

## Acidifier as an Alternative Material to Antibiotics in Animal Feed

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**ABSTRACT :** Dietary acidifiers appear to be a possible alternative to feed antibiotics in order to improve performance of weaning pigs. It is generally known that dietary acidifiers lower gastric pH, resulting in increased activity of proteolytic enzymes, improved protein digestibility and inhibiting the proliferation of pathogenic bacteria in GI tract. It is also hypothesized that acidifiers could be related to reduction of gastric emptying rate, energy source in intestine, chelation of minerals, stimulation of digestive enzymes and intermediate metabolism. However, the exact mode of action still remains questionable. Organic acidifiers have been widely used for weaning pigs' diets for decades and most common organic acidifiers contain fumaric, citric, formic and/or lactic acid. Many researchers have observed that dietary acidifier supplementation improved growth performance and health status in weaning pigs. Recently inorganic acidifiers as well as organic acidifiers have drawn much attention due to improving performance of weaning pigs with a low cost. Several researchers introduced the use of salt form of acidifiers because of convenient application and better effects than pure state acids. However, considerable variations in results of acidifier supplementation have been reported in response of weaning pigs. The inconsistent responses to dietary acidifiers could be explained by feed palatability, sources and composition of diet, supplementation level of acidifier and age of animals. (*Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 7 : 1048-1060*)

**Key Words :** Acidifier, Gastric pH, Weaning Pig, Antibiotics

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### INTRODUCTION

The swine industry has been interested in reduced weaning age in order to maximize annual sow productivity. It seems to save the cost and to increase the productivity of the swine farm. However, weaning at an early age exposes the piglets to nutritional, environmental and social stresses that usually result in a postweaning lag phase manifested by low growth rate, scouring and high mortality (Ravindran and Kornegay, 1993). It is generally demonstrated that weaning pigs have an ill-prepared intestinal system (Easter, 1988) and additionally face a marked reduction of digestive enzyme activity (Lindemann et al., 1986, Figure 1). Abrupt feed change, from sow's milk to solid ingredients in diets such as corn and soybean meal, of weaning piglets having immature digestive systems usually showed a malnutrition syndrome characterized by digestive disorder, pathogenic bacteria over-growth and villus atrophy. In order to overcome the postweaning problems of weaning pigs, prophylactic doses of antimicrobial feed additives such as antibiotics are commonly applied to weaning pig's diets (Partanen and Morz, 1999).

Antibiotics have been used in animal production for over 50 years. The practice of feeding antibiotics was very successfully adopted and become an integral part of

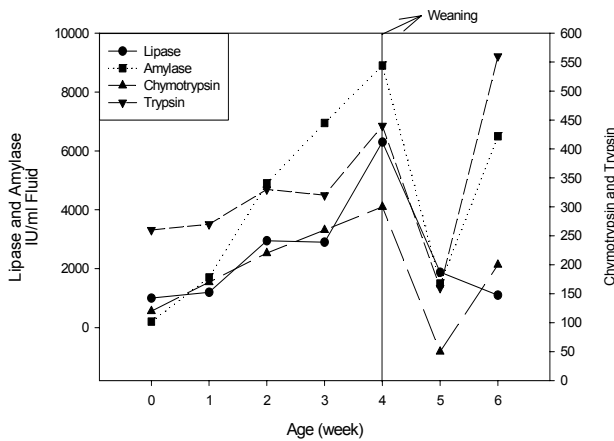
developing nutritional strategies for all farm animals (Close, 2000). Feeding swine with antibiotics has been documented to increase weight gain by 3.3-8.0% and improve feed efficiency approximately by 3% (Doyle, 2001). It is known that the beneficial effects of these compounds result from alteration of the bacterial population primarily within animal's digestive tract and utilization of nutrients in feed. In recent years, however, there is growing concern that the use of antibiotics in livestock feed causes increasing numbers of antibiotic-resistant pathogens and antibiotic residue problems in animal products. As a result, the expanded use of antibiotics, in particular for growth promotion, has led to a partial or total ban of antibiotic application to feed in a number of European countries. Therefore many parts of the world are currently searching for alternative feed additives that can be used to alleviate the problems associated with the withdrawal of antibiotics from animal feed and improve performance (Choct, 2001). Probiotics, prebiotics (mainly oligosaccharide products), enzymes and acidifiers have been referred to be the most common and useful feed additives instead of antibiotics. Many studies have reported several beneficial effects showing improvements not only in the general health of animals, but also in growth rate and feed efficiency when various dietary additives were supplemented to the diet (Pollman et al., 1980; Hesselman et al., 1986; Easter, 1993; Hill et al., 2000). Among these alternatives, acidifiers have been considered as an attractive additive for weaning pigs' diets. It is proposed that dietary acidifiers may provide a prophylactic measure similar to feed antibiotics (Sciopioni et al., 1978; Mathew et al., 1991). While antibiotics are designed to inhibit most microbial growth (Cromwell,

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## ACIDIFIERS IN ANIMAL FEED



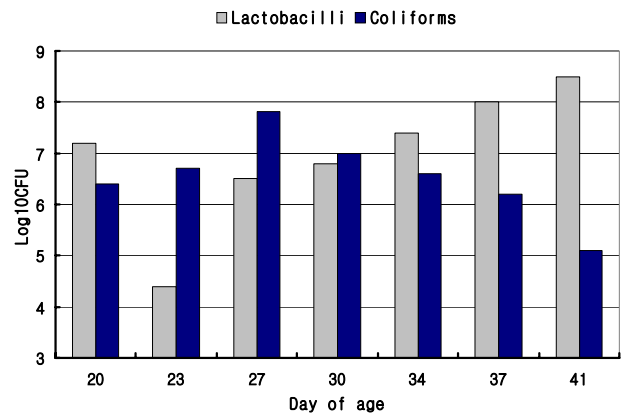
**Figure 1.** Effect of age on pancreatic enzyme activity (weaning at 4 week, Lindemann et al., 1986)

1990), acidifiers would reduce harmful microorganisms and help beneficial microorganisms to dominate in the gastrointestinal tract (Mathew et al., 1991).

Acidifier in animal feed was introduced because young pigs have limited capacity to maintain proper gastric pH (Ravidran and Kornegay, 1993), resulting in a negative effect on digestion (Easter, 1988). Numerous reports are available where attempts have been made to stabilize the pH of the gastrointestinal tract and to improve postweaning lag phase by feeding various acidifiers (Ravidran and Kornegay, 1993). In early research, Cole et al. (1968) found that growth response and feed efficiency in weaning pigs were significantly improved by the addition of 0.8% lactic acid to the drinking water. Moreover, there was a reduction in hemolytic *E. coli* counts both in duodenum and jejunum. Since then, many researchers have observed beneficial effects on performance of weaning pigs by adding acidifiers (Falkowski and Aherne, 1984; Giesting and Easter, 1985; Risley et al., 1992; Schoenherr, 1994; Oh, 2004).

