Asian-Aust. J. Anim. Sci.
Vol. 21, No. 2 : 252 - 261
February 2008

www.ajas.info

Effects of Organic Acids on Growth Performance, Gastrointestinal pH, Intestinal Microbial Populations and Immune Responses of Weaned Pigs

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ABSTRACT : Two experiments were conducted to compare the effects of feeding organic acids and antibiotic growth promoters in weaned pigs. In Exp. 1, 96 nursery pigs (Large White×Landrace; initial weight 7.80±0.07 kg) were randomly allotted into one of four dietary treatments. Pigs in treatment 1 were fed a complex starter diet. Treatments 2 to 4 were the same as treatment 1 but supplemented with antibiotics (200 ppm chlortetracycline plus 60 ppm Lincospectin), 0.5% potassium diformate or 0.5% dry organic acid blend ACTIVATE Starter DA (ASD). During the 4-week post-weaning period, pigs fed ASD or antibiotics had better gain ($p = 0.03$) and feed efficiency ($p = 0.04$) than pigs fed the control diet. On d 14 post-weaning, pigs fed the control diet had the lowest fecal *lactobacilli* count among all dietary treatments ($p = 0.02$), whereas pigs fed ASD or antibiotics had a trend for lower fecal *E. coli* count compared to the control pigs ($p = 0.08$). Serum insulin-like growth factor-1 (IGF-1) of pigs fed ASD did not differ from pigs fed the control diet ($p>0.05$) at d 14 after weaning. In Exp. 2, 24 weaned pigs (Large White×Long White; initial weight 5.94±0.33 kg) were allotted into four groups and housed individually. Pigs were fed a control diet or diets supplemented with antibiotics (100 ppm colistin sulfate, 50 ppm Kitasamycin plus 60 ppm Olaquindox), 0.5% or 1% ASD. All pigs were orally challenged with *E. coli* $K88^+$ on d 5. During d 5 to 14 after challenge, pigs fed antibiotics, 0.5% or 1% ASD had better gain ($p = 0.01$) and feed efficiency ($p = 0.03$) than pigs fed the control diet. On d 14, compared to the control pigs, pigs fed 0.5% ASD had higher *lactobacilli* in the duodenum and pigs fed 1% ASD and antibiotics had a trend for higher *lactobacilli* in the ileum ($p = 0.08$). Pigs fed antibiotics, 0.5% or 1% ASD diets tended to have decreased ileal *E. coli* count compared to those fed the control diet ($p = 0.08$). Serum interleukin-6 and cortisol and digesta pH values were not affected by treatment or time. These results indicate that feeding ASD can improve the growth performance of weaning pigs, mainly via modulating intestinal microflora populations without affecting gastrointestinal pH or immune indices. (**Key Words :** Organic Acids, Antibiotics, Weaned Pigs, Growth Performance, Gastrointestinal pH, Microbial Population)

INTRODUCTION

After weaning, young pigs are submitted to a variety of nutritional, environmental and social stressors, which often lead to growth depression and post-weaning diarrheic syndrome (Ravindran and Kornegay, 1993). Therefore, antibiotic growth promoters are commonly used to help overcome the weaning problems of nursery pigs (Partanen and Morz, 1999). The primary mode of action of antibiotics is antimicrobial and is mediated directly or indirectly through the gastrointestinal microflora (Anderson et al., 1999; Dibner and Buttin, 2002). However, the use of in-feed antibiotics can result in many side effects, including antibiotic resistance and antibiotic residues in meat, which has become a concerning problem for the swine industry. With increasing pressures of antibiotic resistance and food safety concerns, acceptance for the use of non-therapeutic antibiotics in animal feed is eroding in the EU countries, and antibiotics usage is decreasing in North America and other continents as well (Choct, 2001; Dibner and Richards, 2005).

Among a variety of candidates for the replacement of antibiotics, organic acids have been broadly applied worldwide with reasonable success (Partanen and Mroz, 1999; Mroz, 2005). Cole et al. (1968) reported that the supplementation of organic acids in drinking water not only improved the growth performance but reduced the number

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Received February 9, 2007; Accepted June 15, 2007

Table 1. Dietary composition and nutrient profile, % as fed basis (Exp. 1)

Item	Control	Antibiotic	Potassium Diformate	ASD[2]
Corn	51.50	51.50	51.50	51.65
Soybean meal	21.00	21.00	21.00	21.00
Spray dried porcine plasma	3.00	3.00	3.00	3.00
Fish meal	5.00	5.00	5.00	5.00
Soybean protein concentrate	3.00	3.00	3.00	3.00
Whey powder	10.00	10.00	10.00	10.00
Soybean oil	3.00	3.00	3.00	3.00
Dicalcium phosphate	0.90	0.90	0.90	0.90
Limestone	0.60	0.60	0.60	0.60
Salt	0.25	0.25	0.25	0.25
DL-methionine (99%)	0.15	0.15	0.15	0.00
Corn starch	0.50	0.50	0.00	0.00
Vitamin-mineral premix[1]	1.00	1.00	1.00	1.00
Potassium Diformate[2]	-	-	0.50	-
ACTIVATE Starter DA[2]	-	-	-	0.50
Lysine·HCl (98%)	0.10	0.10	0.10	0.10
Total	100	100	100	100
Calculated nutrient composition[3]				
Crude protein (%)	21.00	21.00	21.00	21.00
ME (kcal/kg)	3,234	3,234	3,220	3,220
TID lysine (%)	1.40	1.40	1.40	1.40
TID methionine activity[4] (%)	0.50	0.50	0.50	0.50
TID cysine (%)	0.36	0.36	0.36	0.36
TID tryptophan (%)	0.27	0.27	0.27	0.27
TID threonine (%)	0.94	0.94	0.94	0.94
Ca (%)	0.80	0.80	0.80	0.80
Available P (%)	0.40	0.40	0.40	0.40

[1] Supplied per kilogram of diet: vitamin A, 11,000 IU; vitamin D_3, 1,503 IU; vitamin E, 44.1 IU; menadione, 4.0 mg; riboflavin, 5.22 mg; pantothenic acid, 20.0 mg; niacin, 26.0 mg; choline chloride, 450 mg; vitamin B_{12}, 0.01 mg; Fe, 150 mg; Cu, 150 mg; Zn, 150 mg; Mn, 20 mg; I, 0.25 mg; Se, 0.25 mg; feed antioxidant, 500 mg.

