5-1998

The effect of volatile fatty acid inclusion in drinking water on intestinal microflora, VFA, pH, dry matter, and performance in weanling pigs

Robert Jason Vickers

To the Graduate Council:

I am submitting herewith a thesis written by Robert Jason Vickers entitled "The effect of volatile fatty acid inclusion in drinking water on intestinal microflora, VFA, pH, dry matter, and performance in weanling pigs." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

Alan G. Mathew, Major Professor

We have read this thesis and recommend its acceptance:

Kelly Robbins, Neal Schrick

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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The Effect of Volatile Fatty Acid Inclusion In Drinking Water on Intestinal Microflora, VFA, pH, Dry Matter, and Performance In Weanling Pigs

A Thesis
Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville

Robert Jason Vickers
May 1998

ACKNOWLEDGMENTS

There are many people that I am indebted to for their help and support during my graduate career at the University of Tennessee. I would like to express great appreciation and admiration to my major professor, Dr. Alan Mathew for his guidance and friendship. He provided invaluable advice and encouragement throughout my educational development. His lasting friendship has been genuine and sincere making the past two years exceptional. To my graduate committee members, Dr. Kelly Robbins and Dr. Neal Schrick, I also extend my gratitude. Both have made my graduate career a challenging and a rewarding experience.

I would like to thank Miss Susan Chattin and Miss Rose Clift for their technical support and aid in the laboratory. Their assistance was vital to the completion of my graduate research.

I also wish to thank my fellow graduate students for their friendship which will long be remembered. Special thanks is extended to my office mate, Mark Franklin for helping with the collection of data and for candid discussion of research issues.

Finally, I would like to thank my family for their

support throughout the years. To my parents, thank you for the love and encouragement to pursue my graduate education. Your guidance has enabled me to achieve such high accomplishments. To my wife, Tammy, thank you for the love, patience, and understanding for the past several years. Without your constant encouragement, I would have given up long ago.

ABSTRACT

In a series of three 17 d replicate trials, a total of 36 ileal cannulated pigs were used to determine the effects of volatile fatty acid (VFA)inclusion in drinking water on ileal microflora, hemolytic *Escherichia coli,* VFA concentrations, pH, dry matter (DM), and pig performance. All pigs were weaned at 21 d-of-age and assigned to one of three treatments including: T1)ad libitum access to water, T2)ad libitum access to water containing acetate(50mM), propionate(5mM), and butyrate (3mM), and T3)ad libitum access to water containing acetate(25mM), propionate(2.5mM), and butyrate(1.5mM). Individual water intake was measured daily and feed intake was measured twice weekly. All pigs were individually caged in an environmentally controlled room with ad libitum access to a phase starter diet. Ileal samples were collected at 21, 24, 28, 31, 35, and 38 d-of-age. At 38 d-of-age, pigs were sacrificed and contents were collected from the stomach, duodenum, ileum, cecum, and spiral colon. Digesta were analyzed for total *E. coli*, streptococci, lactobacilli, VFA, pH, and DM. Neither water nor feed intake differed between treatments and no differences were observed in microflora concentrations

concentrations between treatments. Average ileal *E. coli* concentrations of 6.23, 5.87, and 6.15, streptococci concentrations of 7.55, 7.39, and 7.34, and lactobacilli concentrations of 7.32, 7.49, and 7.15 $\log_{10}$ CFU/g were observed for T1, T2, and T3 pigs, respectively. Time(day) effects (P = .0001) were observed for *E.coli*, streptococci, and lactobacilli. Additionally, VFA concentrations were unaffected by treatment; however, day effects were observed (P = .0001). Treatment had no effect on ileal pH or DM; however, pH was observed to increase (P = .0001) by day 3 postweaning for all treatments. Furthermore, treatment had no effect on pH in the various gastrointestinal sites. Results indicate that VFA inclusion in drinking water had no significant effect on ileal microflora, VFA, pH, DM, or performance in weanling pigs.

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1. INTRODUCTION

Modern swine producers are faced with many challenges in today's high intensity operations. One of the most significant challenges is the management of newly weaned pigs. Upon weaning, the young pig must overcome numerous obstacles, such as the stress associated with weaning, dietary changes, and adjustment to a new environment, along with a decrease in maternal immunity. These factors may increase the potential for nutritional disorders, disease, poor feed intake and efficiency, all resulting in what is termed as postweaning lag. The resulting lag is and has been routinely treated with subtherapuetic levels of antibiotics. However, the widespread use of antibiotics has raised concerns about antibiotic residues and the possibility of bacterial resistance to these drugs (Harper et al., 1983). Therefore, alternative means for combating postweaning disorders have been sought.