### IMPORTANCE OF GASTRIC pH FOR WEANING PIGS

Weaning is a stressful process for piglets as the diet is changed from readily digestible sow milk to unfamiliar solid diet. Moreover, weaning pigs generally are not ready to produce enough hydrochloric acid (HCl) in the stomach to digest solid feed. As suggested by Easter (1988), the suckling pigs employ several strategies to overcome the limitation of insufficient acid secretion. The primary strategy involves the conversion of lactose in sow's milk to lactic acid by the *Lactobacilli* bacteria normally located in the stomach. Secondly, the nursing pigs reduce the need for transitory secretion of copious amounts of acid by frequent ingestion of small meals. Finally, the acidification of sow's milk would be relatively easier to reach proper acidic



**Figure 2.** Concentrations of *Lactobacilli* and *Coliforms* in ileal contents of pigs prior to following weaning at 21 day (Mathew et al., 1996a)

condition rather than solid grain ingredients. As the piglets suddenly lose these productive systems after weaning, resulting in high pH levels in the stomach. Furthermore, other possible reasons of reduction of gastric pH of weaning pigs have been discussed by Mahan et al. (1996). The subsequent production of lactic acid converted by *Lactobacillus spp.* can suppress HCl production which is a primarily a reducer of pH in stomach. Large intakes of feed can also reduce HCl releasing in stomach. Some feed ingredients such as milk by-products can neutralize free acids more than cereal grain protein. As a result, pH of the stomach is increased and this high pH is unfavorable for some enzymes' activation such as pepsin (Manners, 1976). Taylor (1959) reported that pepsin has two optimal pHs (2 and 3.5), and its activity declines at pH above 3.6 with no activity over 6. Ravidran and Kornegay (1993) demonstrated several effects derived from an elevated pH of stomach. First, feed protein may enter the small intestine essentially intact with an eventual reduction in efficiency of protein digestion. Secondly, the end-products of pepsin digestion also stimulate the secretion of pancreatic proteolytic enzyme and stomach acid is the primary stimulant for pancreatic secretion of bicarbonate. Finally, acid leaving the stomach plays a role in the feedback mechanism in the regulation of gastric emptying, thus, decreasing the digesta load on the small intestine. Eventually, the failure of optimal enzyme activation and inefficient digestion make fermentable substrates, partly digested feed, that can support the growth of pathogenic bacteria, resulting in poor performance and severe scours (Easter, 1988).

In addition, a high pH environment can be advantageous for certain microorganisms, in particular for the Coliforms (Sissons, 1989). However, increased gastric pH can permit invasion of harmful bacteria, which have been associated with scours and mortality (Smith and Jones, 1963).

**Table 1.** Effect of dietary acidifier addition on pH of intestinal contents of weaning pigs (Bosi et al., 1999)

|         | Acid |      |      | SEM  |
|---------|------|------|------|------|
|         | NO   | FA   | PA   |      |
| Stomach | 3.88 | 3.34 | 3.50 | 0.30 |
| Ileum   | 7.19 | 7.10 | 7.11 | 0.11 |
| Cecum   | 5.90 | 5.69 | 5.73 | 0.09 |

NO: no acid addition, FA: fumaric acid.

PA: protected organic and inorganic mixture.

**Table 2.** Effect of formic acid addition on microorganism counts<sup>1</sup> in different segments of intestinal piglets (Roth and Kirchgessner, 1997)

| Species         | <i>Lactobacillus/<br/>Bifidobacterium</i> |                      | <i>E. coli</i>       |                      |
|-----------------|---|----------------------|----------------------|----------------------|
|                 | 0   | 1.2                  | 0                    | 1.2                  |
| Formic acid (%) |   |                      |                      |                      |
| Duodenum        | 6.4±0.7                                   | 5.5±0.6              | 5.5±0.9 <sup>a</sup> | 3.3±0.7 <sup>b</sup> |
| Jejunum         | 6.7±0.7 <sup>a</sup>                      | 5.8±0.7 <sup>b</sup> | 6.8±0.5 <sup>a</sup> | 5.3±0.9 <sup>b</sup> |
| Ileum           | 7.2±1.3                                   | 6.6±1.4              | 7.9±0.7              | 6.8±1.5              |
| Cecum           | 8.1±0.7                                   | 7.5±0.6              | 6.8±0.6              | 6.9±0.6              |
| Colon           | 8.6±0.8                                   | 8.0±0.7              | 6.3±0.7              | 6.0±1.3              |

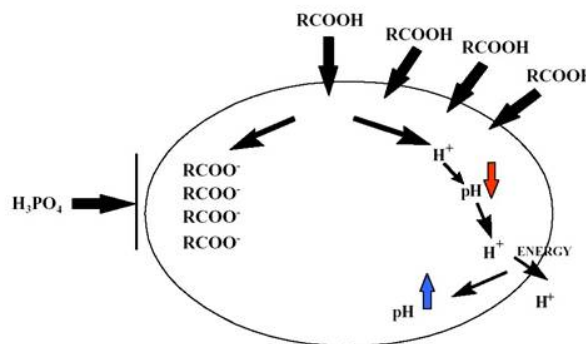
<sup>1</sup> Colony forming units (CFU) in log<sub>10</sub>/g fresh matter.

### POSSIBLE MODE OF ACTION OF ACIDIFIER FOR WEANING PIGS

Application of the potential value of dietary acidifiers requires an understanding of their mode of action although it is not yet clearly established. Their mode of action may be partially related to maintenance of low gastric pH and possible effects on pepsin activation, inhibition of pathogenic bacteria proliferation, energy source in GI tract, gastric emptying rate, endogenous enzyme secretion, morphology, chelation of minerals and stimulatory effects on intermediary metabolism (Ravindran and Kornegay, 1993). There are several hypothesis related to the mode of action of dietary acidifiers.

#### Reduced pH in stomach

It is accepted that dietary acidifier lowers gastric pH following reduced diet pH. Lowering gastric pH with acidifier could induce increased activity of proteolytic enzymes. 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% fumaric acid additions. Some studies also documented that dietary acidifier significantly reduced gastric pH (Giesting and Easter, 1985; Bolduan et al., 1988a,b; Burnell et al., 1988; Radcliffe et al., 1998; Bosi et al., 1999; Oh, 2004, Table 1). To the contrary, Risley et al. (1992) observed no difference in the pH of the digesta of any gastrointestinal tract sections when 4-wk-old pigs were fed 1.5% of either citric or fumaric acid although dietary pH was reduced by the supplementation of organic acid (pH 4.9 and 4.7 versus 6.4).

