Means with different subscripts are significantly different ($p<0.05$). [2] Standard Error of the Mean.

combination, numerically ($p>0.05$) decreased the E. coli counts in the stomach, duodenum, jejunum and ileum, while with the exception of the ileum, E. coli counts were numerically ($p>0.05$) higher in the gastrointestinal tract of pigs fed enzyme. In contrast, pigs fed organic acid had numerically ($p>0.05$) higher concentrations of *lactobacillus* in all sections of the tract, both when the acid was fed alone and also when fed in combination with the enzyme.

Feeding the enzyme additive significantly increased trypsin ($p=0.01$) and chymotrypsin ($p=0.03$) levels in the jejunum (table 5). Amylase levels were also increased ($p=0.08$). Chymotrypsin levels were significantly higher ($p=0.04$) in the ileum of pigs fed the enzyme while amylase levels were also substantially higher in pigs fed enzyme. Because of large sample variability, the difference did not reach statistical significance ($p>0.05$).

There were no significant changes in serum levels of blood urea nitrogen, alanine amino transferase, creatinine kinase or cholesterol in blood samples taken at either day 3 or day 14 (table 6). Serum glucose levels were significantly lower ($p=0.01$) at day 14 in pigs fed the enzyme either alone or in combination with acid.

DISCUSSION

Supplementation with organic acid did not significantly improve either growth rate or feed conversion. Previous attempts to improve the performance of starter pigs by supplementation of the diet with various organic acids have generally been more successful (Kirchgessner and Roth, 1982; Falkowski and Aherne, 1984; Giesting and Easter, 1985; Easter, 1988; Bergstrom et al., 1995; Bergstrom et al., 1996). This apparent discrepancy may be partially explained by the fact that in the present trial, the pigs were weaned at 35 days of age while Bergstrom et al. (1996), reported that the greatest response to acidifiers was obtained with younger pigs. In addition, it has been reported that pigs fed diets with a significant portion of milk or animal-based proteins will show less of a response to organic acids than pigs fed simple diets (Giesting and Easter, 1991, Johnson et al., 1993). All diets fed during the present study contained 10% whey.

Feeding organic acid significantly decreased the pH in the colon and produced numerical reductions in the pH of the stomach, jejunum, ileum and cecum. Burnell et al. (1988), also reported that organic acid addition to a corn-soybean meal-whey based diet resulted in a numerical reduction in the pH of the contents of the stomach, small intestine and large intestine of pigs at 21 d. Scipioni et al. (1978), reported that the pH in the stomach and jejunum was reduced from 4.55 and 6.57 to 3.5 and 6.43 by the addition of citric acid to the diet, but Risley et al. (1992), found no change in the pH of the stomach or jejunum.

Thomlinson et al. (1981) and Scipioni et al. (1978), reported that the incorporation of organic acids in the diet reduced the bacterial burden in the gastrointestinal tract. However, our results showed that supplementing lactic acid only slightly decreased *E. coli* counts compared with the control, and there was no appreciable effect on *Lactobacillus* counts ($p>0.05$). The general health of the pigs in our study was good and there were no problems with post-weaning diarrhea in any of the groups. The effect of an acidifier on intestinal *E. coli* burden may be more significant in situations where colibacillosis is a problem.

Feeding the enzyme additive significantly increased trypsin, chymotrypsin and amylase activity in the jejunum. This shows that the enzymes present in the multi-enzyme product survived passage through the stomach and were active in the small intestine. However, despite this dramatic increase in enzyme activity in the small intestine, neither daily gain nor feed conversion were significantly improved as a result of enzyme supplementation. Owsley et al. (1986), observed that, in weaned pigs between days 31 and 56, the synthesis and secretion of trypsin, chymotrypsin and amylase increased approximately 10 fold. Therefore, it is likely that the 35 to 63 day old pigs used in the present experiment had sufficient endogenous enzyme secretion and therefore no benefit was observed from providing additional enzyme.

The failure of the enzyme cocktail to improve starter pig performance contrasts with earlier work conducted with the same enzyme product that was used in the present experiment (Han et al., 1997). Han et al. (1997), reported an 8.3% improvement in daily gain from including 75 mg/kg Kemzyme in the diet of pigs weaned at 4 weeks of age. The older age of the pigs used in the present experiment may partially account for this inconsistency but several other report involving supplementation with amylases (Cunningham and Brisson, 1957a, Combs et al., 1960 Inboor et al., 1993), proteolytic enzymes (Cunningham and Brisson, 1957b; Combs et al., 1960: or P-glucanase (Thacker et al., 1992; Inboor et al., 1993) have also resulted in only modest or no improvements in pig performance.

IMPLICATIONS

Under the conditions of this experiment, in which the pigs were weaned at 35 days of age and fed diets which included 10% whey, neither organic acid nor enzyme was effective in improving starter pig performance. Therefore, there would appear to be little justification for the routine inclusion of these products in diets fed to pigs weaned at 35 days or later.

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