Diet acidification is one possible alternative to the use of antibiotics. Several studies have been conducted to determine the effects of organic acids on improving the performance of weanling pigs. Organic acids such as citric and fumaric acid have been shown to increase performance;

however, these improvements have been variable (Kirchgessner and Roth, 1982; Falkowski and Aherne, 1984; Giesting and Easter, 1985). Additionally, it has been suggested that fermentation acids, such as propionate, play a role in maintaining the gastrointestinal health of newly weaned pigs (Wolin, 1969). Therefore, the objective of this study was to determine the effects of volatile fatty acid inclusion in drinking water of weanling pigs on performance, intestinal microflora, pH, and VFA concentrations.

2. REVIEW OF THE LITERATURE

Economic Impact of Weaning Disorders

The need to more efficiently utilize expensive farrowing facilities has led swine producers to wean piglets at earlier ages (Ravindran and Kornegay,1993). However, weaning at 3-4 weeks, exposes the pigs to nutritional, environmental, and social stresses that usually result in a postweaning lag phase manifested by slow growth, scouring, and general unthriftiness (Ravindran and Kornegay, 1993). Death of nursery pigs has been estimated at 2.5 million of the 98 million pigs produced annually (USDA, 1992). These nursery losses cost U.S. swine producers over 80 million dollars per year, assuming a production cost of $25 per weaned pig and a potential net profit of $10 per finished pig (Rawls, 1993). In addition to lost income due directly to death, chronic diarrhea (scours), poor feed efficiency, and stunted growth associated with weaning disorders result in untold losses due to higher feed and fuel costs, treatment costs, increased days to market, and an interruption in the flow of production.

Postweaning Diarrhea and the Involvement of Pathogenic *Escherichia coli*

Losses due directly to scouring account for 15 percent of total nursery phase pig deaths (USDA, 1997). Bergeland in 1980 determined that *E. coli* were the etiological agent in 48% of the cases of swine diarrhea. Additionally, K88 *E. coli*, a pathogenic variant, has been implicated as a major source of diarrhea in postweaned pigs, with one study showing 72% of strains isolated from pigs greater than 24 days old being K88 positive (Wilson and Francis, 1986). *Escherichia coli* concentrations rapidly increase following weaning with the majority of strains exhibiting hemolysis (Kenworthy and Crabb, 1963). Simultaneous increases in hemolytic and total *E. coli* correspond with the onset of scours (Kenworthy and Crabb, 1963; Chopra et al., 1964). Kenworthy and Allen (1966) concluded that the role of *E. coli* in pathogenesis was secondary to the disturbance in physiological function created by the change in diet. A reduction in small intestinal absorptive area and the appearance of a less mature enterocyte population also increase the susceptibility of the pig to diarrhea and poor performance during the postweaning period (Hampson, 1986).

Gastrointestinal Microflora In The Young Pig

The intestinal tract of newborn piglets is sterile at birth (Kenworthy and Crab, 1963). The tract quickly becomes colonized shortly after birth, which is thought to be enhanced by the high pH of the intestinal contents prior to gut closure (Kenworthy and Crab, 1963; Smith and Jones, 1963). Throughout its entire length, the intestinal tract of healthy pigs is occupied by bacteria, with concentrations of 10^6 to 10^9 CFU/g in the small intestine and 10^9 to 10^{10} CFU/g in the cecum and colon (Kenworthy and Crabb, 1963; Smith and Jones, 1963). It was established that lactobacilli and streptococci were the major intestinal microflora (Briggs et al., 1954). Subsequent work by Smith and Crabb (1961), Kenworthy and Crabb (1963), and Smith and Jones (1963) established that the "normal" flora of the pig were lactobacilli, streptococci, *Bacteroides* spp., and *Escherichia coli*. After weaning, populations of all groups of bacteria decline, with aerobes, lactobacilli, and bacteroides-clostridia declining to about one tenth of the preweaning concentrations (McAllister et al., 1979). The change in diet following weaning may lead to temporary shifts in indigenous microflora and their metabolites,

which may provide a more conducive environment for pathogenic colonization (McAllister et al., 1979). Mathew et al, (1991) found that by day two postweaning (23 d-old-pigs), ileal lactobacilli concentrations exhibited nearly a 1000 fold decrease, which coincided with an increase in pH and an increase in *E. coli* concentrations. In the same study, streptococci concentrations remained between 10^7 and 10^8 CFU/g of intestinal contents until 20 d postweaning (41 d-old-pigs) when concentrations increased approximately 10-fold. Mathew also found that lactobacilli and *E. coli* concentrations, as well as pH, returned to near preweaning levels over the next several days. In addition, similar observations were made by McAllister et al. (1979) and Hampson et al.(1985). Risley et al. (1993) observed a decrease in jejunal lactobacilli concentrations from 5 to 16 days postweaning, whereas concentrations in the lower colon increased during the same time period.