[2] Antibiotic treatment was fed 200 ppm chloretetracycline (Jinghe Group, Inner Mongolia, China) and 60 ppm lincospectin (Pfizer Animal Health, NJ, USA); Potassium diformate was supplied by Tiaozhan group, Beijing, China; ASD: ACTIVATE Starter DA was a dry organic acid blend composed of calcium salt of 2-hydroxy-4-(methylthio)butanoic acid, fumaric acid, benzoic acid, and carrier with 31% L-Met activity (supplied by Novus International, Inc., St. Louis, MO, USA).

[3] Calculated based on the feed ingredient composition data from our lab and the true ileal digestibility (TID) data from NRC (1998).

[4] For ASD treatment, 0.155% TID methionine activity was supplied by ACTIVATE Starter DA.

of *E. coli* in the gut of post-weaning pigs. Ever since then, many findings have shown that organic acids can provide beneficial effects similar to those of feeding antibiotics (Sciopioni et al., 1978; Mathew et al., 1991). The probable mode of actions of organic acids includes reducing the digesta pH value in the gastrointestinal tract (GIT) (Ravindran and Kornegay, 1993), regulating the balance of microbial populations in the gut, stimulating the secretion of digestive enzyme (Harada et al., 1988; Thaela et al., 1998) and promoting the growth and recovery of the intestinal morphology (Galfi and Bokori, 1990). However, in some research publications, there was no effect of feeding organic acids on growth performance (Omogbenigun et al., 2003; Sacakli et al., 2006) and microbial populations (Risley et al., 1992). Both potassium diformate (Partanen and Morz, 1999) and dry organic acid blend ACTIVATE Starter DA (ASD) (a product of Novus International Inc., St. Louis, MO, USA) (Yi et al., 2006) were found to enhance the growth performance of weaning pigs and modulate the intestinal microbial population and pH of early-weaned pigs, but the studies on the somatotrophic axis and immune response were very limited.

Therefore, two experiments were conducted to evaluate the impact of feeding organic acids or antibiotics on growth performance, digesta pH values and microbial population of GIT, and serum indexes including cortisol, interleukin-6, urea nitrogen, and insulin-like growth factor-1 (IGF-1) of weaned pigs with or without enterotoxigenic *E. coli* $K88^+$ challenge.

MATERIALS AND METHODS

General management

All the pigs in this study were allowed to have *ad libitum* access to dry mash feed an water and pig facility was supplied with 24 h constant lighting and room temperature was maintained between 28 to 30°C. Experimental diets were formulated to meet or exceed the

nutrient requirements of nursery pigs (NRC, 1998) (Table 1, 5).

Experiment 1

A total of 96 crossbred barrows (Large White× Landrace; initial weight 7.80±0.07 kg) were randomly allotted into one of four dietary treatments with eight replicate pens per treatment and three pigs per pen. Pigs in treatment 1 were fed a corn- and soybean-based diet. Treatment 2 to 4 were the same as treatment 1 but supplemented with antibiotics (200 ppm chlortetracycline plus 60 ppm Lincospectin), 0.5% potassium diformate, or 0.5% ASD (Dry organic acid blend ACTIVATE® Starter DA, registered trademark of Novus International Inc., St. Louis, MO; which is composed of calcium salt of 2-hydroxy-4-(methylthio)butanoic acid, fumaric acid, benzoic acid and carrier). Body weight, feed intake and feed consumption for the individual pens were recorded at d 0 and 28. On d 14 and 28 of weaning, blood samples were collected by anterior vena cava puncture and fresh fecal samples were collected (Kim et al., 2007) and immediately frozen at -20°C until analysis.

Experiment 2

Twenty-four 18-day-old crossbred barrows (5.94±0.33 kg) were assigned randomly to one of the four dietary treatments. Dietary treatments including control diets, diets supplemented with antibiotics (100 ppm colistin sulfate, 50 ppm Kitasamycin plus 60 ppm Olaquindox), 0.5% or 1% ASD. Because piglets were weaned one week earlier than the pigs in Exp. 1, therefore, according to the routine feeding program in China pig farms, piglets were provided the higher nutrition level diets.

After feeding pigs experimental diets for 4 days, all pigs were orally challenged with 5 ml culture fluid containing 1.0×10^9 cfu of *E. coli* $K88^+$ by using a syringe attached to a polyethylene tube. The *E. coli* $K88^+$ strain was isolated at the School of Veterinary Medicine of China Agricultural University (Beijing, China) and was confirmed by Polymerase chain reaction (PCR) genotyping as possessing the genes necessary for toxin expression *in vitro*. Blood samples were collected on 6, 12 and 24 h after challenge.

After 14 days experimental period, all pigs were killed to collect digesta samples of stomach, duodenum, jejunum, and ileum for microflora culturing and pH measurement. The liver and spleen were collected to measure the weight.

Determination of microflora counts in feces and gut

1 g of fecal or digesta samples was weighed and diluted to a 1:9 weight:volume ratio in sterile PBS, which was then homogenized for 2 min. Serial dilutions (1:10) were prepared, in which the dilutions used for *E. coli* and aerobic culturing were 10^{-2} to 10^{-5}, and those used for *lactobacilli* and anaerobic culturing were 10^{-4} to 10^{-7}, respectively. Diluted samples were plated on MacConkey agar (DIFCO Laboratories, Detroit, MI) following aerobic incubation at 37°C for 24 h to count *E. coli* bacteria; and plated on Man-Rogosa-Sharp Broth agar (DIFCO Laboratories, Detroit, MI) following anaerobic incubation at 37°C for 24 h to count *lactobacilli* according to the roll tube methods described by Eller et al. (1971); and plated on Brain-Heart Infusion agar (DIFCO Laboratories, Detroit, MI) following anaerobic or aerobic incubation at 37°C for 24 h to count anaerobes or aerobes. All bacteria were counted and expressed as total cfu/ml digesta, and the numbers of colony forming units were expressed as $\log_{10}$-transformed data for statistical analysis.

Determination of blood indexes

Blood were centrifuged at 3,500 rpm (Ciji 800 Model Centrifuge, Surgical Instrument Factory, Shanghai, China) for 10 minutes at 4°C and serum was separated for the analysis of urea nitrogen, IGF-1 in experiment 1 and interleukin-6, cortisol in experiment 2. Serum concentration of IGF-1 was determined by using a commercially available porcine IGF-1 IRMA kit (DSL-2800, Diagnostic Systems Laboratories, Inc., USA). The minimum detection limit was 2 ng/ml, with an intra-assay CV of 4%. Serum urea nitrogen was measured using a Biochemical Analytical Instrument (manufactured by Bayer Diagnostics Manufacturing Ltd., Dublin, Ireland) according to the method of Tao et al. (1982). Serum cortisol was determined using a commercially available ^{125}I RIA kit (Beijing Beimian Dongya Institute of Biological Technology, Beijing, China). The minimum detectable dose of cortisol was 1 ng/ml with an intra-assay coefficient of variation less than 5%. Serum interleukin-6 concentration was determined using a commercially available ^{125}I RIA kit (Beijing Beimian Dongya Institute of Biological Technology, Beijing, China). The minimum detection limit for the kit was 50 pg/ml with an intra-assay CV of 7% and an inter-assay CV of 15%.

