

EFFECT OF FEEDING FUMARIC OR CITRIC ACID ON WEANLING PIG
PERFORMANCE AND SELECTED INTESTINAL DIGESTA MEASUREMENTS AT
VARYING TIMES POSTWEANING

by

Chad Richard Risley

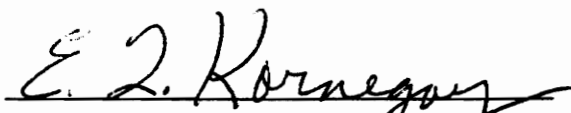
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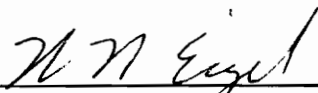

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(ABSTRACT)

Five studies were conducted to elucidate a possible mode of action of dietary organic acids in weanling pigs (n=536). The effect of feeding 1.5% fumaric or citric acid on growth performance, intestinal digesta pH, chloride ion concentration (Cl⁻), VFA profile and microflora populations was studied. In study 1, feeding citric acid improved ADG and feed conversion (F:G), whereas fumaric acid only numerically improved ADG and F:G. No significant dietary effect on intestinal pH, Cl⁻ or VFA profile was observed. In study 2, feeding organic acids with and without a microbial supplement (*Lactobacillus acidophilus*) resulted in no interactive effects, but fumaric acid and the microbial supplement each improved ADG and F:G. In study 3, pigs fed organic acids were killed at -2, 0, 3, 7, 14 and 21 d postweaning. Organic acids had no significant effect on intestinal pH, Cl⁻, VFA profile or microflora populations. Postweaning age affected intestinal digesta measurements in these ways: pH decreased; VFA concentrations increased; *lactobacillus* decreased to d 3 postweaning; *E. coli*

increased to d 7 postweaning; and clostridia decreased to d 21 postweaning. In study 4, pigs were fed organic acids and orally challenged with 10^{10} enterotoxigenic *E. coli* (0157:K88) (ETEC) at d 3, 7 and 14 postweaning. Organic acids had no measurable effect on intestinal pH, Cl⁻ or VFA profile. ETEC challenge increased jejunum lactic acid concentration, lower colon VFA concentration and severity of scouring. Postweaning age affected these intestinal digesta measurements: cecum pH decreased; jejunum Cl⁻ increased; jejunum lactobacillus count decreased; lower colon lactobacillus count increased; and *E. coli* count decreased in all intestinal sections. In study 5, pigs were fed organic acids and challenge with ETEC on d 3 postweaning. Organic acids had no significant effect on severity of scouring or protection against reduced growth performance from the ETEC challenge. The ETEC challenge resulted in an increased severity of scouring and a reduction in ADG of 20% and F:G of 19%. In summary, feeding organic acids generally improved weanling pig growth performance and had little effect on intestinal digesta measurements. Postweaning age did have an effect on intestinal digesta measurements. The primary mechanism of action of dietary organic acids does not seem to be the lowering of intestinal pH or modifications to the intestinal microflora, but may act as an additional energy source.

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CHAPTER I

INTRODUCTORY SECTION

Weaning exposes the pig to environmental, social and dietary stresses that can result in poor growth performance and scouring during the first week. This period is commonly referred to as the postweaning lag phase. Producers have implemented many different management practices to alleviate this lag phase to fully realize the efficiency potential of the weanling pig. The most successful practice has been the feeding of sub-therapeutic levels of antibiotics.

Antibiotics have been shown to increase average daily gain, stimulate feed intake, improve feed efficiency and reduce scouring. However, their continued use is in jeopardy from activists who are questioning food safety and the general use of drugs in livestock. Because of their pressure, the future use of feed antibiotics may be highly regulated or eliminated. Therefore, a need exists to develop effective alternatives to the use of feed antibiotics. Two feed additives which show some promise are organic acids and microbial fermentation products.

Organic acids and microbials have been reported to increase growth performance when added to pig starter diets, but these improvements are not as consistent as those observed with feeding antibiotics. A potential reason for these inconsistencies may be the incomplete knowledge of how

these feed additives exert their growth promoting effects.

Organic acids are hypothesized to lower the gastric pH and shift the gastrointestinal bacterial population to a more favorable population. A lower gastric pH would benefit the weanling pig because until about five weeks of age, the pig is unable to secrete sufficient quantities of hydrochloric acid to effectively lower the stomach pH. An alkaline gastric environment may result in poor feed utilization due to inadequate pepsinogen activation and protein denaturation. In addition, a high intestinal pH will allow the proliferation of coliforms, like *Escherichia coli*, which may produce scouring. Microbials may act similarly to organic acids by producing lactic acid, which lowers intestinal pH, and to antibiotics by secreting antibacterial substances that can inhibit coliforms.

This research was conducted to investigate the growth promoting effects of fumaric and citric acid, as well as a microbial (*Lactobacillus acidophilus*) in weanling pigs. Furthermore, the effects of these organic acids on the intestinal digesta pH, concentration of chloride ion, volatile fatty acid profile and microflora population were investigated to determine a mode of action for organic acids.

CHAPTER II

LITERATURE REVIEW

Weaning

Traditionally, swine farmers have weaned pigs at an age of 6 to 8 weeks; they have currently adopted early weaning at 3 to 4 weeks of age. This enables them to be more productive due to an increased number of pigs per sow per year (Cole et al., 1975) and to make more efficient use of their facilities. However, early weaning is usually associated with a period of poor growth performance and scouring. This postweaning lag, as it is commonly referred to as, normally lasts about a week and is characterized by little or no growth, poor feed conversions and varying degrees of scouring (Tzipori et al., 1980; Barnett et al., 1989).

Early weaning subjects the pig to many stresses at a time when the pig is least able to handle them. The passive immunity obtained from the colostrum is low at 3 weeks and the active immune system of the pig is not completely developed until 5 weeks of age. In addition, at 3 weeks of age, the small physical size of the pig results in a high ratio of body surface area to body volume that is coupled with low reserves of body fat, which leaves the pig unable to regulate its body temperature properly (Fenten et al., 1985). These factors allow pathogenic bacteria, normally

proliferate and thereby cause disease.

Stevens (1963b) described the postweaning lag in 3 week old weaned pigs as a setback in growth rate and an increase in the number of *E. coli* (not a new colonization) in the intestinal tract caused by the change to a dry diet and a low antibody titer (passive and active). Miniats and Roe (1968) suggested that poor postweaning performance is due to immaturity of the pig (size, immune response, gastrointestinal tract) and failure to consume enough feed or water.

The stresses of mixing with other pigs, dietary modifications and environmental changes that accompany weaning (Thomlinson, 1963) may overwhelm the pig and lead to poor postweaning growth performance and reduced resistance to disease. In order to minimize these stresses, producers are recommended to wean pigs by litter and placed the pigs in a warm (30°C), clean and dry environment. The disinfecting of the nursery is necessary to reduce the microbial load to which the weanling pig is exposed. Sanitation is an important aspect in preventing or reducing the severity of the postweaning scour syndrome. Without proper sanitation, potentially pathogenic bacteria can be ingested by the weanling pigs by dung or other tissue which enables these bacteria to increase their pathogenicity and thereby increase herd health problems (Porter et al., 1979).

Stevens (1963a) summarized the management practices necessary to reduce the incidence of postweaning scouring. His suggestions were to reduce the number of pathogenic organisms in the environment by disinfection and antibiotics, to increase the immunity of the sow and to improve overall herd management practices.

Sudden changes in the diet and(or) environmental stress can produce proliferation of pathogenic *E. coli* in the weanling pig (Buxton and Thomlinson, 1961). Diet composition and amount of diet consumed has a profound effect on the ability of *E. coli* to proliferate and colonize the gut. Bertschinger et al. (1979) inoculated weanling pigs with a toxigenic strain of *E. coli* and fed two different diets: high-fiber/low protein nutrient dense and low-fiber/high protein nutrient dense and observed a reduction in the shedding of fecal *E. coli* and death loss in the pigs fed the high-fiber/low nutrient dense diet. They concluded that death losses were proportional to the level of protein in the diet. This observation is of concern in weanling pigs, because in order to achieve greater growth potential, a high nutrient dense diet (normally at least 20% CP) is fed. This diet, according to Bertschinger and co-workers, would cause an increase in postweaning scouring and death.

During the first few days after weaning the pig is

stressed and consumes little feed. However, after the stress of weaning has past, the now famished pig gorges itself on the high nutrient dense starter diet. This overeating can result in scouring (Goodwin, 1957). Scouring caused by overeating usually occurs between 4 to 7 days postweaning. Restriction of feed after weaning has been reported to reduce the incidence of scouring (Palmer and Hulland, 1965; Miniats and Roe, 1968). The restriction of feed after weaning may reduce the severity of scouring, but will also result in poorer growth performance due to an inadequate intake of protein and energy.

Starvation causes changes in the gut microflora such that after one day without food, the population of lactobacillus and bifidobacteria decreased and the number of E. coli and bacteriodes increased in pigs (Morishita and Ogata, 1970). The lactobacillus and bifidobacteria are usually considered to be beneficial bacteria, whereas E. coli and bacteriodes can be associated with a disease state. The reduction in feed consumption that is normally associated with the weaning of pigs could result in this type of microbial population shift and render the weanling pig more susceptible to enteric infections.

Creep Feed

Creep feeding, the feeding of a highly palatable and digestible diet before weaning, allows the suckling pigs access to more energy, protein and nutrients. Creep feeding has been shown to be beneficial as a milk supplement to pigs weaned at 6 to 8 weeks of age, but its usefulness in early weaning is less clear. The milk production of the sow steadily increases to about 3 to 4 weeks and declines thereafter. The energy requirement for maximum growth of the pig increases with age and surpasses the ability of the sow to provide this energy at 4 to 5 weeks of age. Thus pigs weaned at 6 to 8 weeks of age need an additional source of energy (creep feed or sow feed) to maximize their growth rate. However, early weaned pigs eat little creep feed before 21 days of age, and therefore the labor and expense involved in creep feeding is of questionable value.

Hampson and Kidder (1986) reported that the average daily intake of creep feed was 236 grams between 18 - 28 days of age. Corring and co-workers (1978) reported that by 4 weeks of age the nursing pig consumes less than 100 grams of creep feed per day, but by 5 weeks of age consumes over 500 grams per day. Okai et al. (1976) and Barnett et al. (1989) found similar results in 3 week old pigs which consumed 13 - 71 and 13 - 194 grams per pig per day, respectively. Low intake of creep feed is a concern because

of the possibility that a transient dietary hypersensitivity to antigens present in the creep-feed may be the underlying cause of the postweaning lag (Newby et al., 1983).

Creep feed has been postulated to increase the postweaning feed intake and growth performance in pigs, but Hampson and Kidder (1986) observed that non-creep fed pigs consumed more feed the first five days postweaning than did creep-fed pigs. Contrary to these findings, Okai et al. (1976) and Barnett et al. (1989) observed more scouring in creep-fed pigs. Both groups also reported no differences in postweaning growth performance between creep-fed and non creep-fed pigs. Hampson et al., (1985) observed that creep feeding increased fecal water as did age, but *E. coli* and rotavirus did not influence fecal water.

Consumption of creep feed appears to induce the intestinal enzymes (Okai et al., 1976) and reduce gastric pH (Hampson et al., 1985) needed to properly digest a corn-soybean based diet. At birth, lactase activity is high and sucrase activity is low, but as the pig matures, the activity of these enzymes reverse (Kenworthy and Crabb, 1963). In addition, the stomach pH of the nursing pig is high and production of hydrochloric acid is nominal. An acidic gastric environment is necessary to denature protein and reduce the possible occurrence of pathogens. Creep feed intake may hasten this normal transition of activities and

may induce other digestive enzymes. This provides the pig with a more mature gastrointestinal tract that is more able to digest and utilize the starter diet.

The feeding of complex diets to pigs weaned at 3 weeks of age produces better growth performance than feeding a simple diet, but this improvement is not as great in heavier pigs or pigs weaned at 4 weeks of age (Himmelburg et al., 1985). Also, increases in growth obtained with the complex diet is not carried over into the growing-finishing phase (Himmelburg et al., 1985). The feeding of a complex starter diet results in increased postweaning performance and may be related to increases in protease and amylase activities in pigs fed dried whey (Graham et al., 1981). The addition of dried skim milk increases the digestibility of energy and nutrients in starter diets (Hays et al., 1959) and is probably related to milk protein rather than milk carbohydrates (Turlington et al., 1989).

Feed Additives

Antibiotics

Many different management practices have been implemented to alleviate the postweaning lag. The feeding of subtherapeutic levels of antibiotics in the starter diet has produced the most consistent results. Feed antibiotics can increase feed intake, improve feed conversion and reduce

scouring. However, even antibiotics can produce inconsistent improvements in growth performance. Fuller et al. (1960) reported that not all pigs responded to the feeding of the growth promotants penicillin, chlorotetracycline or copper sulphate.

The future of feed antibiotics is uncertain because of health concerns about of antibiotic-resistant microorganisms and antibiotic residues in meat, therefore, their future use may be reduced and(or) tightly regulated. Therefore, the developement of safe and effective alternatives to the use of antibiotics is imperative.

Probiotics

Probiotics, the use of bacteria or a bacterial fermentation product, has been an area of interest due to their proposed action against microorganisms which produce enteric infections. Probiotics are either an extract from a mixture of bacteria or actual viable bacteria which are fed to the animal (Lyons, 1987). Probiotics have been reported to reduce scouring, improve the general health, and increase the general performance of the animal (Hale and Newton, 1979; Lessard and Brisson, 1987). However, these results have not been consistently observed. The mode of action of probiotics has yet to be elucidated, but is believed to be associated with promotion of 'good' bacteria and reduction

in the number of 'bad' bacteria within the gastrointestinal tract. Most probiotics rely on lactobacillus species as the 'good' bacteria, but may also contain streptococcus and(or) bifidobacterium organisms. These bacteria produce acid and other undefined factors which supposedly suppress the growth of 'bad' bacteria (i.e. E. coli, bacteriodes and clostridia) and, thereby, help promote growth. Another possible mode of action is in the preventing of the colonization of the gastrointestinal tract by the harmful bacteria. Feeding a viable probiotic prior to exposure to the pathogenic bacteria allows probiotic bacteria to occupy attachment sites on the intestinal lining that are also utilized by the harmful bacteria and thereby prevent attachment and colonization. Also the low pH of the extracts and the production of acids by the probiotic organisms may lower the pH of the gut producing a less desirable environment for pathogenic bacteria (Lyons, 1987).

Organic Acids

Acidified diets have also been used as an alternative to antibiotics in starter diets, however, their mode of action is yet to be determined. The lowering of the gut pH is one hypothesized action. As with probiotics, a lower gut pH is a more desirable environment for good bacteria and suppresses the proliferation of harmful bacteria. The

weanling pig's ability to produce acid in the stomach is limited until about 5 weeks of age, therefore, the 3 week weaned pig is unable to lower the gut pH substantially. This higher gut pH allows the bad bacteria to colonize and proliferate within the gastrointestinal tract. Acidified diets, due to their acid content, may directly lower the gut pH and stimulate the stomach to secrete hydrochloric acid.

The addition of acid may also increase the digestibility of the diet. Calcium, iron, copper and zinc all have greater apparent absorption in acidic environments. Protein is also denatured by acid which allows proteases to work more efficiently. Partially hydrolysis of the diet during storage due to the activity of the acid may also ease digestion.

Acidic diets can cause problems with use. The corrosive nature of the acid may lead to a shorter lifespan of feeders and feed mixers and thereby cause greater maintenance costs. The feed may also be less palatable if too high a percentage of acid is added to the diet.

Variations to the responses observed with feeding acidic diets have been reported, and depend upon what form of acid, organic or inorganic, as well as what type, formic, citric or hydrochloric acid, is fed. Research indicates that the most consistent positive growth response are from the acids fumaric, citric and propionic with varying results

from other acids.

Kirchgessner and Roth (1982) reported in their review of fumaric acid that feeding fumaric acid promotes weight gain in weanling pigs by an average of 10% above that of control pigs and that feed efficiency improves about 4%. They also state that at the level of 1.5 - 2.0%, fumaric acid can also stimulate feed intake in weanling pigs. Fumaric acid has also been observed to increase energy and protein digestibility, N-retention, and Ca and P balance (Kirchgessner and Roth, 1982). In contrast, Radecki et al. (1988) observed no positive effect from 1.5% fumaric acid supplementation on energy, metabolizable energy, N balance, N digestibility or calcium and phosphorous balance.

Radecki et al. (1988) observed no benefit on growth performance with the feeding of 1.5% citric acid, but observed an improvement in ADG and F:G with the addition of 1.5% fumaric acid. Kornegay et al. (1976) observed no benefit in growth performance in feeding 7 day old weanling pigs citric acid. Burnell et al. (1988) observed an improvement in ADG and F:G with the addition 1.0% citric acid in a simple diet, but observed no benefit from acid supplementation in a complex diet (15% dried whey). In addition, Burnell et al. (1988) reported a slight reduction in intestinal pH with diets containing citric acid and dried whey.

The feeding of 1 or 2% fumaric or citric acid in a complex diet (fish meal and dried skim milk) improved F:G and numerically increased ADG 4 - 7% in a 4-wk trial (Falkowski and Aherne, 1984). This level of supplementation had no affect on the apparent digestibility of protein or dry matter. Giesting and Easter (1985) fed 2% propionic, fumaric or citric acid in a simple diet to weanling pigs and observed an improvement ($P < .07$) in F:G while propionic acid depressed feed intake. Additionally, increasing the level of fumaric acid between 1, 2, 3 and 4% produced a linear inprovement in ADG and F:G. Giesting and Easter (1985) were unable to observe a protein sparing effect with fumaric acid supplementation, contrary to the report of Kirchgessner and Roth (1982), and did not observe any improvement in growth performance in finishing pigs fed 1.5 or 3% fumaric acid.

Henry and co-workers (1985) fed weanling pigs either citric or fumaric acid and found that citric acid, not fumaric acid, increased ADG and feed intake. Simarily, Scipioni et al. (1978) observed an increase in ADG and improvements in apparent digestibility of dry matter, nitrogen and minerals. Furthermore, these researchers were unable to observe any benefit on weanling pig performance with the addition of fumaric or malic acid. Scipioni et al. (1978) also reported a decrease in gastric and duodenal pH

as well as a decrease in total *E. coli* and anaerobic bacteria with the feeding of citric acid. The feeding of propionic acid resulted in poorer growth performance due to a decrease in feed intake (palatability problem), however propionic acid was directly incorporated into fatty acids due to the increase in odd-chain fatty acids in the backfat of the pigs (Thacker and Bowland, 1980).

In summary, the supplementation of weanling pig diets with the organic acids, fumaric or citric acid, has generally resulted in improvements in ADG and(or) F:G. However, improvements in apparent digestibility of protein, dry matter, energy, nitrogen or minerals have been less consistent. Also, the ability of organic acids to lower intestinal pH and modify intestinal microflora has not been completely substantiated in the current literature.

Enzymes

At 3 weeks of age the digestive system of the pig is geared for a diet of milk and not a diet based on cereal grains. The enzymes, lipase and lactase, are at their highest activities, whereas the amylases and proteases, necessary for the breakdown of a corn-soybean meal diet, have yet to be fully induced and active. These enzyme activities are one reason for the incorporation of milk products into the creep feed and starter diets.

Early pig growth rate reflects the development of pancreatic digestive enzymes up to 21 days old, such that heavier pigs have a more developed gastrointestinal tract than lighter weight pigs (de-Passille et al., 1989). The activities of the digestive enzymes lactase, maltase and sucrase peak at two weeks of age and are located in the jejunum and ileum, but not in the duodenum (Aumaitre and Corring, 1978) suggesting that the starter diet must be tailored to the digestive capabilities of the pig. However, Kidder and Manners (1980) reported that the enzyme levels of the weanling pig approaches that of a mature pig by 8 weeks of age and that age, not weight, was better correlated with the level of enzymes.

Weaning causes a large loss of digestive enzymes (lactase and sucrase) at the brush border which coincides with the reduced ability of the pig to absorb xylose and a decrease in the growth rate (Hampson and Kidder, 1986). The reduction in the ability to absorb xylose indicates intestinal damage and malabsorption. Risley et al. (1988) related a decreased ability of weanling pigs to absorb xylose and an increase in the malabsorption of ovalbumin to intestinal damage.

As stated previously, the ingestion of creep feed may hasten the normal transition of intestinal enzymes. In support of this hypothesis, Corring et al. (1978) reported

the level of intestinal enzyme activity parallels the intake of creep feed. These researchers also indicated that pancreatic adaptation may occur with the intake of creep feed, but that the humoral signals in the milk, i.e. decreasing prolactin, should not be ignored.

Because of the low activities of the intestinal enzymes in the weanling pig, the supplementation with creep feed or the starter diet with these intestinal enzymes seems an attractive way to improve weanling pig performance. Ivy and co-workers (1936) suggested that adding amylases to a starter diet may increase digestibility. However, Cunningham and Brisson (1957) added amylases to the diet and observed no increases in starch digestibility. Few studies have reported beneficial responses from the addition of enzymes to weanling diets.