Microbial Fermentation

All species of bacteria ferment some component of the digesta and together produce the microbial bodies and VFA used by the host (Bergman, 1990). Diet can change the metabolic activities of the microorganisms by providing new

or different substrates, thus influencing the amount and nature of the fermentative end products (Hungate, 1968; Schwartz and Gilchrist, 1975). The major substrates for fermentation are complex carbohydrates which consist of cellulose, hemicellulose, pectin, starches, dextran, and soluble carbohydrates such as mono- and disaccharides, as reviewed by Bergman (1990). The principal end products of fermentation are VFA, especially acetate, propionate, butyrate, and gases including carbon dioxide and methane. Lactate can be produced, but it usually is not an important intermediate (Glinsky et al., 1976). However, the primary carbohydrate in sow's milk is lactose which can be converted to lactic acid by lactobacilli present in the stomach, and this appears to be the primary method of gastric acidification in suckling pigs (Easter, 1988).

Volatile Fatty Acid Production

Over the past 50 years, animal studies have shown that VFA are found in the gastrointestinal tracts of all herbivores, most omnivores, and depending on the diet, some carnivores (Bergman, 1990). Elsden et al. (1946) first demonstrated that the concentrations of VFA at different sites in the gastrointestinal tract are a direct function of

the bacterial population and are proportional to the time or extent to which digesta are retained. Total concentrations of VFA in the cecum, large colon, rectum, and feces of all animals have been measured in the range of 30-240 mM but more commonly average 70-120 mM (Bergman, 1990). Barcroft et al. (1944) and Elsden et al. (1946) found the chief sites for production of VFA in the pig to be the cecum and colon; however, significant amounts of VFA were present in the stomach and the small intestine. A more recent study also showed that in the pig, VFA (mainly acetate, propionate, and butyrate) are produced chiefly in the large intestine (Imoto and Namioka, 1978). The molar proportions of acetate to propionate to butyrate in the cecum, colon, or rectum of horses, sheep, and pigs have been found to approximate 70:20:10 (Elsden et al., 1946; Glinsky et al., 1976; Stevens, 1978). Total VFA concentrations have been found to be lower in the upper GI tract (stomach and jejunum) than in the lower GI tract (cecum and lower colon) (Risley et al., 1992). Volatile fatty acid concentrations in pigs have been shown to increase as the proportion of fiber reaching the hindgut increases (Kass et al., 1980). Friend et al. (1962) found that cellulose-supplemented diets generally resulted

in a lower total organic acid content than whey-supplemented diets. Young pigs (12.5 kg) fed a conventional high-concentrate, low fiber diet indicated a substantial degree of microbial digestion in the stomach 2 and 4 h after feeding, with mean VFA concentrations of 40 mM in the cranial half of the stomach and 20 mM in the caudal half (Argenzio and Southworth, 1974). These values returned to normal levels by 8 h after feeding. These researchers also discovered that the lowest concentrations of VFA were found in the cecum 2 h after feeding, but levels increased to 212 mM at 4h.

Volatile Fatty Acid Absorption

Barcroft et al. (1944) showed that the chief sites of VFA production and absorption in the pig are the cecum and colon, but some production and absorption occur in the stomach and small intestine. Among the three major VFA found in the pig large intestine, acetate is produced and absorbed more than propionate or butyrate, and acetate absorption amounts to at least one-half of the total caloric value of the three (Imoto and Namioka, 1978). Each VFA is readily absorbed from all segments of the lower digestive tract, and absorption appears to be mostly passive and

increases linearly with corresponding decreases in pH or increases in VFA concentration (Hollander et al., 1986). The rate of absorption of VFA from the rumen increases with increasing chain length (Danielli et al., 1945). Because VFA are weak acids with a pK of 4.8 and because the pH of the gastrointestinal fermentation chambers is near neutral, 90-99% of the VFA are present as anions rather than as free acids (Bergman, 1990). Absorption of the nondissociated or acid form of the VFA can occur with luminal accumulation of bicarbonate and an increase in pH. The dissociated form is absorbed with the aid of sodium, but with no associated appearance of bicarbonate in the lumen (Stevens et al., 1980). Furthermore, Stevens et al. (1980) determined that both mechanisms have been observed in the colon of ponies, pigs, and humans as well as in the rumen of sheep.