Statistical analyses

All the data were subjected to ANOVA appropriate for a randomized complete block design by using the GLM procedures of SAS (SAS Inst., Inc., Cary, NC). Statistical differences of treatment mean comparisons were made with Duncan's Multiple Range Test. An alpha level of $p<0.05$ was used as the criterion for statistical significance, and $0.05<p<0.10$ was considered as trends.

RESULTS

Experiment 1

No difference was observed in ADFI among dietary treatments during the 4-week post-weaning period (p =

Table 2. Effects of feeding antibiotic and organic acids on growth performance of weaned pigs (4 weeks postweaning period, Exp. 1)

Item	Dietary treatments				SEM[1]	p-value
	Control	Antibiotic[2]	Potassium Diformate[2]	0.5% ASD[2]		
ADG (g/d)	323[b]	395[a]	354[ab]	371[a]	16.27	0.03
ADFI (g/d)	539	576	542	545	31.33	0.82
Feed:gain (g/g)	1.68[a]	1.46[b]	1.52[ab]	1.47[b]	0.06	0.04

[1] Standard error of mean.

[2] Antibiotic treatment was fed 200 ppm chloretetracycline (Jinghe Group, Inner Mongolia, China) and 60 ppm lincospectin (Pfizer Animal Health, NJ, USA); Potassium diformate was supplied by Tiaozhan group, Beijing, China; ASD: ACTIVATE Starter DA was a dry organic acid blend composed of calcium salt of 2-hydroxy-4-(methylthio)butanoic acid, fumaric acid, benzoic acid, and carrier with 31% L-met activity (supplied by Novus International, Inc., St. Louis, MO, USA).

[a, b] Means in a row without a common superscript differ ($p<0.05$). ADG = average daily gain; ADFI = average daily feed intake.

Table 3. Effects of feeding antibiotic and organic acids on fecal microbial counting of *lactobacilli*, *E. coli*, anaerobes, and aerobes (Exp. 1)

Item	Time	Control	Antibiotic[1]	Potassium Diformate[1]	ASD[1]	SEM	p-value
Lactobacilli	d 0	7.39	7.31	7.31	7.33	0.23	0.92
	d 14	6.81[b]	6.99[a]	7.03[a]	7.01[a]	0.04	0.02
	d 28	7.06	7.27	7.03	7.11	0.07	0.11
E .coli	d 0	3.70	3.66	3.68	3.66	0.08	0.96
	d 14	4.14	3.71	3.90	3.79	0.09	0.08
	d 28	4.13	3.66	3.87	3.74	0.14	0.23
Anaerobes	d 0	7.07	7.05	7.15	7.02	0.50	0.91
	d 14	7.25	6.77	7.02	6.96	0.13	0.33
	d 28	7.32	6.72	6.97	6.95	0.17	0.32
Aerobes	d 0	4.65	4.73	4.72	4.86	0.07	0.68
	d 14	4.57	4.44	4.35	4.39	0.44	0.94
	d 28	4.81	4.37	4.40	4.68	0.36	0.23

[a, b] Means in a row without a common superscript differ ($p<0.05$).

[1] Antibiotic treatment was fed 200 ppm chloretetracycline (Jinghe Group, Inner Mongolia, China) and 60 ppm lincospectin (Pfizer Animal Health, NJ, USA); Potassium diformate was supplied by Tiaozhan group, Beijing, China; ASD: ACTIVATE Starter DA was a dry organic acid blend composed of calcium salt of 2-hydroxy-4-(methylthio)butanoic acid, fumaric acid, benzoic acid, and carrier with 31% L-met activity (supplied by Novus International, Inc., St. Louis, MO, USA).

Table 4. Effect of feeding antibiotic and organic acids on serum urea nitrogen and insulin-like growth factor-1 concentration of weaned pigs (d 14 and 28, Exp. 1)

Item	Dietary treatments				SEM	p-value
	Control	Antibiotic[1]	Potassium Diformate[1]	ASD[1]		
d 14						
Urea nitrogen (mg/dl)	19.5	16.8	16.8	20.0	2.21	0.59
IGF-1 (ng/ml)	123.9[b]	231.2[a]	168.0[ab]	188.0[ab]	22.08	0.03
d 28						
Urea nitrogen (mg/dl)	22.1	24.0	18.1	22.5	2.60	0.43
IGF-1 (ng/ml)	198.4	260.3	201.3	250.0	23.39	0.15

[a, b] Means in a row without a common superscript differ ($p<0.05$); IGF-1 = Insulin-like growth factor-1.

[1] Antibiotic treatment was fed 200 ppm chloretetracycline (Jinghe Group, Inner Mongolia, China) and 60 ppm lincospectin (Pfizer Animal Health, NJ, USA); Potassium diformate was supplied by Tiaozhan group, Beijing, China; ASD: ACTIVATE Starter DA was a dry organic acid blend composed of calcium salt of 2-hydroxy-4-(methylthio)butanoic acid, fumaric acid, benzoic acid, and carrier with 31% L-met activity (supplied by Novus International, Inc., St. Louis, MO, USA).

0.82). However, pigs fed antibiotics or 0.5% ASD had better weight gain ($p = 0.03$) and feed efficiency ($p = 0.04$) than pigs fed the control diet (Table 2).

At the beginning of the experiment (d 0), there were no differences in fecal *lactobacilli*, *E. coli*, anaerobes and aerobes counts among dietary treatments ($p = 0.68$). On d 14 post-weaning, pigs fed the control diet had the lowest fecal *lactobacilli* count among all dietary treatments ($p = 0.02$), whereas pigs fed antibiotics or 0.5% ASD had a trend for lower fecal *E. coli* count compared to the control pigs ($p = 0.08$). However, there were no differences in the counts of anaerobes and aerobes among dietary treatments at d 14 after weaning ($p = 0.23$). On d 28 post-weaning, no difference was detected in fecal *lactobacilli*, *E. coli*, anaerobes and aerobes among any dietary treatments ($p = 0.11$) (Table 3).