However, other feed additives may have a positive effect on intestinal enzymes. The increase in growth performance observed in pigs fed copper sulphate may be due partially to the ability of Cu^{2+} to stabilize pepsin which would increase protein digestion (Kirchgessner et al., 1976). Also, adding acid to the diet may stimulate the secretion of hydrochloric acid and(or) increase the activation of pepsinogen to pepsin.

Microflora

The gastrointestinal tract of the newborn pig is basically sterile and receives enteric bacteria from the surrounding environment (udder, cage, diet and feces) (Ducluzeau, 1985). The composition of this bacterial population is important in relation to the health status of the pig. If the pig is exposed to primarily pathogenic organisms, the pig will probably succumb to these bacteria and be a 'poor-doer' and(or) die. However, if proper sanitation has been implemented, a non-pathogenic bacterial population should populate the intestines of the pig.

The microbial population of the gastrointestinal tract is a complex ecosystem about which little is understood. Research in this area is now beginning to shed light on the interactions and interdependence of bacteria and host (Byrant, 1974; March, 1979). Early attempts to characterize the microflora of the gut utilized techniques that could only isolate facultative anaerobes, i.e. oxygen tolerant, and therefore defined this ecosystem accordingly (Fuller et al., 1960). However with the advent of anaerobic techniques, the microflora of the gut was discovered to be predominately obligate anaerobes and that facultative anaerobes were only a minor population (Salanito et al., 1977).

The isolation and characterization of the bacteria in order to define the normal bacterial population is an area

of much research. Moore et al. (1987) characterized the fecal microflora of finishing swine and observed the following microbial population: streptococcus (27.5%), lactobacillus (12.7%), fusobacterium (10.7%), eubacteria (8.6%), bacteriodes (3.6%), clostridia (2.6%) and E. coli (1.7%). Robinson et al. (1981) found the following microbial population in the cecum of adult swine: bacteriodes (42.5%), selenomonas (20.8%) and lactobacillus (10.1%). In addition, Robinson et al. (1981) noticed that the colon and cecal bacterial populations differed in that the cecal bacteria were strict anaerobes. Russel (1979) isolated anaerobic bacteria from the large intestine of pigs and found predominately streptococcus, lactobacillus, eubacteria, bacteriodes and clostridia. Salanito (1977) isolated streptococcus, eubacteria, clostridia and propionbacteria. These results indicate that there is variation in the microbial population of the lower gastrointestinal tract in adult swine and that the most predominate bacterial species isolated are streptococcus, lactobacillus, bacteriodes and clostridia.

The microflora population of the upper gastrointestinal tract has mainly been described in terms of oxygen tolerant species, lactobacillus, streptococcus and E. coli. Streptococcus, and especially lactobacillus, are considered 'beneficial' bacteria due to their production of lactic acid

and other beneficial metabolites as well as their non-pathogenicity, whereas *E. coli* and other coliforms are considered 'harmful' bacteria due their production of enterotoxin and pathogenicity (Schaedler et al., 1965).

Streptococcus, *Lactobacillus* and *E. coli* must be able to adhere to the mucosal wall of the intestine in order to be established in the gastrointestinal tract (Dubos et al., 1965; Svendsen et al., 1977). If adherence is not accomplished, or is prevented, the bacteria can be removed from the gut by peristaltic contractions. Bacteria accomplish adherence via special projections from their cell membrane, called pili, that have affinity for specialized receptors on the intestinal lining (Nagy et al., 1976; Moon et al., 1979).

McAllister et al. (1977) examined the microflora of the upper gastrointestinal tract in young pigs and reported that it consisted mainly of *Streptococcus*, *Lactobacillus*, *E. coli*, clostridia-like bacteria and strict anaerobes. After weaning, the total number of bacteria decreased, however, if the pigs exhibited signs of postweaning scouring, the number of *Streptococcus* and hemolytic *E. coli* increased. Smith and Jones (1963) reported approximately the same microbial composition as McAllister (1977) and also observed a decrease in the microflora population after weaning. Smith and Jones (1963) also observed the microflora of scouring

pigs and reported an increase in *E. coli* and streptococcus and a decrease in lactobacillus numbers. To the contrary, Barrow et al. (1977) failed to observe a decrease in lactobacilli and an increase in *E. coli* and streptococcus in scouring weaned pigs, but did notice more lactobacillus and streptococcus in suckling pigs than weaned pigs. Generally, weaning causes a decrease in the number of bacteria in the gastrointestinal tract, especially streptococcus, *E. coli* and lactobacillus, but the disease state of scouring results in proliferation of *E. coli* and streptococcus.

Many anaerobic techniques have been used to study the intestinal bacteria, however the best method as reported by Vervaeke et al. (1972) is the roll tube method. These researchers compared roll tubes, ring plates and petri dishes in anaerobic jars.

Intestinal bacteria fall into three distinct groups: fermentative, hydrogen forming (acetogenic) and methanogenic (Smith and Bryant, 1979). The majority of intestinal bacteria are in the rumen, cecum and large intestine. These bacteria are capable of partial digestion of organic material to carbon dioxide, methane, hydrogen and VFA (Smith and Bryant, 1979). These bacteria accomplish digestion via the production of intra- and extra- cellular enzymes. Rumen bacteria release extracellular enzymes (Wolin, 1974) which

could release nutrients from feedstuffs that benefit the bacteria population and possibly the host. The colon in the non-ruminant has a similar function (Wolin, 1979) and, therefore, the colon bacteria, as well as the rest of the enteric microflora, may be of similar benefit to the host. One enzyme that is beneficial to the host animal is urease, which is released by *Lactobacillus* in the stomach (pH 4.0) and by *Bacteriodes multocodus* in the small intestine (pH 8.0) (Suzuki et al., 1979). This enzyme enables the ruminant, and possibly the non-ruminant, to utilize nitrogen and ammonium more efficiently.

Total fecal bacteria counts have been reported by Salanitio and co-workers (1977) to range from $4.48 - 7.40 \times 10^{10}$. They also reported fecal VFA to be 48 - 59% acetate: 10 - 20% propionate: 4 - 13% butyrate, and fecal pH to be 6.8. They hypothesized that several factors could influence the intestinal microflora: media and methods, sampling site, diet and nutritional state, environmental conditions, sex and age of the animal, and the individual variation of the animal. Russell (1979) reported fecal bacteria numbers to be 13.3×10^{10} (dry weight) with 56.2% recovery compared with direct microscopic clump counts.

Moore and co-workers (1987) fed three diets to finishing hogs: control, high-fiber and high-oil, and observed no changes in the fecal microflora due to the

different diets. Etheridge et al. (1984a) also observed no changes in fecal bacteria counts due to dietary treatments (corn-soybean meal diet; groats-casein diet or suckling pigs). Varel (1987) observed no change in the total number of colonic bacteria in pigs fed a control or high fiber diet (1×10^{11} wet tissue). Therefore, this data suggests that the intestinal bacteria population is stable and resists changes due to diet alterations.

Gastric lactobacillus $\log_{10}$ counts 7.20 - 8.01, coliforms 5.05 - 6.88 and pH 4.21 - 5.10 were reported by White et al. (1969) for weanling pigs. Fecal lactobacillus $\log_{10}$ counts were reported to be 9.7 - 9.9 and E. coli 6.2 - 6.4 and that lactobacillus inhibited the growth of E. coli (Danielson et al., 1989). Fecal $\log_{10}$ counts of E. coli and anaerobes in rats were 6.75 and 10.42, respectively (Pond et al., 1989).

Escherichia coli

E. coli are responsible for common enteric problems observed in pigs, but require a predisposing factor before producing a disease state (Sojka et al., 1960). Some common predisposing factors are weaning, dietary changes or a previous rotavirus infection (Lecce et al., 1983). In support, Chopra et al. (1964a) observed that E. coli can proliferate after the stress of weaning and Shimizer and Terashima (1982) observed an increase in E. coli after a change in diet. The disease state normally associated with E. coli, i.e. scouring, is not always associated with E. coli, but may associated with a "non-infective" diarrhea that can be attributed to the presence of rotavirus, over-eating or a dietary hypersensitivity (Smith and Jones, 1963). Stevens (1963a) described the events to an E. coli infection: ingestion of the organism, balance between organism and host immunity, changes in intestinal substrate, weaning, diet and(or) environment, E. coli proliferation and then scouring.

The adhesion of E. coli to the epithelium of the anterior portion of the small intestine is necessary for colonization (Nagy et al., 1976). E. coli can be prevented from adhering to the intestinal lining by prior infection by the K-88 positive E. coli (active immunity) or by ingestion of colostrum containing K-88 antibodies (Nagy et al., 1976).

E. coli is able to proliferate in an alkaline environment (Smith and Jones, 1963). Thomlinson and Lawrence (1981) delayed the multiplication of *E. coli* in pigs by lowering gastric pH by feeding bran fiber, lactic acid or a liquid feed.

Pigs vary in their ability to handle an *E. coli* challenge as well as their ability to handle dietary changes (Kenworthy and Allen, 1966). Weaned pigs (18 - 38 days of age) are more resistant to *E. coli* enterotoxin with increasing body weight (Stevens et al., 1972).

Oral challenge of suckling pigs with enterotoxigenic *E. coli* produced less adhesion as pigs aged from 4 to 5 weeks (Nagy et al., 1979). The antibodies in the colostrum of sows vaccinated against *E. coli* did not prevent the adhesion of this bacterium in nursing pigs, but properties of the milk itself were able to prevent adhesion, therefore vaccination does not provide adequate protection against an *E. coli* challenge (Nagy et al., 1979).

Pili

Much research has focused on the significance of the pili in the pathogenicity of *E. coli*. *E. coli* is an opportunistic organism that can proliferate rapidly and lead to a disease state, scours, when the proper conditions are present (Palmer and Hulland, 1965; Richards and Fraser,

1961). Two types of pili are normally associated with pathogenic *E. coli*, namely the K88⁺ and K99⁺ pili (Stevens, 1963a; Rutter et al., 1976; Nagy et al., 1979). These pili specifically bind to the enterocyte membrane which allows the bacterium to adhere and thereby colonize the gastrointestinal tract (Moon et al., 1979).

Piliated *E. coli* are a major cause of enteric disease in neonatal swine (Evans et al., 1986). *E. coli* are capable of adhering to the villous epithelium of the small intestine and normally colonize the anterior portion of the jejunum (Svendsen et al., 1977). Pathogenic *E. coli* were located along the villi from tip to base and were contiguous to the brush border, whereas non-pathogenic *E. coli* are located in the central lumen near the tips of the villi and were occasionally contiguous with the brush border (Bertschinger et al., 1972).

After colonizing the anterior portion of the small intestine, *E. coli* are able to release enterotoxins. These enterotoxins result in the loss of fluid into the gut and thereby cause diarrhea, dehydration and possibly death.

Enterotoxin

Enterotoxins increase the level of cAMP which changes the ion transport (chloride secretion and sodium absorption) and produces an accumulation of fluid in the lumen of the gut (Stevens et al., 1972; Kohler, 1972). This imbalance leads to an influx of fluid into the intestinal lumen, which exceeds the absorptive capacity of the large intestine and therefore causes scours (Hamilton and Roe, 1977).

Milk

Research has been directed into preventing the attachment of *E. coli* by 'colostrum management' or by blocking the intestinal attachment sites for *E. coli* with a beneficial organism (*Lactobacillus* and/or *Streptococcus*). The idea behind colostrum management is to expose the dam to the bacteria or virus and allow her immune system to make antibodies. These antibodies are then secreted in her 'first milk', or colostrum. The newborn pig is able to directly absorb these antibodies from the gastrointestinal tract into the systemic circulation. This ability of the neonate to absorb these antibodies lasts for about 48 h after birth.

Vaccinating the dam against *E. coli* and other pathogenic bacteria six weeks and then two weeks pre-farrowing has become a common management technique.

However, research has yet to conclusively prove this practice to be an effective measure to transmit passive immunity against enteric infections to piglets. Nagy et al. (1979) orogastrically vaccinated sows prefarrowing with a pathogenic strain of *E. coli* (0149:K91(B)K88ac(L)) and observed the adherence of *E. coli* in the gut lining of the litters. They reported that the antibodies present in colostrum were ineffective in preventing *E. coli* adhesion, but they did conclude that milk itself could prevent *E. coli* adhesion. Rutter et al. (1976) vaccinated sows with *E. coli* strain K88 and observed the same bacteriocidal and bacteriostatic activity in the milk from vaccinated and non-vaccinated sows.

Milk contains several other antibacterial factors beside passive antibodies found in colostrum (Stephens et al., 1979). One such factor is secretory IgA, which is an important intestinal antibody that bathes the intestinal lining and also binds antigenic material (Ogra, 1976). Secretory IgA binds to the receptors that *E. coli* can utilize and thereby prevents colonization (Porter et al., 1973).

Secretory IgA in the milk provides the suckling pig with constant protection against enteric infections; the pig begins to synthesize its own secretory IgA by 2 weeks of age (Porter et al., 1973). Constant ingestion of milk is

essential to afford the pig protection against enteric infections because milk loses its ability to prevent adhesion of *E. coli* after 4 hours at 37°C (Stephens et al., 1979). The prevention of antigenic material entering the systemic circulation is dependent partially on the integrity of the mucous membranes and it may also be due to the function of secretory IgA to complex with antigenic material and retain this complex in the gut lumen until it is digested (Stokes et al., 1975).

Volatile Fatty Acids

Bacteria in the gastrointestinal tract produce and utilize volatile fatty acids (VFA) (Wolin, 1974). Due to the large number of bacteria in the colon, and their metabolic activity, VFA are the major anions (sodium the major cation) in the lower gastrointestinal tract (Argenzio, 1981; Argenzio and Southworth, 1974). VFA are important to the nutrition, secretory and absorptive function of the large intestine (Stevens, 1978).

Studies in humans suggest that the short-chained VFA formed in the colon have a great capacity to be absorbed and metabolized such that acetate and butyrate enter the citric acid cycle as acetyl-CoA and propionate via other routes that result in a net synthesis of glucose (Hoverstad et al., 1982).

Recent evidence indicates that VFA from the colon of the pig, analogous to VFA in the rumen, are rapidly absorbed and utilized as an energy source (Argenzio and Southworth, 1978). Imoto and Namioka (1978) determined that VFA can supply about 10% of the energy requirement of the pig. Kass et al. (1980) also reported similar findings of 7 - 12%. Kennelly and co-workers (1981) reported considerable bacterial fermentation in the hindgut of swine that were cecal cannulated such that 9 - 23% of the energy requirements for maintenance were met by VFA absorption.

Besides augmenting sodium and water reabsorption and supplying the pig with energy, VFA also have a bacteriocidal effect. Prokaszka and Baron (1982) reported that VFA have antibacterial activity in the cecum and colon which is dependent on gut pH. They noticed that at a pH of 6.8 or greater, VFA lost their bacteriocidal effects. At a low pH, the proportion of non-dissociated VFA increases which allows absorption by bacteria due to their lipophilic nature. Once absorbed, the neutral pH of the cytosol results in the dissociation of the VFA which accounts for the bacteriocidal effect (Prohaszka and Baron, 1982). The VFA exert their effects mainly in the lower gastrointestinal tract due to a greater concentration of VFA; $190 - 210 \text{ mmol} \cdot \text{l}^{-1}$ lower gastrointestinal tract compared to $0 - 40 \text{ mmol} \cdot \text{l}^{-1}$ in the upper gastrointestinal tract in grower pigs (Stevens, 1978).

The pH of the colon is around 6.5 - 7.1 (Prohaszka and Baron, 1982). The pH of the intestinal tract of growing swine was reported to be: stomach 3.51 - 4.00; small intestine 6.12 - 6.43; cecum 5.8 - 6.05 and; colon 5.80 - 5.95 (Kass et al., 1980). Stevens (1978) reported the pH of the intestinal tract of mature swine to be: stomach 2.5 - 4.0; small intestine 5.8 - 6.2; cecum 6.2 and; colon 5.9 - 6.1. Argenzio and Southworth (1974) reported the pH of the intestinal tract to be: stomach 2 - 4; duodenum/jejunum 3.5 - 6.0; cecum 6.5; and colon 6.2. Farrell and Johnson (1970) reported the pH of the cecum to be 6.4 - 6.8. pH has a major effect on microbial metabolism such that the production of VFA is greater at pH 6.7 than at 5.7 in the rumen (Peters et al., 1989).

VFA are readily absorbed from the intestinal lumen such that 70% of acetate, 20% of propionate and 5% of butyrate were detected in the arterial/portal blood (Guisi-Perier et al., 1989). The liver has a large capacity to absorb these VFA: propionate, butyrate, isovalerate and valerate (Rerat et al., 1987). The epithelium of the large intestine metabolizes VFA suggesting that VFA may have a role in the function of the large intestine (Imoto and Namioka, 1978b).

VFA production in pigs is proportional to the intake of NDF (Stanogias and Pearce, 1985) and feed intake (Rerat et al., 1987). The cellulolytic activity of fecal bacteria in

gestating swine was increased by feeding increasing levels of fiber and suggests that modifications in the VFA profile indicate an altered bacterial metabolism (Varel and Pond, 1985).

pH

The pH of the gut is of importance to the proper digestion and utilization of feedstuffs. The low pH of the stomach is necessary for denaturing protein, activation of pepsinogen and killing of ingested bacteria. The gastric acidity is caused by the production of hydrochloric acid by the parietal cells. These cells are sensitive to various stimuli including the sight, smell and taste of food as well as the act of swallowing, chewing and stomach distention. The low gastric pH causes a cascade effect of activating other digestive enzymes.

The acidic nature of the stomach prevents bacteria from colonizing this organ. Any bacteria that may be contaminating the feed, or originating from the oral cavity, are normally killed or are damaged enough by the acid to prevent their growth and proliferation. Problems arise when the diet has a high buffering capacity, like soybean meal and(or) the stomach is unable to produce acid in sufficient amounts, as is the case with weanling pigs.

The small intestine is normally at a higher pH, but is still acidic, pH 5 to 6.5. This higher pH is necessary for

the proper function of the enzymes secreted in this region of the gastrointestinal tract. The neutralization of HCl from the stomach is achieved by the base, bicarbonate, which is produced by the pancreas in response to the hormone secretin. If insufficient acid is produced in the stomach, the pH of the lower gastrointestinal tract is more alkaline, which allows greater bacterial proliferation and poorer utilization of feedstuffs due to improper induction and activation of digestive enzymes.

In pigs weaned at 2 days of age, Barrow and co-workers (1977) observed a negative correlation between the concentration of lactate and intestinal pH and a positive correlation between pH and the number of *E. coli*. These observations suggest that increasing the level of lactate and(or) lowering the pH will result in a reduction of *E. coli*.

Lactic acid is the major contributor in lowering gastric pH at 4 - 5 days of age and hydrochloric acid is at 11 - 12 days of age. Cranwell et al. (1976) reported that lactic acid inhibits the secretion of HCl. Cranwell also reported the stomach pH 3 - 6.5, chloride concentration 20 - 130 mmol and lactic acid 0 - 170 mmol in 20 - 24 day old pigs (suckling). These researchers further suggested that the weanling pig is unable to secrete sufficient quantities of HCl due to inadequate development of secretory stimulatory

mechanisms, inhibition by organic acids and(or) alterations of the mucosa by the microflora.

The pH of cecum and colon contents of pigs fed lactose was 5.6 - 6.6 and 5.7 - 6.5, respectively (Kim et al., 1978). Walker (1959) reported the pH in the stomach of the young pig to be 1.5 - 5.6 and in the small intestine 5.3 - 7.0 and that there was no free HCl in the stomach. Wilson and Leibholz (1981) reported pH ranges in pigs given milk or soy proteins and observe little differences between diets: stomach - milk 4.08 and soy 4.10; jejunum - milk 5.73 and soy 6.10; cecum - milk 6.06 and soy 5.83; and colon - milk 5.88 and soy 6.55.

Morphology

The blunting of the villi in the small intestine results in less time for the enterocytes to mature and therefore poor enzyme secretion and nutrient absorption. Morphological changes in the intestinal villi height and crypt depth were associated with impairment of intestinal absorptive function (Hall and Parsons, 1983). Also the surface receptors of the enterocytes change as these cells migrate to the villous tip (Etzler, 1979). This change in the surface receptors along with the reduced villous height may allow *E. coli* to attach to the intestinal lining more efficiently.

The villous height to crypt depth ratio is depressed at 7 - 8 days postweaning which was related to a dietary change (Kenworthy, 1976). The reduction in the small intestinal absorptive area and the appearance of a less mature enterocyte population may partially explain the postweaning growth check and scouring (Hampson, 1986b). The villous height to crypt depth ratio was lowest at day 5 postweaning with recovery by day 11 postweaning and the changes in this ratio were not related to creep-feed consumption (Hampson, 1986b). The feeding of a hydrolyzed casein diet resulted in less change in the villous height to crypt depth ratio than non-hydrolyzed casein diet which was hypothesized to be due to better digestibility and lower diet antigenicity (Hampson, 1986c). Weaning caused the reduction of villous height and an increase in crypt depth and therefore an older animal is more capable of handling the stress of weaning due to the larger intestinal absorptive area compared with a younger animal (Miller et al., 1986).

Villous height was decreased by feeding soybean meal and was decreased from day 3 to day 12 postweaning (Dunsford et al., 1989). This blunting of the villi would result in less mature enterocytes and therefore decrease digestive capacity. Dunsford et al. (1989) also stated that feeding corn may lessen the detrimental effects of soybean meal.