Metabolisim of Volatile Fatty Acids

Metabolism of VFA first occurs at their site of absorption and then by the liver or by peripheral tissues. Stevens (1970) showed that metabolism by the rumen epithelium during absorption accounted for 45% of the acetate, 65% of the propionate, and 85% of the butyrate absorbed from the lumen bath. Most of butyrate is oxidized

to CO_2 or to ketone bodies by rumen epithelium (Pennington, 1952) and by colonic mucosa of pigs (Imoto and Namioka, 1978) during its transport to the blood stream. Between 10-15% of propionate is metabolized by the rumen of cattle; whereas, approximately 50% of propionate is metabolized by the rumen of sheep, giving rise to lactate, CO_2, and probably alanine (Bergman, 1990). Butyrate and propionate not metabolized by epithelial tissue along with most of the acetate are transported to the liver via the portal vein (Bergman, 1990). Because of direct absorption of VFA through the gut epithelium of pigs, portal blood has a higher concentration of total VFA than does hepatic or arterial blood (Imoto and Namioka, 1978). Most of the propionate (95%) is removed from the portal blood by the liver where it is the only VFA that can be used as a major source for glucose production (Steinhour and Bauman, 1988). Ninety to ninety-eight percent of acetate present in the liver enters arterial and peripheral venous blood where it is utilized by peripheral tissues (Bergman, 1990). Additionally, there is substantial gut utilization of acetate from the blood with the majority being used by smooth muscle in the gut wall (Pethick et al., 1981).

Contributions of Volatile Fatty Acids

Investigations have shown that short chain fatty acids (SCFA) play several important roles in maintaining the health of nonruminants. It has been reported that VFA act as the primary energy source for the intestinal mass, contributing up to 70% of its required maintenance needs (Henning and Hird, 1972). Large intestine VFA production has been estimated to contribute between 5 and 28% of the total maintenance energy requirement to the pig (Friend et al., 1964; Imoto and Namioka, 1978). In the ruminant, VFA production has been estimated to supply 70-80% of the animals energy requirement (Bergman et al., 1965).

In addition to contributing energy, VFA may also perform other functions. Several studies have shown SCFA are more conducive to the growth of commensal organisms while hindering the growth of pathogenic species (Wolin, 1969; Lee and Gemmell, 1972; Fay and Farias, 1975). Malbert (1994) reported ileal VFA aid in the regulation of gastric emptying. As reviewed by Bergman (1990), VFA have been shown to be potent stimulators of insulin secretion, cholesterol metabolism, and of GI blood flow, as well as epithelial cell proliferation.

Postweaning Changes In Gastrointestinal pH and Volatile Fatty Acid Concentrations

It has been adequately demonstrated that the weanling pig is ill-prepared, enzymatically, to digest the complex carbohydrates found in most cereal-based weaning diets (Corring et al., 1978). Additionally, HCl production in the stomach of early weaned pigs is also inadequate, which results in gastric pH being higher than acidic values found in the mature animal (Kidder and Manners, 1978). Pigs weaned at 21 d-of-age exhibited an increase in ileal pH, and a decrease in acetate, butyrate, isovalerate, and total VFA concentrations within two days postweaning (Mathew et al., 1993; Mathew et al., 1994). Ileal pH was greater from 23 d-of-age through 27 d-of-age, and pH change was greater from the last d preweaning (20 d- of-age) to the first day postweaning (23 d-of-age) (Mathew et al., 1994). These findings support the work done by Smith and Jones (1963), who also reported an increase in intestinal pH following weaning. Mathew et al. (1994) postulated that the increase in pH could be due to the initial fasting period observed with many newly weaned pigs. It has also been reported that the toxin produced by some enterotoxigenic *E. coli* can

increase pH by increasing cAMP activity in mucosal enterocytes, resulting in a flow of electrolytes and fluids out of the cells thus increasing lumen pH (Moon et al., 1986). Concentrations of VFA in the ileum are higher prior to weaning compared to any other time postweaning (Mathew et al., 1993). The decrease in ileal VFA corresponds with an increase in lactate concentrations (Mathew et al., 1994). Concentrations of acetic and propionic acids decrease immediately following weaning with concentrations beginning to increase by day 3 postweaning and generally stabilizing thereafter (Risley et al., 1991). The change in VFA patterns may be indicative of a shift in metabolic pathways as enteric microflora adapt to a change in the pigs diet. Short chain fatty acids have been shown to be affected by diet in the pig (Friend et al., 1963).