On d 14 post-weaning, serum IGF-1 of pigs fed antibiotics was greater than pigs fed the control diet ($p = 0.03$), but no difference was observed between two organic acid treatments and control treatment. Moreover, serum

Table 5. Dietary composition and nutrient profile, % as fed basis (Exp. 2)

Items	Control	Antibiotic	0.5% ASD[2]	1% ASD[2]
Corn	46.30	46.30	46.45	46.60
Soybean meal	16.00	16.00	16.00	16.00
Spray dried porcine plasma	5.00	5.00	5.00	5.00
Fish meal	6.00	6.00	6.00	6.00
Soybean protein concentrate	5.00	5.00	5.00	5.00
Whey powder	15.00	15.00	15.00	15.00
Soybean oil	3.00	3.00	3.00	3.00
Calcium phosphate	0.80	0.80	0.80	0.80
Limestone	0.40	0.40	0.40	0.40
Salt	0.20	0.20	0.20	0.20
DL-methionine (99%)	0.30	0.30	0.15	-
Corn starch	1.00	1.00	0.50	-
Vitamin-mineral premix[1]	1.00	1.00	1.00	1.00
ACTIVATE Starter DA[2]	-	-	0.50	1.00
Total	100	100	100	100
Calculated nutrient composition[3]				
Crude protein (%)	23.66	23.66	23.66	23.66
ME (kcal/kg)	3,273	3,273	3,259	3,244
TID lysine (%)	1.53	1.53	1.53	1.53
TID methionine activity[4] (%)	0.66	0.66	0.66	0.67
TID cysine (%)	0.40	0.40	0.40	0.40
TID tryptophan (%)	0.30	0.30	0.30	0.30
TID threonine (%)	1.00	1.00	1.00	1.00
Ca (%)	0.80	0.80	0.80	0.80
Available P (%)	0.50	0.50	0.50	0.50

[1] Supplied per kilogram of diet: vitamin A, 11,000 IU; vitamin D_3, 1,503 IU; vitamin E, 44.1 IU; menadione, 4.0 mg; riboflavin, 5.22 mg; pantothenic acid, 20.0 mg; niacin, 26.0 mg; choline chloride, 450 mg; vitamin B_{12}, 0.01 mg; Fe, 150 mg; Cu, 150 mg; Zn, 150 mg; Mn, 20 mg; I, 0.25 mg; Se, 0.25 mg; feed antioxidant, 500 mg.

[2] Antibiotic treatment was fed 100 ppm colistin sulfate (Elanco Animal Health IN, USA), 50 ppm kitasamycin (Heruikang Animal Health, Chongqing, China) and 60 ppm olaquidox (Zhongmuanda group, Hubei, China); ASD: ACTIVATE Starter DA was a dry organic acid blend composed of calcium salt of 2-hydroxy-4-methylthiobutanoic acid, fumaric acid, benzoic acid, and carrier with 31% L-met activity (supplied by Novus International, Inc., St. Louis, MO, USA).

[3] Calculated based on the feed ingredient composition data from our lab and the true ileal digestibility (TID) data from NRC (1998).

[4] For 0.5% and 1% ACTIVATE treatment, 0.155% and 0.31% TID methionine activity was supplied by ACTIVATE Starter DA.

Table 6. Effects of feeding antibiotics and organic acids on growth performance of weaned pigs after *E. coli* $K88^+$ challenge (Exp. 2)

Item	Dietary treatments				SEM	p-value
	Control	Antibiotics[1]	0.5% ASD[1]	1% ASD[1]		
Days 0-4 (prechallenge)						
ADG (g/d)	221	246	221	205	14.40	0.29
ADFI (g/d)	270	283	279	251	20.49	0.71
Feed:gain (g/g)	1.21	1.15	1.26	1.24	0.06	0.56
Days 5-14 (postchallenge)						
ADG (g/d)	280[a]	341[b]	318[b]	315[b]	11.30	0.01
ADFI (g/d)	432	461	443	435	20.70	0.77
Feed:gain (g/g)	1.53[a]	1.34[b]	1.39[b]	1.40[b]	0.04	0.03
Days 0-14 (prechallenge and postchallenge)						
ADG (g/d)	270	307	290	281	9.22	0.07
ADFI (g/d)	390	406	396	383	16.87	0.79
Feed:gain (g/g)	1.44	1.32	1.36	1.36	0.04	0.26

[a, b] Means in a row without a common superscript differ ($p<0.05$); ADG = average daily gain; ADFI = average daily feed intake.

[1] Antibiotic treatment was fed 100 ppm colistin sulfate (Elanco Animal Health IN, USA), 50 ppm kitasamycin (Heruikang Animal Health, Chongqing, China) and 60 ppm olaquidox (a domestic supplier (Zhongmuanda group, Hubei, China); ASD: ACTIVATE Starter DA was a dry organic acid blend composed of calcium salt of 2-hydroxy-4-(methylthio)butanoic acid, fumaric acid, benzoic acid, and carrier with 31% L-met activity (supplied by Novus International, Inc., St. Louis, MO, USA).

IGF-1 on d 28 and urea nitrogen concentration on d 14 and 28 post-weaning were not affected by dietary treatments ($p = 0.15$) (Table 4).

Experiment 2

No differences were detected in ADG, ADFI, and feed: gain ratio among dietary treatments before the challenge (d

Table 7. Effects of feeding antibiotics and organic acids on organ indexes and digesta pH values of gastrointestinal tract after oral *E. coli* K88$^+$ challenge (d 14, Exp. 2)

	Dietary treatments				SEM	p-value
	Control	Antibiotics[1]	0.5% ASD[1]	1% ASD[1]		
Liver index[2]	35.02	31.28	31.24	31.06	1.22	0.09
Spleen index[2]	2.18	1.71	1.95	2.01	0.25	0.62
Stomach	3.27	2.98	2.82	2.93	0.20	0.45
Duodenum	5.72	5.17	5.25	5.62	0.34	0.60
Proximal jejunum	5.41	5.02	5.04	5.39	0.43	0.86
Middle jejunum	5.75	5.69	5.10	5.77	0.40	0.59
Distal jejunum	6.55	6.54	5.90	6.61	0.36	0.48
Ileum	6.94	6.92	6.03	6.54	0.27	0.11

[a, b] Means in a row without a common superscript differ ($p<0.05$).