Rotavirus

Rotavirus damages the epithelium of the small intestine which changes the intestinal environment to one that favors the colonization of *E. coli* by interfering with non-specific intestinal protectors or by altering the binding sites for *E. coli* to adhere (Lecce et al., 1982) and it may damage the intestinal tissue which allows *E. coli* to colonize (Lecce et al., 1983). A rotavirus infection causes a reduction in intestinal absorption due to an increase in stomach retention and a decrease in intestinal transit time, i.e. increase in gut motility. This was determined indirectly via the D-xylose absorption test by Woode et al. (1978) in rotavirus infected calves.

Pigs are more susceptible to an *E. coli* infection if they were previously infected with rotavirus (Tzipori et al., 1980). Tzipori and co-workers (1980) reported that weanling pigs have a period of susceptibility to *E. coli* induced scouring around 4 days postweaning which lasted 3 to 4 days. Also, they reported antibody titers to rotavirus were lowest at 3 - 5 weeks of age in pigs weaned at 4 week of age. Lecce et al. (1982) observed scouring in weanling pigs previously infected with rotavirus occurred within 3 - 5 days postweaning (Lecce et al., 1982).

Scouring

Etheridge and co-workers (1984b) fed three diets: corn-soybean meal, rolled oat groats-casein or suckling, to weanling pigs and observed a lower fecal pH in scouring pigs which was related to a greater bacterial fermentation which caused an increase in fecal osmolarity resulting in impaired water reabsorption in the colon. Therefore, they believe that the mineral concentration of the starter diet is an important consideration when trying to alleviate the postweaning scour syndrome because excess minerals will add to the increase in fecal osmolarity.

Diet regime can influence the enteroenvironment and therefore the severity of scours (Lecce et al., 1983). Lecce and co-workers (1983) fed a high solid diet 3 times a day or hourly and observed scouring, but the severity was less in the pigs fed hourly. Additionally, pigs were fed the same high solid diet that was diluted six fold 3 times per day and hourly. Scouring was reduced with the hourly treatment resulting in the least amount of scouring compared to the other three treatments.

Richard and Fraser (1961) prevented scouring in weanling pigs by a gradual change from a low nutrient dense diet to a high nutrient dense diet. Palmer and Hulland (1965) also reported that the incidence of postweaning scouring could be reduced by restricting feed intake after

weaning. Scouring is related to the high nutrient intake the first week after weaning and that abruptly weaned pigs did not scour due to their reduced feed intake postweaning (Hampson, 1986a).

Immune Response

The antibody IgA is able to limit intestinal uptake of macromolecules and other non-replicating antigens from the intestinal lumen and therefore prevent direct or indirect immunologically mediated tissue damage (Ogra, 1976). This is important because ingested antigens can result in a local and systemic immune response (Bellamy and Nielsen, 1974). This antibody may be able to prevent the uptake of the lipopolysaccharide membrane of *E. coli* and prevent the immune response by forming an antibody-antigen complex that prevents the absorption of this antigen (Andre et al., 1974). Weanling pigs can become hypersensitive to the lipopolysaccharide membrane by absorbing small amounts of this compound which can result in a localized immune response, edema and hemorrhagic gastro-enteritis (Buxton and Thomlinson, 1961; Thomlinson, 1963).

The immune response becomes tolerant to antigens that are constantly presented, however before tolerance is achieved, a transitory delayed hypersensitivity is observed (Stokes et al., 1983). At weaning the pig has the greatest

susceptibility to the adverse effects of cell-mediated and humoral immune responses, and if the pig does not readily develop tolerance to dietary antigens, profound and long term effects on the intestinal tract may occur due to this hypersensitivity (Stokes et al., 1983). Miller and co-workers (1984a) suggested that a misdirected immune response may be the underlying cause of the postweaning lag and that the consumption of 400 grams of creep-feed is needed to prevent this dietary immune response.

Miller and co-workers (1984b) postulated that a transient dietary hypersensitivity is responsible for the postweaning scouring. These researchers suggested that the ingestion of sufficient quantities of creep-feed would prevent the postweaning scouring caused by this immune response because the pig would be 'tolerized' to the dietary antigens and would not illicit an immune response that would result in intestinal damage. Newby and co-workers (1983) observed slight scouring in abruptly weaned pigs, no scouring in creep-fed pigs but severe scouring in pigs given little creep-feed (primed), thereby giving support to the hypersensitivity theory. However, Risley et al. (1988) and Barnett et al. (1989) were unable to substantiate this dietary hypersensitivity theory. Risley et al. (1988) observed a decrease in xylose and an increase in ovalbumin absorption the first week postweaning indicating intestinal

damage, but no differences in absorption between creep fed or non-creep fed pigs. Barnett et al. (1989) was unable to detect differences in the systemic production of antibodies to a dietary antigen (ovalbumin) between creep fed and non-creep fed pigs.

A delayed hypersensitivity in the intestine to dietary and bacterial antigens may cause intestinal damage (Newby et al., 1980). At a high intestinal pH, intestinal administration of antigens may result in a systemic cellular immune reaction (Periotto et al., 1974). Sensitivity to a food antigen results in a hypersensitivity reaction that causes intestinal inflammation and increases in the absorption of other dietary antigens which is aggravated by a deficiency in IgA (Thomas et al., 1973). Each of these factors, high intestinal pH, dietary hypersensitivity and a deficiency in IgA, can occur in the early weaned pig and thereby cause the postweaning lag.

A localized cell-mediated immune reaction in the intestine may cause villous atrophy and crypt hyperplasia which would result in malabsorption (MacDonald and Ferguson, 1976). This reaction is observed when pigs become hypersensitive to *E. coli*, which causes a local intestinal inflammation that causes alterations in the mucosal barrier, and increases in the uptake of macromolecules from the intestinal lumen (Walker and Isselbacher, 1974). This

immune response results in a localized inflammation and damage to the intestinal lining (Thomlinson and Buxton, 1962). The absorption of macromolecules across the intestinal lining is nutritionally insignificant but are important immunologically (Swarbrick et al., 1979).

Kilshaw and Slade (1980) reported that in the preruminant calf, the absorption of abnormally large quantities of food antigens and(or) bacterial products during a hypersensitivity reaction in the gut might perpetuate the hypersensitivity reaction and initiate secondary pathogenic reactions. Kilshaw and Sissons (1979) observed that the soya proteins glycinin and β -conglycinin were associated with the development of digestive disturbances and the production of serum antibodies in the preruminant calf. They suggested that the suitability of soyabean products for nutrition could be assessed by immunological methods.

Summary

The weanling pig is faced with many challenges at the time of weaning, which include a new environment, a different diet and the mixing with other pigs. These changes usually result in a postweaning lag in growth and scouring. The traditional practice of feeding antibiotics to weanling pigs to alleviate this lag phase will probably be prohibited or

restricted in the near future. Therefore, a need exists to discover alternatives to the feeding of antibiotic. One possible alternative is the incorporation of organic acids into the weanling diet.

Organic acids seem to have the potential to replace antibiotics in starter diets because they have been reported to improve the growth performance of weanling pigs. This growth improvement is believed to be related to the ability of organic acids to reduce the intestinal pH and therefore prevent the proliferation of enteric coliforms. However, this hypothesis has not been substantiated by the literature. Before the full growth-improving potential of organic acids can be realized, a better understanding of the mode of action of organic acids is necessary.

The causes of postweaning lag in pigs present a complex an interrelated problem that faces the swine industry today. No one management practice will be able to allievate this problem. However, with better understanding of the changes that occur in the weanling pig, a group of practices, which includes the feeding of organic acids, may be developed that will overcome the postweaning lag.

CHAPTER III

OBJECTIVES

The purpose of this research project was to investigate the influence of feeding organic acids on the intestinal pH, chloride ion concentration, volatile fatty acid profile and microflora population in weanling pigs. These measurements were chosen because of their hypothesized relationship to a possible mechanism of action for organic acids. The specific objectives for each of the studies were the following:

Study 1 - Growth Performance

a) to investigate the improvement in growth performance (ADG, ADFI and F:G) in weanling pigs fed diets supplemented with 1.5% fumaric or citric acid.

b) to investigate the effects of these diets on intestinal pH, chloride ion concentration and VFA profile.

Study 2 - Microbial/Organic Acid

a) to investigate the growth promoting effects of starter diets containing 1.5% fumaric or citric acid with and without a microbial product (*Lactobacillus acidophilus*).

Study 3 - Serial Slaughter

a) to investigate the intestinal microflora population (total anaerobes, lactobacillus, clostridia and *E. coli*) as influenced by the day of sampling (-2, 0, 3, 7, 14 and 21 days postweaning) and(or) by the feeding of the organic acids, fumaric or citric to weanling pigs.

b) to investigate the intestinal pH, chloride ion concentration and VFA profile as influenced by the day of sampling (-2, 0, 3, 7, 14 and 21 days postweaning) and(or) by the feeding of the organic acids, fumaric or citric to weanling pigs.

Study 4 - *Escherichia coli* Challenge

a) to investigate the effect of feeding weanling pigs a diet containing 1.5% fumaric or citric acid on intestinal pH, chloride ion concentration, VFA profile and the incidence of scouring when orally challenged with *E. coli*.

b) to investigate the effect of feeding a diet containing 1.5% fumaric or citric acid on the intestinal lactobacillus and *E. coli* population in weanling pigs orally challenged with *E. coli*.

Study 5 - *Escherichia coli* Challenge Growth Study

a) to investigate if feeding a diet containing fumaric or citric acid can prevent the decrease in growth performance in weanling pigs challenged with *E. coli*.

CHAPTER IV

GROWTH PROMOTING EFFECTS OF ORGANIC ACIDS WITH AND WITHOUT A MICROBIAL FED TO WEANLING PIGS

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Abstract

The effect of dietary fumaric and citric acid on avg daily gain (ADG), avg daily feed intake (ADFI) and efficiency of feed utilization (F:G), gastrointestinal pH, concentration of chloride ion (Cl^-) and VFA of 25-d-old crossbred weanling pigs was evaluated in one study ($n = 144$) and the growth promoting effect of fumaric and citric acid in combination with a microbial, Lactobacillus acidophilus, was evaluated in a second study ($n = 192$). A 20% CP corn-soybean meal basal diet was used. In study 1, pigs fed 1.5% citric acid had improved ADG and F:G during the first 4-wk with no effect on ADG during wk 5; 1.5% fumaric acid only numerically improved F:G. ADFI was unaffected by organic acids. The pH, Cl^- and VFA concentration of intestinal contents from the stomach, jejunum, cecum and lower colon of 27 pigs killed at the end of study 1 were not measurably affected ($P < .70$) by dietary organic acids. With the exception of fumaric acid, the concentration of non-volatile acids was not affected by diet. The stomach and jejunum

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for pigs fed fumaric acid had a greater concentration ($P < .05$) of fumaric acid compared with the control and citric acid diets. In study 2, 1.5% fumaric acid supplementation increased ($P < .03$) ADG and microbial addition improved ADG ($P < .08$) and F:G ($P < .02$) during the 5-wk test; 1.5% citric acid had no effect. In conclusion, the addition of citric or fumaric acid and(or) a microbial produced variable improvements in ADG and F:G 4 and 5 wk postweaning with little effect on intestinal digesta measurements.

Introduction

Early weaning exposes pigs to stresses (environmental, dietary and social) that usually result in a postweaning lag phase characterized by poor growth rate, scouring and general unthriftiness (Tzipori et al., 1980; Barnett et al., 1989). The digestive tract of the pig is not fully developed until around five wk of age and the ability to produce sufficient quantities of HCl to lower gastric pH is limited (Manners, 1976). Improvements in postweaning growth and efficiency of feed utilization have been achieved by supplementing starter diets with fumaric or citric acids (Kirchgessner and Roth, 1982; Falkowski and Aherne, 1984; Giesting and Easter, 1985) or with microbials, such as Lactobacillus acidophilus (Hale and Newton, 1979; Lessard and Brisson, 1987).

One proposed mechanism of action for organic acid

supplementation is the reduction of the gastrointestinal tract pH, which may aid in protein digestion and shift microbial activity in the gastrointestinal tract (Scipioni et al., 1978; Radecki et al., 1988). Microbials may similarly decrease the pH of the gut by producing lactic acid and control other bacterial activity by the production of antibiotic substances (Lyons, 1987).

The purpose of these studies was to further evaluate the effects of supplementing starter diets with organic acids alone and in combination with a microbial, and to determine the effects of organic acids on gastrointestinal pH, chloride ion, and VFA.

Experimental Procedures

Growth Studies

Study 1. Two 5-wk trials were conducted to determine the effect of organic acid supplementation on the growth performance of early weaned pigs. A total of 144 crossbred pigs (mean initial age and wt of 25 d and 6.3 kg, respectively) was assigned to blocks by weight and gender and then randomly allotted within blocks to three dietary treatments with the restriction that littermates be balanced across treatments. Dietary treatments consisted of a 20% CP corn-soybean meal based diet formulated to meet or exceed the nutrient guidelines of weanling pigs (NRC, 1988) with 1) no supplementation (control), 2) 1.5% fumaric acid added or

3) 1.5% citric acid added (Table 1). Pigs (three per pen) were housed in temperature controlled nurseries with wire bottom pens (.6 m x .9 m). Temperature and ventilation rates were maintained at recommended levels (Hinkle et al., 1978; Lubinus and Murphy, 1979). Water and feed were available ad libitum. At weekly intervals, pigs were weighed and pen feed consumption was recorded. Average daily gain (ADG), avg daily feed intake (ADFI) and efficiency of feed utilization (F:G) were calculated.

Study 2. Two 5-wk trials were conducted to evaluate the main and interactive effects of supplemental organic acids (fumaric and citric) and a microbial³ on the growth performance of weanling pigs. A total of 192 pigs (mean initial age and wt of 25 d and 7.0 kg, respectively) was assigned to blocks by weight and gender and randomly allotted within blocks to a 2 x 3 arrangement of treatments with the restriction that littermates be balanced across treatments. Treatments consisted of two levels of microbial (with and without) and three types of organic acid supplementation (none, 1.5% fumaric acid or 1.5% citric acid). Pigs (four per pen) were housed in temperature controlled nurseries with plastic-coated wire bottom pens (.9 m x 1.2 m). As in study 1, temperature and ventilation

³Lactobacillus acidophilus, strain # BT 1386 supplied by Bio Techniques Laboratories, Inc., Redmond, WA. Added at a level to supply 5×10^6 organisms per pig daily.

rates were maintained at recommended levels. To prevent possible cross-contamination between microbial and non-microbial fed pigs, the pigs fed the microbial were housed in one nursery and non-microbial fed pigs were housed in a separate, similar nursery. Nursery and microbial treatment was alternated in the second trial; the uniformity of performance in the two nurseries had been previously demonstrated (Gore et al., 1986). Feed and water were available ad libitum. Pigs and feeders were weighed weekly and feed consumption calculated.

Small vials containing the correct number of freeze-dried colony-forming units for a two-day feeding for each treatment were prepared and kept frozen until the day of feeding. The freeze-dried microbial was rehydrated in water and mixed with the appropriate amount of each diet every other day to ensure uniform pig inoculation. At the end of each two-day interval, the residual feed was weighed and discarded. The amount of feed used at each mixing was estimated to be slightly greater than the amount of feed that the pigs would eat based on the previous two day's intake and the increased needs of the pigs.

Gastrointestinal Measurements.

At the conclusion of study 1, 27 pigs (9 from trial 1, 18 from trial 2) were randomly selected across all pens and treatments to evaluate the pH and concentration of chloride

ion (Cl^-) and VFA in the stomach, jejunum, cecum and lower colon. Pigs, in a full-fed state, were killed at approximately 0900 via electrocution followed by exsanguination. The viscera were exposed via a midline incision and the following sections isolated, doubly ligated and removed: entire stomach, 1 m section of jejunum, entire cecum and .3 m section of lower colon (near rectum). The digesta were collected, and within 30 min, pH^4 and chloride ion⁵ concentration were determined by using 2 g of digesta, which were diluted in 18 ml of deionized water and suspended via a magnetic stir bar and electromagnetic mixer. The pH and Cl^- concentration were measured using the appropriate electrodes and standards. Additionally, 1 g of digesta was acidified with 50% (v/v) H_2SO_4 for later determination of VFA concentration according to the procedures of Moore (1977).

Diet pH was determined in triplicate by weighing 20 g of diet into a 250 ml beaker and adding 100 ml of deionized water. This mixture was continuously mixed with a stir bar and electromagnetic mixer while the pH was determined via an electronic pH meter⁶ (Radecki et al., 1988).

Statistical Analysis. Performance data were analyzed

⁴Fisher Scientific Accu-pHast combination electrode # 13-620-281.

⁵Orion Research Ionalyzer chloride electrode # 94-17-B and double junction reference electrode # 90-02.

⁶Fisher Scientific Accumet pH meter # 620.

by using the general linear model program (GLM) of the Statistical Analysis System (SAS, 1985) using pen means as the experimental unit. Gastrointestinal measurements were analyzed by GLM, but by using the individual pig as the experimental unit. Data from study 2 were analyzed as a 2 x 3 arrangement of treatments. For study 1, the model contained the main effects of diet, replicate within trial, trial and the appropriate interactions. For study 2, the model contained the main effects of acid, microbial, replicate within trial, trial and the appropriate interactions. Non-significant interactions were removed from the models based on results of preliminary analysis. Comparisons between dietary treatments or intestinal sections were made using non-orthogonal contrasts or Tukey's studentized range test, respectively.

Results

Growth Studies

Study 1. During the first 4 wk postweaning, feeding citric acid increased ($P < .09$) ADG compared with the control-fed pigs, whereas no effect ($P < .45$) was observed for feeding fumaric acid (Table 2). During wk 5, however ADG were similar for pigs fed citric or fumaric acids compared with the controls. The overall ADG were not different ($P < .34$) among control-fed pigs and those fed diets with added citric or fumaric acid. Dietary treatments did not affect ADFI ($P < .40$) during any period of the study.

The addition of citric acid improved F:G ($P < .05$) compared with the controls during the first 4 wk. Also numerical means, although not significant ($P < .30$), favored pigs fed the citric acid diet during wk 5. Thus, a resulting overall trend ($P < .09$) for improved F:G was observed for pigs fed diets with added citric acid. Numerically, F:G for pigs fed fumaric acid were similar to those obtained for pigs fed citric acid, but were not statistically different ($P < .11$) from F:G for control-fed pigs.

Two pigs in trial 1 were removed due to poor health which was believed to be unrelated to treatments; one pig was fed the citric acid diet and the other was fed the

fumaric acid diet.

Study 2. There were no significant interactive effect ($P < .75$) of organic acids and microbial; thus main effects will be discussed. Contrary to results of study 1, the addition of organic acids had no effect on ADG during the first 4 wk (Table 3); ADG was increased ($P < .07$) during wk 5 for pigs fed fumaric acid (diets 2 and 5) compared with the controls (diets 1 and 4). A resulting overall improvement ($P < .03$) in ADG was observed for pigs fed fumaric acid diets. Again, in contrast to results of study 1, pigs fed citric acid had ADG during all periods which were similar ($P < .70$) to those of control-fed pigs. Therefore, the overall acid effect obtained ($P < .03$) was due to fumaric acid. ADFI and F:G means were not influenced ($P < .76$) by either fumaric or citric acid during any of the periods.

The addition of the microbial did not improve ADG during the first 4 wk, but ADG was greater ($P < .02$) during wk 5 and the resulting overall ADG was larger ($P < .08$) for microbial-fed pigs compared with control-fed pigs. ADFI was not influenced by feeding the microbial during any of the periods. The addition of the microbial did not affect F:G during the first 4 wk, but F:G was reduced during wk 5 ($P < .01$) and overall ($P < .02$) for microbial-fed pigs compared with control-fed pigs.

Gastrointestinal Measurements (Study 1 only).

The pH of the organic acid diets was lower ($P < .001$) than that of the control diet (Table 2). At the end of the 5-wk study, however the organic acid diets did not substantially reduce the pH in either the gastric digesta or in the digesta of the other intestinal sections measured ($P < .30$; Table 4). The pH of the stomach digesta was lowest followed by cecum and lower colon digesta pH; the jejunum digesta had the highest pH ($P < .05$). The Cl^- concentration of digesta from the various intestinal sections also was not affected by dietary treatments ($P < .24$), but Cl^- concentrations steadily decreased ($P < .05$) from a high in the stomach to a low in the lower colon (Table 4).

No differences ($P < .70$) in the VFA concentration were observed among dietary treatments. Acetic acid was the primary VFA detected in the gastrointestinal tract, but the cecum and lower colon exhibited increased ($P < .05$) levels of propionic acid, butyric acid and total VFA concentrations compared with the stomach and jejunum (Table 5).

Non-volatile acid concentrations were generally low throughout the gastrointestinal tract, excluding fumaric acid in the fumaric acid fed pigs. Fumaric acid concentration, was greater ($P < .05$) in the stomach and jejunum of fumaric acid fed pigs and hence, total non-volatile acid concentration ($P < .05$) in the stomach and

jejunum in pigs fed the fumaric acid diet than that of pigs fed either of the diets without fumaric acid.