Acidification of Weanling Pig Diets

The interest in acidifying weanling pig diets began with the discovery that young pigs have limited capacity to maintain proper gastric pH (Manners, 1976). However, the first attempt to use acidification in pig diets was directed at the alleviation of postweaning scours (Cole et al., 1968). Since then, numerous studies have attempted to

stabilize GI pH and to enhance postweaning performance by using a variety of acidifying agents. Organic acids such as citric, fumaric, formic, propionic, malic, and lactic acids have been evaluated by a number of researchers (Sciopioni et al., 1978; Kirchgessner and Roth 1982; Giesting and Easter, 1985; Mathew et al., 1991). Use of the inorganic acids such as hydrochloric, sulfuric, and phosphoric acids was investigated by Giesting and Easter (1986); however, attempts were met with disappointing results.

Microfloral Response to Diet Acidification

Cole et al. (1968) showed that the addition of 0.8% lactic acid to drinking water reduced the number of hemolytic as well as total *E. coli* concentrations in the duodenum and jejunum of weanling pigs. When utilized in Antibiotic Medium 3, a combination of 60 µmoles of acetate, 20 µmoles of propionate, and 15 µmoles of butyrate per ml resulted in significant inhibition of *E. coli* growth at various pH values (Wolin, 1969). Sciopioni et al. (1978) noted reduced numbers of *E. coli* along with anaerobic microflora in the GI tract of starter pigs after diet supplementation with fumaric or citric acid. The

multiplication of *E. coli* 0141:K85 was reduced by acidification with a corresponding reduction in mortality (Thomlinson and Lawrence, 1981). In ileal cannulated pigs, the addition of 1% propionic acid in the feed decreased *E. coli* concentrations (Mathew et al., 1991). In contrast to the previous studies, Risley et al.(1993) did not see a response of enterotoxigenic *E. coli* challenged pigs to organic acids. Additionally, organic acid supplementation did not affect lactobacilli or *E. coli* concentrations throughout the GI tract (Risley et al., 1992).

Influence of Acid Supplementation on Performance

The effects of acid supplementation on performance have been highly variable, with some researchers reporting improvements of up to 14% in postweaning growth and up to 11% in feed efficiency, while others have reported depressions in both growth and feed efficiency (Ravindran and Kornegay, 1993). Cole et al.(1968) found that drinking water consisting of 0.8 to 1% lactic acid significantly improved growth rate and feed efficiency. Sciopioni et al. (1978) reported improved performance and increased dry matter and protein digestibility in early weaned pigs fed diets supplemented with 1% citric acid. Additionally,

increased gains were also observed for the first 3 weeks, but total gains were not different by 6 weeks postweaning. Giesting et al.(1991) observed that the addition of fumaric acid improved feed efficiency in both corn-soybean and dried whey diets for at least 4 weeks postweaning.

In contrast to these studies, Kornegay et al.(1976) reported no beneficial effects from the addition of 1% citric acid to the diets of 7-d-old weaned pigs. The inclusion of fumaric or citric acids to the diets of pigs weaned at 4 weeks of age did not significantly affect daily feed intake (Falkowski and Aherne, 1984). This study also indicated acid inclusion had no significant effect on apparent digestibility of protein or dry matter. Sciopioni et al.(1978) noted depressions in performance upon the supplementation of malic acid to weanling pig diets. Giesting and Easter (1985) reported that the addition of 2% propionic acid in the feed had no beneficial effects on growth or feed efficiency in weanling pigs, but resulted in depressed feed intake. Furthermore, Giesting and Easter (1986) demonstrated that the addition of hydrochloric, sulfuric, and phosphoric acids resulted in severe depressions in intake and growth.

Influence of Acid Supplementation on Intestinal pH

Inadequate activation of pepsinogen for protein digestion in the newly weaned pig is the result of insufficient HCl production by the parietal cells of the stomach (Manners, 1976). Furthermore, low acid levels allow the intestinal pH to remain high, providing a suitable environment for the proliferation of opportunistic bacteria. It has been speculated that supplementation with organic acids may decrease stomach and intestinal pH preventing the spread of pathogenic bacterial species. However, Burnell et al.(1988) reported little or no decrease in pH of the stomach or small intestine when 1% citric acid was given to weanling pigs for 7 to 21 days. Risley et al.(1991) noted similar results when fumaric or citric acid was included in starter pig diets. Sciopioni et al.(1978) reported a nonsignificant reduction in stomach and jejunal pH of weanling pigs by feeding diets containing 1% citric acid. The feeding of 1.5% fumaric or citric acid resulted in nonsignificant reduction in stomach and jejunal pH (Risley et al., 1992). Similar results were reported by Burnell et al.(1988) when 7-week-old weanling pigs were fed a starter diet with 1% sodium citrate. These findings suggest that

the supplementation of starter diets with organic acids does not substantially reduce GI pH.