[1] Antibiotic treatment was fed 100 ppm colistin sulfate (Elanco Animal Health IN, USA), 50 ppm kitasamycin (Heruikang Animal Health, Chongqing, China) and 60 ppm olaquidox (Zhongmuanda group, Hubei, China); ASD: ACTIVATE Starter DA was a dry organic acid blend composed of calcium salt of 2-hydroxy-4-(methylthio)butanoic acid, fumaric acid, benzoic acid, and carrier with 31% L-met activity (supplied by Novus International, Inc., St. Louis, MO, USA).

[2] Liver/spleen index = liver/spleen weight×1,000/body weight.

Table 8. Effects of feeding antibiotics and organic acids on gastrointestinal digesta microflora of weaned pigs after oral *E. coli* K88$^+$ challenge (d 14, Exp. 2)

	Dietary treatments				SEM	p-value
	Control	Antibiotics[1]	0.5% ASD[1]	1% ASD[1]		
Stomach						
Lactobacilli	6.99	7.04	7.00	6.99	0.08	0.95
E. coli	4.93	4.93	4.88	4.96	0.04	0.53
Anaerobes	7.06	7.10	7.02	6.95	0.05	0.24
Aerobes	5.09	5.21	5.22	5.18	0.04	0.18
Duodenum						
Lactobacilli	5.85	6.02	6.09	6.01	0.06	0.08
E. coli	3.91	3.92	3.95	3.89	0.04	0.78
Anaerobes	6.19	6.04	6.18	6.04	0.08	0.51
Aerobes	4.96	5.02	5.03	5.00	0.07	0.88
Jejunum						
Lactobacilli	6.59	6.72	6.78	6.79	0.08	0.24
E. coli	3.60	3.60	3.57	3.59	0.07	0.98
Anaerobes	6.89	6.93	6.85	6.78	0.07	0.52
Aerobes	4.99	5.02	4.93	4.90	0.07	0.69
Ileum						
Lactobacilli	6.89	7.05	7.00	7.07	0.05	0.08
E. coli	4.04[b]	3.91[a]	3.93[a]	3.93[a]	0.04	0.08
Anaerobes	6.96	6.88	6.93	6.91	0.06	0.82
Aerobes	4.95	5.06	5.03	5.07	0.06	0.48

[a, b] Means in a row without a common superscript differ ($p<0.05$).

[1] Antibiotic treatment was fed 100 ppm colistin sulfate (Elanco Animal Health IN, USA), 50 ppm kitasamycin (Heruikang Animal Health, Chongqing, China) and 60 ppm olaquidox (Zhongmuanda group, Hubei, China); ASD: ACTIVATE Starter DA was a dry organic acid blend composed of calcium salt of 2-hydroxy-4-(methylthio)butanoic acid, fumaric acid, benzoic acid, and carrier with 31% L-Met activity (supplied by Novus International, Inc., St. Louis, MO, USA).

0 to 4) ($p = 0.29$). During the post-challenge period with *E. coli* K88$^+$ (d 5 to 14), pigs fed 0.5% or 1% ASD and antibiotics had improved weight gain ($p = 0.01$) and feed efficiency ($p = 0.03$) compared to pigs fed the control diet. However, during the combined pre- and post-challenge period (d 0 to 14), only pigs fed antibiotics seemingly improved the body weight gain compared with the control pigs ($p = 0.07$).

The indexes of the liver and spleen and digesta pH values of GIT on d 14 were shown in Table 7. The liver index was tended to decrease in pigs fed antibiotics, 0.5% or 1% ASD diets compared to pigs fed the control diet ($p = 0.09$). However, there were no differences in spleen index ($p = 0.62$) or digesta pH values across different segments of GIT ($p = 0.11$) among all dietary treatments.

As shown in Table 8, at the end of post-challenge period (d 14), compared to pigs fed the control diet, pigs fed 0.5% ASD had a trend for higher *lactobacilli* count in the duodenum and pigs fed antibiotics and 1% ASD had a trend for greater *lactobacilli* count in the ileum ($p = 0.08$). Pigs

Table 9. Effects of feeding antibiotics and organic acids on serum interleukin-6 and cortisol concentrations after oral *E. coli* K88$^+$ challenge

Items	Dietary treatments				SEM	p-value
	Control	Antibiotics[1]	0.5% ASD[1]	1% ASD[1]		
6 h						
Interleukin-6 (pg/ml)	303.3	251.9	294.6	299.7	35.48	0.72
Cortisol (ng/ml)	118.6	89.0	121.6	98.6	17.70	0.51
12 h						
Interleukin-6 (pg/ml)	242.9	236.8	203.5	251.2	21.81	0.45
Cortisol (ng/ml)	148.0	123.0	102.8	124.1	12.54	0.12
24 h						
Interleukin-6 (pg/ml)	270.4	249.9	226.0	264.6	23.63	0.56
Cortisol (ng/ml)	114.8	120.5	132.6	116.2	18.45	0.90

[1] Antibiotic treatment was fed 100 ppm colistin sulfate (Elanco Animal Health IN, USA), 50 ppm kitasamycin (Heruikang Animal Health, Chongqing, China) and 60 ppm olaquidox (Zhongmu'anda group, Hubei, China); ASD: ACTIVATE Starter DA was a dry organic acid blend composed of calcium salt of 2-hydroxy-4-methylthiobutanoic acid, fumaric acid, benzoic acid, and carrier with 31% L-met activity (supplied by Novus International, Inc., St. Louis, MO, USA).

fed antibiotics, 0.5% or 1% ASD diets tended to decrease ileal *E. coli* count in relation to those fed the control diet ($p = 0.08$). Serum interleukin-6 and cortisol were not affected by all treatments at 6, 12, 24 h after oral *E. coli* K88 challenge ($p = 0.11$, Table 9).

DISCUSSION

The positive effects of the use of antibiotics in pig diets have been widely documented (Doyle, 2001). However, recent concerns regarding the antibiotic residues in feedstuffs and potential antibiotic resistance indicate the need to find alternatives to antibiotics and promote antibiotic-free pig production. Dietary supplementation of organic acids has been reported to improve growth performance of weaning pigs (Namkung et al., 2004; Kluge et al., 2006). However, the results were variable and sometimes no benefit was observed in weaned pigs (Omogbenigun et al., 2003; Kil et al., 2006; Kommera et al., 2006).