Discussion

The variable response of the organic acids in improving ADG and F:G in weanling pigs is consistent with previous research. Radecki and co-workers (1988) observed a positive response on ADG and F:G by feeding fumaric acid but not with citric acid, whereas Henry et al. (1985) reported increases in ADG in pigs fed citric acid but not in pigs fed fumaric acid. Falkowski and Aherne (1984) and Giesting and Easter (1985) reported a numerical increase ($P < .07$) in ADG and an improvement ($P < .05$) in F:G with the addition of 2% fumaric or citric acid. Kirchgessner and Roth (1982) reported that the addition of 1 to 2% fumaric acid improved ADG, F:G and stimulated ADFI. This increase in ADFI, however, is inconsistent with our results and the findings of Falkowski and Aherne (1984) and Giesting and Easter (1985), who observed a non-significant depression in ADFI.

In our first study, improvements in ADG and F:G were observed primarily during the first 4 wk of each trial. But in our second study, improvements in ADG were also observed during wk 5 for pigs fed fumaric acid. The duration of the experiments reported by Falkowski and Aherne (1984), Giesting and Easter (1985), Burnell et al. (1988) and Radecki et al. (1988) was 4 wk.

Few reports have extended their experiments past 4 wk. Our results from study 1 suggest most of the response to organic acids occurs during the first 4 wk postweaning. In agreement, Scipioni et al. (1978) reported significant increases in ADG by feeding 1% citric acid for the first 3 wk, but this increase in ADG was lost during wk 4-6 of the experiment. This transitory response to organic acid supplementation may be related to the increasing maturity of the gastrointestinal tract of the weanling pig, which may overcome any possible buffering effects of the organic acid.

We do not have an explanation for the lack of consistency in the response to citric acid and fumaric acid from study to study. In study 1, citric acid gave the best response, whereas in study 2, fumaric acid gave the best response.

The use of a microbial in improving postweaning performance in pigs (Hale and Newton, 1979; Lessard and Brisson, 1987) has had less consistent results than that of diet acidification. However, our results indicate that the addition of a microbial improves F:G and tends to improve ADG. As reported by Lyons (1987), the success of a microbial is dependent upon the gastrointestinal environment. Generally, microbial organisms (*Streptococcus* and *Lactobacillus*) prefer an acidic environment, whereas coliforms (*E. coli*) thrive in a more alkaline environment.

This observation indicates that using a microbial in diets for the young pig whose ability to lower gastric pH is limited (Manners, 1976) may not result in an optimal microbial response. If an acidic gastrointestinal environment is not maintained, the microbial organism may not be able to establish itself in the gut and thus coliforms may be allowed to proliferate and cause scouring and poor growth performance. The combination of a microbial with an organic acid should result in a more favorable acidic environment and result in better growth performance in weanling pigs. In study 2, however we were unable to detect any interactive effects between organic acid supplementation and microbial addition, suggesting that the 1.5% level of organic supplementation is unable to substantially improve the effectiveness of the microbial.

Lowering the gastric pH has also been suggested as a possible mode of action of diet acidification (Scipioni et al., 1978; Burnell et al., 1988; Radecki et al., 1988). The newly weaned pig may be unable to substantially reduce gastric pH due to limited HCl secretion and therefore may be unable to adequately activate pepsinogen to pepsin. Low pepsin activation may lead to poor diet utilization and undigested feed particles may reach the lower gastrointestinal tract, which results in a higher pH and more rapid rate of passage (Kidder and Manners, 1978). The

addition of an organic acid to a starter diet might aid the pig in activating pepsinogen and stimulating HCl secretion.

Our results, however, suggest that the inclusion of organic acids in the starter diet does not substantially lower the gastrointestinal pH at the end of a 5-wk period. Burnell et al. (1988) also observed little or no decrease in stomach, small intestinal and large intestinal pH by feeding 1.0% citric acid to weanling pigs for 7 or 21 days. These data suggest that reducing gastric pH may not be a major mode of action of acidified diets. Additionally, the level of Cl^- was expected to reflect the production of hydrochloric acid, but the Cl^- concentration of the digesta more probably indicated the level of chloride in the diet.

The decrease in fumaric acid concentration from the stomach to the jejunum suggests that this acid may be directly absorbed from the stomach and thus serve as an additional energy source. At weaning, the pig utilizes its already low lipid stores for energy, which adds to the poor postweaning weight gain and stress (Fenten et al., 1985). Kirchgessner and Roth (1982) suggested that fumaric acid could be used as an energy source with an efficiency close to that of glucose. Therefore, organic acids may act as an energy source, which could assist the weanling pig in maintaining its lipid stores. More research is required to substantiate this hypothesis however.

Scipioni et al. (1978) reported that citric acid, but not fumaric acid, tended to reduce gastric and duodenal pH and lowered total E. coli and anaerobic microflora. Our results, however, indicate that organic acids had little or no effect on the intestinal microflora metabolism as measured by the VFA concentrations (Varel and Pond, 1985), therefore, suggesting little change in the microflora population.

Conclusions

The supplementation of a starter diet with organic acids and(or) a microbial generally improved F:G and tended to increase ADG of weanling pigs, although the magnitude of response to citric or fumaric acid was not consistent from study to study. However, our results do not support the hypothesis that organic acids can lower the gastrointestinal pH or alter bacterial activity as measured by VFA concentrations at the end of the 5-wk trials. Further investigation into the mode of action and their interactive effects of these feed additives is warranted.

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TABLE 1. PERCENTAGE COMPOSITION OF DIETS

Item	Diet ^a		
	Control	Fumaric acid	Citric acid
Corn	64.20	62.50	62.50
Soybean meal (44% CP)	33.11	33.31	33.31
Limestone	.60	.58	.58
Defluorinated phosphate	1.42	1.44	1.44
Salt	.30	.30	.30
Trace mineral premix ^b	.07	.07	.07
Vitamin premix ^c	.25	.25	.25
Selenium premix ^d	.05	.05	.05
Fumaric/citric acid	—	1.50	1.50

^aCalculated to supply 20% crude protein, .80% Ca and .65% P.

^bSupplied per kg of diet: 105 mg Zn, 123 mg Fe, 42 mg Mn, 12 mg Cu, and 1.4 mg I.

^cSupplied per kg of diet: 4,400 IU vitamin A, 440 IU vitamin D, 11 IU vitamin E, 4.4 mg riboflavin, 22 mg d-pantothenic acid, 22 mg niacin, 489.5 mg choline, .022 mg vitamin B₁₂, .5 mg menadione and .44 mg d-biotin.

^dSupplied .3 mg Se per kg of diet.

TABLE 2. EFFECTS OF ORGANIC ACID ON THE DIET pH AND PERFORMANCE OF WEANLING PIGS (STUDY 1)

Item ^a	Diet			SEM
	Control	Fumaric acid	Citric acid	
Initial wt, kg	6.3	6.3	6.3	.046
Final wt, kg	18.9	19.1	19.3	.353
ADG, g				
wk 1-4 ^b	297	308	321	9.3
wk 5	577	576	567	25.9
wk 1-5	359	367	372	9.9
ADFI, g				
wk 1-4	537	513	523	19.3
wk 5	1003	996	963	25.0
wk 1-5	638	618	619	18.4
F:G				
wk 1-4 ^c	1.83	1.68	1.64	.067
wk 5	1.98	1.76	1.72	.172
wk 1-5 ^b	1.78	1.69	1.67	.041
Diet pH ^d	6.42	4.70	4.90	.004

^aSixteen pens (three pigs per pen) per treatment mean.

^bMeans for control diet vs citric acid diet differ ($P < .09$).

^cMeans for control diet vs citric acid diet differ ($P < .05$).

^dMeans for control diet vs fumaric acid or citric acid differ ($P < .001$).

TABLE 3. EFFECTS OF ORGANIC ACID WITH AND WITHOUT A MICROBIAL ON PERFORMANCE OF WEANLING PIGS (STUDY 2)

	Diet						SEM
	1	2	3	4	5	6	
Fumaric acid %		1.5			1.5		
Citric acid %			1.5			1.5	
Microbial ^a	-	-	-	+	+	+	
Item ^{b,c}							
Initial wt, kg	7.0	7.0	7.0	7.0	7.0	7.0	.02
Final wt, kg ^d	19.0	19.9	19.0	19.7	20.2	19.5	.35
ADG, g							
wk 1-4	313	330	304	318	330	315	12.6
wk 5 ^e	460	531	487	544	563	533	27.3
wk 1-5 ^d	342	370	341	363	377	359	10.1
ADFI, g							
wk 1-4	494	509	478	502	505	469	17.9
wk 5	944	1024	971	992	995	987	38.7
wk 1-5	588	611	583	593	601	573	19.4
F:G							
wk 1-4	1.58	1.54	1.56	1.58	1.53	1.49	.039
wk 5	2.12	2.02	2.07	1.78	1.79	1.85	.118
wk 1-5	1.70	1.66	1.69	1.65	1.60	1.59	.035

^a*Lactobacillus acidophilus*, strain # BT 1386. Added at a level to supply 5×10^6 CFU per pig daily.

^bEight pens (four pigs per pen) per treatment mean.

^cInteraction of acid and microbial not significant ($P < .75$).

^dContrasts, diets 1 and 4 vs diets 2 and 5 differ ($P < .03$).

^eContrasts, diets 1 and 4 vs diets 2 and 5 differ ($P < .07$).

TABLE 4. GASTROINTESTINAL PH AND CHLORIDE ION CONCENTRATION OF EIGHT WEEK OLD PIGS FED EITHER THE CONTROL, 1.5% FUMARIC OR 1.5% CITRIC ACID DIETS (STUDY 1)

Item	Diet ^a			Diet SEM	Pooled Mean
	Control	Fumaric	Citric		
pH					
Stomach	4.73	4.30	4.83	.318	4.63 ^b
Jejunum	7.06	7.01	7.00	.196	7.02 ^c
Cecum	5.96	6.04	6.05	.139	6.02 ^d
L. colon	6.51	6.53	6.47	.133	6.50 ^e
Chloride ion (M)					
Stomach	.082	.091	.082	.014	.085 ^b
Jejunum	.082	.061	.092	.017	.078 ^b
Cecum	.028	.024	.045	.010	.032 ^c
L. colon	.014	.016	.016	.002	.015 ^c

^aEach treatment mean represents 9 observations.

^{bcd}e Pooled means with different superscript letters differ ($P < .05$).

Tukey's studentized range test was used.

TABLE 5. MEANS OF INTESTINAL VOLATILE FATTY ACIDS AND NON-VOLATILE ACIDS OF EIGHT WEEK OLD PIGS FED EITHER THE CONTROL, 1.5% FUMARIC OR 1.5% CITRIC ACID DIETS (STUDY 1)

Item	Diet ^a			Diet SEM	Pooled Mean
	Control	Fumaric	Citric		
Volatile Fatty Acids (meq/dl) ^b					
Stomach					
Acetate	82.5	83.5	79.6	17.06	81.9 ^d
Propionate	6.9	1.8	1.7	2.88	3.5 ^e
Butyrate	2.7	1.4	.9	1.48	1.7 ^d
Total	92.8	87.4	82.8	20.88	87.7 ^d
Jejunum					
Acetate	37.3	61.8	36.1	23.64	45.1 ^d
Propionate	0.0	0.0	0.0	0.00	0.0 ^e
Butyrate	0.0	0.0	0.0	0.00	0.0 ^d
Total	43.3	82.2	49.8	33.18	58.4 ^d
Cecum					
Acetate	235.9	205.8	232.2	35.68	224.6 ^c
Propionate	106.9	92.1	122.7	15.21	107.2 ^c
Butyrate	56.8	54.8	61.5	10.06	57.7 ^c
Total	415.9	365.5	433.8	57.79	405.1 ^c
Lower colon					
Acetate	277.9	284.8	249.4	34.45	270.7 ^c
Propionate	87.0	77.4	92.6	11.93	85.7 ^d
Butyrate	51.8	53.3	63.0	8.97	56.0 ^c
Total	451.7	439.6	440.9	52.11	444.0 ^c
Non-volatile acids (meq/dl)					
Stomach					
Pyruvate	0.0	0.0	.4	.30	.1
Lactate	1.5	.1	.1	.56	.5
Fumarate	2.5 ^g	444.2 ^f	29.1 ^g	63.53	158.6
Succinate	2.8	4.2	.6	2.18	2.6
Total	6.7 ^g	448.6 ^f	30.1 ^g	63.21	161.8
Jejunum					
Pyruvate	0.0	0.0	0.0	0.00	0.0
Lactate	.6	.6 ^f	.6	.31	.6
Fumarate	0.0 ^g	93.6 ^f	18.6 ^g	27.44	37.4
Succinate	0.0	0.0	2.0	.73	.7
Total	.6 ^g	94.1 ^f	21.1 ^g	27.47	38.6
Cecum					
Pyruvate	0.0	0.0	0.0	0.00	0.0
Lactate	0.0	0.0	0.0	0.00	0.0
Fumarate	5.8	0.0	2.8	3.26	2.9
Succinate	0.0	1.0	.6	.50	.5
Total	5.8	1.0	3.4	3.30	3.4
Lower colon					
Pyruvate	0.0	0.0	.4	.16	.1
Lactate	0.0	0.0	0.0	0.00	0.0
Fumarate	12.8	12.9	12.6	5.91	12.7
Succinate	1.4	4.9	.9	1.96	2.4
Total	14.2	17.8	13.9	6.17	16.7

^aEach treatment mean represents 9 observations.

^bTotal volatile fatty acid concentration also includes the acids: formate, isobutyrate, isovalerate, valerate, isocaproate and caproate.

^{cde}Pooled means within a specific acid between intestinal sections with different superscript letters differ ($P < .05$). Tukey's studentized range test was used.

^f^gMeans of dietary treatments within a row with different superscript letters differ ($P < .05$). Tukey's studentized range test was used.

Chapter V

RUNNING HEAD: ORGANIC ACID EFFECT ON INTESTINAL DIGESTA

EFFECT OF FEEDING ORGANIC ACIDS ON SELECTED INTESTINAL DIGESTA MEASUREMENTS AT VARYING TIMES POSTWEANING IN PIGS¹

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Abstract

Pigs weaned at 21 d of age (n=72) were fed a 20% CP corn-soybean meal based diet (control) with 1.5% fumaric or 1.5% citric acid added to observe the effect of these acids on the intestinal digesta pH, chloride ion concentration (Cl⁻), VFA profile and microflora population in the stomach, jejunum, cecum and lower colon at -2, 0, 3, 7, 14 and 21 d postweaning. Feeding organic acids had no appreciable effect ($P < .10$) on stomach, jejunum, cecum or lower colon pH, Cl⁻, VFA profile or microflora populations, except for increasing the concentration of fumaric acid in the stomachs of pigs fed fumaric acid. The pH of the gastrointestinal

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tract generally decreased from -2 to 21 d postweaning with no corresponding change in Cl⁻ over time. Stomach anaerobes decreased from -2 to 3 d postweaning. No age effects on anerobic bacterial numbers were observed in the other intestinal sections. Clostridia counts decreased from -2 to 21 d postweaning in all intestinal sections. Lactobacillus counts were lowest at d 0 and 3 and greatest at d 7 postweaning in the stomach, jejunum and lower colon, but no age effect was observed for concentration of cecum lactobacilli. Escherichia coli counts generally increased from -2 to 3 and 7 d postweaning. The feeding of organic acids to weanling pigs had little effect on these intestinal digesta measurements, but postweaning age did affect these intestinal measurements.

(Key words: Pigs, pH, Organic Acids, Volatile Fatty Acids, Microorganisms.)

Introduction

The first week after weaning 3 to 4 wk old pigs is usually associated with a period of poor growth, general unthriftiness and often scouring (Tzipori et al., 1980; Barnett et al., 1989) which may have a lasting detrimental effect on performance. This postweaning lag phase may be related to the inability of the weanling pig to secrete sufficient quantities of hydrochloric acid in the stomach to lower gastric pH (Manners, 1976), to properly digest a corn-

soy bean meal diet (Kidder and Manners, 1978), or to inhibit the proliferation of coliforms (Cranwell et al., 1976).

Improvements in postweaning growth rate and efficiency of feed utilization have been achieved by supplementing starter diets with fumaric or citric acids (Falkowski and Aherne, 1984; Giesting and Easter, 1985; Risley et al., 1990). This improvement in growth performance has been hypothesized to be related to the lowering of gastric pH and modifying the intestinal microflora by these acids (Scipioni et al., 1978; Burnell et al., 1988; Radecki et al., 1988).

The present study was conducted to evaluate the hypotheses concerning the mechanism of action of organic acids by measuring the postweaning changes in intestinal digesta pH, chloride ion concentration, VFA profile and microflora populations in pigs fed fumaric or citric acid.

Methods and Materials

Four trials of 18 pigs each were conducted to determine the effect of organic acid supplementation on the intestinal digesta pH, chloride ion concentration (Cl⁻), VFA profile and microflora population in weanling pigs. In each trial, female crossbred pigs were randomly selected from each of six paternal half-sib litters (three pigs per litter) and were randomly assigned to a dietary treatment and an intestinal digesta sampling day, such that within and across litters, no dietary treatment and sampling day combination

was repeated. Pigs were weaned at 21 d of age. Dietary treatments consisted of a 20% CP corn-soybean meal based diet formulated to meet or exceed the nutrient guidelines for weanling pigs (NRC, 1988) with 1) no organic acid supplementation (control); 2) 1.5% fumaric acid added; or 3) 1.5% citric acid added (Table 1). Sampling days were 2 d preweaning (-2 d postweaning), 0, 3, 7, 14 and 21 d postweaning. Pigs assigned to d -2 postweaning were removed from the sow at 19 d of age and necropsied, whereas pigs assigned to d 0 postweaning were removed from the sow at 21 d of age and allowed access to the starter diet for 6 h and then necropsied.

Pigs were housed individually in an environmentally-controlled nursery with wire bottom pens (.6 m x .9 m). Temperature (Hinkle et al., 1978) and ventilation rates (Lubinus and Murphy, 1979) were maintained at recommended levels. Water and feed were available ad libitum.

Gastrointestinal Measurements. On each sampling day, the assigned pig in a full-fed state was killed (at approximately 0900) by electrocution followed by exsanguination. Immediately, the viscera were exposed via a midline incision and the following sections were aseptically isolated, doubly ligated and removed: entire stomach, 1 m section of jejunum, entire cecum and .3 m section of lower colon (near the rectum). Two g of intestinal digesta were

diluted in 18 ml of deionized water and mixed and suspended on an electromagnetic mixer. The pH and Cl⁻ concentration were determined within 30 min of collection using the appropriate electrodes⁵ and standards. Additionally, 1 g of intestinal digesta was acidified with 50% (v/v) H₂SO₄ for later determination of VFA concentration according to the procedures of Holdeman et al. (1977).

Intestinal digesta for microbial determinations were collected in plastic zip-lock bags and immediately flushed three times with oxygen-free CO₂. Intestinal digesta were serially diluted in pre-reduced salt media (Holdeman et al., 1977) and plated on selective media. For total anaerobic bacterial counts, samples were serially diluted and cultured in roll tubes containing rumen fluid-glucose-cellubiose agar (Holdeman et al., 1977) with pig fecal extract replacing the rumen fluid (Moore et al., 1987) and were incubated for five days at 37°C. Lactobacillus were enumerated on tomato juice agar⁶ plates and incubated for 72 h. Clostridia were enumerated on McClung Toabe agar plus 10% egg yolk with 10 µg/liter neomycin⁷ plates and incubated for 48 h.

⁵Fisher Scientific Accu-pHast combination electrode # 13-620-281; Fisher Scientific Accumet pH meter # 620; Orion Research Ionalyzer chloride electrode # 94-17-B and double junction reference electrode # 90-02.

⁶Bacto tomato juice agar, Difco laboratories, # 0031-01-2.

⁷Bacto McClung Toabe agar, Difco laboratories, # 0941-01; Bacto egg yolk enrichment, Difco laboratories, # 3347-72; Neomycin sulfate, Sigma, # N 6386.

Escherichia coli were enumerated on MacConkey agar⁸ (trials 1 and 2) or Levine eosin methylene blue agar⁹ (trials 3 and 4) plates and incubated for 72 h. All plates were incubated anaerobically at 37½C

Statistical Analysis. Data were analyzed by the general linear model program (GLM) of the statistical analysis system (SAS, 1988) using the individual pig as the experimental unit. For each intestinal section, the model contained the main effects of sampling day, diet, trial and the interactions. Non-significant interactions were pooled into the error. Comparisons between d -2 postweaning, considered the baseline value, and d 0, 3, 7, 14 or 21 postweaning, were conducted by using non-orthogonal contrasts. Comparisons across intestinal sections and among dietary treatments were made with the Tukey's studentized range test. Bacterial counts that were below detectable levels (less than 10⁵ per g of wet digesta) were considered as missing values. Frequency of detection of E. coli and clostridia was determined by the SAS Logist procedure (1988).

Results

pH and Cl⁻. The pH of the control diet was decreased (P < .001) by the addition of the organic acids from 6.42 to

⁸Bacto MacConkey agar, Difco laboratories, # 0075-05-5.

⁹Bacto Levine eosin methylene blue agar, Difco laboratories, # 0075-01-9.

