Effects of Organic or Inorganic Acid Supplementation on Growth Performance, Nutrient Digestibility and White Blood Cell Counts in Weanling Pigs*

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ABSTRACT : Four experiments were conducted to investigate the effect of organic or inorganic acid supplementation on the growth performance, nutrient digestibility, intestinal measurements and white blood cell counts of weanling pigs. In growth trial (Exp I), a total of 100 crossbred pigs ({Landrace×Yorkshire}×Duroc), weaned at 23±2 days of age and 7.25±0.10 kg average initial body weight (BW), were allotted to 5 treatments by body weight and sex in a randomized complete block (RCB) design. Three different organic acids (fumaric [FUA], formic [FOA] or lactic acid [LAA]) and one inorganic acid (hydrochloric acid [SHA]) were supplemented to each treatment diet. Each treatment had 5 replicates with 4 pigs per pen. During 0-3 wk, average daily gain (ADG), average daily feed intake (ADFI) and feed efficiency (G/F ratio) were not significantly different among treatments. However, pigs fed LAA or SHA diet showed improved ADG by 15 or 13% respectively and 12% greater ADFI in both treatments compared to CON diets. Moreover, compared to organic acid treatments, better ADG (p = 0.07) and ADFI (p = 0.09) were observed in SHA diet compared to pigs that were fed the diet containing organic acids (FUA, FOA or LAA). However, during 4-5 wk, no differences in ADG, ADFI and G/F ratio were observed among treatments. Overall, ADG, ADFI and G/F ratio were not affected by acidifier supplementation. Although it showed no significant difference, pigs fed LAA or SHA diets showed numerically higher ADG and ADFI than pigs fed other treatments. In metabolic trial (Exp II), 15 pigs were used to evaluate the effect of acidifier supplementation on nutrient digestibility. The digestibility of dry matter (DM), crude protein (CP), crude fat (CF), crude ash (CA), calcium (Ca) and phosphorus (P) was not improved by acidifier supplementation. Although the amount of fecal-N excretion was not different among treatments, that of urinary-N excretion was reduced in acid-supplemented treatments compared to CON group (p = 0.12). Subsequently, N retention was improved in acid-supplemented groups (p = 0.17). In anatomical trial (Exp III), the pH and Cl^- concentrations of digesta in gastrointestinal (GI) tracts were not affected by acidifier supplementation. No detrimental effect of intestinal and lingual (taste bud) morphology was observed by acidifier supplementation particularly in inorganic acid treatment. In white blood cell assay (Exp IV), 45 pigs were used for measuring white blood cell (WBC) counts. In all pigs after LPS injection, WBC counts had slightly declined at 2 h and kept elevating at 8 h, then returned to baseline by 24 h after injection of lipopolysaccharide (LPS). However, overall WBC counts were not affected by acidifier supplementation. In conclusion, there was no difference between organic and inorganic acidifier supplementation in weanling pigs' diet, however inorganic acidifier might have a beneficial effect on growth performance and N utilization with lower supplementation levels. Furthermore, inorganic acidifier had no negative effect on intestinal measurements and white blood cell counts in weanling pigs. These results suggested that inorganic acidifier might be a good alternative to organic acidifiers in weanling pigs. ***(Asian-Aust. J. Anim. Sci. 2006. Vol 19, No. 2 : 252-261)***

Key Words : Weanling Pig, Organic and Inorganic Acidifier, Growth, Nutrient Digestibility, White Blood Cell Counts

INTRODUCTION

At weaning, young pigs are exposed to many stressors such as loss of mothering, mixing with other pigs, end of lactational immunity, change in their environment and diet (Tsiloyiannis et al., 2001). Abrupt change of feed, from sow's milk to solid grain source diet could be the most critical in digestive immaturity of weanling pigs. Therefore, weanling pigs usually showed a malabsorption syndrome characterized by villus atrophy, digestive enzyme disorder and pathogenic bacterial over-growth (Hampson, 1986; Hampson and Kidder, 1986, Tsiloyiannis et al., 2001), which resulted in poor growth performance and increased mortality. Thus, antibiotics have been commonly applied in weaner diets in order to prevent diarrhea and to improve growth performance. In recent years, however, public concern over development of resistant pathogenic strains and antibiotic residue in animal products has led to pressure to search for alternative materials or methods to antibiotics in animal industry.

Organic acids normally used as an acidifier in animal feeds have been considered to be attractive alternatives for improving nutrient digestibility and growth performance of weanling pigs (Falkowski and Aherne, 1984; Giesting and Easter, 1985; Risley et al., 1992). Dietary supplementation with organic acids has been hypothesized to improve growth performance by lowering gastrointestinal pH and subsequent modification of the intestinal microflora (Scipioni et al., 1978; Kirchegessner and Roth, 1982; Burnell et al., 1988). However, although organic acid

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Received July 17, 2005; Accepted October 17, 2005

Table 1. Composition of experimental diet (Experiment I, II)