3. MATERIALS AND METHODS

Surgical Procedure

In each of three replicate trials, twelve 15-d-old nursing pigs were surgically fitted with T-cannulas in the medial ileum, in accordance with the University of Tennessee Office of Laboratory Animal Care. The cannulas were made at Purdue University Mechanical Engineering Department (West Lafayette, IN) from Delrin 600 plastic and were similar in design to those described by Walker et al.(1986).

After the pigs were fasted for 24 hours and moved to the surgical suite, they were pre-anesthetized by intramuscular injection of 1 ml (100 mg) of ketamine hydrochloride (Ketaset, Fort Dodge Laboratories, Fort Dodge, IA) plus .1 ml (1 mg) acepromazine malate and .3 ml (3 mg) of atropine sulfate (Butler Company, Columbus, OH). Halothane (Fluotane, Fort Dodge Laboratories) anesthetic gas was delivered by a non-rebreathing delivery system, initially at 2% level and a flow rate of 1.5 1/min of O_2. Initial delivery of halothane was made using a cone mask until the proper plane of anesthesia was reached, after which pigs were intubated with a 3.0 mm I.D., cuffed, Murphy Eye tracheal tube (Mallincrodt Critical Care, Glens Falls,

NY). The left flank of the pig was prepared for surgery by shaving, followed by 3 betadine and alcohol scrubs. A 3 cm dorsoventral incision, using a number 10 scalpel blade attached to a number 3 scalpel handle, was made approximately 2 cm posterior to the last rib. A laparotomy was performed to locate the distal end of the cecum and the mesenteric attachment of the distal loop of the ileum. The ileum was exteriorized and placed on a sterile 4x4 gauze pad soaked with sterile saline to keep the ileum from drying during the surgical procedure. A purse string suture was placed in the muscularis layer on the anti-mesenteric surface of the ileum approximately 4 cm anterior to the ileo-cecal junction using 3-0 PDS II monofilament suture with a Taper RB-1 needle (Ethicon, Somerville, NJ). A 1 cm incision was made into the lumen betwen the stitches of the purse string suture, the flange of the cannula was inserted into the incision and the purse string suture was drawn up to secure the intestinal wall to the cannula. The purse was tied using a surgeon's knot followed by a minimum of 3 square knots.

A hole, 1 cm in diameter, was cut into the body wall approximately 1.5 cm dorsal to the incision using a brass

cork borer. The stem of the cannula was brought out through this opening by securing the stem of the cannula with Allis forceps and pulling it through the hole. A washer threaded onto the exposed cannula was turned down in order to draw the intestine, muscle layers and body wall securely together without twisting the intestine or applying too much pressure to the skin or muscle.

The incision was closed using 1-0 PDS II monofilament suture with a Taper TP-1 needle (Ethicon) in a simple continuous pattern for the peritoneum and muscle layers. A separate skin closure was performed using 3-0 PDS II monofilament with a Taper RB-1 needle (Ethicon) in a continuous subcuticular pattern. All sutures were secured with a surgeon's knot and a minimum of 3 square knots.

At the end of surgery, the use of halothane was discontinued and the endotracheal tube was removed following evidence of a swallowing reflex. Antibiotic ointment was applied prior to placing a 4x4 gauze pad over the incision. Pigs were wrapped with 2 inch cling gauze and 3 inch Elasticon tape (Johnson and Johnson, New Brunswick, NJ) in order to protect the incision and cannula. The pigs were observed until fully recovered from the effects of the

anesthesia, at which time they were returned to the Blount Swine Farm, placed on their respective sow for a 5 d recovery period. Pigs were observed daily and bandages were changed as necessary until weaning.