4.70 (fumaric acid) and 4.90 (citric acid), but no significant effect ($P < .10$) of organic acid supplementation was observed on intestinal pH (Table 2). The pH of the stomach digesta was lowest followed by cecum and jejunum digesta pH; the lower colon digesta had the highest pH ($P < .05$). The Cl^- concentration of digesta from the various intestinal sections was also not significantly affected by dietary treatments ($P < .27$), but Cl^- concentration steadily decreased ($P < .05$) from a high in the stomach and jejunum to a low in the cecum and lower colon.

In the stomach, pH of the digesta decreased ($P < .01$) with age (Table 3). Postweaning age had less of an effect ($P < .08$) on the pH of the jejunum digesta. Age affected ($P < .001$) the pH in the cecum and lower colon digesta, with the pH decreasing with age. Chloride ion concentration was not measurably affected ($P < .10$) by postweaning age for any of the intestinal sections.

VFA and NVA. The total concentration of VFA was lower in the upper gastrointestinal tract (stomach and jejunum) compared with the lower gastrointestinal tract (cecum and lower colon), with acetic acid being the primary VFA detected in each of the intestinal sections (Table 4). The increase in total VFA concentration in the lower gastrointestinal tract reflects a modest increase in acetic acid and a larger increase in propionic and butyric acid.

No appreciable diet effect was observed on the concentration of VFA in the intestinal digesta.

Throughout the gastrointestinal tract, the concentration of VFA increased from -2 to 21 d postweaning (Table 5). Concentration of acetic, propionic and total VFA in the stomach digesta increased by d 3 postweaning and generally stabilized thereafter. Jejunum digesta VFA exhibited quite similar concentration patterns of VFA as that of the stomach digesta. Cecum digesta VFA were generally stable across days with the exception of an increased concentration of propionic and total VFA on d 14 postweaning compared with the baseline VFA concentration. Lower colon digesta concentration of acetic acid increased from baseline values to d 3 and remained relatively stable thereafter. Concentration of propionic and total VFA in the lower colon digesta increased from baseline values to d 14 and 21 postweaning.

Pigs fed fumaric acid, as compared with pigs fed the control or citric acid diet, had an overall greater concentration of fumaric acid, and therefore total non-volatile acids (NVA), in the stomach digesta. In the jejunum, only the concentration of fumaric acid was greater ($P < .05$; Table 6). The concentration of the other NVA were not affected ($P < .10$) by the dietary treatments. Lactic acid was generally low in the lower gastrointestinal tract,

however lactic acid was a major NVA in the stomach and in the jejunum. The concentration of total NVA, excluding fumaric acid, was generally low throughout the gastrointestinal tract in relation to the VFA concentration.

Postweaning age affected the concentration of lactic ($P < .001$) and fumaric acid ($P < .002$) in the stomach digesta and the concentration of lactic ($P < .01$) and total NVA ($P < .002$) in the jejunum digesta (Table 7). Lactic and total NVA concentration of the stomach digesta decreased after weaning. The concentration of fumaric acid increased from the baseline level in the stomach and jejunal contents, only in pigs fed fumaric acid. Jejunum digesta lactic acid concentration decreased to near non-detectable levels by d 0 postweaning and increased thereafter. These increases in the jejunum digesta concentration of lactic and fumaric acid resulted in the concentration of the total NVA being greater on d 14 postweaning than the baseline concentration. The concentration of fumaric and total NVA in the cecal and lower colon contents increased to d 7 postweaning and generally leveled off thereafter.

Microflora. The anaerobic cultural count, lactobacillus counts, and E. coli counts all were greater in the lower gastrointestinal tract than in the upper gastrointestinal tract, whereas the number of clostridia was not significantly greater in the lower gastrointestinal

tract (Table 8). No appreciable dietary effect on intestinal bacterial numbers was observed.

In trials 1 and 2, E. coli were enumerated on MacConkey agar. However, this medium did not support the growth of typical E. coli cultures under the conditions of this experiment. Therefore, in trials 3 and 4, Levine eosin methylene blue agar was used. This was found to be satisfactory; hence, the data presented for E. coli counts in Tables 8 and 9 are from trials 3 and 4.

Stomach digesta anaerobic bacteria were affected ($P < .004$) by postweaning age; anaerobe numbers decreased within six h of weaning and generally rose thereafter (Table 9). Anaerobes in the jejunum digesta exhibited a somewhat similar pattern to that of the stomach digesta, whereas cecum and lower colon digesta anaerobe counts were generally stable from -2 to 21 d postweaning. As indicated by direct microscopic clump counts (DMCC), anaerobic colony counts were satisfactory and ranged from 70 to 105% of the DMCC.

Number of lactobacilli in the stomach ($P < .04$), jejunum ($P < .004$) and lower colon digesta ($P < .02$) were influenced by postweaning age; no age effect was observed for numbers of lactobacilli in the cecum (Table 9). Numbers of lactobacilli in the stomach digesta decreased from the baseline value to d 0 postweaning and then numerically increased to d 7 postweaning, and decreased ($P < .05$) to

below baseline levels at d 14 and 21 postweaning. Jejunum digesta lactobacillus counts exhibited a similar pattern to that of lactobacilli in the stomach, but the decline in numbers at d 14 and 21 postweaning was not as pronounced. Lower colon digesta lactobacillus numbers increased from baseline counts to d 7 postweaning and stabilized thereafter.

Clostridia numbers in the stomach ($P < .04$), jejunum ($P < .01$), cecum ($P < .02$) and lower colon ($P < .06$) were affected by postweaning age (Table 9). The clostridia counts generally decreased from d -2 to d 3 postweaning, numerically increased on d 7 and then decreased to undetectable levels ($< 10^5$ organisms per gram wet digesta) by d 21 postweaning in all of the intestinal sections. The frequency of detection of clostridia was not affected ($P < .10$) by the dietary treatments for any of the intestinal sections. Enumeration of clostridia was not conducted during trial 3, due to limited incubator space.

No effect of postweaning age was detected, but the pattern of E. coli counts was similar for all the intestinal sections, such that E. coli numbers were greatest on d 3 or 7 and then generally decreased to d 21 postweaning. The frequency of detection of E. coli was not affected ($P < .40$) by the dietary treatments for any of the intestinal sections.

Discussion

pH and Cl⁻. An improvement in ADG and feed efficiency has been observed with the supplementation of weanling pig diets with organic acids (Falkowski and Aherne, 1984; Giesting and Easter, 1985; Risley et al., 1990). Improvement in growth performance has been hypothesized to be a direct result of the reduction of intestinal pH and the modification of intestinal microflora by the organic acids (Scipioni et al., 1979; Burnell et al., 1988). In the present study, no improvement in growth performance was observed during the first 21 d postweaning by feeding the organic acids (data not shown).

Scipioni et al. (1979) reported a numerical reduction in the stomach and jejunum pH of weanling pigs from 4.55 and 6.57 to 3.50 and 6.43, respectively, by feeding diets containing 1.0% citric acid. Burnell et al. (1988) observed a numerical decrease in the pH of the small intestine from 6.76 to 6.19 in 7-wk old weanling pigs fed a starter diet with 1.0% sodium citrate. Both investigators used complex diets containing dried whey which resulted in a lower diet pH than diets which do not contain dried whey. We also observed a non-significant decrease in gastric and jejunal pH by feeding 1.5% fumaric or citric acid in a simple diet (Table 2). Our data, and the data of Scipioni et al. (1979) and Burnell et al. (1988), suggest that the supplementation

of starter diets with organic acids does not substantially reduce the intestinal pH; therefore, decreasing the intestinal pH is probably not a primary mode of action of organic acids.

Reduction of intestinal pH might be beneficial to the weanling pig. Weanling pigs do not secrete sufficient quantities of hydrochloric acid to significantly reduce gastric pH until 5-wk of age (Manners, 1976; Cranwell, 1985). Inability to lower intestinal pH may limit the activation of pepsinogen, cause poor protein denaturation and allow the proliferation of coliforms which may contribute to the poor growth performance commonly observed the first week postweaning. The increasing ability of the weanling pig to lower the intestinal pH as it gets older was demonstrated in our study by the intestinal pH decreasing from d -2 to d 21 postweaning (Table 3).

The organic acids were expected to stimulate the secretion of HCl, but this was not confirmed in our study (Table 2). If HCl secretion was stimulated, the stomach concentration of Cl⁻ should have increased from a low at d 0 to a high at d 21 postweaning. The concentration of Cl⁻ remained stable from d 7 to d 21 postweaning however (Table 3). This observation suggests that the Cl⁻ concentration is more a reflection of the level of sodium chloride ingested in the diet, rather than gastric HCl secretion.

VFA and NVA. The lack of a dietary effect on the VFA profile suggests that the organic acids were unable to modify the intestinal microflora population and(or) their metabolism (Varel and Pond, 1985). However, the increase in succinic acid in the stomach of fumaric acid fed pigs may indicate some modification of microbial metabolism. Lack of a dietary effect on the VFA profile was also observed in previous work conducted by our lab (Risley et al., 1990) and suggests that the mode of action of organic acids on growth performance may not be related to changes in the intestinal microflora.

The concentration of NVA decreased from the upper to the lower gastrointestinal tract. This decrease in NVA, notably fumaric, suggests that fumaric acid may be directly absorbed by the pig and(or) utilized by the intestinal microflora. If fumaric acid is directly absorbed by the pig, this NVA may serve as an additional energy source. Kirchgessner and Roth (1982) reported that fumaric acid could be used as an energy source with the energy efficiency equal to that of glucose. This additional energy would be important to the newly weaned pig whose intake of feed and body reserves are limited (Fenten et al., 1985). Our observation suggests that organic acids may promote growth in weanling pigs by serving as a readily available source of energy.

The increase in VFA and NVA concentrations in the gastrointestinal tract coincides with the increase in feed consumption (Rerat et al., 1987) that is normally observed a few days after weaning. Newly weaned pigs normally have a period of depressed feed intake shortly after weaning and then increase their feed consumption by d 7 postweaning (Fenten et al., 1985). Burnell et al. (1988) reported that the average feed consumption is 168 g/d during the first week postweaning. Similarly, related research in our lab has found the feed consumption of weanling pigs to be 22.5 g the first 24 h postweaning, 93 g/d for the first 3 d postweaning and 230.5 g/d for the first 7 d postweaning (Ogunbameru et al., unpublished data). In the present study, the low feed intake was evident from the small amount of digesta that was found in the stomach on d 0, 3 and 7 postweaning. Low feed intake following weaning may be the underlying cause of the poor growth performance observed during the first week postweaning.

Microflora. The addition of organic acid had no major effect on the bacterial populations, in contrast to the findings of Scipioni et al. (1979) who observed a decrease in total anaerobes and E. coli by feeding citric acid to weanling pigs. The difference between studies may be due to different media used; Scipioni et al. (1979) used Violet Red Bile agar for E. coli and RCM (Oxoid) for anaerobes and used

Gas-pak jars for incubating anaerobic bacteria. We used Levine eosin methylene blue agar in Gas-pak jars for E. coli and roll-tubes with pig fecal extract-glucose-cellubiose agar for enumeration of total anaerobic count. The general lack of a dietary effect on intestinal microflora in our study suggests these acids may promote growth by another mechanism.

Total anaerobic counts generally decreased from -2 to 3 d postweaning and then increased by d 7 in all the intestinal sections. The change in these bacterial numbers in the stomach and jejunum corresponds to the level of feed intake, (Ogunbameru et al., unpublished data) whereas in the cecum and lower colon these bacteria numbers reflect the relative percent of dry matter in the digesta. As stated previously, the level of feed intake is low shortly after weaning, which would result in little intestinal digesta to support bacterial growth (Morishita and Ogata, 1970). By d 7 postweaning, however the feed intake would be sufficient to support a larger bacterial population. Our anaerobic counts for the cecum and colon were similar to those reported by Varel (1987) and Moore et al. (1987), respectively.

Clostridia numbers linearly decreased to undetectable levels by 14 and 21 d postweaning. This decline in numbers may be due to the change in diet from a liquid milk diet

(high clostridia counts) to a dry corn-soybean meal based diet (low clostridia counts). The biological significance of the modest changes in clostridial numbers is questionable due to the fact that this population of clostridia (lipase positive and neomycin resistant) made up less than .01% of the total intestinal microflora.

Lactobacillus and E. coli numbers were not affected by the supplementation of organic acid to the weanling diet. Our bacterial counts of lactobacillus and E. coli are similar to previous reports: fecal lactobacillus counts 9.7 to 9.9 and E. coli counts 6.2 to 6.4 (Danielson et al., 1989). It was expected that the organic acids would lower the intestinal pH, which would result in a more favorable environment for lactobacillus and inhibit the proliferation of E. coli (Cranwell et al., 1976). This effect was not observed and may be attributed to the lack of an intestinal pH change with the 1.5% level of organic acid supplementation.

The population of lactobacilli did decline after weaning, d 0 and 3 postweaning, and then increased by d 7 postweaning. Concurrently, E. coli counts were generally greater on d 3 and 7 postweaning. These bacteria are normally considered antagonistic, i.e. lactobacillus inhibits the colonization and proliferation of E. coli by blocking possible intestinal receptors of E. coli and

secreting toxic metabolites (Danielson et al., 1989). It is during this time, the first week postweaning, when the numbers of lactobacillus are low and the number of E. coli are high, that the pig is most susceptible to scouring and poor growth performance.

Implications

The supplementation of organic acids in diets for weanling pigs lowered dietary pH, but did not lower the intestinal pH, increase chloride ion concentration, modify the VFA profile nor significantly change the intestinal bacterial populations that we measured. These results suggest that the mode of action of organic acids may not be related to reducing gastric pH and(or) altering the intestinal microflora, but suggests that organic acids may act as an additional energy source for the weanling pig. However more research is needed to substantiate this hypothesis.

TABLE 1. PERCENTAGE COMPOSITION OF DIETS

Item	Diet ^a		
	Control	Fumaric acid	Citric acid
Ground corn	64.20	62.50	62.50
Soybean meal (44% CP)	33.11	33.31	33.31
Limestone	.60	.58	.58
Defluorinated phosphate	1.42	1.44	1.44
Salt	.30	.30	.30
Trace mineral premix ^b	.07	.07	.07
Vitamin premix ^c	.25	.25	.25
Selenium premix ^d	.05	.05	.05
Fumaric/citric acid	—	1.50	1.50

^aCalculated to supply 20% crude protein, .80% Ca and .65% P.

^bSupplied per kg of diet: 105 mg Zn, 123 mg Fe, 42 mg Mn, 12 mg Cu, and 1.4 mg I.

^cSupplied per kg of diet: 4,400 IU vitamin A, 440 IU vitamin D, 11 IU vitamin E, 4.4 mg riboflavin, 22 mg d-pantothenic acid, 22 mg niacin, 489.5 mg choline, .022 mg vitamin B₁₂, .5 mg menadione and .44 mg d-biotin.

^dSupplied .3 mg Se per kg of diet.

TABLE 2. MEANS OF THE INTESTINAL DIGESTA pH AND CHLORIDE ION CONCENTRATION IN WEANLING PIGS FED EITHER THE CONTROL, 1.5% FUMARIC OR 1.5% CITRIC ACID DIET

Item ^{a,b}	Intestinal Section			
	Stomach	Jejunum	Cecum	Lower colon
pH				
Control	4.07	6.76	6.36	7.06
Fumaric	3.87	6.42	6.16	6.89
Citric	3.82	6.69	6.19	6.93
SEM	.189 ^g	.106 ^g	.101	.082
Pooled Means	3.92 ^f	6.63 ^d	6.24 ^e	6.96 ^c
Chloride ion concentration (M)				
Control	.144	.131	.033	.016
Fumaric	.088	.128	.031	.016
Citric	.118	.111	.038	.018
SEM	.014	.020	.005	.003
Pooled Means	.107 ^c	.123 ^c	.034 ^d	.016 ^d

^aEach mean value represents 24 observations.

^bNo diet or day by diet interaction was observed ($P < .10$).

^{cdef}Pooled means within a row with different superscript letters differ ($P < .05$). Tukey's studentized range test was used.

TABLE 3. MEANS OF THE INTESTINAL DIGESTA pH AND CHLORIDE ION CONCENTRATION ON -2, 0, 3, 7, 14 AND 21 DAYS POSTWEANING IN TREATED AND UNTREATED PIGS^a

Item ^b	Intestinal Section			
	Stomach	Jejunum	Cecum	Lower colon
pH				
d -2	4.82	6.58	6.79	7.19
0	3.98*	6.70	6.72	7.01
3	3.92*	6.79	6.36*	7.16
7	3.71**	6.80	6.05***	7.17
14	3.47***	6.35	5.79***	6.76**
21	3.62**	6.54	5.73***	6.47***
SEM	.267	.150	.143	.116
Chloride ion concentration (M)				
d -2	.126	.124	.044	.019
0	.096	.113	.019	.023
3	.088	.092	.030	.014
7	.111	.123	.030	.014
14	.110	.142	.045	.015
21	.111	.147	.030	.016
SEM	.020	.027	.007	.004

^aNo treatment or day by treatment interaction was observed ($P < .10$), therefore all age data were pooled across treatments.

^bEach mean value represents 12 observations.

*, **, *** Mean differs from day -2 ($P < .05, .01, .001$). Determined by using non-orthogonal contrasts.

TABLE 4. MEANS OF INTESTINAL DIGESTA VFA (MEQ/DL) IN WEANLING PIGS FED EITHER THE CONTROL, 1.5% FUMARIC OR 1.5% CITRIC ACID DIETS^a

Item ^b	Intestinal Section			
	Stomach	Jejunum	Cecum	Lower colon
Acetic acid				
Control	70.4	34.4	159.7	180.2
Fumaric	74.6	26.0	199.3	190.9
Citric	60.9	43.9	184.5	172.1
SEM	6.66	6.39	17.73	18.60
Pooled Means	68.6 ^d	34.8 ^e	181.2 ^c	181.1 ^c
Propionic acid				
Control	4.0	.4	75.7	58.4
Fumaric	4.6	.9	90.7	55.6
Citric	2.9	3.8	78.7	62.2
SEM	1.60	2.20	6.66	7.11
Pooled Means	3.8 ^e	1.7 ^e	81.7 ^c	58.7 ^d
Butyric acid				
Control	1.7	.2	46.5	28.7
Fumaric	2.2	.5	55.0	29.4
Citric	5.2	3.8	48.2	32.0
SEM	1.59	2.20	8.79	4.15
Pooled Means	3.0 ^e	1.5 ^e	49.9 ^c	30.0 ^d
Total ^f				
Control	78.3	38.2	285.7	292.5
Fumaric	83.8	28.2	368.0	295.8
Citric	61.8	53.6	335.8	290.8
SEM	8.69	10.30	26.82	29.46
Pooled Means	74.6 ^d	40.0 ^d	329.8 ^c	293.0 ^c

^aNo treatment or treatment by day interaction was observed ($P < .10$).

^bEach mean value represents 24 observations.

^{cde}Pooled means within a row with different superscript letters differ ($P < .05$). Tukey's studentized range test was used.

^fTotal volatile fatty acid concentration also includes formic, isobutyric, isovaleric, valeric, isocaproic and caproic acids.

TABLE 5. MEANS OF THE INTESTINAL DIGESTA VFA (MEQ/DL) ON -2, 0, 3, 7, 14 AND 21 DAYS POSTWEANING IN TREATED AND UNTREATED PIGS

Item ^{a,b}	Intestinal Section			
	Stomach	Jejunum	Cecum	Lower colon
Acetic acid				
d -2	5.7	6.3	148.5	88.7
0	1.8	2.4	165.2	85.2
3	84.2***	44.1**	198.4	185.1**
7	86.8***	45.8**	190.2	218.7***
14	116.3***	53.4**	195.3	282.8***
21	117.0***	56.6***	189.4	225.9***
SEM	9.41	9.04	25.07	26.31
Propionic acid				
d -2	0.0	0.0	74.1	29.5
0	0.0	0.0	60.8	26.9
3	6.8*	2.8	56.2	34.0
7	8.0*	7.4	81.1	55.8
14	6.9*	0.0	122.4***	118.5***
21	1.4	0.0	95.7	87.8***
SEM	2.26	1.18	9.42	10.06
Butyric acid				
d -2	0.0	0.0	57.5	14.4
0	.3	0.0	32.4	11.5
3	2.0	1.6	24.1	24.2
7	10.4**	7.6	54.2	29.4
14	5.1	0.0	74.8	60.0***
21	.5	0.0	56.3	40.6**
SEM	2.25	3.11	12.43	5.87
Total^c				
d -2	5.7	6.3	296.6	153.2
0	2.6	2.4	287.2	139.6
3	81.5***	55.1*	294.2	264.9
7	112.4***	63.6**	334.5	325.8**
14	128.4***	53.4*	412.6*	500.8***
21	118.9***	59.0**	353.8	373.8***
SEM	12.29	14.56	37.93	41.66

^aEach mean value represents 12 observations.^bNo treatment or treatment by day interaction was observed ($P < .10$).
*, **, *** Mean differs from day -2 ($P < .05, .01, .001$). Determined by using non-orthogonal contrasts.^cTotal volatile fatty acid concentration also includes formic, isobutyric, isovaleric, valeric, isocaproic and caproic acids.