In Exp. 1, potassium diformate and ASD were used to compare their impact on the performance and intestinal microbial populations with antibiotics in weaned pigs. Similar to the effect of antibiotics, feeding 0.5% ASD significantly improved the weight gain and feed efficiency of weaned pigs whereas potassium diformate resulted in numeric increase in growth performance. In agreement with our current study, Yi et al. (2006) reported that 0.5% dry ASD comparable growth promoting effect with antibiotics (i.e., carbadox) in different nursery pig feeding program of North America. In contrast to our observations, Paulicks et al. (1996) reported that the addition of potassium diformate in weaning pig diet significantly increased weight gain and feed efficiency. The variable effects of potassium diformate and some other organic acids can be attributed to the difference in dietary buffering capacity, dietary organic acids supplementation dosage, combination of feed ingredients and additives, weaning age, and feeding duration (Paulicks et al., 2000; Dibner and Buttin, 2002).

Some previous studies showed that the number and species of intestinal micro-organisms changed dramatically during the first few days after weaning (Barrow et al., 1977; Mathew et al., 1996). Collier et al. (2003) reported that feeding nursery pigs with antibiotics for 5 weeks suppressed the growth of the small intestinal micro biota and reduced the total bacteria population, consistent with the findings by Dibner et al. (2007), who indicated that gut immune cells not only fight against the pathogenic bacteria and toxin but also suppress the overgrowth and colonization of normal microflora. Some documents recorded that dietary supplementation with organic acids can maintain a lower pH in the gastric digesta of weaned pigs and therefore reduce the proliferation of pathogenic bacteria such as *E. coli* in the upper GIT (Richards et al., 2005). In Exp. 1, we did not measure the digesta pH values of GIT; however, on d 14 post-weaning, feeding antibiotics and 0.5% ASD decreased fecal counts of pathogenic gram-negative *E. coli* and increased beneficial *lactobacilli*, which indicated the favorable modulatory effect of antibiotics and ASD on gut microbial population and the similarity of ASD with antibiotics in regard to the antimicrobial activity in the GIT. The reduction of pathogenic bacteria in the GIT can be expected to improve amino acid and energy digestibility and absorption by reducing the microflora competition with the host for nutrients and reducing endogenous losses (Dibner and Buttin, 2002). These were probably why the growth performance of pigs fed antibiotics and ASD was improved in response to the modulated gut microflora. However, at d 28 post-weaning, there was no treatment effect on the fecal microflora population, indicating that the stability of gastrointestinal microflora increased with age (Collier et al., 2003; Richards et al., 2005). Therefore, it is possible that there was less opportunity to detect the impact of antibiotics and organic acids on intestinal microflora

modulation and fecal microbial excretion as the pigs aged. Partanen et al. (1998) reported that organic acids can lower the microbial proliferation and then reduce the competition of nitrogen between the microflora and the host and therefore improve nitrogen retention in pigs. IGF-1 plays a vital role in the modulation of animal postnatal growth. Some researches documented that the concentration of IGF-1 was increased in pigs fed antibiotics (Hathaway et al., 2003; Jenkins et al., 2004). However, no differences were observed on the IGF-1 levels between organic acid groups and control. The result indicated that organic acid mainly exerted its effect on growth via modulating gut microbial but had little effects on the somatotrophic axis. Serum urea nitrogen was not affected by dietary treatments, which indicated that organic acid might have minimal effect on nitrogen retention in the current study.

In Exp. 1, we didn't find the concentration of serum IGF-1 was altered by ASD. Therefore, we didn't repeat it again in Exp. 2. Enterotoxigenic *E. coli* strains that express K88 fimbrial antigen are a major cause of diarrhea and death problem of neonatal and weaned pigs for swine producers (Francis et al., 1998). Because of the positive effects of feeding ASD on the intestinal microbial modulation and performance observed in Exp. 1, two different dietary supplementation levels of 0.5% and 1% ASD were used to compare with antibiotics after *E. coli* $K88^+$ challenge in Exp. 2. After 10 days challenge with *E. coli* $K88^+$ (d 5 to 14), similar to the effects of antibiotics, pigs fed both 0.5% and 1% ASD diets had improved growth performance in relation to pigs fed the control diet, which was in agreement with the findings of Exp. 1. However, the feed intake was not affected after *E. coli* $K88^+$ challenge, which indicated that the growth depression was mainly caused by impaired intestinal digestion and absorption of nutrients. Organic acids not only possess antimicrobial activity, but also can reduce dietary buffering capacity and pH (Dibner and Buttin, 2002). Weaning pigs are usually not ready to produce enough hydrochloric acid, resulting in a high pH in the stomach and upper GIT. The high pH can be favorable for certain microbial proliferation, particularly coliform bacteria (Sissons, 1989). It is accepted that the organic acids can lower gastric pH to inhibit the growth of bacteria (Giesting and Easter, 1985; Bosi et al., 1999). We observed numeric digesta pH reduction (about 0.3 to 0.9) across different GIT segment after feeding 0.5% ASD. In line with our observation, Kirchgessner and Roth (1988) reported that the effects of organic acids on the growth performance of pigs are partly associated with the reduction of pH in diet and digesta. Consistent with the findings in Exp. 1, after oral *E. coli* $K88^+$ challenge, pigs fed ASD or antibiotics tended to decrease ileal *E. coli* count and increase duodenal and ileal *lactobacilli* count in Exp. 2. Similar to our findings, Kluge et al. (2006) reported that the organic aicds supplemented diet mainly reduced the number of pathogenic bacteria in ileum and affected the duodenal microbial balance. The favorable intestinal microbial modulation after feeding ASD and antibiotics can be attributed to the enhanced growth performance of pigs after oral *E. coli* $K88^+$ challenge.

The reduction of detrimental bacteria in the GIT can be expected to improve nutrient digestibility and absorption and growth performance by alleviating weaning stress and lowering the inflammatory responses to sub clinical infections (Anderson et al., 1999; Blank et al., 2001; Dibner and Buttin, 2002). Roura et al. (1992) reported that antibiotics may function by preventing immune activation and decreasing the magnitude of immune response after enteric challenge. In Exp. 2, we hypothesized that organic acids and antibiotics might reduce the magnitude of immune stimulation and decrease inflammatory cytokine production of orally *E. coli* $K88^+$ challenged pigs. As a result, the oral challenge of *E. coli* $K88^+$ led to changes in the relative weight of the organs, in which the liver index was decreased by feeding antibiotics or ASD. However, we did not observe an increase of serum interleukin-6 after the oral *E. coli* challenge, which is consistent with Burkey et al. (2004) and Yi et al. (2006) who did not observe any elevation after an oral challenge with *Salmonella typhimurium* or *E. coli* $K88^+$. The studies on the effect of organic acid on the response of weaned pigs after *E. coli* $K88^+$ challenge were very limited. Lai et al. (2005) reported that congjugated linoleic acid show anti-inflammatory role via inhibits the product of IL-6 and other cytokines after lipopolysaccharide challenge, which was different with organic acids and antibiotics. Moreover, cortisol concentrations in serum after *E. coli* challenge did not increase, which is in accordance with Jones et al. (2001) that reported no elevation of serum cortisol concentration after *E. coli* $K88^+$ challenge. Therefore, the modulation of intestinal microbial population after feeding antibiotics and organic acid blend may have more impact on local immune response in the GIT instead of systemic inflammatory cytokine production and hypothalamic-pituitary-adrenal axis activation, which warrants further research.