Item	d 0-7	d 7-21	d 21-35
Ingredient (%)			
Corn[1]	44.01	55.16	67.59
SBM-46	13.00	24.00	27.00
SDPP	3.50	1.00	1.00
SPC	15.00	6.50	1.00
Lactose	20.00	10.00	0.00
DSM	1.00	0.00	0.00
Soy bean oil	0.50	0.50	0.50
TCP	1.20	1.30	1.19
Limestone	0.65	0.48	0.48
L-lysine-HCl	0.00	0.00	0.20
DL-methionine	0.24	0.16	0.14
Vit. mixture[2]	0.20	0.20	0.20
Min. mixture[3]	0.20	0.20	0.20
Salt	0.20	0.20	0.20
Antibiotics	0.10	0.10	0.10
ZnO	0.10	0.10	0.10
Choline-chloride	0.10	0.10	0.10
Total	100.00	100.00	100.00
Chemical compositon[4]			
ME (kcal/kg)	3,519.87	3,390.73	3,295.70
CP	23.33	21.07	19.52
Lysine	1.40	1.15	1.05
Ca	0.80	0.75	0.70
Total P	0.65	0.63	0.60

[1] Organic or inorganic acidifier was premixed in corn and add to the diet at the appropriate treatment level at the expense of corn.

[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}.

[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).

[4] Calculated values.

supplementation could improve the growth response, its cost has been too high for utilization in animal feed, and most feed companies are forced to use a limited amount of organic acid for acidification of diet. As a result, utilization of cheaper inorganic acid instead of organic acid is desirable in order to reach proper acidification of weanling pigs' diet. However, Easter (1993) suggested that inorganic acid could reduce the palatability of feed and destroy the electrolyte balances, and result in a severe depression in growth. On the contrary, Mahan et al. (1996, 1999) reported that addition of hydrochloric acid as the source of chloride to the starter diets resulted in improved daily gains and feed efficiency. Schoenherr (1994) also reviewed that phosphoric acid-based acidifier gave similar growth performance enhancement compared with fumaric acid. However, there have been few studies to investigate comparative effects between organic and inorganic acid in practical aspect. Therefore, the object of this study was to compare the effect of organic or inorganic acid supplementation on growth performance, nutrient digestibility and WBC counts of weanling pigs.

MATERIALS AND METHODS

Growth trial (Exp I)

A total of 100 crossbred pigs ((Landrace×Yorkshire) ×Duroc), weaned at 23±2 days of age and average initial body weight (BW) 7.25±0.10 kg, were used in a 5-week feeding trial. Pigs were allotted to dietary treatments based on body weight and sex in a randomized complete block (RCB) design with 5 replicates, 4 pigs per pen. Treatments included 1) control (CON) : basal+no acid supplementation, 2) fumaric acid (FUA): fumaric acid 0.2%+scoria 0.47%, 3) formic acid (FOA): formic acid 0.2%+scoria 0.47%, 4) lactic acid (LAA): lactic acid 0.2%+scoria 0.47%, 5) hydrochloric acid (SHA): hydrochloric acid 0.1% and scoria 0.57%. The levels of organic acids used in this experiment were determined according to general dosage amount of the feed company and inorganic acid was based on previous research. Treatments had one basal diet (CON), three organic acidifiers (FUA, FOA, and LAA) and one inorganic form acidifier (SHA). The basal diet was formulated to contain approximately averaging 3,400 ME kcal/kg for the young period (d 0-35) and 1.40%, 1.15%, and 1.05% lysine for d 0-7, d 7-21, and d 21-35, respectively. Other dietary nutrients met or exceeded NRC (1998) standard. The ingredient and chemical composition of experimental diets are presented in Table 1. Pigs were housed in half-slotted concrete floor pen (0.90×2.15 m^2 for four pigs) and allowed *ad libitum* access to water and diet during 5-week trial. Environmental temperature was maintained in the range of 30°C (at the beginning of experiment) to 25°C (at the end of experiment). Blood samples were collected directly from the jugular vein, weekly between 08:00 and 08:30 before pigs were fed. In order to avoid individual differences blood was collected from the same pigs each week during the whole experimental period. This method was considered appropriate because one previous study (Phillip et al., 1998) suggested that it would be desirable to take blood from just a few pigs rather than selecting all pigs for blood sampling. After blood sampling, all samples were quickly transferred to a centrifuge tube and then centrifuged for 15 minutes at 3,000 rpm in a cold chamber (4°C). The sera were carefully removed to plastic vials and stored at -20°C for blood urea nitrogen (BUN) and chloride concentration analyses. Total BUN concentration was analyzed by blood analyzer (Ciba-Corning model, Express Plus, Ciba Corning Diagnosis Co.), and blood chloride (Cl^-) concentration was analyzed by blood analyzer (Toshiba 200FR).