TABLE 6. MEANS OF INTESTINAL DIGESTA NON-VOLATILE ACIDS (MEQ/DL) IN WEANLING PIGS FED EITHER THE CONTROL, 1.5% FUMARIC OR 1.5% CITRIC ACID DIETS

Item ^a	Intestinal Section			
	Stomach	Jejunum	Cecum	Lower colon
Pyruvic acid				
Control	0.0	0.0	.1	.5
Fumaric	0.0	0.0	.4	0.0
Citric	0.0	0.0	.1	6.0
SEM	0.00	0.00	.19	2.50
Pooled Means	0.0	1.4	.2	2.2
Lactic acid				
Control	16.3	13.2	0.0	.2
Fumaric	7.6	10.7	0.0	.1
Citric	20.3	11.0	4.5	0.0
SEM	5.82	4.03	2.59	.13
Pooled Means	14.7 ^b	11.7 ^b	1.5 ^c	.1 ^c
Fumaric acid				
Control	.2	.2	7.8	11.8
Fumaric	83.5***	18.7*	10.4	12.0
Citric	.9	.4	7.6	13.5
SEM	11.26	4.70	4.32	3.80
Pooled Means	28.2 ^b	6.4 ^c	8.6 ^c	12.4 ^{bc}
Succinic acid				
Control	6.8	.7	.3	.2
Fumaric	17.6	1.3	.4	.2
Citric	5.6	2.7	1.2	1.4
SEM	4.30	1.24	.37	.52
Pooled Means	10.0 ^b	1.6 ^c	.6 ^c	.6 ^c
Total				
Control	23.4	14.1	8.1	12.6
Fumaric	108.0***	30.8*	11.3	12.4
Citric	26.8	18.3	13.4	20.9
SEM	12.85	6.07	5.00	4.62
Pooled Means	52.7 ^b	21.1 ^c	10.9 ^c	15.3 ^c

^aEach mean value represents 24 observations.

^{bc}Pooled means within a row with different superscript letters differ ($P < .05$). Tukey's studentized range test was used.

*, **, *** Mean differs from control diet ($P < .05, .01, .001$).

Determined by non-orthogonal contrasts.

TABLE 7. MEANS OF THE INTESTINAL DIGESTA NON-VOLATILE ACIDS (MEQ/DL) ON -2, 0, 3, 7, 14 AND 21 DAYS POSTWEANING IN TREATED AND UNTREATED PIGS

Item ^a	Intestinal Section			
	Stomach	Jejunum	Cecum	Lower colon
Pyruvic acid				
d -2	0.0	0.0	0.0	0.0
0	0.0	0.0	.2	1.4
3	0.0	0.0	.1	8.3
7	0.0	0.0	.4	0.0
14	0.0	0.0	.6	2.3
21	0.0	0.0	0.0	1.0
SEM	0.00	0.00	.27	3.53
Lactic acid				
d -2	55.3	16.5	0.0	.3
0	10.2***	.1*	0.0	0.0
3	5.0***	3.7	9.0	0.0
7	12.1***	11.2	0.0	0.0
14	5.5***	29.0	0.0	.3
21	.1	9.4	0.0	0.0
SEM	8.23	5.71	3.66	.18
Fumaric acid				
d -2	0.0	0.0	0.0	.3
0	0.0	0.0	0.0	1.9
3	35.0	2.4	0.0	.6
7	1.7	1.0	19.6*	29.3***
14	56.3*	22.2*	15.5	19.9**
21	76.4***	13.1	16.4	22.4**
SEM	15.93	6.64	6.11	5.38
Succinic acid				
d -2	11.5	4.5	0.0	0.0
0	3.4	.2	.9	1.7
3	9.5	0.0	.2	1.4
7	22.1	2.0	.9	0.0
14	12.2	2.0	1.7	.5
21	1.2	.7	0.0	0.0
SEM	6.08	1.76	.53	.73
Total				
d -2	66.8	21.0	0.0	.6
0	12.3*	.3	1.1	5.1
3	49.5	6.0	9.2	10.4
7	36.0	22.5	20.9*	29.3**
14	74.1	53.2**	17.9	23.1**
21	77.7	23.3	16.4	23.4**
SEM	18.17	8.58	7.07	6.53

^aEach treatment mean represents 12 observations.

*, **, *** Mean differs from day -2 ($P < .05$, $.01$, $.001$). Determined by using non-orthogonal contrasts.

TABLE 8. MEANS OF THE INTESTINAL DIGESTA ANAEROBIC BACTERIA, LACTOBACILLUS, CLOSTRIDIA AND ESCHERICHIA COLI IN WEANLING PIGS FED EITHER THE CONTROL, 1.5% FUMARIC OR 1.5% CITRIC ACID DIETS^a

Item ^b	Intestinal Section			
	Stomach	Jejunum	Cecum	Lower colon
Total Anaerobes ^c				
Control	8.4	8.3	10.6	10.8
Fumaric	8.5	8.5	10.8	11.0
Citric	8.8	8.6	10.7	11.0
SEM	.16	.16	.06	.08
Pooled Means	8.6 ^e	8.5 ^e	10.7 ^d	10.9 ^d
Lactobacillus ^c				
Control	7.5	7.4	8.9	8.9
Fumaric	7.7	7.6	9.0	9.0
Citric	8.0	7.9	9.2	9.2
SEM	.18	.22	.16	.16
Pooled Means	7.7 ^e	8.5 ^e	9.1 ^d	9.1 ^d
Clostridia ^f				
Control	5.8 (.33) ^g	4.9 (.52)	6.7 (.33)	6.4 (.30)
Fumaric	5.7 (.38)	5.6 (.27)	6.7 (.35)	6.6 (.35)
Citric	6.3 (.29)	5.7 (.27)	6.7 (.30)	6.7 (.35)
Pooled Means	5.5 ^{de}	5.3 ^e	6.0 ^d	6.0 ^d
Escherichia coli ^h				
Control	5.4 (.25)	6.0 (.44)	7.0 (.28)	7.3 (.29)
Fumaric	6.0 (.45)	6.4 (.41)	7.0 (.31)	7.4 (.31)
Citric	6.0 (.36)	5.7 (.39)	6.8 (.29)	7.2 (.31)
Pooled Means	5.3 ^e	5.6 ^e	6.9 ^d	7.2 ^d

^aNo treatment or treatment by day interaction was observed ($P < .10$).

^bMicrobial counts are expressed as the $\log_{10}$ of the cultural count per gram of wet digesta.

^cEach mean value represents 24 observations.

^{de}Pooled means with different superscript letters differ ($P < .05$).

^fTukey's studentized range test was used.

^gEach mean value represents 9 observations.

^hNumber within parentheses is the standard error of the mean.

^hEach mean value represents 6 observations.

TABLE 9. MEANS OF THE INTESTINAL DIGESTA ANAEROBIC BACTERIA, LACTOBACILLUS, CLOSTRIDIA AND ESCHERICHIA COLI CULTURAL COUNTS ON -2, 0, 3, 7, 14 AND 21 DAYS POSTWEANING IN TREATED AND UNTREATED PIGS

Item ^{a,b}	Intestinal Section			
	Stomach	Jejunum	Cecum	Lower colon
Total anaerobic cultural counts ^c				
d -2	8.9	8.7	10.8	11.0
0	8.1**	8.1	10.9	10.9
3	8.7	8.4	10.6	11.0
7	9.2	8.9	10.8	11.0
14	8.0**	8.5	10.6	10.9
21	8.4	8.3	10.6	10.8
SEM	.23	.22	.09	.11
Lactobacilli ^c				
d -2	8.3	8.2	9.2	8.4
0	7.3**	6.9**	9.0	9.0
3	7.6	7.1*	8.6	9.1*
7	8.2	8.4	9.4	9.4**
14	7.4*	7.8	9.3	9.4**
21	7.5*	7.4	8.8	9.2*
SEM	.25	.31	.22	.22
Clostridia ^d				
d -2	6.5 (.34) ^e	6.2 (.30)	7.6 (.31)	7.6 (.32)
0	5.6 (.37)	4.9** (.35)	6.8 (.31)	6.9 (.30)
3	5.0 (.39)	4.1** (.47)	6.0** (.34)	6.5* (.38)
7	6.4** (.56)	6.4 (.51)	6.1** (.44)	6.3** (.35)
14	ND ^f	ND	ND	5.6* (.96)
21	ND	ND	7.0 (.94)	ND
Escherichia coli ^g				
d -2	6.2 (.75)	5.3 (.87)	6.9 (.43)	7.3 (.46)
0	5.6 (.32)	6.0 (.42)	7.2 (.39)	7.5 (.41)
3	5.6 (.28)	6.5 (.36)	7.5 (.39)	8.0 (.41)
7	6.2 (.44)	6.2 (.42)	7.4 (.43)	7.0 (.41)
14	5.4 (.78)	5.3 (.89)	6.6 (.39)	7.3 (.41)
21	5.8 (.55)	6.2 (.85)	7.0 (.43)	6.7 (.46)

^aNo treatment or day by treatment interaction was observed ($P < .10$).

^bMicrobial counts are expressed as the $\log_{10}$ of the cultural count per gram of wet digesta.

^cEach mean value represents 12 observations.

^d*, **, *** Mean differs from day -2 ($P < .05, .01, .001$). Determined by using non-orthogonal contrasts.

^eEach mean value represents 9 observations.

^fNumber within parentheses is the standard error of the mean.

^gND = not detectable ($< 10^5$ organisms per gram of wet digesta).

^hEach mean value represents 6 observations.

Chapter VI

RUNNING HEAD: INTESTINAL EFFECTS OF ORGANIC ACID/E. COLI

EFFECT OF FEEDING ORGANIC ACIDS ON SELECTED INTESTINAL
DIGESTA MEASUREMENTS AT VARIOUS TIMES POSTWEANING IN PIGS
CHALLENGED WITH ENTEROTOXIGENIC ESCHERICHIA COLI¹

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Abstract

In Study 1, pigs (n=72) weaned at 21 ± 2 d were fed a 20% CP corn-soybean meal based diet (control) with 1.5% fumaric or 1.5% citric acid. Half of these pigs were orally challenged on d 3, 7 or 14 postweaning with 10¹⁰ E. coli (0157:K88+) to observe the effect of these treatments on intestinal digesta pH, chloride ion concentration (Cl⁻), VFA profile and microflora in the stomach, jejunum, cecum and lower colon and on scouring when necropsied on 5, 9 and 16 d postweaning. Organic acids had no appreciable effect (P < .18) on intestinal measurements except for fumaric and

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succinic acids were greater in the stomach ($P < .001$) and jejunum ($P < .05$) of pigs fed fumaric acid. Cecum pH decreased ($P < .002$) and jejunum Cl^- increased ($P < .03$) from 5 to 16 d postweaning. E. coli challenge had no significant effect on pH, Cl^- , lactobacillus or E. coli counts, but did increase ($P < .05$) the concentration of lactic acid in the jejunum, total VFA in the lower colon and the severity of scouring. Jejunum lactobacillus counts decreased ($P < .001$), whereas lower colon lactobacillus counts increased ($P < .004$) from 5 to 16 d postweaning. E. coli counts decreased in the jejunum ($P < .07$), cecum ($P < .02$) and lower colon ($P < .001$) from 5 to 16 d postweaning.

In Study 2, pigs ($n=96$) weaned at 21 ± 2 d were similarly fed organic acids. Half of these pigs were orally challenged with E. coli on d 3 postweaning to observe the effect of these treatments on growth performance and severity of scouring during the first 15 d postweaning. The E. coli challenge decreased ($P < .05$) ADG by 20% and F:G 19% from 7 to 15 d postweaning and increased ($P < .001$) the severity of scouring between 4 and 11 d postweaning, but feeding the organic acids had no measurable effect on growth performance or scouring.

(Key words: Pigs, pH, Organic Acids, Volatile Fatty Acids, Lactobacillus, Escherichia coli.)

Introduction

The stress that accompanies the weaning of 3 wk old pigs usually results in a period of poor growth performance and scouring (Tzipori et al., 1980; Barnett et al., 1989). Scouring is normally associated with the proliferation of enterotoxigenic Escherichia coli (Kenworthy and Crabb, 1963; McAllister et al., 1979) which may be due to immaturity of the gut (Miniats and Roe, 1968), high gastric pH (Smith and Jones, 1963), or a change in diet (Thomlinson, 1963). Antibiotics are usually fed to reduce the scouring and to improve growth performance (Chopra et al., 1964a; Chopra et al., 1964b), but with public concern over food safety, alternatives to antibiotics are needed.

Organic acids may be an alternative for improving growth performance when added to weanling diets (Falkowski and Aherne, 1984; Giesting and Easter, 1985; Risley et al., 1990). Organic acids are hypothesized to improve growth performance by reducing gastrointestinal pH and decreasing coliform numbers (Scipioni et al., 1979; Kirchgessner and Roth, 1982; Burnell et al., 1988). The purpose of this study was to observe the effect of feeding organic acids on the severity of scouring, intestinal digesta measurements, and growth performance of weanling pigs challenged orally with an enterotoxigenic E. coli dose.

Methods and Materials

Study 1. Three trials were conducted to determine if organic acid supplementation of weanling diets afforded any protection against scouring or influenced the intestinal digesta pH, chloride ion concentration, VFA profile and microflora population in early weaned pigs challenged with enterotoxigenic Escherichia coli. Each trial included 18 pigs, six from each of three paternal half-sib litters weaned at 21 ± 2 d of age. Pigs were randomly assigned to a dietary organic acid treatment and challenge day (3, 7 or 14 d postweaning), with the restriction that littermates be balanced across all treatments. These 18 pigs were then randomly assigned to an oral challenge treatment: 1) non-challenged - 5 ml of trypticase soy broth⁵, and 2) challenged - 5 ml of trypticase soy broth with approximately 10^{10} E. coli (0157:K88+)⁶. Dietary treatments consisted of a 20% CP corn-soybean meal based diet formulated to meet or exceed the nutrient guidelines for weanling pigs (NRC, 1988) with 1) no supplementation (control), 2) 1.5% fumaric acid added or 3) 1.5% citric acid added (Table 1).

Pigs were housed six per pen in an environmentally-controlled nursery with plastic coated wire bottom pens (.9 m x 1.2 m). Prior to oral challenging with E. coli, pigs were moved to an environmentally-controlled isolation

⁵Trypticase soy broth, BBL Microbiology Systems # 11768.

⁶Penn State Reference Center: ETEC 0157:K88ac:H13.

trailer with expanded metal bottom pens (.9 m x 1.2 m) to prevent possible cross-contamination. Temperature (Hinkle et al., 1978) and ventilation rates (Lubinus and Murphy, 1979) were maintained at recommended levels in both facilities. Water and feed were available ad libitum.

All pigs were scored daily for the incidence of scouring using the following 5-point system: 1=hard feces (rarely seen), 2=normal consistency of feces (no scours), 3=soft, partially-formed feces (mild scours), 4=loose, semi-liquid feces (moderate scours), and 5=watery feces (severe scours). After being challenged, the pigs were observed every 8 h for 48 h for the incidence of scouring. Two days after the challenge, pigs were killed by electrocution followed by exsanguination. Viscera were exposed by a midline incision and the following sections were aseptically isolated, doubly ligated and removed: entire stomach, 1 m section of jejunum, entire cecum and .3 m section of lower colon (near rectum). The intestinal digesta contents were collected, and within 30 min, pH and chloride ion concentration were determined. Two g of intestinal digesta were diluted in 18 ml of deionized water and suspended via a magnetic stir bar and electromagnetic mixer. The pH and Cl⁻ concentration were measured using the appropriate electrodes⁷ and standards. Additionally, 1 g of intestinal

⁷Fisher Scientific Accu-pHast combination electrode # 13-620-281; Fisher Scientific Accumet pH meter # 620; Orion

digesta was acidified with 50% (v/v) H_2SO_4 for later determination of VFA concentration according to the procedures of Holdeman et al. (1977).

Intestinal digesta from the jejunum, cecum and lower colon were collected in plastic zip-lock bags for microbial determination and were immediately flushed with oxygen-free CO_2 . Microbial analysis were not conducted on the stomach digesta. Intestinal digesta were serially diluted in pre-reduced salt media (Holdeman et al., 1977) and then plated onto selective media. Total lactobacillus and *E. coli* were plated onto tomato juice agar⁸ and Levine eosin methylene blue agar⁹, respectively and incubated anaerobically at 37°C for three days.

Data were analyzed with the general linear model program (GLM) of the Statistical Analysis System (SAS, 1988) using the individual pig as the experimental unit. The model contained the main effects of postweaning age, diet, challenge treatment, trial and the interactions. Non-significant interactions were pooled with the error. Comparisons among intestinal sections were conducted by Tukey's studentized range test. Comparisons between challenge days and between dietary treatments were conducted

Research Ionalyzer chloride electrode # 94-17-B and double junction reference electrode # 90-02.

⁸Bacto tomato juice agar, Difco laboratories, # 0031-01-2.

⁹Bacto Levin eosin methylene blue agar, Difco laboratories, # 0075-01-9.

by orthogonal contrasts.

Study 2. Two 15-d trials were conducted to determine whether or not dietary organic acid would decrease the effects of an enterotoxigenic E. coli challenge on growth performance of early weaned pigs. In each trial, 48 crossbred pigs (mean initial age and wt of 21 ± 2 d and 5.5 kg, respectively) were assigned to blocks according to weight and gender and randomly allotted to three dietary treatments and two challenge treatments with the restriction that littermates be balanced across treatments. Dietary organic acid and E. coli challenge treatments were the same as in Study 1 but the challenge was only administered at 3 d postweaning. Pigs were evaluated daily for the incidence of scouring using the aforementioned 5-point scoring system. Pigs were housed two per pen in environmentally-controlled nurseries with wire bottom pens (.6 m x .9 m). Non-challenged and challenged pigs were housed in separate, similar nurseries which were reversed in the second trial. Water and feed were available ad libitum. At 3 d intervals, pigs were weighed and pen feed consumption were recorded. Average daily gain (ADG), avg daily feed intake (ADFI) and efficiency of feed utilization (F:G) were calculated.

Performance data were analyzed using the general linear model program (GLM) of the Statistical Analysis System (SAS, 1988) using pen means as the experimental unit. Scour

scores were similarly analyzed by using pigs as the experimental unit. The model contained the main effects of diet, challenge treatment, trial and the interactions as well as adjustments made for ADG from 1 to 3 d postweaning and body weight at 3 d postweaning. Non-significant interactions were pooled with the error.

Results

pH and Cl⁻. The pH of the control diet was decreased ($P < .001$) by the addition of the organic acids from 6.42 (control diet) to 4.70 (fumaric acid) and to 4.90 (citric acid). Neither organic acids nor E. coli challenge had an appreciable effect ($P < .18$) on the intestinal pH or chloride ion (Cl⁻) concentration for any of the intestinal sections measured (Tables 2 and 3). No significant effect ($P < .34$) of postweaning age was observed for pH in the stomach, jejunum or lower colon digesta. Cecum digesta pH decreased ($P < .002$) from 5 to 16 d postweaning. No treatment interactions were noted ($P < .13$) for intestinal pH (Table 2). The pH of the intestinal digesta increased ($P < .05$) from a low in the stomach digesta to a high in the jejunum and lower colon digesta; the cecum digesta pH was intermediate.

Chloride ion concentration increased ($P < .03$) from 5 to 9 and 16 d postweaning in the jejunum digesta. A diet by postweaning age interaction ($P < .01$) was observed for cecum

digesta Cl⁻ concentration. The Cl⁻ concentration of the control diet was highest on d 5 and lowest on d 16 postweaning compared with the fumaric and citric acid diets. No other diet or postweaning age effects on pH and Cl⁻ were observed for any of the intestinal sections. The Cl⁻ concentration decreased ($P < .05$) from a high in the stomach and jejunum digesta to a low in the cecum and lower colon.

NVA and VFA. Pigs fed the fumaric acid diet had a greater concentration of fumaric and total non-volatile acids (NVA) in the stomach, jejunum and cecum digesta and a greater concentration of succinic acid in the stomach and jejunum digesta compared with pigs fed either the control or citric acid diets (Table 4). The concentration of succinic acid in the lower colon digesta was greater in pigs fed the citric acid diet than pigs fed either the control or fumaric acid diets. The concentration of lactic acid was greater in the upper gastrointestinal tract (stomach and jejunum) as compared with the lower gastrointestinal tract (cecum and lower colon), whereas the concentration of fumaric, succinic and total NVA were greater only in the stomach digesta compared with the other intestinal sections.

The concentration of lactic, succinic and total NVA in the jejunum digesta and the concentration of fumaric acid in the lower colon digesta increased from 5 to 16 d postweaning (Table 5). No effect ($P < .17$) of E. coli challenge

treatment, or dietary treatment by E. coli challenge treatment interaction was observed for NVA, except for an increase ($P < .05$) in the concentration of lactic acid in the jejunum digesta of pigs challenged with E. coli as compared to non-challenged pigs (18.6 vs 9.6 meq/dl - data not shown).

Organic acids supplementation had no effect on the concentration of volatile fatty acids, except for pigs fed the fumaric acid diet having a lower concentration of acetic acid in the jejunum digesta than pigs fed either the control or citric acid diets (Table 6). The concentrations of VFA were greater in the lower gastrointestinal tract than the upper gastrointestinal tract.