**Figure 3.** Mode of action of organic acids on pH-sensitive bacteria (*Coliforms, Clostridia, Salmonella, Listeria spp.*) (Gauthier, 2002).

#### Reduced number of pathogenic bacteria

Stress associated with weaning pigs is known to disturb the balance of intestinal microflora and adversely affect gastrointestinal functions (Miller et al., 1985). Also increased pH of stomach and undigested feed due to an immature digestive system could accelerate the proliferation of pathogenic bacteria. At 2 days postweaning of pigs, large numbers of Coliforms were found to proliferate in their intestinal tract while counts of *Lactobacilli* were depressed (Barrow et al., 1977). In the studies of Mathew et al. (1996a), *Lactobacilli* in ileal contents were reduced almost to zero within 2 days of weaning. Conversely, numbers of Coliforms increased significantly and were strongly correlated to increased pH of ileal contents (Figure 2). It is well-known that low luminal pH could markedly inhibit growth of undesirable microbes along the whole gastrointestinal tract (Maxwell and Stewart, 1995). It has been also shown that acidic conditions favor the growth of *Lactobacilli* in the stomach, which possibly inhibits the colonization and proliferation of *E. coli* by blocking the sites of adhesion or by producing lactic acid and other metabolites which lower the pH and inhibit *E. coli* (Fuller, 1977). It is also known that *Lactobacilli* could produce hydrogen peroxide which has antimicrobial effects (Reither et al., 1980). Several reports have shown that the use of organic acidifiers reduced the number of Coliform bacteria along the intestinal tract (Cole et al., 1968; Scipioni et al., 1978; Roth and Kirchgessner, 1997; Canibe et al., 2001, Table 2).

Moreover, acidifiers have shown a strong bactericidal effect without reducing pH value in GI tract. Non-dissociated (non-ionized, more lipophilic) organic acids can penetrate the bacterial cell wall and disrupt the normal physiology of certain types of bacteria. As described by Lambert and Stratford (1999), after penetrating the bacteria cell wall, the non-dissociated organic acids will be exposed to the internal pH of the bacteria and dissociate, releasing H<sup>+</sup> and anions (A<sup>-</sup>). The internal pH will decrease and because pH sensitive bacteria such as Coliforms, *Clostridia*,

and *Listeria spp.*, do not tolerate a large spread between the internal and the external pH, a specific mechanism ( $H^+$ -ATPase pump) will act to bring the pH inside the bacteria to a normal level. This phenomenon consumes energy and eventually can stop the growth of the bacteria or even kill it. A lowering of the internal pH of the bacteria also involves other mechanisms, such as inhibition of glycolysis, prevention of active transport and interference with signal transduction (Gauthier, 2002). The anionic ( $A^-$ ) part of the acid is trapped inside the bacteria because it will diffuse freely through the cell wall only in its non-dissociated form. The accumulation of  $A^-$  becomes toxic to the bacteria by complex mechanisms involving anionic imbalance leading to internal osmotic problems for the bacteria (Roe et al., 1998, Figure 3). On the contrary, the non-pH sensitive bacteria like *Lactobacilli* and *Bifidobacterium spp.* will tolerate a larger differentiation between the internal and the external pH, if the internal pH becomes low enough, organic acids will re-appear in a non-dissociated form and exit the bacteria by the same route that they went in. An equilibrium will be created and the bacteria will not suffer from that situation. It is important to note that, even in a non-dissociated form, inorganic acids cannot penetrate the bacteria cell wall (Gauthier, 2002).

Bolduan et al. (1988) explained that antibiotics and acidifier probably act on different populations of bacteria. Consequently, acidifiers could protect the growth of harmful bacteria in the GI tract in virtue of reduced gastric pH and direct bactericidal effect.

#### Energy source in GI tract

Organic acids, which are intermediates of tricarboxylic acid cycle, may act as energy sources and help to reduce the tissue wastage resulting from high rates of gluconeogenesis and lipolysis (Giesting and Easter, 1985; Partanen and Morz, 1999). Bosi et al. (1999) hypothesized that growth promoter effect of organic acids could be derived from the energy value of them when absorbed, particularly at high levels of addition. It is supported by the data of Kirchgessner and Roth (1982), which suggested that pigs could utilize fumaric acid as an energy source with an efficiency close to that of glucose. They determined that the gross energy of fumaric acid, 11.5 MJ/kg, is fully metabolizable in the body. There is another possibility that fumaric acid, as a readily available energy source, may have a local trophic effect on the musosa in the small intestine and lead to an increase in the absorptive surface and capacity in the small intestine due to faster recovery of the gastrointestinal epithelial cells after weaning (Blank et al., 1999).

#### Gastric emptying rate

There is another hypothesis that dietary acidifiers may also affect gastric emptying rate. The high pH of pyloric region stimulates its emptying rate (Kidder and Manners,

1978; Mayer, 1994). Increased acidity of digesta reduces the rate of gastric emptying, which allows more time to digest nutrients in the stomach (Mayer, 1994). However, available data did not support this presumption. Risley et al. (1992) and Roth et al. (1992) failed to find any effect of dietary acidifier on stomach dry matter content in the stomach, which is highly related to the rate of gastric emptying. Therefore, although addition of acidifiers to diets has consistently decreased diet pH (Falkowski and Aherne, 1984; Giesting and Easter, 1985; Radecki et al., 1988), it does not always result in lowered gastric pH (Burnell et al., 1988; Risley et al., 1992; Roth et al., 1992). Consequently, it is difficult to elucidate the direct correlation between gastric emptying and supplementation of acidifiers.

#### Endogenous enzyme secretion and morphology

Acidifiers possibly influence the stimulation of pancreatic secretions and mucosal morphology. Both pancreatic exocrine secretion and biliary excretion are stimulated via the release of secretin (Harada et al., 1986, 1988). As was shown recently by Thaela et al. (1998), supplementation of 2.5% lactic acid to a weaner diet increased the volume and protein content of pancreatic juice as well as trypsin and chymotrypsin. In addition, at weaning time the small intestine of piglets generally showed a reduction in villous height and an increase in crypt death because of physical damage by hard grains in the diets. Several short-chain fatty acids (acetic, propionic and n-butyric acid) produced by microbial fermentation of carbohydrate stimulated epithelial cell proliferation (Lupton and Kurtz, 1993; Sakata et al., 1995). Increased epithelial cell proliferation has also been observed when short-chain fatty acids have been given orally or provided by intravenous or gastrointestinal infusion (Sakata et al., 1995), as dietary organic acidifiers can influence fermentation patterns in the small intestine, and may indirectly influence intestinal morphology. Galfi and Bokori (1990) demonstrated an increase in the length of the microvilli in the ileum and the depth of the crypts in the cecum in growing pigs when 0.17% of n-butyrate was provided.