Experimental Design

Pigs were weaned at 21 d-of-age and moved to individual cages in the Brehm Animal Science Building on the Knoxville campus, where they were randomly assigned to one of three treatments including: T1)ad libitum access to water, T2) ad libitum access to water containing acetate (50 mM), propionate (5mM), and butyrate (3mM), and T3) ad libitum access to water containing acetate (25mM), propionate (2.5mM), and butyrate (1.5mM). Water intake was measured daily by weighing each individual's water container. All pigs were allowed ad libitum access to a typical Phase I starter diet (Table 1) with antibiotics omitted. Pigs were fed the starter diet from weaning until the end of the trial. Feed was collected and weighed twice weekly to determine feed intake. Pigs were weighed weekly to determine body weight, rate of gain and feed efficiency. Fecal scour scores were taken daily by the same individual in order to maintain consistency. Scores ranged from 1 to

Table 1

Composition of Creep and Nursery Diet

Nutrient	kg/100kg
Ground corn	55.95
Soybean meal	24.30
Blood meal	3.00
Dried whey	10.00
Fish meal	2.00
Fat	2.00
Limestone	0.65
Dicalcium Phosphate	1.20
Salt	0.35
Vitamin/mineral premix*	0.50
DL Methionine	0.05

*Nutrient (amount/kg of feed): Ca, 849 mg; Zn, 150 mg; Fe, 132 mg; Mn, 20 mg; Cu, 12 mg; Se, 0.31 mg; Vit. A, 1298 IU; Vit. D, 3260 IU; Vit. E, 2.4 IU; Menadione (sodium bisulphite form), 143 µg; Vit. B12, 3.3 µg; Riboflavin, 880 µg; d-Pantothenic Acid, 2.6 mg; Niacin, 4.4 mg.

4, with 1 being little or no scouring and 4 being profuse watery scours. Room temperature was maintained at approximately 30°C with humidity ranging from 65 to 70%. Artificial lighting was provided by florescent fixtures, with a photoperiod consisting of 12 h on and 12 h off. On day 3 postweaning, all pigs were orally challenged with 2 ml of suspension containing a minimum of 10^{10} cfu/ml of wild type enterotoxigenic *E. coli* variant 0157:K88:H13 (*E. coli* Reference Center, University Park, PA). The challenge organism was grown overnight in 50 ml of naldixic acid (Rep 1) or spectinomycin (Rep 2-3) treated Luria-Bertani (LB) (Bertani, 1952) broth at 37°C in a G24 Environmental Incubator Shaker (New Brunswick Scientific Co., Edison, NJ). Following incubation, the culture was centrifuged at 15000 x g in a Beckman J2-Hs centrifuge (Beckman Instruments, Palo Alto, CA) using a JA-20 rotor to pellet the cells. The supernatant was poured off and 2 wash cycles were performed on the pellet using 50 ml of phosphate buffered saline (PBS). The cells were resuspended by vortexing. Two milliliters of the mixture were then orally administered to the pigs using a 3 ml syringe attached to a 4 inch piece of tygon tube. To enumerate bacteria in the challenge

suspension, a portion of the mixture was serially diluted and plated on naldixic (Rep 1)or spectinomycin (Rep 2-3) treated lactose MacConkey agar (Difco, Detroit, MI) and incubated overnight at 37°C in a Precision Scientific (Chicago, IL) model 4EM convection incubator. Colonies were visually counted to verify that a minimum of 10^{10} cfu/ml of culture was administered to each pig.

Sample Collection and Analysis

Ileal samples were collected at 21, 24, 28, 31, 35, and 38 d-of-age with the 21 d collection being a preweaning sample. Samples were collected by attaching a sterile balloon to the open cannula. Because the interval between outflows of digesta varied widely between pigs, balloons were replaced frequently and immediately placed on ice until sufficient sample could be collected for analysis. This procedure was followed to minimize the possibility of bacterial proliferation and (or) fermentation in the balloons. When sufficient material had been collected, samples were taken immediately to the laboratory, assayed for pH and prepared for microbial and SCFA analysis. Sample pH was determined using a Corning #345 pH meter (Corning, New York, NY) with a high performance glass electrode (cat.

#476390). Dry matter was determined by drying approximately 5 g of digesta at 90^{0} C for 24 h.