Table 2. Composition of experimental diet (Experiment III, IV)

Item	d 0-7	d 7-21	d 21-35
Ingredient (%)			
Corn[1]	41.69	52.89	67.69
SBM-46	12.87	26.54	24.50
SDPP	3.00	1.10	1.00
HP300	17.50	6.09	3.31
Lactose	15.00	10.00	0.00
Whey	5.00	0.00	0.00
DSM	1.50	0.00	0.00
Soy bean oil	0.50	0.50	0.50
TCP	1.10	1.31	1.29
Limestone	0.70	0.50	0.46
L-lysine-HCl	0.00	0.00	0.20
DL-methionine	0.24	0.17	0.15
Vit. mixture[2]	0.20	0.20	0.20
Min. mixture[3]	0.20	0.20	0.20
Salt	0.20	0.20	0.20
Antibiotics	0.10	0.10	0.10
ZnO	0.10	0.10	0.10
Choline-chloride	0.10	0.10	0.10
Total	100.00	100.00	100.00
Chemical compositon[4]			
ME (kcal/kg)	3,503.37	3,378.86	3,298.22
CP	23.07	21.14	19.35
Lysine	1.36	1.15	1.05
Ca	0.80	0.75	0.70
Total P	0.65	0.63	0.60

[1] Organic or inorganic acidifier was premixed in corn and add to the diet at the appropriate treatment level at the expense of corn.
[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}.
[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).
[4] Calculated value.

Metabolic trial (Exp II)

Fifteen pigs (6.21±0.16 kg, average initial body weight) were housed individually in metabolic crates. Pigs were allotted to 5 treatments with 3 replicates according to a completely randomized design (CRD). During the experiment, diet was provided twice per day at 08:00 and 20:00 h. Pigs were given four days of collection period after three days of adaptation and this process was repeated three times duringa 21-day experiment. Total feed intake and fecal samples were recorded daily. Collected fecal samples were pooled, sealed in plastic bags and stored at -20°C then dried in an air-forced drying oven at 60°C for 72 h. Dried fecal samples were ground with 1 mm Wiley mill for chemical analysis. Chemical analyses of proximate nutrients in diets, feces and urine were conducted according to the method of AOAC (1990).

Anatomical trial (Exp III)

A total of 20 pigs (7.25±0.23 kg, average initial body weight) were divided into 5 treatments to evaluate gastric pH, Cl^- concentration, intestinal morphology according to the method as described by Overland et al. (2000). All pigs were fed each treatment diet (presented Table 2) *ad libitum* and fed restricted feed for 3 days before slaughtering. Pigs were provided twice daily with a fixed amount of feed that could be consumed within about 30 minutes without producing any leftover. At slaughter day, pigs were killed after 4 h from the time when pigs ate all of given feed. Clemens et al. (1975) reported gastrointestinal pH values showed significant changes with time postfeeding and within 2 h after feeding, and gastric pH was the highest at 4 h postfeeding. Therefore, 4 h postfeeding was considered the best slaughtering time to evaluate exactly the effect of acidifiers on pH of intestinal tracts. At the d 10, d 20 and d 30 from beginning of experiment, one pig of each treatment group was slaughtered and samples collected to evaluate pH, Cl^- concentration in gastrointestinal tracts and morphology of small intestine and tongue. Samples were taken from 4 sites in gastrointestinal tracts; stomach, small intestine, cecum and rectum. The contents of each intestinal section were collected and mixed. Then 3 g of sample was diluted in 27 ml of PBS buffer solution (pH = 7), mixed and suspended on an electromagnetic mixer.

White blood cell assay (Exp IV)

A total of 45 pigs (6.97±0.15 kg, average initial body weight) were divided into 5 treatments to investigate immune responses by measuring WBC counts. Pigs were housed in groups of three in pens and supplied feed as same as used in anatomical trial *ad libitum*. At d 7 of the study, pigs were injected intraperitoneally with a solution of lipopolysaccharide (LPS, *E. coli* serotype 0.55:B55, Sigma Chemical, St. Louis, MO) at a concentration of 0.1 ml/kg BW. LPS was used as the challenge agent. LPS is a component of cell wall of gram-negative bacteria and can enter the circulation via the gastrointestinal tract (Wan et al., 1989) under certain conditions such as gut epithelium damage. Blood samples were taken from four pigs per treatment at initial, 2, 4, 8, 12 and 24 h after LPS injection. After blood samples were collected, all samples were directly transferred into heparinized syringes and stored at 4°C before analysis. WBC counts were analyzed by hematology analyzer (HEMAVET850, CDC Tech., USA).

Statistical analyses

All analyses of data were carried out by comparing means according to least significant difference (LSD) multiple range tests using the General Linear Model (GLM) procedure of SAS (1990). Data of growth performance was analyzed as a randomized complete block design and that of nutrient digestibility trial and WBC counts were analyzed as

Table 3. Effect of various acidifier supplementation on growth performance of weanling pigs[a]