#### Chelation of minerals

Some acidifiers could be formed complexes with various cations, thus helping the absorption of cationic minerals, such as calcium (Ca) and zinc (Zn), to be easily absorbed in the digestive tract. Kirchgessner and Roth (1982) reported that apparent absorption and retention of Ca, P and Zn were improved by the addition of fumaric acid.

Jongbloed et al. (1987) reviewed that lowered intestinal pH increased the solubility of P and phytate; thus improved P absorption in the small intestine. Jongbloed et al. (2000) also suggested that microbial phytase is known to be favorable to low pH, therefore it is more activated by

**Table 3.** Summary of published data of the effects of citric acid and fumaric acid on the performance of weaning pigs (Ravindran and Kornegay, 1993)

| Reference                  | Level of citric acid<br>(g/kg) | % changes in |             |           |
|----------------------------|--------------------------------|--------------|-------------|-----------|
|                            |                                | Daily gain   | Feed intake | Gain/Feed |
| Radecki et al. (1988)      | 15                             | -8.3         | -5.0        | -6.0      |
|                            | 30                             | -0.9         | -3.0        | +5.3      |
| Clark and Batterham (1989) | 10                             | +2.7         | +10.1       | 0         |
| Risley et al. (1991)       | 15                             | +11.4        | +10.3       | +0.8      |
| Johnson (1992)             | 15                             | +11.4        | +10.3       | +0.8      |
|                            | 30                             | +9.6         | +10.3       | -1.6      |
|                            | 50                             | +1.7         | 2.6         | -3.1      |

| Reference                   | Level of fumaric acid<br>(g/kg) | % changes in |             |           |
|-----------------------------|---------------------------------|--------------|-------------|-----------|
|                             |                                 | Daily gain   | Feed intake | Gain/Feed |
| Falkowski and Aherne (1984) | 10                              | +5.9         | -0.8        | +6.0*     |
|                             | 20                              | +4.7         | -0.35       | +8.1*     |
| Giesting and Easter (1985)  | 10                              | 0            | -3.4        | -3.7      |
|                             | 20                              | -1.5         | -11.2       | +9.6      |
|                             | 30                              | 13.4         | -1.6        | +15.4     |
| Radecki et al. (1988)       | 15                              | -0.4         | +0.6        | -0.4      |
|                             | 30                              | -11.8        | -6.7        | -5.9      |
| Giesting et al. (1991)      | 20                              | +10.7        | +1.7        | +7.4**    |
|                             | 30                              | +7.6         | -1.3        | +7.4**    |
| Risly et al. (1991)         | 15                              | +2.2         | -3.1        | +5.1      |

\*, \*\* Significantly different from the control group ( $p < 0.05$ ,  $p < 0.01$ , respectively).

supplementation of organic acid. Nonetheless, they didn't observe the synergistic effect between microbial phytase and organic acid in P utilization.

#### Stimulatory effects on intermediary metabolism

It is also suggested that metabolic reactions could be affected by the addition of acidifiers (Ravindran and Kornegay, 1993). Kirchgessner and Roth (1982) proposed that organic acids stimulated intermediary metabolism, resulting in improved energy or protein/amino acid utilization. Grassmann et al. (1992) found that formic acid addition to weaner diets increased the activities of  $\alpha$ -ketoglutaric dehydrogenase (EC 1.2.4.2) and glutamate-pyruvate transaminase (EC 2.6.1.15). And Tschierschwitz et al. (1982) observed increased activity of aspartate transferase (EC 2.6.1.1) and succinate dehydrogenase (EC 1.3.5.1) in blood with the addition of fumaric acid to rat diets, suggesting that this compound may modify intermediary metabolism of protein and energy. However this conjecture is not supported with consistent results.

### THE USE OF ORGANIC ACIDIFIER FOR WEANING PIGS

Organic acids (C1-C7) are widely distributed in nature as normal constituents of plant or animal tissue. They are also formed through microbial fermentation of carbohydrates, predominantly in the large intestine. Organic acidifier using organic acid for weanling pigs is not a new concept in the swine industry. Dietary organic acidifiers generally seemed to improve the growth performance and feed efficiency of weaning pigs presumably due to

supportive explanation of increased nutrient digestibility and reduced scours.

#### Fumaric acid and citric acid

Fumaric and citric acids are the most commonly studied organic acidifiers in weaner diets. Fumaric and citric acids are both crystalline and odorless. Fumaric acid has a tart flavor and citric acid has a pleasant sour taste. Fumaric and citric acid formed in the intermediary metabolism, as well as those of dietary origin, are possibly directed to the citric-acid cycle where they serve as important intermediary metabolites (Stryer, 1988). Since the report of Kirchgessner and Roth (1982), fumaric or citric acid became the most preferred organic acids for weaning pig's diet. Falkowski and Aherne (1984) demonstrated that ADG (average daily gain) was 4 to 7% greater and feed conversion ratio was also improved 5 to 10% when fumaric or citric acid was provided to pigs weaned at 4 weeks of age. They also reported that protein digestibility coefficients of diets containing acid tended to be higher, especially during the first week. Giesting and Easter (1985) concluded that addition of graded levels of fumaric acid (0, 1, 2, 3 and 4%) resulted in linear increase in gain/feed, daily gain and feed utilization efficiency regardless of dietary protein level. Blank et al. (1999) suggested that the inclusion of fumaric acid to the diet during the first 3 to 4 week postweaning increased the ileal digestibility of gross energy (GE), crude protein (CP) and the majority of amino acids. However, the beneficial effects of fumaric or citric acid are not always consistent. Kornegay et al. (1976) reported no beneficial effect from the addition of 1% citric acid to the diets of 7-day-old weanling pigs. Walz and Pallauf (1997) observed that supplementation of citric or fumaric acid did not affect