IMPLICATION

Feeding ASD is useful for alleviating growth depression of weaned pigs with or without *E. coli* $K88^+$ challenge. Additionally, ASD improves growth performance might mainly via reducing pathogenic bacteria and increasing beneficial bacterial population in the gut and feces.

ACKNOWLEDGMENT

The investigation was financially supported by the

Nature Science Foundation of China (30121004) and the National Basic Research Program (2004 CB 117503). The authors show appreciation to Prof. Phil Thacker in university of Sakatchewan (Saskatoon, Canada), Ariel Keating and Dr. Dong Bing in China Agricultural Univeristy for their excellent checking and improving of English of this paper.

REFERENCES

Anderson, D. B., V. J. McCracken, R. I. Aminov, J. M. Simpson, R. I. Mackie, M. W. A. Verstegen and H. R. Gaskins. 1999. Gut microbiology and growth-promoting antibiotics in swine. Pig News Inf. 20:115N-112N.

Barrow, P. A., R. Fuller and M. J. Nweport. 1977. Changes in the microflora and physiology of the anteriorintestinal tract of pigs weaned at 2 days with special reference to the pathogenesis if diarrhea. Infect. Immun. 18:586-595.

Blank, R., W. C. Sauer, R. Mosenthin, J. Zentek, S. Huang and S. Roth. 2001. Effect of fumaric acid supplementation and dietary buffering capacity on the concentration of microbial metabolites in ileal digesta of young pigs. Can. J. Anim. Sci. 81:345-353.

Bosi, P., H. J. Jung, In K. Han, S. Perini, J. A. Cacciavillani, L. Casini, D. Creston, C. Gremokolini and S. Mattuzzi. 1999. Effects of dietary buffering characteristic and protected or unprotected acids on piglet growth, digestibility and characteristics of gut content. Asian-Aust. J. Anim. Sci. 12: 1104-1110.

Burkey, T. E., S. S. Dritz, J. C. Nietfeld, B. J. Johnson and J. E. Minton. 2004. Effects of dietary mananoligosaccharide and sodium chlorate on the growth performance, acute-phase response, and bacterial shedding of weaned pigs challenged with *Salmonella enterica* serotype *Typhimurium*. J. Anim. Sci. 82:397-404.

Choct, M. 2001. Alternatives to in-feed antibiotics in monogastric animal industry. ASA Technical Bulletin. AN30:1-6.

Cole, D. J. A., R. M. Beal and J. R. Luscombe. 1968. The effect on perfoemance and bacterial flora of lactic acid, propionic acid, calcium propionate and calcium acrylate in the drinking water of weaned pigs. Vet. Rec. 83:459-464.

Collier, C. T., M. R. Smiricky-Tjardes, D. M. Albin, J. E. Wubben, V. M. Gabert, B. Deplancke, D. Bane, D. B. Anderson and H. R. Gaskins. 2003. Molecular ecological analysis of porcine ileal microbiota responses to antimicrobial growth promoters. J. Anim. Sci. 81:3035-3045.

Dibner, J. J. and P. Buttin. 2002. Use of organic acids as a model to study the impact of gut microflora on nutrition and metabolism. J. Appl. Poult. Res. 11:453-463.

Dibner, J. J. and J. D. Richards. 2005. Antibiotic growth promoters in Agriculture: history and mode of action. Poult. Sci. 84:634-643.

Dibner, J. J., C. Knight, G. F. Yi and J. D. Richards. 2007. Gut development and health in the absence of antibiotic growth promoters. Asian-Aust. J. Anim. Sci. 20:1007-1014.

Doyle, M. E. 2001. Alternatives to antibiotic use for growth promotion in animal husbandary. Food Res. April:1-17.

Eller, C., M. R. Crabill and M. P. Bryant. 1971. Anaerobic roll tube media for nonselective enumeration and isolation of bacteria in human feces. Appl. Microbiol. 22:522-529.

Francis, D. H., P. A. Grange, D. H. Zeman, D. R. Baker, R. G. Sun and A. K. Erickson. 1998. Expression of mucin-type glycoprotein K88 receptors strongly correlates with piglet susceptibility to K88 enterotoxigenic *Escherichia coli*, but adhesion of this bacterium to brush border does not. Infec. Immun. 66:4050-4055.

Galfi, P. and J. Bokori. 1990. Feeding trial in pigs with a diet containing sodium n-butyrate. Acta. Vet. Hung. 38:3-17.

Giesting, D. W. and R. A. Easter. 1985. Response of starter pigs to supplementation of corn soybean meal diets with organic acids. J. Anim. Sci. 60:1288-1294.

Harada, E., K. Hiroko, E. Kobayashi and H. Tsuchita. 1988. Postnatal development of biliary and pancreatic exocrine secretion in piglets. Comp. Biochem. Phsiol. 91A:43-51.

Hathaway, M. R., W. R. Dayton, M. E. White and M. S. Pampusch. 2003. Effects of antimicrobials and weaning on porcine serum insulin-like growth factor binding protein levels. J. Anim. Sci. 81:1456-1463.

Jenkins, N. L., J. L. Turner, S. S. Dritz, S. K. Durham and J. E. Minton. 2004. Changes in circulating insulin-like growth factor-1, insulin-like growth factor binding proteins, and leptin in weaned pigs infected with *Salmonella enterica* serovar *Typhimurium*. Domest. Anim. Endocrinol. 26:49-60.

Jones, P. H., J. M. Roe and B. G. Miller. 2001. Effects of stressors on immune parameters and on the fecal shedding of enterotoxigenic *Escherichia coli* in piglets following experimental inoculation. Res. Vet. Sci. 70:9-17.

Kelley, K. W. 2004. From hormones to immunity: The physiology of immunology. Brain Behav. Immun. 18:95-113.