Items	CON	Organic acid (OA)			SHA	SEM[b]	Contrast[c]		
		FUA	FOA	LAA			CON vs. others	OA vs. SHA	LAA vs. SHA
Body weight (kg)									
Initial	7.47	7.27	7.52	7.39	7.49	-			
3 week	13.58	13.66	13.56	14.55	14.50	0.41	NS	0.07	NS
5 week	23.22	22.74	22.37	24.15	23.60	0.59	NS	0.08	NS
ADG (g)									
0-3 week	291	304	288	341	334	11.64	NS	0.07	NS
3-5 week	689	649	629	686	650	14.99	NS	NS	NS
0-5 week	450	442	424	479	460	11.98	NS	NS	NS
ADFI (g)									
0-3 week	445	470	446	504	506	15.05	NS	0.09	NS
3-5 week	1,026	1,001	944	1,071	1,010	29.17	NS	NS	NS
0-5 week	678	682	645	731	708	20.18	NS	NS	NS
Gain/feed									
0-3 week	0.654	0.649	0.646	0.675	0.654	0.009	NS	NS	NS
3-5 week	0.673	0.651	0.667	0.640	0.652	0.008	NS	NS	NS
0-5 week	0.665	0.650	0.659	0.655	0.652	0.005	NS	NS	NS

[a] A total of 100 crossbred pigs was fed from average initial body weight (BW) 7.25±0.10 kg.
[b] Standard error of mean. [c] NS indicates, p>0.10.

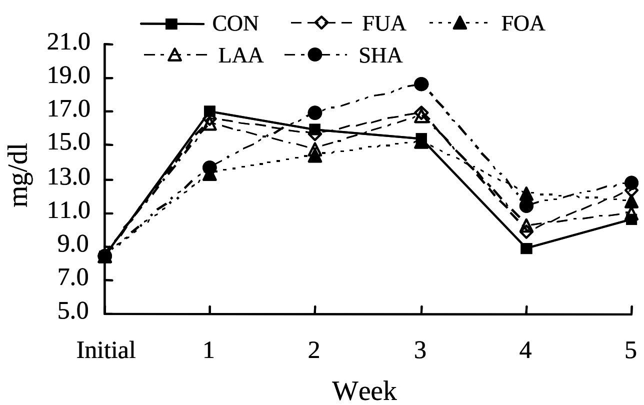

Figure 1. Effect of various acidifier supplementation on blood urea nitrogen (BUN) concentration of weanling pigs.

a completely randomized design. Pen was used as an experimental unit for the performance data, whereas individual pig data served as the experimental unit in nutrient digestibility trial and WBC counts data. Means were separated with the orthogonal contrasts: 1) CON vs. acidifiers, 2) organic acid vs. inorganic acid, and 3) LAA vs. SHA.

RESULTS AND DISCUSSION

Growth trial (Exp I)

The effect of organic or inorganic acid supplementation on the growth performance of weanling pigs was shown in Table 3. During 0-3 weeks, ADG, ADFI and G/F ratio were not significantly different among treatments. However, pigs fed LAA or SHA diets showed improved ADG by 15 or 13%, respectively and 12% greater ADFI in both treatments compared to pigs fed CON diet. Pigs fed SHA diet showed better ADG (p = 0.07) and ADFI (p = 0.09) than the pigs fed the diets containing organic acid (FUA, FOA or LAA). However, there was no significant difference in growth performance among pigs fed, CON vs. acid-supplemented group and LAA vs. SHA. Overall, ADG, ADFI and G/F ratio were not affected by acidifier supplementation. However, pigs fed LAA or SHA diet had numerically higher ADG and ADFI than pigs fed other diets. LAA treatment showed the best growth performance among all treatments even though there was no significant difference.

It has been generally reported that dietary organic acidifiers have shown improvements in growth performance of weanling pigs (Falkowski and Aherne, 1984; Giesting and Easter, 1985; Henry et al., 1985). Moreover, the influence of acidifier on growth performance in weanling pigs was found clearly in younger age of pigs and declined slowly as pigs became older (Straw et al., 1991; Mahan et al., 1999; Chung et al., 2000) because the ability of young pigs to synthesize hydrochloric acid (HCl) seemed to be limited until 4 to 5 week of age (Cranwell et al., 1976). These reports agreed with present study that pigs fed LAA or SHA diet gained faster especially during first 0-3 weeks, although pigs fed FUA or FOA diets showed no improvement in growth performance. Until now, inorganic acidifier has been generally considered to have negative effect on growth performance in weanling pigs by compromising feed intake (Giesting and Easter, 1986), However, there was no detrimental effect on ADFI by addition of inorganic acidifier in present study. On the contrary to previous reports (Henry et al., 1985; Radecki et al., 1988), pigs fed SHA diet tended to be higher ADFI compared to the pigs fed CON diet in present study.

Table 4. Effect of various acidifier supplementation on nutrient digestibility and N utilization of weanling pigs[a]

Items	CON	Organic acid (OA)			SHA	SEM[b]	Contrast[c]		
		FUA	FOA	LAA			CON vs. others	OA vs. SHA	LAA vs. SHA
Nutrient digestibility (%)									
Dry matter	90.66	91.86	90.52	91.51	90.26	0.34	NS	NS	NS
Protein	89.67	90.44	90.00	91.17	89.65	0.38	NS	NS	NS
Fat	51.12	54.83	60.88	62.24	57.94	1.80	NS	NS	NS
Ash	67.39	66.61	61.06	66.74	59.78	1.46	NS	NS	NS
Calcium	66.55	71.16	66.45	69.31	68.23	1.07	NS	NS	NS
Phosphorus	52.86	61.26	56.28	60.76	52.64	2.13	NS	NS	NS
N utilization (g/day)									
N intake	4.64	4.64	4.64	4.64	4.64	-	-	-	-
Fecal N	0.50	0.45	0.48	0.41	0.48	0.02	NS	NS	NS
Urinary N	1.74	1.41	1.51	1.58	1.21	0.08	0.12	NS	NS
N retention[d]	2.40	2.77	2.65	2.64	2.95	0.09	0.17	NS	NS