## ACIDIFIERS IN ANIMAL FEED

**Table 4.** Effect of formic acid supplementation on growth performance and protein accretion of pigs (Kirchgessner et al., 1992)

| Formic acid (%)                | 0                       | 0.6                     | 1.2                      | 1.8                     | 2.4                      |
|--------------------------------|-------------------------|-------------------------|--------------------------|-------------------------|--------------------------|
| Live weight 6 to 12 kg         |                         |                         |                          |                         |                          |
| Weight gain (g)                | 334 <sup>c</sup> ±53    | 412 <sup>ab</sup> ±48   | 439 <sup>a</sup> ±59     | 431 <sup>ab</sup> ±45   | 372 <sup>bc</sup> ±74    |
| Feed intake (g)                | 389 <sup>ab</sup> ±61   | 451 <sup>a</sup> ±48    | 451 <sup>a</sup> ±60     | 426 <sup>ab</sup> ±41   | 382 <sup>b</sup> ±60     |
| Feed/gain                      | 1.16 <sup>a</sup> ±0.06 | 1.10 <sup>a</sup> ±0.08 | 1.03 <sup>bc</sup> ±0.04 | 0.99 <sup>c</sup> ±0.06 | 1.04 <sup>bc</sup> ±0.10 |
| Diarrhea (d)                   | 45                      | 13                      | 5                        | 5                       | 4                        |
| Live weight 13 to 25 kg        |                         |                         |                          |                         |                          |
| Weight gain (g)                | 434 <sup>b</sup> ±94    | 516 <sup>a</sup> ±48    | 498 <sup>a</sup> ±71     | 369 <sup>c</sup> ±37    | 276 <sup>d</sup> ±56     |
| Feed intake (g)                | 803 <sup>a</sup> ±141   | 910 <sup>a</sup> ±56    | 892 <sup>a</sup> ±109    | 807 <sup>a</sup> ±65    | 605 <sup>b</sup> ±85     |
| Feed/gain                      | 1.87 <sup>c</sup> ±0.16 | 1.77 <sup>c</sup> ±0.13 | 1.81 <sup>c</sup> ±0.26  | 2.19 <sup>b</sup> ±0.17 | 2.47 <sup>a</sup> ±0.40  |
| Diarrhea (d)                   | 23                      | 2                       | 0                        | 0                       | 1                        |
| Accretion and utilization      |                         |                         |                          |                         |                          |
| Protein accretion              | 54.8 <sup>b</sup> ±8.6  | 68.6 <sup>a</sup> ±5.2  | 68.4 <sup>a</sup> ±10.4  | 61.6 <sup>a</sup> ±6.7  | 52.4 <sup>b</sup> ±8.7   |
| N utilization <sup>1</sup> (%) | 53.1 <sup>b</sup> ±2.3  | 57.8 <sup>a</sup> ±2.6  | 59.5 <sup>a</sup> ±5.5   | 57.5 <sup>a</sup> ±5.1  | 58.1 <sup>a</sup> ±3.9   |

<sup>1</sup> N accretion/N intake×100; values on the same line with a different superscript are significantly different.

utilization of amino acids. Numerous experiments demonstrated that nutrient digestibility was not affected by feeding of citric or fumaric acid (Radecki et al., 1988; Giesting and Easter, 1991). Gabert and Sauer (1995) observed a negative effect on ileal digestibility of crude protein and amino acid with increasing levels of fumaric acid supplementation to a wheat-soybean meal diets in early-weaned pigs. The comparative efficacy of citric and fumaric acid as acidifiers in weaner diets has been evaluated. Scipioni et al. (1978) reported that diets including citric acid depressed pH and bacterial numbers in the stomach and was greater in duodenum when pigs were fed fumaric acid. From the research of Falkowski and Adherne (1984), it is probable that citric acid would depress gastric pH more than fumaric acid and so facilitate improved growth performance more efficiently. Henry et al. (1985) also reported that inclusion of citric acid is more effective than that of fumaric acid. On the contrary, growth performance was improved during 1 to 2 weeks for pigs fed fumaric acid-supplemented diets however, citric acid supplementation had no beneficial effect on ADG during the 4-week trial (Radecki et al., 1988). It is difficult to determine which organic acid is more useful for weanling pigs.

However, it is suggested that fumaric acid was the preferred dietary acidifier since it is of lower cost as well as solid form (Partanen and Morz, 1999).

### Formic acid

Formic acid is a colorless, transparent liquid with a pungent odor. It is commonly used as a preservative in ensiling forage and various by-products which contain less substrate for the desirable production of lactic acid by *Lactobacilli*. Formic acid is an effective acidulant, but it can also inhibit microbial decarboxylase and enzymes such as catalase (Partanen and Morz, 1999). The antibacterial activity of formic acid is primarily against yeasts and some bacteria, whereas lactic acid bacteria and moulds are

relatively resistant to its effects (Lueck, 1980). Kirchgessner et al. (1992) studied the effect of formic acid supplementation (6-24 g/kg diet) on protein, fat, ash and energy retention in weaning piglets. They found that all formic acid-supplemented diets resulted in increased carcass protein content, compared to control group, and the retention of protein was higher (averaged 61 g/day) when pigs were fed diets with 6-18 g formic acid/kg diet. Also, at low levels of supplementary formic acid (6-12 g/kg diet), energy retention was enhanced (Table 4). In another experiment, formic acid supplementation of the prestarter diet, which was used from 6 to 12 kg body weight, induced improved growth rate, feed intake and feed conversion ratio to a maximum of 31, 16 and 15%, respectively. And the most efficient supplementation level of formic acid level was 1.2%, lower or higher levels were less efficient. However, Garbert et al. (1995) did not observe an effect of formic acid supplementation on apparent ileal digestibility of CP and amino acids for weanling pigs. Although some results with formic acid have been effective (Ward et al., 1987; Bolduan et al., 1988b), formic acid has a strong odor and flavor, and an increasing dietary acid level could show a detrimental effect on feed intake, as reflected by lower daily gains (Ekel et al., 1992a). Addition of excessive formic acid to the diet may also disturb the acid-base status of pigs leading to metabolic acidosis, which results in decreased feed intake and slower growth rate (Giesting et al., 1991; Ekel et al., 1992a).

### Lactic acid

Lactic acid is produced by many bacterial species, primarily those of genera *Lactobacillus*, *Bifidobacterium*, *Streptococcus*, *Pediococcus* and *Leuconostoc*. It is a natural constituent of some feedstuffs and among the oldest of the preservatives of food. The antimicrobial action of lactic acid is directed primarily against bacteria, whereas many moulds and yeasts can metabolize it. In early research, the addition of lactic acid in concentrations of 0.8% to weaning

**Table 5.** Effect of various acidifiers<sup>1</sup> supplementation on growth performance of weaning pigs (Kil, 2004)

| Items                 | CON | FUA | FOA | LAA | SHA | SEM <sup>2</sup> |
|-----------------------|-----|-----|-----|-----|-----|------------------|
| Daily gain (g)        |     |     |     |     |     |                  |
| 0-3 week              | 291 | 304 | 288 | 341 | 334 | 11.64            |
| 0-5 week              | 450 | 442 | 424 | 479 | 460 | 11.98            |
| Daily feed intake (g) |     |     |     |     |     |                  |
| 0-3 week              | 445 | 470 | 446 | 504 | 506 | 15.05            |
| 0-5 week              | 678 | 682 | 645 | 731 | 708 | 20.18            |

<sup>1</sup> CON: control diet, FUA: 0.2% fumaric acid, FOA: 0.2% formic acid, LAA: 0.2%; lactic acid, SHA: 0.1% hydrochloric acid.