Kil, D. Y., L. G. Piao, H. F. Long, J. S. Lim, M. S. Yun, C. S. Kong W. S. Ju, H. B. Lee and Y. Y. Kim. 2006. Effects of organic or inorganic acid supplementation on growth performance, nutrient digestibility and white blood cell counts in weaning pigs. Asian-Aust. J. Anim. Sci. 19:252-261.

Kim, Y. G., J. D. Lohakare, J. H. Yun, S. Heo and B. J. Chae. 2007. Effect of feeding levels of microbial fermented soy protein on the growth performance, nutrient digestibility and intestinal morphology in weaned piglets. Asian-Aust. J. Anim. Sci. 20: 399-404.

Kirchgessner, M. and F. X. Roth. 1988. Ergotrope Effekte durch Sauren in der Ferkelaufzucht und Schweinemast. Ubers Tierernahrung. 16:93-108.

Kluge, H., J. Broz and K. Eder. 2006. Effect of benzoic acid on growth performance, nutrient digestibility, nitrogen balance, gastrointestinal microflora and parameters of microbial metabolism in piglets. J. Anim. Physiol. Anim. Nutr. 90:316-324.

Kommera, S. K., R. D. Mateo, F. J. Neher and S. W. Kim. 2006. Phytobiotics and organic acids as potential alternatives to the use of antibiotics in nursery pig diets. Asian-Aust. J. Anim. Sci. 19:1784-1789.

Lai, C. H., J. D. Yin, D. F. Li, L. D. Zhao, S. Y. Qiao and J. J. Xing. 2005. Conjugated linoleic acid attenuates the production and gene expression of proinflammatory cytokines in weaned pigs challenged with lipopolysaccharide. J. Nutr. 135:239-244.

Mathew, A. G., A. L. Sutton, A. B. Scheidt, D. M. Forsyth, J. A. Patterson and D. T. Kelly. 1991. Effects of a propionic acid

containing feed additive on performance and intestinal microbial fermentation of the weanling pigs. In: Proc Sixth Int Symposium on the Digestive Physiology in Pigs. PUDOC. Wageningen, The Netherlands, pp. 464-469.

Mathew, A. G., M. A. Franklin, W. G. Upchurch and S. E. Chattin. 1996. Influence of weaning age on ileal microflora and fermentation acids in young pigs. Nutr. Res. 16:817-827.

Mroz, Z. 2005. Organic acids as alternatives to antibiotic growth promoters for pigs. In: Advances in Pork Production, (Ed. G. Foxcroft). University of Alberta Press, Edmonton, Alberta. pp. 169-182.

Namkung, H., M. Li, J. Gong, H. Yu, M. Cottrill and C. F. M. de Lange. 2004. Impact of feeding blends of organic acids and herbal extracts on growth performance, gut microbiota and digestive function in newly weaned pigs. Can. J. Anim. Sci. 84:697-704.

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

Omogbenigun, F. O., C. M. Nyachoti and B. A. Slominski. 2003. The effect of supplementing microbial phytase and organic acids to a corn-soybean based diet fed to early-weaned pigs. J. Anim. Sci. 81:1806-1813.

Partanen, K. H. and Z. Morz. 1999. Organic acids for performance enhancement in pig diets. Nutr. Res. Rev. 12:117-145.

Partanen, K., J. Valaja, J. Siljander-Rasi, T. Jalava and S. Panula. 1998. Effects of carbadox or formic acid and diet type on ileal digestion of amino acids by piglets. J. Anim. Feed. Sci. 7:199-203.

Paulicks, H. R., F. X. Roth and M. Kirchgessner. 1996. Dose effects of potassium diformate (Formi™ LHS) on the performance of growing piglets. Agribiol Res 49:318-326.

Paulicks, H. R., F. X. Roth and M. Kirchgessner. 2000. Effects of potassium diformate (Formi™ LHS) in combination with different grains and energy desities in the feed on growth performance of weaned piglets. J. Anim. Physiol. Anim. Nutr. 84:102-111.

Ravindran, V. and E. T. Kornegay. 1993. Acidification of weaner pig diets: A review. J. Sci. Food Agric. 62:313-322.

Richards, J. D., J. Gong and C. F. M. de Lange. 2005. The gastrointestinal microbiota and its role in monogastric nutrition and health with an emphasis on pigs: current understanding, possible modulations, and new technologies for ecological studies. Can. J. Anim. Sci. 85:421-435.

Risley, C. R., E. T. Kornegay, M. D. Lindemann, C. M. Wood and W. N. Eigel. 1992. Effect of feeding organic acids on selected intestinal content measurements at varying times postweaning in pigs. J. Anim. Sci. 70:196-206.

Roura, E., J. Homedes and K. C. Klasing. 1992. Prevention of immunologic stress contributes to the growth-pronoting ability of dietary antibiotics in chicks. J. Nutr. 122:2283-2290.

Sacakli, P., A. Sehu, A. Ergün, B. Genc and Z. Selcuk. 2006. The effect of phytase and organic acid on growth performance, carcass yield and tibia ash in quails fed diets with low levels of non-phytate phosphorus. Asian-Aust. J. Anim. Sci. 19:198-202.

Sciopioni, R., G. Zaghini and B. Biavati. 1978. Researches on the use of acidified diets for early weaning of piglets. Zootechnol Nutr. Anim. 4:201-218.

Sissons, J. W. 1989. Potential of probiotic organisms prevent diarrhea and promote digestion in farm animals - a review. J. Sci. Food Agric. 49:1-13.

Tao, Y., C. Ying, Z. Ni, J. Li and L. Li. 1982. Clinical Biochemical Analysis, 2nd edn. Shanghai: Shanghai Scientific Technical Press, pp. 102-103.

Thaela, M. J., M. S. Jensen, S. G. Pierzynowski, S. Jakob and B. B. Jensen. 1998. Effect of lactic acid supplementation in pigs after weaning. J. Anim. Feed Sci. 7:181-183.

Yi, G. F., J. A. Carroll, G. L. Allee, A. M. Gaines, D. C. Kendall, J. L. Usry, Y. Toride and S. Izuru. 2005. Effect of glutamine and spray-dried plasma on growth performance, small intestinal morphology, and immune responses of *Escherichia coli* K88$^+$-challenged weaned pigs. J. Anim. Sci. 83:634-643.

Yi, G. F., R. Harrell, J. J. Dibner, C. S. Schasteen, J. Wu, K. R. Perryman and C. D. Knight. 2006. Evaluation of 2-hydroxy-4-(methylthio) butanoic acid (HMTBa) and HMTB-containing organic acid blends in different nursery pig feed programs. (submitted to J. Anim. Sci.).