[a] Fifteen pigs were used from an average initial BW of 6.21±0.16 kg to an average final BW of 8.34±0.19 kg.
[b] Standard error of mean. [c] NS indicates p>0.20. [d] N retention (g) = N intake (g)-Fecal N (g)-Urinary N (g).

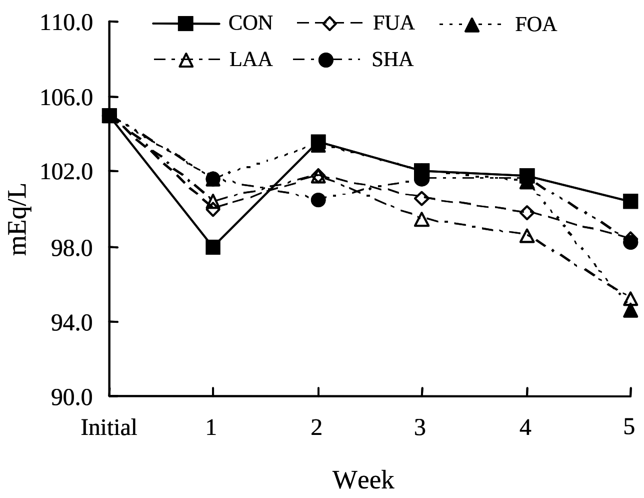

Figure 2. Effect of various acidifier supplementation on blood chloride ion (Cl^-) concentration of weanling pigs.

Tsiloyiannis et al. (2001) reported that organic acids, especially lactic acid, were the most useful dietary material in controlling post-weaning diarrhea syndrome (PWDS) and increased growth performance.

In blood assays, overall all pigs had inconsistent BUN concentration patterns (Figure 1). However, pigs fed SHA diet tended to have higher BUN value during 2-3 weeks of experiment compared to other treatments even though there was no significant difference. In general, BUN concentration has been used as an indicator of maximal amino acid utilization (Eggum, 1970) and it was related directly to protein intake, inversely to protein quality (Eggum, 1970; Hahn et al., 1995) and retained dietary nitrogen in the body (Whang and Easter, 2000). Numerous researchers reported that acidifier supplementation to pigs' diet had changed protein utilization (Scipioni et al., 1978; Kirchgessner and Roth, 1980; Blank et al., 1999) and this change could have an effect on BUN concentration (Scheuermann, 1993; Whang and Easter, 2000). However, BUN value of pigs was not altered by acidifier supplementation in this experiment. Also, blood serum chloride (Cl^-) concentration was not significantly affected by acidifier supplementation during whole experimental periods (Figure 2). Honeyfield et al. (1985) reported that weight gain, feed efficiency, basic amino acid metabolism and plasma electrolyte balance of growing-finishing pigs were influenced by dietrary Na and Cl^- concentration. It was reported that additional Cl^- supplementation could induce metabolic acidosis, suppressing feed intake and weight gain of pigs (Yen et al., 1981; Patience and Wolynetz, 1990). Also, Giesting and Easter (1986) observed that the use of HCl treatment of diets led to an increase of 1.3% of dietary Cl^- level that produced a dEB of -6.7 meq, which might have influenced feed intake and subsequent performance. However, Patience and Chaplin (1997) concluded that serum Cl^- level was not affected by dietary Cl^- level and increased Cl^- intake showed higher ADG and G/F ratio than those fed the control or compensated diets. In the present experiment, when HCl was used as a source of inorganic acidifier, it induced approximate 0.1% increased dietary Cl^- level compared to other treatments. However, it did not cause any detrimental effect on growth performance of pigs and abnormal blood Cl^- concentration compared to normal value from 93 to 126 meq/L (Hannon et al., 1990).

Therefore, these results demonstrated that supplementation of LAA or SHA showed beneficial effect on growth performance especially in younger age. Moreover, LAA supplementation had the most dominant effects on growth performance of weanling pigs. There were no significant differences in BUN and blood Cl^- concentrations of pigs among all treatments.