<sup>2</sup> Standard error of mean.

pig's diets effectively reduced the levels of *E. coli* in the duodenum and jejunum of 8 weeks old piglets (Cole et al., 1968). In another study (Kershaw et al., 1966), lactic acid was added to drinking water resulting in improved growth rate and feed efficiency of weaning pigs. The acidification of the drinking water reduced hemolytic *E. coli* counts in tested pigs and was considered the primary reason that growth performance was enhanced and piglet scours were reduced. Furthermore, lactic acid delayed the multiplication of an enterotoxigenic *E. coli* and reduced the mortality rate of animals (Thompson and Lawrence, 1981). In a recent report, Kil (2004) observed the best performance in weaning pigs fed lactic acid compared to other acidifiers (Table 5). Similarly Tsiloyiannis et al. (2001) also reported that lactic acid were the most useful tool in controlling post-weaning diarrhea syndrome (PWDS) and improving growth performance (Table 6).

#### Other organic acidifiers

Many organic acidifiers beside above described acidifiers have been used for diet acidifiers. Propionic acid is frequently used in pig nutrition research. Mathew et al. (1991) found improvements in growth and feed efficiency and a similar response between propionic acid and antibiotics addition. In another experiment, Bolduan et al. (1988) added Luprosil-NC (product containing 53.5% propionic acid) at levels of 0.3 and 1% to weaner diet. Luprosil-NC did not affect pH, lactic acid concentration or SCFA (short chain fatty acid) content in the stomach and small intestine, but decreased *E. coli* counts in the stomach at concentration of 1%. Benzoic acid is not yet approved as an additive or preservative for pig feed, but is extensively used as a food preservative in human nutrition. The supplementation of pigs' diets with benzoic acid resulted in significantly lower counts of lactic acid bacteria, *Lactobacilli* and yeast throughout the entire gastrointestinal tract and the number of Coliforms was numerically lowered as compared to the control diet (Maribo et al., 2000). The effects of malic acid were investigated by Sciopioni et al. (1978) who reported depression in performance at a supplementation level of 0.9%. Bokori et al. (1989) observed improved performance of weanling pigs fed diets

**Table 6.** Effect of different organic acidifiers on diarrhea scores<sup>1</sup> of weaning pigs (Tsiloyiannis et al., 2001)

| Day <sup>3</sup> | Group <sup>2</sup> |      |      |      |      |      |      |
|------------------|--------------------|------|------|------|------|------|------|
|                  | NC                 | PA   | LA   | FOA  | MA   | CA   | FA   |
| 1-7              | 4.57               | 3.11 | 1.07 | 1.79 | 2.86 | 2.50 | 2.00 |
| 1-14             | 7.98               | 5.57 | 2.77 | 3.66 | 4.84 | 4.38 | 4.34 |
| 1-28             | 5.63               | 4.41 | 1.94 | 2.50 | 3.49 | 3.21 | 3.00 |

<sup>1</sup> Scored by the scales as : 0 = no diarrhea, 1 = soft feces, 2 = fluid feces, 3 = projectile diarrhea.

<sup>2</sup> NC: negative control, PA: propionic acid, LA: lactic acid, FOA: formic acid, MA: malic acid, CA: citric acid, FA: fumaric acid.

<sup>3</sup> after weaning.

containing 1.7% sodium-n-butyrate. However, It is true that there have been few experiments to explain the effect of these organic acidifiers on pig nutrition.

### THE USE OF INORGANIC ACIDIFIER FOR WEANING PIGS

The most widespread benefit from acidification of weaner diets has been obtained with organic acidifiers. Although organic acidifier addition appeared to improve the growth response, its cost was an obstacle for extensive utilization in animal feed. Therefore, most feed companies are forced to use a limited amount of organic acidifier for acidification of diet. As inorganic acidifier is cheaper than organic acidifier, inorganic forms have received much attention in order to reach proper acidification of weaning pigs' diet with low cost. Several studies were conducted with inorganic acids such as hydrochloric, sulfuric and phosphoric acids (Giesting, 1986; Roth and Kirchgessner, 1989; Oh, 2004). In the pure state, these are extremely corrosive and dangerous liquids. They are strong acids but also have either a large chloride, phosphate or sulphate component in the molecule. Giesting (1986) attempted to demonstrate growth responses to the addition of hydrochloric, phosphoric and sulfuric acids in amounts calculated to provide acidification similar to that obtained with 3% fumaric acid. Supplementation of concentrated hydrochloric acid to weaning pigs' diet resulted in a severe depression in growth probably due to an unfavorable electrolyte balance in the feed. Sulfuric acid addition also depressed performance, probably for the same reason. Of the three inorganic acids tests, only phosphoric acid did not result in a growth depression with even no improvement. It does not upset the electrolyte balance as does the other inorganic acids and can be a source of available phosphorus for the piglet. Similarly Roth and Kirchgessner (1989) reported that inorganic acids such as ortho-phosphoric acid or hydrochloric acid induced no comparable results although they lower the pH value of diet. Most researchers generally demonstrated the use of inorganic acid could have a negative effect on growth performance probably



## ACIDIFIERS IN ANIMAL FEED

**Table 7.** Effect of added dietary hydrochloric acid on performance and N utilization of weaning pigs (Mahan et al., 1999)

| Item                              | Dietary chloride level (%) |      |      |      |      | SEM               |
|-----------------------------------|----------------------------|------|------|------|------|-------------------|
|                                   | 0.20                       | 0.26 | 0.32 | 0.38 | 0.42 |                   |
| Daily gain (g)                    |                            |      |      |      |      |                   |
| 0-7 d                             | 120                        | 123  | 156  | 147  | 138  | 11 <sup>a</sup>   |
| 7-14 d                            | 294                        | 316  | 342  | 351  | 310  | 10 <sup>b</sup>   |
| 14-21 d                           | 372                        | 422  | 447  | 418  | 427  | 12 <sup>b</sup>   |
| N retention, g/d                  | 6.09                       | 6.58 | 6.66 | 6.80 | 6.36 | 0.11 <sup>b</sup> |
| Apparent digest. <sup>d</sup> , % | 88.6                       | 90.7 | 91.4 | 93.2 | 94.9 | 0.54 <sup>c</sup> |

<sup>a</sup> Quadratic response (p<0.10).<sup>b</sup> Quadratic response (p<0.01).<sup>c</sup> Linear response (p<0.01).<sup>d</sup> Apparent digestibility, % = {(N intake-fecal N)/N intake}×100.

attributable to alteration of electrolyte balance or to feed palatability.