The concentration of acetic acid increased in the stomach and cecum digesta from 5 to 16 d postweaning (Table 7). The concentration of propionic acid increased in the cecum and lower colon digesta from 5 to 16 d postweaning. Similarly, the concentration of butyric acid increased in cecum and lower colon digesta from 5 to 16 d postweaning. The total concentration of VFA increased in the stomach, cecum and lower colon digesta from 5 to 16 d postweaning.

Pigs challenged with E. coli had greater lower colon concentrations of propionic (76.4 vs 54.0 meq/dl; $P < .01$), butyric (38.3 vs 23.1 meq/dl; $P < .01$) and total VFA (389.8 vs 296.0 meq/dl; $P < .03$) than non E. coli challenged pigs.

No other effects of the E. coli challenge on VFA concentration were observed (data not shown).

Microflora. The number of lactobacilli in the jejunum digesta decreased from 5 to 16 d postweaning, whereas in the lower colon digesta, lactobacilli numbers increased from 5 to 16 d postweaning (Table 8). No significant effect of postweaning age ($P < .18$) was observed for the numbers of lactobacilli in the cecum digesta. No significant effect of organic acid treatment or of E. coli challenge was observed ($P < .13$) for numbers of lactobacilli in any of the intestinal sections.

The number of E. coli generally decreased in the jejunum, cecum and lower colon from 5 to 16 d postweaning (Table 9). Across all challenge days, there was a tendency ($P < .08$) for the E. coli-challenged pigs to have a greater population of E. coli than the non-challenged pigs in the lower colon digesta (means = 8.1 vs 7.8, respectively).

Pigs challenged with E. coli on d 3, 7 and 14 postweaning scoured more severely ($P < .05$) than pigs not challenged; however the difference between the two groups was less after 14 d postweaning than after 3 or 7 d postweaning (Figure 1).

Study 2. Supplementation with organic acids had no effect on growth performance, ADG, avg daily feed intake

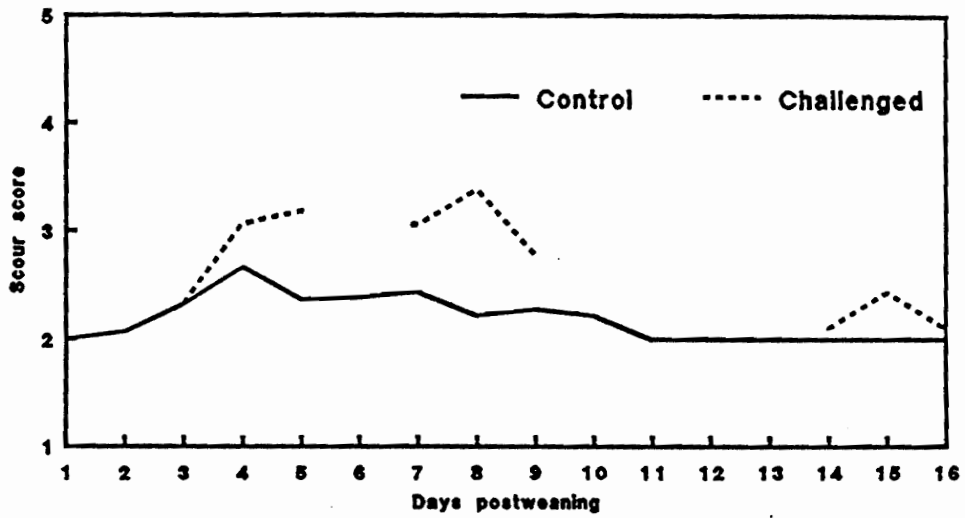


Figure 1. The scour scores of pigs challenged with enterotoxigenic *E. coli* on d 3; 7 or 14 postweaning (Study 1).

(ADFI) or efficiency of feed utilization (F:G), of the challenged and non-challenged weanling pigs (Table 10). The organic acids did not greatly reduce the negative effects of the enterotoxigenic E. coli challenge as indicated by a non-significant ($P < .25$) diet by challenge treatment interaction. The enterotoxigenic E. coli challenge depressed growth performance during 7 to 15 d postweaning; ADG was depressed by 20% and F:G by 19%.

Pigs challenged with E. coli scoured more severely ($P < .001$) than pigs not challenged, with the severity of scouring being greatest between 4 and 11 d postweaning (Figure 2). Organic acid supplementation had no significant effect on the severity or the incidence of scouring.

Discussion

pH and Cl⁻. Burnell et al. (1988) and Scipioni et al. (1979) observed a numerical decrease in small intestinal pH with the incorporation of citric acid into the starter diet. We did not, however, observe any changes in pH of the gastrointestinal tract digesta when fumaric or citric acid was added to the diet even though dietary pH was lowered with the addition of these organic acids. These findings are in agreement with our previous report (Risley et al., 1990). An explanation of the difference between our findings and those reported by Burnell et al. (1988) and Scipioni et al. (1979) may be related to the type of diet

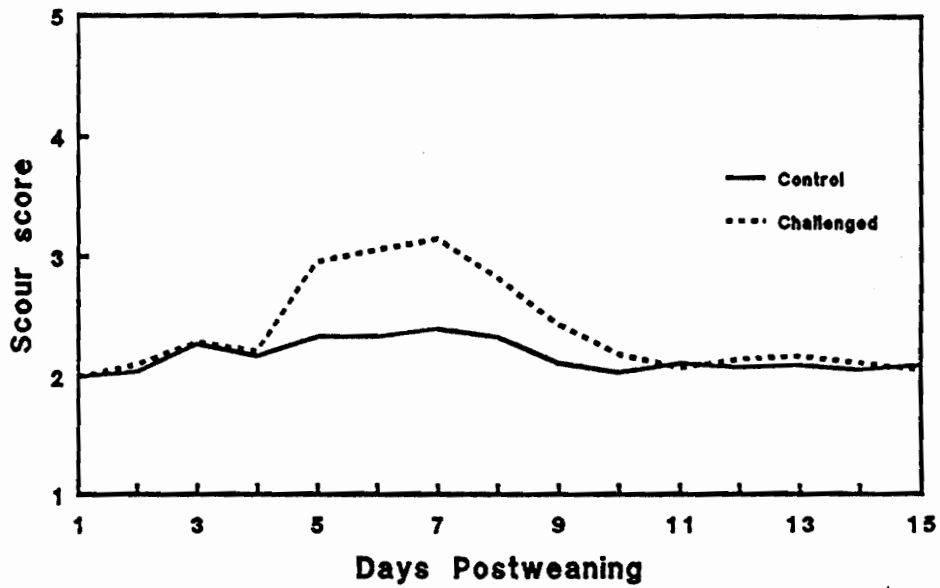


Figure 2. The scour scores of pigs challenged with enterotoxigenic *E. coli* on d 3 postweaning (Study 2).

used. In our studies, a simple diet (no added whey) was fed, whereas they fed a complex diet that included whey. The incorporation of the whey probably increased the level of digestibility of the diet (Tokach et al., 1989), and therefore reduced the buffering capacity of the soybean meal (Maner et al., 1962) and allowed the citric acid to reduce the intestinal pH.

The chloride ion concentration of the intestinal digesta was expected to reflect the secretion of gastric hydrochloric acid, but probably was more indicative of the level of chloride ingested with the diet. If chloride ion concentration reflects HCl secretion, then we would have expected an increase in stomach Cl⁻ with increasing age (Kidder and Manner, 1978), but this trend was not observed.

NVA and VFA. As described by Kenworthy and Crabb (1963), the increase in the concentration of VFA indicates the increasing availability of fermentable substrate with increased feed consumption and the establishment of a fermentation flora in the gastrointestinal tract. The increased VFA concentration in the lower colon of E. coli-challenged pigs may reflect the increased rate of passage associated with scouring and(or) a transient colonization. Increasing the rate of passage would limit the time for absorption of VFA and result in the washing of VFA into the lower colon (Argenzio, 1981). Etheridge et al. (1984) also

observed increased VFA concentrations in the feces of scouring pigs which compares with our measurements from the lower colon.

Newly weaned pigs normally have a reduced level of feed intake shortly after weaning (Fenten et al., 1985; Burnell et al., 1988; Ogunbameru et al., unpublished data). In the pigs fed fumaric acid, the concentration of this acid in the stomach digesta should indicate the relative amount of diet consumed. The concentration of fumaric acid of the stomach digesta of these pigs decreased from d 5 to d 9 and then increased to d 16 postweaning, indicating feed consumption followed the same pattern. Decrease in fumaric acid and probably feed consumption, occurred concurrently with the greatest incidence of scouring. Coincident and probably interrelated, a reduction in feed intake can result in scouring (Richard and Fraser, 1961). Decrease in feed consumption may be the underlying cause for the postweaning lag phase. The subsequent increases in the concentration of lactic, succinic and total NVA and VFA over time is a direct result increased feed consumption resulting in more available substrate for bacterial fermentation and the establishment of an active gastrointestinal flora.

The E. coli challenge treatment did not change the concentration of the NVA, except for increasing the concentration of lactic acid in the jejunum. This increase

in lactic acid may reflect E. coli metabolic activity. The increase in the concentration of lactic acid may be a direct result of the enterotoxigenic E. coli proliferating in the gut due to lactic acid being a major product of E. coli (Clark, 1989).

The feeding of organic acids may serve as an energy source for the weanling pig. This hypothesis is supported by the observation that the concentration of fumaric acid steadily decreased from the stomach to the lower colon, thus suggesting intestinal absorption and(or) microbial utilization. Fumaric acid has been reported to have the energy efficiency equal to that of glucose (Kirchgessner and Roth, 1982) and therefore fumaric acid and the other NVA could serve as an energy supplement to the weanling pig. Microbial utilization of fumaric acid is demonstrated by the increase in succinic acid in the stomach of pigs fed fumaric acid.

Microflora. The E. coli challenge did not affect the concentration of lactobacilli, but generally increased the population of E. coli in the intestinal digesta. Although enteropathogenic E. coli can displace non-pathogenic bacteria (Stevens, 1963; Bertschinger et al., 1972), our data suggest that the challenged E. coli were transient. The increase in scouring was indicative that the enteropathogenic E. coli challenge was active in the gut and

as described by Richard and Fraser, (1961) the small intestine was flaccid and dilated and contained large amounts of fluid in scouring pigs. We are unable to determine whether the total number of bacteria increased in the challenged pigs because total gut contents was not measured.

Pigs challenged with E. coli scoured more severely than non-challenged pigs, but the severity of scouring decreased as the age at challenging increased. This decrease in scouring with increasing age suggests that as the pig matures, the pig is more capable of handling the stress of the E. coli challenge and probably indicates that the pig is no longer under the stresses of weaning by 14 d postweaning.

Study 2. The E. coli challenge treatment produced scouring, which probably caused the reduction in ADG and F:G. However, organic acid supplementation did not prevent the E. coli challenge from causing scouring or a growth lag. Unlike an earlier report (Risley et al., 1990), we were unable to confirm an increased growth performance. The apparent discrepancy in results may be related to the duration of the experiments, 15 d vs 35 d.

Implications

The feeding of fumaric and citric acids, have been reported to improve growth performance in weanling pigs, but their effect on the incidence of postweaning scouring or

other mechanisms of action is not well documented. Findings from this research indicate that supplemental organic acids are unable to reduce gastric pH, change intestinal chloride ion concentration, reduce the population of E. coli or prevent the adverse effects (scouring and reduction in growth performance) in weanling pigs orally challenged with an enterotoxigenic E. coli.

TABLE 1. PERCENTAGE COMPOSITION OF DIETS

Item	Diet ^a		
	Control	Fumaric acid	Citric acid
Ground corn	64.20	62.50	62.50
Soybean meal (44% CP)	33.11	33.31	33.31
Limestone	.60	.58	.58
Defluorinated phosphate	1.42	1.44	1.44
Salt	.30	.30	.30
Trace mineral premix ^b	.07	.07	.07
Vitamin premix ^c	.25	.25	.25
Selenium premix ^d	.05	.05	.05
Fumaric/citric acid	—	1.50	1.50

^aCalculated to supply 20% crude protein, .80% Ca and .65% P.

^bSupplied per kg of diet: 105 mg Zn, 123 mg Fe, 42 mg Mn, 12 mg Cu, and 1.4 mg I.

^cSupplied per kg of diet: 4,400 IU vitamin A, 440 IU vitamin D, 11 IU vitamin E, 4.4 mg riboflavin, 22 mg d-pantothenic acid, 22 mg niacin, 489.5 mg choline, .022 mg vitamin B₁₂, .5 mg menadione and .44 mg d-biotin.

^dSupplied .3 mg Se per kg of diet.

TABLE 2. INTESTINAL PH OF NON-CHALLENGED AND CHALLENGED WEANLING PIGS ON DAYS 5, 9 OR 16 POSTWEANING

Item ^{a,b}	Intestinal section			
	Stomach	Jejunum	Cecum ^c	Lower colon
Day 5				
Control	3.75	6.60	6.01	7.16
Challenged	3.58	6.68	5.94	6.72
SEM	.263	.210	.103	.218
Pooled means	3.66	6.64	5.97	6.94
Day 9				
Control	4.37	6.96	5.77	6.80
Challenged	3.71	6.43	5.56	6.86
SEM	.263	.210	.103	.218
Pooled means	4.04	6.69	5.66	6.83
Day 16				
Control	3.74	6.68	5.47	6.74
Challenged	4.01	6.50	5.73	6.63
SEM	.263	.210	.103	.218
Pooled means	3.88	6.59	5.60	6.68

^aEach mean value represents nine observations.

^bNo diet, E. coli challenge or interactive effects were observed ($P < .13$).

^cPostweaning age effect ($P < .002$) based on pooled means of sample days.

TABLE 3. INTESTINAL CHLORIDE ION CONCENTRATION (M) OF NON-CHALLENGED AND CHALLENGED WEANLING PIGS ON DAYS 5, 9 OR 16 POSTWEANING

Item ^{a,b}	Intestinal section			
	Stomach	Jejunum ^c	Cecum ^d	Lower colon
Day 5				
Control	.101	.082	.035	.025
Challenged	.078	.079	.037	.035
SEM	.015	.023	.008	.008
Pooled means	.089	.080	.036	.030
Day 9				
Control	.079	.104	.040	.028
Challenged	.086	.141	.039	.029
SEM	.015	.023	.008	.008
Pooled means	.083	.122	.040	.028
Day 16				
Control	.100	.114	.041	.031
Challenged	.105	.140	.046	.037
SEM	.015	.023	.008	.008
Pooled means	.103	.127	.043	.034

^aEach mean value represents nine observations.

^bNo interaction of postweaning age by E. coli challenge treatment or diet by E. coli challenge treatment was observed ($P < .18$).

^cPostweaning age effect ($P < .03$) based on pooled means of sample days.

^dDiet by postweaning age interaction was observed ($P < .01$).

TABLE 4. MEANS OF NON VOLATILE ACIDS (MEQ/DL) OF NON-CHALLENGED AND CHALLENGED WEANLING PIGS FED EITHER THE CONTROL, 1.5% FUMARIC OR 1.5% CITRIC ACIDS DIETS

Item ^a	Intestinal section			
	Stomach	Jejunum	Cecum	Lower colon
Pyruvic acid				
Control	0.0	0.0	.1	0.0
Fumaric	0.0	0.0	.4	0.0
Citric	.1	0.0	.3	.4
SEM	.03	.01	.28	.15
Pooled means	0.0	0.0	.3	.1
Lactic acid				
Control	11.3	10.5	0.0	.3
Fumaric	9.2	19.3	.1	0.0
Citric	11.1	12.4	3.1	0.0
SEM	3.90	3.79	1.74	.17
Pooled means	10.5 ^b	14.1 ^b	1.0 ^c	.1 ^c
Fumaric acid				
Control	0.0	0.0	.5	9.5
Fumaric	84.4***	25.5***	15.5**	13.3
Citric	.3	.2	1.5	3.3
SEM	10.98	4.72	3.49	3.30
Pooled means	28.2 ^b	8.6 ^c	5.8 ^c	8.7 ^c
Succinic acid				
Control	7.6	1.9	.9	.4
Fumaric	19.1*	2.8	2.2	1.1
Citric	7.0	0.0	2.9	4.4*
SEM	3.26	.78	1.37	1.15
Pooled means	11.3 ^b	1.6 ^c	2.0 ^c	1.9 ^c
Total				
Control	18.9	12.4	1.4	10.2
Fumaric	112.7***	47.6**	18.2*	14.4
Citric	18.5	12.6	7.7	8.0
SEM	10.55	5.94	4.73	3.65
Pooled means	50.0 ^b	24.2 ^c	9.1 ^c	10.9 ^c

^aEach mean value represents three observations.

^{b,c}Means within a row with different superscript letters differ (P < .05). Tukey's studentized range test was used.
 *, **, *** Mean differs from control diet (P < .05, .01, .001).
 Determined by using non-orthogonal contrasts.

TABLE 5. MEANS OF NON VOLATILE ACIDS (MEQ/DL) OF NON-CHALLENGED AND CHALLENGED WEANLING PIGS ON DAYS 5, 9 OR 16 POSTWEANING

Item ^a	Intestinal section			
	Stomach	Jejunum	Cecum	Lower colon
Pyruvic acid				
d 5	0.0	0.0	0.0	.1
9	0.0	0.0	.3	0.0
16	.1	0.0	.5	.3
SEM	.03	.01	.28	.15
Lactic acid				
d 5	7.0	4.6	3.1	0.0
9	17.4	15.1*	0.0	0.0
16	7.2	22.5***	.1	.3
SEM	3.90	3.79	1.74	.17
Fumaric acid				
d 5	24.9	7.6	.9	1.0
9	8.6	2.3	9.9	14.5**
16	51.1	15.9	6.6	10.7*
SEM	10.98	4.72	3.49	3.30
Succinic acid				
d 5	7.3	0.0	.6	2.9
9	15.3	1.9	2.2	1.7
16	11.2	2.8*	3.3	1.3
SEM	3.26	.79	1.37	1.15
Total				
d 5	39.2	12.2	.1	4.0
9	41.3	19.3	.4	16.2
16	69.6	41.2**	.3	12.5
SEM	10.55	5.94	.28	3.65

^aEach mean value represents 3 observations.

*, **, *** Mean differs from d 5 (P < .05, .01, .001).

Determined by using non-orthogonal contrasts.

TABLE 6. MEANS OF VOLATILE FATTY ACIDS (MEQ/DL) OF NON-CHALLENGED AND CHALLENGED WEANLING PIGS FED EITHER THE CONTROL, 1.5% FUMARIC OR 1.5% CITRIC ACIDS DIETS

Item ^a	Intestinal section			
	Stomach	Jejunum	Cecum	Lower colon
Acetic acid				
Control	104.1	73.5	209.5	222.3
Fumaric	93.2	29.0**	197.5	214.1
Citric	111.7	65.4	190.3	233.0
SEM	12.01	11.39	18.38	24.33
Pooled means	103.0 ^c	56.0 ^d	199.1 ^b	223.1 ^b
Propionic acid				
Control	12.6	1.3	69.5	57.1
Fumaric	9.9	2.9	85.1	66.4
Citric	10.4	8.0	71.1	72.1
SEM	3.76	2.99	7.92	7.60
Pooled means	11.0 ^c	4.1 ^c	75.2 ^b	65.2 ^b
Butyric acid				
Control	2.9	.2	29.0	26.0
Fumaric	5.2	1.7	44.1	32.9
Citric	9.6	7.5	46.6	33.4
SEM	2.65	4.09	8.82	4.89
Pooled means	5.9 ^c	3.1 ^c	39.9 ^b	30.7 ^b
Total ^e				
Control	121.7	81.0	320.1	335.9
Fumaric	113.1	36.5	340.8	333.2
Citric	133.9	86.1	324.0	359.5
SEM	15.54	16.32	30.50	35.65
Pooled means	122.9 ^c	67.8 ^c	328.3 ^b	342.9 ^b

^aEach mean value represents three observations.

*, **, *** Mean differs from control diet (P < .05, .01, .001).

Determined by using non-orthogonal contrasts.

^{bcd}Means within a row with different superscript letters differ (P < .05). Tukey's studentized range test was used.

^eTotal volatile fatty acid concentration also includes formic, isobutyric, isovaleric, valeric, isocaproic and caproic acids.

TABLE 7. MEANS OF VOLATILE FATTY ACIDS (MEQ/DL) OF NON-CHALLENGED AND CHALLENGED WEANLING ON DAYS 5, 9 OR 16 POSTWEANING

Item ^a	Intestinal section			
	Stomach	Jejunum	Cecum	Lower colon
Acetic acid				
d 5	75.6	44.4	161.9	187.2
9	102.9	49.2	211.7	214.8
16	130.5**	74.3	223.7*	267.3
SEM	12.01	11.39	18.38	24.33
Propionic acid				
d 5	10.0	6.1	46.4	43.2
9	11.0	6.1	68.1	48.9
16	11.9	0.0	111.2***	103.6***
SEM	3.76	2.99	7.92	7.60
Butyric acid				
d 5	4.1	2.4	23.0	21.8
9	11.2	7.0	35.3	25.2
16	2.4	0.0	61.4**	45.2**
SEM	2.65	4.09	8.82	4.89
Total ^b				
d 5	92.4	61.9	247.6	266.1
9	130.1	64.6	324.2	310.0
16	146.2*	77.1	413.2***	454.5***
SEM	15.54	16.32	30.50	35.65

^aEach means value represents 3 observations.