Metabolic trial (Exp II)

The effect of organic and inorganic acid

Table 5. Effect of various acidifier supplementation on pH of gastrointestinal tracts of weanling pigs[a]

pH[b]	CON	FUA	FOA	LAA	SHA
Feed					
Phase I	6.83	6.80	6.73	6.78	6.80
Phase II	6.83	6.74	6.72	6.76	6.78
Phase III	6.84	6.75	6.72	6.77	6.82
Stomach					
10 d	6.70	6.69	6.73	6.74	6.76
20 d	6.73	6.85	6.75	6.81	6.74
30 d	6.77	6.87	6.76	6.69	6.81
Small intestine[c]					
10 d	7.00	7.01	6.99	6.99	7.02
20 d	7.05	7.03	7.02	7.04	7.04
30 d	6.97	6.97	7.01	7.01	7.04
Cecum					
10 d	6.72	6.80	6.85	6.74	6.84
20 d	6.85	6.93	6.85	7.00	6.85
30 d	6.81	6.92	6.93	6.86	6.87
Rectum					
10 d	7.01	6.99	6.93	6.96	7.02
20 d	6.96	6.92	6.92	7.01	6.91
30 d	7.00	6.92	6.87	6.92	6.84

[a] One pig per treatment was slaughtered at 10 d, 20 d, and 30 d, which weighed average 9.85±0.17 kg, 12.70±0.21 kg, and 16.39±0.23 kg, respectively.
[b] Mean value of three samples which were collected in each tract.
[c] All of digesta in small intestine were collected and mixed for determination of pH.

Table 6. Effect of various acidifier supplementation on chloride (Cl^-) concentration of gastrointestinal tracts of weanling pigs[a]

Cl^- (%)[b]	CON	FUA	FOA	LAA	SHA
Stomach					
10 d	0.55	0.44	0.41	0.55	0.58
20 d	0.56	0.68	0.45	0.55	0.73
30 d	0.41	0.25	0.46	0.21	0.28
Small intestine[c]					
10 d	0.35	0.54	0.47	0.18	0.52
20 d	0.46	0.49	0.40	0.32	0.34
30 d	0.31	0.54	0.54	0.17	0.34
Cecum					
10 d	0.28	0.33	0.35	0.27	0.38
20 d	0.38	0.16	0.36	0.11	0.32
30 d	0.17	0.16	0.25	0.09	0.19
Rectum					
10 d	0.28	0.35	0.32	0.30	0.34
20 d	0.48	0.55	0.31	0.34	0.33
30 d	0.31	0.10	0.23	0.12	0.15

[a] One pig per treatment was slaughtered at 10 d, 20 d, and 30 d, which weighed average 9.85±0.17 kg, 12.70±0.21 kg, and 16.39±0.23 kg, respectively.
[b] Mean value of three samples which were collected in each tract.
[c] All of digesta in small intestine were collected and mixed for determination of Cl^- concentration.

supplementation on the nutrient digestibility and N utilization was presented in Table 4. The digestibility of DM, CP, CF, CA, Ca and P was not improved by acidifier supplementation. A positive effect of acidifier supplementation on nutrient digestibility has been reported by some researchers (Kirchgessner and Roth, 1980; Blank et al., 1999). However, other researchers have failed to observe any favorable effect by supplemented acidifier (Falkowski and Aherne, 1984; Radecki et al., 1988; Giesting and Easter, 1991). These inconsistent results may be involved with some factors including experimental pigs' age, diet composition (dietary buffering capacity) and amount of supplemented acidifiers (Ravindran and Kornegay, 1993). Kirchgessner and Roth (1982) had reported that addition of fumaric acid in the range of 1.5 to 2.0% to be optimal. Eckel et al. (1992) concluded that the most efficient dosage level had been 1.2% fumaric acid, moreover lower and higher levels were less effective. Thus, it is supposed that the dosage level in the present experiment was too low to affect nutrient digestibility of weanling pigs. Although the amount of fecal N excretion was similar among treatments, urinary N excretion was decreased in acid-supplemented groups compared to CON group ($p = 0.12$) and resulted in increasing N retention in acid-supplemented groups ($p = 0.17$). These results were similar to Mahan et al. (1999) who demonstrated that added dietary hydrochloric acid had increased N retention and improved apparent digestibility of N. In the present experiment, N utilization was improved by acidifier supplementation although protein digestibility was not significantly different among treatments. One possible reason for improved N utilization regardless of protein digestibility is that all acid-supplemented groups used scoria as a carrier. Scoria formed by the alteration of volcanic ash could be effective on reduced excretion of N and P (Yang et al., 2000). Poulsen and Oksbjerg (1995) reported Klinofeed which has similar characteristics to scoria changed the excretion pattern of nitrogen towards increased excretion in the feces and decreased excretion in the urine. They implied that binding of NH_4^+ to Klinofeed reduced N-absorption by the conversion of ammonium to urea, which resulted in reduced excretion of urinary nitrogen. These results could agree with present results showing that pigs fed SHA containing 0.57% of scoria excreted lower urinary N and retained more N in the body compared to pigs fed other diets containing 0.47% of scoria (FUA, FOA or LAA). In conclusion, these results demonstrated that nutrient digestibility was not affected by acidifier supplementation, but N utilization was improved by inorganic acidifier supplementation.