However, despite these unfavorable aspects, several investigators have attained noticeable results. Straw et al. (1991) reported that reduced dietary pH by supplementation of hydrochloric acid increased the ADG and ADFI during first 3 week and overall (0-6 week) within adequate dEB value. In addition, Schoenherr (1994) found positive results of using phosphoric acid-based acidifiers for pigs immediately following weaning. The advantage of using phosphoric acid-based products was similar to the advantage of using fumaric acid when compared with non-acidified diets. There were another studies using inorganic acids used for sources of minerals. Mahan et al. (1996) reported that addition of hydrochloric acid as the source of chloride to the starter diets resulted in improved daily gains linearly during the initial 2-week postweaning and feed efficiency was also increased linearly in first week without any reduced feed intake. In another study, (Table 7), addition of hydrochloric acid improved ADG and feed efficiency in the first week and also demonstrated a weekly decrease in the fecal N excretion and improved N retention during the initial two week of postweaning (Mahan et al., 1999). Based on this result, additional chloride supplementation was needed in weaning pigs and assumed higher dietary chloride from hydrochloric acid could be a source of HCl production of stomach. Even though inorganic acid might be useful in pig nutrition, few studies were conducted so that it was difficult to evaluate the effect of inorganic acid.

#### THE USE OF EXTENDED ACIDIFIER FOR WEANING PIGS

Recently an increasing interest has been directed towards various salts of organic acidifiers. Salts of organic acids, such as formates, diformates, calcium propionate, have advantage over free acids because they are generally odorless and easier to handle in the feed manufacturing process, owing to their solid and less-volatile form. They

**Table 8.** Effect of hydrochloric acid complex<sup>1</sup> on growth performance of weaning pigs (Oh, 2004)

| Item      | CON                 | H0.1                | H0.2               | H0.3               | SEM  |
|-----------|---------------------|---------------------|--------------------|--------------------|------|
| ADG (g)   |                     |                     |                    |                    |      |
| 0-3 week  | 322 <sup>a</sup>    | 396 <sup>b</sup>    | 288 <sup>a</sup>   | 298 <sup>a</sup>   | 20   |
| 0-5 week  | 397 <sup>ab</sup>   | 442 <sup>a</sup>    | 352 <sup>b</sup>   | 357 <sup>b</sup>   | 20   |
| ADFI, g   |                     |                     |                    |                    |      |
| 0-3 week  | 485 <sup>ab</sup>   | 561 <sup>a</sup>    | 420 <sup>b</sup>   | 418 <sup>b</sup>   | 20   |
| 0-5 week  | 649 <sup>ab</sup>   | 731 <sup>a</sup>    | 619 <sup>ab</sup>  | 555 <sup>b</sup>   | 40   |
| Gain/Feed |                     |                     |                    |                    |      |
| 0-3 week  | 0.668               | 0.706               | 0.684              | 0.713              | 0.02 |
| 0-5 week  | 0.614 <sup>ab</sup> | 0.612 <sup>ab</sup> | 0.570 <sup>b</sup> | 0.648 <sup>a</sup> | 0.02 |

<sup>1</sup> salt form of hydrochloric acid mixed with scoria.<sup>a,b</sup> means with different superscripts in the same row differ (p<0.05).

are also less corrosive and may be more soluble in water than free acids (Partanen and Morz, 1999). In particular, salts of formic acid have received much attention. It is assumed that the combination of formic acid with various formates was more effective than the application of formic acid alone (Roth et al., 1996). Kirchgessner and Roth (1987a, 1990) also reported results of experiments that piglets supplemented with calcium formate in combination with formic acid had better performance data than piglets which got pure formic acid addition. Other researchers found the beneficial effect on the growth performance and feed efficiency in weaning pigs by feeding salts of formic acid (Øverland et al., 2000; Paulicks et al., 2000).

In recent study, however, Canibe et al. (2001) demonstrated that the addition of K-diformate to a starter diet for piglets did not show increased growth performance and decreased total anaerobic bacteria, lactic acid bacteria, coliforms and yeasts in feces and digesta from various segments of the gastrointestinal tract without affecting the gastric or intestinal pH. Moreover, the addition of organic acids sometimes successfully increased stomach acidity, but no further effect was found in the lower part of the digestive tract (Aumaitre et al., 1995). Therefore, another method using a slow-release form of acidifier has been introduced. It consists of organic acids with fatty acids and mono- and diglycerides mixed to form microgranules. Results of experiments showed that use of these acidifiers, as compared to other free acids, resulted in greater feed intake and growth (Cerchiari, 2000).

Recently, Oh (2004) and Kil (2004) used salts of hydrochloric acid carried with scoria which is formed by alteration of volcanic ash in order to evaluate the effect of inorganic acids for weaning pigs. They observed that the inorganic acidifier (scoria-hydrochloric acid complex) were less corrosive and volatile, moreover it improved the growth performance of weaning pigs (Oh, 2004) and showed better growth performance than fumaric or formic acid (Kil, 2004, Tables 5 and 8). In their experiments, growth performance was increased when hydrochloric acid containing inorganic acidifier was provided to weaning pigs



**Table 9.** Acid buffering capacity (ABC) for each feed category (Lynch et al., 1998)

| Feed category       | ABC-4 <sup>1</sup> | ABC-3 |
|---------------------|--------------------|-------|
| Milk products       | 644                | 840   |
| Cereals             | 87                 | 217   |
| Root products       | 145                | 383   |
| Amino acids         | 101                | 747   |
| Vegetable proteins  | 389                | 652   |
| Meat and fish meals | 866                | 1,839 |
| Minerals            | 2,919              | 5,568 |

<sup>1</sup> ABC was calculated as the amount of acid in milliequivalents (meq) required to lower the pH of 1kg of feed a) pH 4 (ABC-4) b) pH 3 (ABC-3).

but its response was only observed within 3 wks postweaning. Moreover, the level of blood IgA was lowered when pigs were fed inorganic acidifiers (Oh, 2004). This result implied that supplementation of hydrochloric acid showed bacteriocidal effect on harmful bacteria in GI tract, resulting in the reduction of the population of pathogenic bacteria subsequent released less IgA in the body.