*, **, *** Mean differs from d 5 (P < .05, .01, .001).

Determined by using non-orthogonal contrasts.

^bTotal volatile fatty acid concentration also includes formic, isobutyric, isovaleric, valeric, isocaproic and caproic acids.

TABLE 8. INTESTINAL LACTOBACILLUS NUMBERS OF NON-CHALLENGED AND CHALLENGED WEANLING PIGS ON DAYS 5, 9 OR 16 POSTWEANING

Item ^{b,c}	Intestinal section ^a		
	Jejunum ^d	Cecum	Lower colon ^e
Day 5			
Control	8.78	9.16	8.66
Challenged	8.73	9.17	9.01
SEM	.250	.172	.224
Pooled means	8.75	9.16	8.83
Day 9			
Control	7.87	9.59	9.54
Challenged	7.97	9.13	9.31
SEM	.250	.172	.224
Pooled means	7.92	9.36	9.43
Day 16			
Control	7.60	9.56	9.42
Challenged	7.54	9.42	9.72
SEM	.250	.172	.224
Pooled means	7.62	9.49	9.57

^aMicrobial determination was not conducted on stomach digesta.

^bEach mean value represents nine observations.

^cMicrobial counts are expressed as the log₁₀ of the cultural count per gram of wet digesta.

^dPostweaning age effect ($P < .001$) based on pooled means of sample days.

^ePostweaning age effect ($P < .004$) based on pooled means of sample days.

TABLE 9. INTESTINAL ESCHERICHIA COLI NUMBERS OF NON-CHALLENGED AND CHALLENGED WEANLING PIGS ON DAYS 5, 9 OR 16 POSTWEANING

Item ^{b, c}	Intestinal section ^a		
	Jejunum ^d	Cecum ^e	Lower colon ^f
Day 5			
Control	6.43	7.69	8.14
Challenged	6.82	8.46	8.97
SEM	.404	.302	.242
Pooled means	6.63	8.08	8.55
Day 9			
Control	6.21	8.10	7.72
Challenged	5.82	7.97	7.80
SEM	.404	.302	.242
Pooled means	6.01	8.03	7.76
Day 16			
Control	5.57	7.19	7.55
Challenged	5.71	7.46	7.66
SEM	.404	.302	.242
Pooled means	5.64	7.33	7.61

^aMicrobial determination was not conducted on stomach digesta.

^bEach mean represents nine observations.

^cMicrobial counts are expressed as the log₁₀ of the cultural count per gram of wet digesta.

^dPostweaning age effect ($P < .07$) based on pooled means of sample days.

^ePostweaning age effect ($P < .02$) based on pooled means of sample days.

^fPostweaning age effect ($P < .001$) based on pooled means of sample days.

TABLE 10. PERFORMANCE DATA OF NON-CHALLENGED AND CHALLENGED WEANLING PIGS FED EITHER THE CONTROL, 1.5% FUMARIC OR 1.5% CITRIC ACID DIET

Item ^{a,b}	Diet			Pooled Means	SEM
	Control	Fumaric	Citric		
Initial wt, kg	5.50	5.49	5.48	5.49	.015
Final wt, kg	7.75	7.80	7.75	7.76	.109
ADG, g					
Days 1 - 6					
Non-challenged	49	55	56	54	.004
Challenged	55	54	56	55	.004
Days 7 - 15 ^c					
Non-challenged	141	142	114	132	.015
Challenged	107	112	97	105	.015
Feed Intake, g					
Days 1 - 6					
Non-challenged	116	126	112	118	.009
Challenged	114	113	107	111	.009
Days 7 - 15					
Non-challenged	217	228	205	217	.015
Challenged	201	222	193	205	.015
Feed to Gain					
Days 1 - 6					
Non-challenged	2.37	2.29	2.00	2.19	1.54
Challenged	2.07	2.09	1.91	2.02	1.54
Days 7 - 15 ^c					
Non-challenged	1.54	1.61	1.80	1.64	.476
Challenged	1.88	1.98	1.99	1.95	.476

^aEach mean value represents eight pen means (two pigs per pen).

^bPigs were challenged with 10^{10} of *E. coli* on d 3 postweaning.

^cChallenge treatment effect ($P < .05$).

Chapter VII

SUMMARY AND CONCLUSIONS

The supplementation of fumaric or citric acid to diets of weanling pigs produced variable improvements in growth performance (ADG and feed efficiency) as did the incorporation of a microbial product (*Lactobacillus acidophilus*). However, the ability of the organic acids to significantly lower intestinal pH, increase chloride ion (Cl^-) concentration or modify the metabolism of the microflora in weanling pigs was not substantiated in Study 1.

Fumaric or citric acid did not significantly change the intestinal pH, Cl^- concentration, microflora or the metabolism of the microflora in weanling pigs in Study 3. Changes in these intestinal measurements were observed as the pig matured from 2 d pre-weaning to 21 d postweaning. Most notable was the decrease in stomach, cecal and lower colon pH. The stomach and jejunum counts of lactobacilli and stomach anaerobic cultural counts decreased after weaning (d 3 postweaning). Stomach and jejunum counts of *Escherichia coli* increased at d 7 postweaning. Counts of clostridia decreased over time for all intestinal sections measured. These changes in microflora populations reflect the normal transition of intestinal bacteria from an

bacteria population.

The concentration of VFA was not significantly changed by feeding the organic acids, but changes were observed with increasing age. The concentration of acetic acid and total VFA increased in the stomach, jejunum and lower colon from pre-weaning to 21 d postweaning. Increases in propionic acid in the cecum and lower colon and increases in butyric acid in the lower colon were also observed as the pig matured, indicating the establishment of a more mature fermentation flora in the gut.

Pigs fed fumaric acid had an increased concentration of fumaric acid in the stomach, however the concentration of fumaric acid decreased in the jejunum to the lower colon. The decrease in fumaric acid suggests the direct absorption of fumaric acid as an energy source by the pig and(or) the utilization of fumaric acid by the intestinal flora.

The ability of dietary fumaric or citric acid to favorably alter the effects of an oral challenge of an enteropathogenic *E. coli* on intestinal pH, Cl⁻ concentration, microflora, scouring or growth performance in weanling pigs was not observed in Studies 4 and 5. No significant effect of dietary organic acid or *E. coli* challenge treatment was observed on intestinal pH, Cl⁻ concentration or counts of lactobacilli. The *E. coli* challenge treatment tended to increase the number of *E. coli*

in the intestinal sections, but these increases were not significant. Similar effects, as observed in Study 3, from increasing age on intestinal pH and counts of lactobacilli and *E. coli* were observed also.

The *E. coli* challenge treatment increased the severity of scouring in Studies 4 and 5, and in Study 5, the *E. coli* challenge depressed ADG and feed efficiency. Feeding organic acids had no significant effect on growth performance or reduced the severity of scouring from the *E. coli* challenge. The severity of scouring, induced by the *E. coli* challenge, was greater after d 3 and 7 than d 14 postweaning. The differences in scouring suggest that the weanling pig is more susceptible to an additional stress on d 3 and 7 postweaning than on d 14 postweaning.

No significant effect of dietary organic acid or *E. coli* challenge treatment was observed on the concentration of intestinal VFA, except for increases in the concentration of acetic, propionic, butyric and total VFA in the lower colon in pigs challenged with *E. coli*. The increases in VFA concentrations are probably due to the washing out of the VFA by the increase in scouring.

The concentration of fumaric and succinic acid was greater in pigs fed fumaric acid than control or citric acid fed pigs. No other observable dietary effects on intestinal NVA concentration were noted. The concentration of jejunal

lactic acid was increased in pigs challenged with *E. coli*, indicating that the challenge was metabolically active in the gut.

In conclusion, the supplementation of weanling pig diets with fumaric or citric acid can improve growth performance. However, the generally hypothesized mechanism of action of organic acids, namely the lowering of intestinal pH and the reduction of *E. coli*, was not substantiated. An alternative mode of action was suggested by this research that organic acids may serve as an additional energy source for the weanling pig. However, more research is needed in this area.

Results discussed also suggest that the low feed intake that normally occurs after weaning may be a direct cause for the poor growth performance and scouring. Therefore, management practices that increase the energy intake of weanling pigs should reduce the severity of the postweaning lag.

CHAPTER VIII

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APPENDIX TABLES

DIETS

APPENDIX TABLE 1. COMPOSITION OF SOW RATION.^a

Ingredients	Percentage
Ground corn	81.64
Soybean meal (44% CP)	15.60
Defluorinated phosphate	1.57
Limestone	0.64
Salt, plain	0.25
Vitamin-selenium premix ^b	0.25
Trace mineral premix ^c	0.05
Total	100.00

^aCalculated to contain 14.0% crude protein, 0.8% Ca and 0.6% P.

^bSupplied per lb of vitamin premix: 800,000 IU vitamin A, 80,000 IU vitamin D₃, 2,000 IU vitamin E, 200 mg vitamin K, 800 mg riboflavin, 4,000 mg pantothenic acid, 4,000 mg niacin, 4,000 ug vitamin B₁₂, 80 g choline, 80 mg biotin and 18.2 mg selenium. Supplied per kg of vitamin premix: 1,763,698 IU vitamin A, 176,370 IU vitamin D₃, 4,409 IU vitamin E, 441 mg vitamin K, 1,764 mg riboflavin, 8,818 pantothenic acid, 8,818 mg niacin, 8,818 ug vitamin B₁₂, 176 g choline, 176 mg biotin, and 40.1 mg selenium.

^cContained 20% Zn, 10% Fe, 5.5% Mn, 1.1% Cu and 0.15% I.

APPENDIX TABLE 2. LEAST SQUARES MEANS OF THE PH OF THE
CONTROL, 1.5% FUMARIC AND 1.5% CITRIC ACID DIETS

Item ^a	Diet			SEM
	Control	Fumaric Acid	Citric Acid	
pH	6.38 ^b	4.70 ^c	4.90 ^d	.004

^aEach treatment mean represents 3 replicates per diet.

^{bcd}Diets with different superscripts differ ($P < .001$).

PROCEDURES

APPENDIX TABLE 3. PROCEDURE FOR SACRIFICING ANIMAL AND OBTAINING INTESTINAL DIGESTA SAMPLES

-
- 1) Electrocute pig with 120 volts for 20 seconds and exsanguinated by severing the vena cava.
 - 2) Rinse pig and place on an alcohol-cleaned stainless steel table. Make an incision into the abdominal cavity on the midline from the sternum to the rectum.
 - 3) Remove the viscera, isolate, and doubly ligate the various intestinal sections, entire stomach, 1 m of jejunum, entire cecum and .3 m of large intestine near rectum.
 - 4) Aseptically remove the digesta contents of the intestinal section and place in either a 50 ml centrifuge tube or a plastic zip-lock bag.
-

APPENDIX TABLE 4. PROCEDURE FOR PH DETERMINATION OF
INTESTINAL DIGESTA SAMPLES

-
- 1) Add 2 g of intestinal digesta sample to 18 ml of dH₂O and mix well.
 - 2) Read pH by placing electrode^a into sample.
 - 3) Be sure to standardize pH meter^b before reading samples.
-

^aFisher Scientific Company, combination pH electrode, model # 13-639-90.

^bFisher Scientific Company, Accumet pH meter, model # 620.

APPENDIX TABLE 5. PROCEDURE FOR DETERMINATION OF CHLORIDE ION CONCENTRATION IN INTESTINAL DIGESTA SAMPLES

Important: daily check electrodes¹ for proper levels of filling solutions.

1) Prepare 10^{-2} , 10^{-3} and 10^{-4} standards by serial dilution of 0.1 M NaCl. Add 2 ml of ISA² per 100 ml of standard.

2) Place electrodes in 10^{-4} dilution and switch meter³ in the millivolt mode to read function and wait for a stable reading.

3) Rinse electrodes and blot dry. Read the rest of the standard dilutions.

4) Plot standard curve on semilogarithmic paper (millivolt vs concentration).

5) Transfer 2 g of intestinal sample to 18 ml of dH₂O and add 1 ml of ISA.

6) Determine millivolt reading of each sample and determine chloride concentration from standard curve.

¹Orion Ionalyzer, double junction reference electrode, model # 90-02 and Orion Research, specific chloride ion electrode, model # 94-17-B.

²Ion strength adjuster: 5M NaNO₃.

³Fisher Scientific Company, Accumet pH meter, model # 620.

APPENDIX TABLE 6. PROCEDURE FOR GRAM STAINING (KOPELOFF MODIFICATION) OF INTESTINAL DIGESTA SAMPLES

- a) Smear .1 ml of 10^{-3} dilution of digesta sample evenly on microscope slide in a 1 cm square. Air dry and heat fix.
 - b) Flood slide with crystal violet and add 5 drops of a 5% (w/v) solution of sodium bicarbonate. Wait 2 minutes and then wash with distilled water.
 - c) Flood slide with gram's iodine. Wait 2 minutes and then wash with distilled water.
 - d) Wash slide with decolorizing solution and then wash with distilled water.
 - e) Flood slide with safranin. Wait 45 seconds and then wash with distilled water.
 - f) Air dry - do not blot.
 - h) Calculate the percentage of gram positive cocci and rods and gram negative cocci and rods present.
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APPENDIX TABLE 7. PROCEDURE FOR DIRECT MICROSCOPIC CLUMP
COUNTS (DMCC)

- a) Dispense .1 ml of 10^{-3} dilution of digesta onto a bacterial counting chamber.
 - b) Place a cover slip on chamber and view under a phase contrast microscope.
 - c) Count the bacteria in 20 squares and determine average number of bacteria per square.
 - d) Multiply average number of bacteria per square by 2×10^7 (correction factor) and the dilution used to determine number of bacteria per gram.
-

APPENDIX TABLE 8. PROCEDURE FOR DETERMINATION OF TOTAL ANAEROBIC BACTERIA, LACTOBACILLUS, CLOSTRIDIA AND ESCHERICHIA COLI FROM INTESTINAL DIGESTA SAMPLES

- 1) Aseptically place approximately 5 g of intestinal sample into a plastic zip-lock bag.
- 2) Flush bag 3 times with oxygen-free CO₂ and then knead sample throughout bag.
- 3) Under CO₂ flushing, transfer 1 g of sample to 9.0 ml of pre-reduced salts media and make 10⁻², 10⁻³, 10⁻⁵ and 10⁻⁷ dilutions.
- 4) Pipet into roll tubes containing pig fecal extract agar, dilutions 10⁻⁶ to 10⁻⁹ for determination of total bacteria.
- 5) Plate out dilutions 10⁻⁵ to 10⁻⁸ onto the various media for determination of lactobacillus¹, clostridia² and E. coli³.
- 6) Incubate media plates anaerobically at 35°C for clostridia for 48 h and count colonies that exhibit lipase and(or) lecithinase activity and stain as gram positive rods with or without spores.
- 7) Incubate the rest of the media plates anaerobically at 35°C for 3 d and count colonies:
 - a) lactobacillus - use plates containing 30 to 300 colonies and only count colonies that are small to medium size, rough, raised, opaque color and stain as gram positive rods.
 - b) E. coli - use plates containing 30 to 300 colonies and only count colonies that are small, round, and have a greenish metallic sheen and stain as gram negative rods.
- 8) Incubate roll tubes for 5 d at 35°C and count tubes containing 30 to 300 colonies and correct the number of bacteria for the amount of sample used.

¹Bacto tomato juice agar, Difco laboratories, # 0031-01-2.

²Bacto McClung Toabe agar, Difco laboratories, # 0941-01, plus 10% Bacto egg yolk enrichment, Difco laboratories, # 3347-72 and 10 mg/liter Neomycin sulfate, Sigma, #N 6386.

³Bacto Eosin methylene blue, Levine Difco laboratories, # 0075-01-9.

APPENDIX TABLE 9. PROCEDURE FOR DETERMINATION OF VOLATILE FATTY ACIDS FROM INTESTINAL DIGESTA SAMPLES

A. Volatile fatty acids

1) Prepare ether extract

i) pipet 1 ml of the acidified sample into test tube (12 x 75 mm). Add 0.6 ml of 50% H_2SO_4 , 0.4 g NaCl and 1 ml ethyl ether; stopper tube.

ii) Mix by inverting 20 times.

iii) Break emulsion, if necessary, by brief centrifugation.

iv) Pipet ether layer off. Add anhydrous $MgSO_4$ or $CaCl_2$ to equal one half the volume of ether, stopper and wait 10 minutes.

2) Inject about 14 microliters of ether extract into column.

B. Methyl derivatives of pyruvate, lactate, fumarate and succinate

1) Prepare methyl derivatives

i) Pipet 1 ml of the acidified sample into test tube (12 x 75 mm).

ii) Add 2 ml of methanol and 0.6 ml of 50% H_2SO_4 ; stopper tube and heat at $55^{\circ}C$ for 30 minutes or at room temperature overnight.

iii) Add 1 ml water and 0.5 ml chloroform, replace stopper and invert 20 times.

iv) Break emulsion, if necessary, by brief centrifugation.

v) Draw off chloroform layer.

vi) Inject 14 microliters into column. After 20 samples recondition column by injecting 14 microliters of methanol.

APPENDIX TABLE 10. PROCEDURE FOR PREPARING ENTEROTOXIGENIC
ESCHERICHIA COLI PIG CHALLENGE

Use one of two strains obtained from the Penn State
Reference Center: ETEC 0157:K88ac:H13 or 0149:K91:K88ac:NM

Preparation of cells:

- 1) Inoculate 7 ml of trypticase soy broth (TSB) with ETEC from a stock plate and incubate aerobically at 37°C for 18 - 24 h.
 - 2) Use culture from step 1 and inoculate a 4 l flask containing 2 l of TSB and a stir bar. Check for contamination on a trypticase soy agar (TSA) plate.
 - 3) Incubate for approximately 18 h at 37°C while stirring.
 - 4) Harvest cells by centrifugation (8,000 x g) for 10 min.
 - 5) Suspend pellets in 4x TSB plus 10% glycerol and store 1 ml aliquots at -70°C in cryovials. Test one vial for viability, purity and plate count.
 - 6) Prior to challenging, test one vial for purity and plate count.
 - 7) Remove an appropriate volume of ETEC culture to inoculate the pig with approximately 10^{11} organisms and suspend into 5 ml of TSB.
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APPENDIX TABLE 11. ABBREVIATIONS USED

ADFI	- average daily feed intake
ADG	- average daily gain
Cl-	- chloride ion
d	- day
dH ₂ O	- distilled water
ETEC	- enterotoxigenic Escherichia coli
F:G	- feed to gain ratio (feed efficiency)
ISA	- ion strength adjuster
NVA	- non-volatile acid
TSA	- trypticase soy agar
TSB	- trypticase soy broth
VFA	- volatile fatty acids

ANIMAL HUSBANDRY

Gestating sows, housed on partially slotted concrete floors in a ventilated confinement or curtain sided building, were limit-fed 1.8 to 2.7 kg per day of a corn-soybean basal diet (Appendix Table 1) with water available at all times. These sows were thoroughly washed, disinfected with Novasil solution and then moved to individual farrowing crates 108 to 111 d post-coitum. After farrowing, the basal diet was increased to 6.5 to 7.5 kg per day and fed twice daily.

Farrowing crates consisted of solid flooring under the sow with plastic coated slotted flooring in the front and back of the crates. The farrowing room was maintained at a temperature of 18 to 22°C with proper ventilation and space heaters. Supplemental heat was provided to the creep area by overhead heat lamps and rubber mats.

Gilts were vaccinated four weeks prior to breeding for Leptospirosis (5-way vaccine), parvovirus, Bordetella bronchiseptica, Pasteurella multocida, Erysipelas and Escherichia coli. This was repeated two weeks prior to breeding for gilts, as well as sows, with the addition of fenbendazole to the feed for deworming. Piglets were given 2 ml of iron dextran (100 mg of iron per ml) at 3 d of age. At 7 and 21 d of age, piglets were vaccinated for Bordetella bronchiseptica, Pasteurella multocida and Erysipelas. Prior

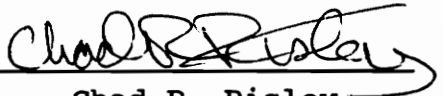
to weaning, litters were dewormed with fenbendazole.

All nursing pigs were weaned into environmentally-controlled nurseries which were maintained at 27 to 30°C for the first two weeks after weaning and then decreased 2°C each week thereafter. Feed and water was available ad libitum for all the studies.

VITA

Chad R. Risley, son of Charles R. and Dora A. Risley, was born on August 29, 1963 in Albany, New York. In 1981, he graduated from Columbia High School, located in East Greenbush, New York. He continued his education at the College of Agriculture and Life Sciences at Cornell University in Ithaca, New York. He graduated from Cornell University in 1985 with a Bachelor's degree in Animal Science. He then relocated to Blacksburg, Virginia and attended the College of Agriculture and Life Sciences at Virginia Polytechnic Institute and State University. He received a Master's degree in Animal Science in 1987 and his Doctorate in Animal Science in 1990.

He is an active member of several professional societies including American Society of Animal Sciences, American Association for the Advancement of Science, American Registry of Professional Animal Scientists, Virginia Academy of Science, Gamma Sigma Delta and Phi Sigma Society-Alpha Psi chapter.


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