Anatomical trial (Exp III)

The effect of organic or inorganic acid supplementation on pH and chloride ion (Cl^-) concentration of gastrointestinal (GI) tracts in weanling pigs is presented in Table 5 and 6, respectively. There was no difference in pH

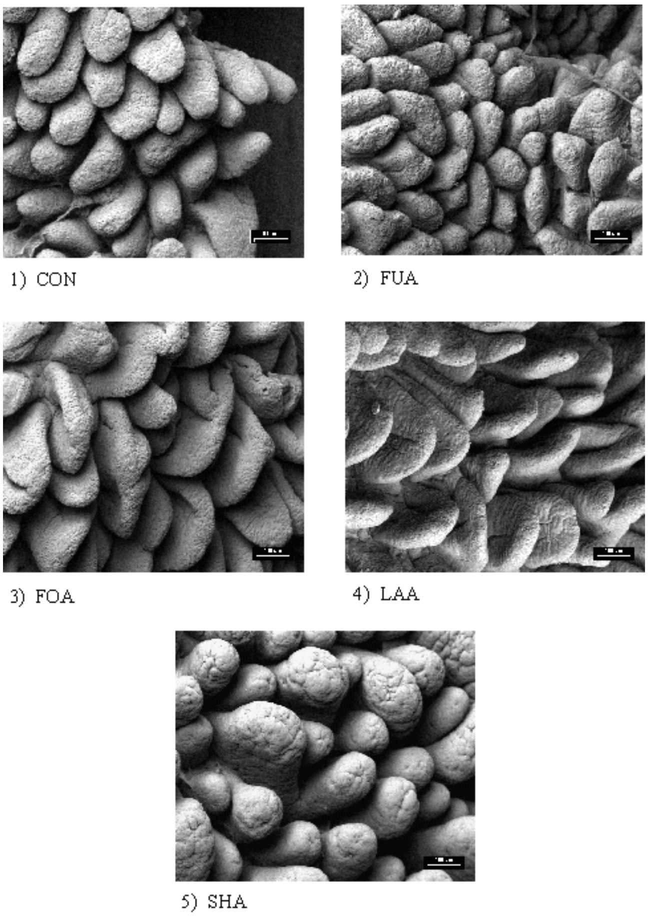

Figure 3. Effect of various acidifier supplementation on intestinal morphology of weanling pigs.

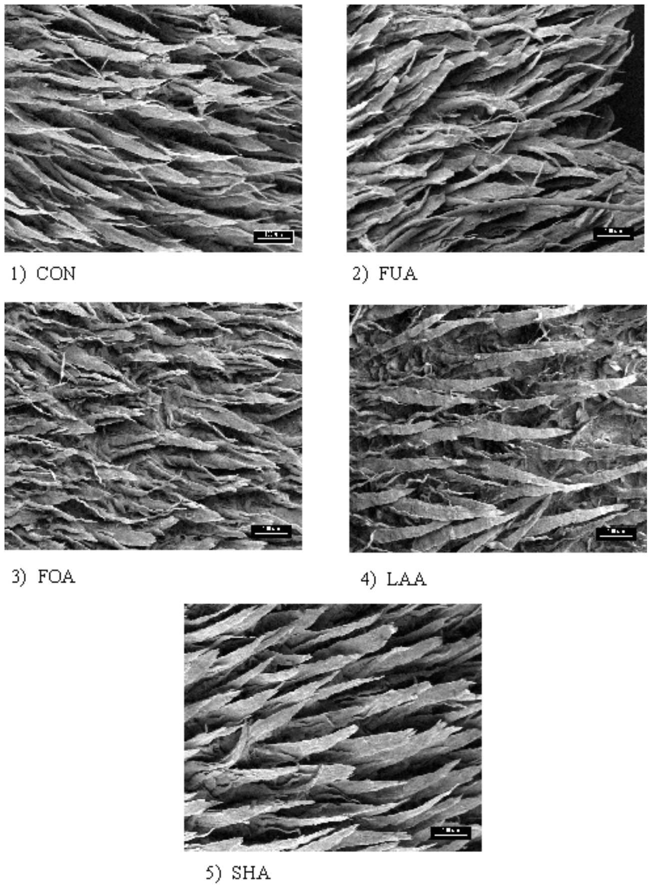

Figure 4. Effect of various acidifier supplementation on lingual morphology (taste bud) of weanling pigs.

of GI tract at 10, 20 and 30 d of age among treatments. Several researchers (Scipioni et al., 1978; Burnell et al., 1988; Radecki et al., 1988) suggested that the beneficial effects of acidification of weanling diets on performance could be mediated through a lowering of gastrointestinal digesta acidity. Although Scipioni et al. (1978) reported a reduction in stomach pH from 4.6 to 3.5 by 1% citric acid and from 4.6 to 4.2 by 0.7% FUA additions to diets fed to weanling pigs, other researchers using organic acids and inorganic acids (Straw et al., 1991; Risley et al., 1992) had failed to show a relationship between performance and stomach pH. Risley et al. (1992) found no differences in the pH of digesta of any gastrointestinal tract sections when 4-week-old pigs were fed 1.5% of either citric or fumaric acid for a similar period of time, although dietary pH was reduced by the addition of organic acid (pH 4.9 and 4.7 vs. 6.4). Even though it had clearly been shown that diet acidity could be manipulated by using a variety of organic and inorganic acids, the potential for lowering stomach and intestinal tract digesta acidity seemed to be difficult to obtain, or at least to measure (Kornegay et al., 1994). Moreover, Risley et al. (1993) suggested that dietary organic acids at the concentrations used in some studies which reported non-decreased pH in GI were unable to lower the pH of GI digesta. Kim et al. (2004) also reported that dietary HCl did not reduce urine pH in growing-finishing pigs.