#### FACTORS AFFECTING RESPONSES TO DIETARY ACIDIFIER

There were considerable variations in responses to dietary acidifier. The discrepancy is thought to be related to various experimental methods and dietary ingredients. Most potential reasons for varying results might be related to differences in feed palatability, source and character of diet, supplementation level of acidifier and the age of animal (Ravindran and Kornegay, 1993).

##### Feed palatability

The growth-promoting effects of dietary acidifier seemed to depend highly on how to increase feed intake. Improved growth of piglets fed acidified diets has been ascribed to a better diet palatability (Cole et al., 1968). However, Henry et al. (1985) reported pigs fed non-acidified diets showed significantly increased feed intake when compared with pigs fed acidified diets. Folkowki and Aherne (1984) adversely demonstrated that inclusion of fumaric or citric acid to the diets did not significantly affect daily feed intake. Partanen and Morz (1999), in the review of the varied effects of organic acid on feed intake, reported that different organic acids could have different effects on feed intake. Generally, dietary formic acid had a positive effect, fumaric acid had no effect and citric acid had a negative effect. Moreover, apart from referred adverse effects on feed palatability of inorganic acids, Oh (2004) and Kil (2004) showed inorganic acidifier had no detrimental effect on feed intake of weaning pigs if inorganic acid was combined with a proper carrier like scoria.

##### Sources and composition of diet

Effects of dietary acidifier supplementation may be affected by some sources of diet. Performance studies showed that a greater response to acidifier was observed when cereal-oilseed meal diets were used compared with diets containing with milk products (Giesting, 1986). It is supposed that lactose in milk products may have been converted to lactic acid and may have decreased the need for dietary acidifier (Easter, 1988). Furthermore, in another aspect using milk by-products, the multiplication of *Lactobacilli spp.* in the stomach of nursing pigs and the subsequent production of lactic acid can suppress HCl production (Cranwell et al., 1968, 1976; Mahan et al., 1996). Burnell et al. (1988) also observed higher improvements in average daily gain and feed conversion when citric acid was added to diets based on corn-soybean meal compared with corn-soybean meal-whey diets. Moreover, they observed that the addition of acidifier to the diet containing copper (Cu) improved growth rate proposing that acid would have accentuated the response to Cu. A similar response was also reported by Edmonds et al. (1985). However, they observed only a small increase of feed efficiency in pigs fed acidifier with Cu and antibiotics. Therefore, effects of acidifier could be influenced by other supplemented ingredients and growth promoters

Another factor affecting the response to acidifier would be the characteristics of diet such as buffering capacity because it compensated for the reduction in gastric pH (Table 9). This would be one of the reasons for the conflicting results obtained in studies with acidifiers. High buffering capacity of milk products (Bolduan et al., 1988a) could be partly responsible for ameliorated effect of acidifier (Giesting et al., 1991). They also reported that although the addition of fumaric acid to weaner diets improved performance irrespective of the inclusion of skim milk, a higher level of fumaric acid was necessary to maximize performance immediately after weaning when diets contained skim milk. Results reported by Jung and Bolduan (1986) demonstrated that a high mineral content in the diet also increased gastric pH and microbial activity in the stomach. As shown by Bolduan et al. (1988a) and Lawlor et al. (1994), the buffering capacity is strongly related to the amount and source of protein as well as minerals in the diet. Lawlor et al. (1993) showed that excluding Ca and P sources from starter diets for a short period of postweaning or feeding 2 g/kg fumaric acid in the diet both reduced diet buffering capacity and improved pig performance. A high buffering capacity of the diet also decreased the ileal amino acid digestibility by 1 to 10% units compared with diets having the low buffering capacity (Blank et al., 1999). However, Roth and Kirchgessner (1989) found no direct relationship between pig performance and reduction in dietary buffering capacity.

### Supplementation level of acidifier

In addition, the magnitude of response to acidifier is influenced by supplementation level of acidifier employed. The difference in dissociation constants and solubility in water of different acidifiers (Gardner, 1972) may be expected to be partly responsible for the variable responses obtained. Several studies have attempted to determine the optimal supplementation levels of different acidifiers (Giesting and Easter, 1985; Radecki et al., 1988; Eckel et al., 1992a). However, the ranges over growth response obviously varied with supplemented levels of acidifier and age of animals. In general, the growth performance tended to depend on dose; the responses tended to improve at higher levels of inclusion and increasing chain length of acidifier. According to several experiments, growth performance was improved when animals were fed above 1% of dietary acidifier (Falkowski and Aherne, 1984; Radcliffe et al., 1998; Thaela et al., 1998; Tsilyiannis et al., 2001). Although the supplementation of acidifiers in young animals' diets showed beneficial effects, high levels of acidifier could not be used in the feed industry because of its cost.

### Age of animal

Most studies have supplied acidifier to pigs aged between 7 and 32 days when pigs had a limited capacity to maintain low gastric pH. The response to dietary acidifier is often most noteworthy especially after immediate weaning time and tends to decline with age. In several studies, the response to acidifier occurred during the first few weeks postweaning (Sciopini et al., 1978; Radecki et al., 1988; Giesing et al., 1991; Risley et al., 1992; Mahan et al., 1996; Kil, 2004; Oh, 2004), but did not show after 3-4 weeks. Early research on the development of pepsin activity in neonatal pigs suggested low acid secretion until pigs reach 2 to 4 weeks of age (Lewis et al., 1957; Hartman et al., 1961). By 4 weeks postweaning, the pig is adapted enzymatically (Cranwell, 1985; Lindemann et al., 1986) to the diets imposed at weaning which may have masked any beneficial effects of dietary acidifier (Ravindran and Kornegay, 1993). Therefore, the lack of response in older pigs to acidifiers is possibly associated with their increased acid secretion and matured gastric function. Otherwise, it should not be thought that younger pigs always responded to acidifier more efficiently. Because it is expected that younger piglets are more sensitive to the change of diet palatability by addition of acidifier, subsequently feed intake and growth performance could be affected.

### CONCLUSION

Dietary acidifiers have been accepted as potential alternatives to antibiotics in order to improve the

performance and health status of weaning pigs. Acidifiers also helped to increase nutrient digestibility and reduce scouring. This improvement has been obtained by lowering gastric pH and subsequent modification of the intestinal microflora. Recently, inorganic acidifiers as well as organic acidifiers have been used and have produced observable effects on performance of weaning pigs. The mode of action and different results of acidifiers cannot be solely ascribed to a specific factor. Thus, at the present time, there are clearly more questions than answers in the area of acidifier application. If we correctly understand and use acidifiers, they can be a powerful tool in maintaining the health of weaning pigs and improving swine productivity.

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