In the present experiment, therefore, the dosage level of acidifier supplementation was too low to change pH of diet acidity and subsequently that of gastrointestinal digesta. There was also no difference in Cl^- concentrations of GI tract at 10, 20 and 30 d of age among all treatments. These results are similar to those of Risley et al. (1993), who reported no observable change of Cl^- concentrations in GI tract by organic acidifier supplementation. However, pigs fed SHA diet showed higher Cl^- concentration in the stomach than those of other treatments at 10 and 20 d of age. The Cl^- concentration of the intestinal digesta was expected to reflect the secretion of gastric hydrochloric acid (Cranwell et al., 1976), however it might be easily determined by the level of Cl^- ingested in the diet (Risley et al., 1993). Therefore increased Cl^- concentration in stomach of pig fed SHA diet was likely to be affected by supplementation of HCl. However, Straw et al. (1991)

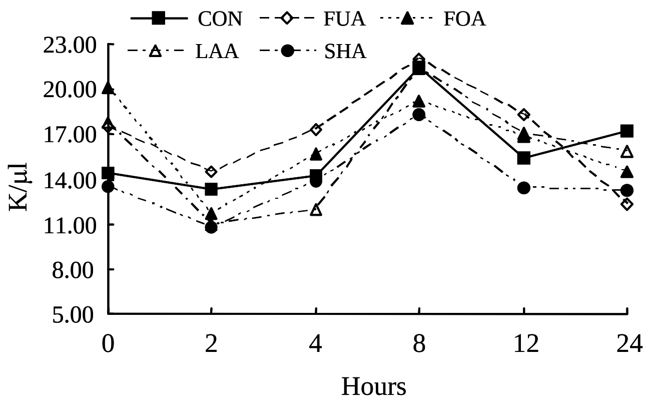

Figure 5. Effect of various acidifier supplementation on white blood cell (WBC) counts of weanling pigs (LPS injected at 0 h).

observed that acidified diet with HCl did not significantly affect Cl^- concentration of the various gastrointestinal sections without stomach.

Figures 3 and 4 showed effects by organic and inorganic acid supplementation on intestinal and lingual (taste bud) morphology. Normal type of villi, a long and finger-shape, was found in intestine regardless of dietary treatments. Taste buds also showed the similar shape among all treatments. These results suggested that supplementation of acidifiers, especially HCl, to weaner diets did not show any detrimental effects on villi in GI tract and taste bud of tongue in weanling pigs.

White blood cell assay (Exp IV)

The effect of organic and inorganic acid supplementation on white blood cell (WBC) counts in weanling pigs was presented in Figure 5. In all pigs, WBC counts was slightly declined at 2 h and kept elevating at 8 h, then returned to baseline by 24 h after injection of LPS. This result was very similar to the changing pattern of immunologically active molecules following immunological challenge that was reported by Webel et al. (1997). In general, sick or immune-challenged animals cannot grow well because nutrients are redistributed away from the growth process and toward support of immune system function. Amino acids are liberated from muscle breakdown (Klasing and Austic, 1984a, b; Webel et al., 1997) and could be utilized for the synthesis of acute phase proteins in the liver and as an energy source (Wannemacher, 1977). Klasing and Roura (1991) reported that a 13% increase in the requirement for methionine or lysine following a period of immunogenic stress in chicks indicating increased need for these amino acids due to compensatory growth. Amino acid deficiency could have resulted in a decreased metabolic response to immunogenic challenge (Klasing and Barnes, 1988). It was generally known that dietary acidifier could improve protein digestibility and N utilization; thus it might be assumed that increased protein utilization could support sudden protein catabolism in muscle after immune challenge. Heugten et al. (1994) also presumed that additional protein supplementation could alleviate growth reduction in immunologically challenged pigs by altering total amino acid supplies. In the present experiment, WBC counts and BUN concentrations were not changed although N retention was improved by acidifier supplementation in weanling pigs.

IMPLICATIONS

Supplementing LAA or SHA to weanling pigs could have beneficial effects on growth performance especially in younger pigs. LAA supplementation showed the most dominant effect on growth performance of weanling pigs. There was no significant effect on BUN and blood Cl^- concentration of pigs among treatments. Nutrient digestibility was not affected by acidifier supplementation, however N utilization was improved in acid-supplemented groups, especially in SHA group. No changes of intestinal measurements and WBC counts were found due to acidifier supplementation. In conclusion, there was no difference between organic and inorganic acidifier supplementation in weanling pigs' diet, however inorganic acidifier might have a beneficial effect on growth performance and N utilization although lower level of acid was provided compared to organic acid treatments. Furthermore, inorganic acidifier had no detrimental effect on tongue and intestine. These results suggested that inorganic acidifier could be utilized as an alternative to organic acidifier in weanling pig's diets.

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