Microbial Analysis

For bacterial assays, 10-fold serial dilutions were made from 1-g aliquots of ileal contents, using PBS as a diluent. One hundred microliters of each dilution were spread in duplicate on Petri dishes containing growth media specific for each bacterial type. Total *E. coli* were determined by growth on lactose MacConkey agar (Difco, Detroit, MI). *Escherichia coli* were confirmed by subjecting two typical colonies per pig per d to biochemical analysis (API 20E, BioMerieux Vitek, Syosset, NY). Total lactobacilli were determined by growth on Rogosa agar (Difco) using an overlay method to minimize oxygen exposure during growth. In that method, 100 µl sample aliquots were spread on solid Rogosa agar (30 ml) and plates were allowed to dry at 37°C for 30 min in a convection incubator. Following drying, approximately 10 ml of liquid Rogosa agar (42°C) was poured over the cultures and allowed to solidify, prior to returning plates to the incubator for the remainder of the incubation period. Total streptococci were

determined by growth on Streptosel agar (Becton Dickinson, Cockeysville, MD). Bacterial samples were incubated at 37° C for 24 h (*E. coli*) or 72 h (lactobacilli and streptococci). All bacteria were enumerated by visual counting of colonies, using the best replicate set from dilutions that resulted in 20 to 200 colonies per plate.

Hemolytic *E. coli* Detection

For detection of the challenge organism, alpha and beta hemolysis was determined. The challenge organism was grown in naldixic acid or spectinomycin treated LB. To determine hemolysis one hundred *E. coli* colonies per pig for each day were transferred from the lactose MacConkey plates to blood agar plates covered with either naldixic or spectinomycin. Plates were then incubated at 37°C for 24 h. Alpha hemolysis was determined by a zone of partial clearing around the colony; whereas, beta hemolysis was determined by a zone of total clearing around the colony. Only those *E. coli* colonies exhibiting beta hemolysis were determined to be hemolytic.

Short-chain Fatty Acid Analysis

Volatile fatty acid concentrations were determined using a gas chromatographic method adapted from Playne

(1985). In the analysis, approximately 10 g of intestinal content was centrifuged at 15,000 x g at 4°C for 15 min in a Beckman, model J2-HS centrifuge with a JA-20.1 rotor (Beckman Instruments). One and one-half milliliters of supernatant were mixed with 300 µl of 25% metaphosphoric acid (H_3PO_4) (5:1 ratio) and incubated at room temperature for 30 min. Following centrifugation to remove the precipitate, 1 µl of sample was injected into a Hewlett Packard model 5890 gas chromatograph (Hewlett Packard, Avodale, PA) with an HP-FFaP 10-m x 0.53-mm x 1-µm capillary column packed with cross-linked polyethylene glycol-TPA. A flame ionization detector was used with an oven temperature of 200°C and a detector temperature of 250°C for determination of acetate, propionate, butyrate, isobutyrate, valerate, and isovalerate concentrations.

Collection of Gastrointestinal Contents and Tissues

At 38 d-of-age, pigs were sacrificed by lethal injection of 1200 mg of sodium pentobarbitol via the anterior vena cava. A midline incision was made and GI samples were immediately obtained from the stomach, duodenum, ileum, cecum, and spiral colon. Samples were

analyzed for microflora and SCFA as described previously. Tissue samples were taken from the duodenum and ileum and preserved in 10% buffered formalin. Tissue samples were fixed and stained, using a general staining combination of hematoxylin and eosin, on slides for histological examination.

Statistical Analysis

The statistical model consisted of a randomized complete block design using repeated measures analysis with individual pig serving as the experimental unit. Data were analyzed using the Mixed Model Procedure of SAS (1996). Differences between least square means were separated using pairwise t-tests. Differences between days were separated using Pdmix procedures. Microbial concentrations were transformed (log_{10}) prior to statistical analysis.

4. RESULTS

Performance

Addition of VFA to drinking water tended (P = .11) to increase the consumption of water in T2 pigs compared to T3 pigs; however, intake in T2 and T3 pigs did not differ from the controls. Feed intake for the first week on trial tended (P =.07) to be greater for T2 pigs compared to T3 pigs; however, intake in T2 and T3 pigs did not differ from the controls (Table 2). No intake differences (P = .33) were observed among treatments during the second week; however, there was a tendency (P = .09) for higher intake in T2 pigs compared to T3 pigs. Gain tended (P = .09) to be greater during week 1 for T2 compared to T3. No differences (P = .46) were observed between treatments for overall gain. Feed efficiencies were not different (P = .23, P = .39, and P = .51) between treatments for week 1, week 2, and total, respectively.

Daily fecal scores indicated minimal scouring with the lowest score recorded being a 2 (data not shown). From all visual indications, pigs remained relatively healthy throughout the study.

Table 2

Effect of VFA inclusion in drinking water on performance of weanling pigs[a]

	Week 1			Week 2			Total		
Treatment	Feed Intake	Gain	Gain/Feed	Feed Intake	Gain	Gain/Feed	Feed Intake	Gain	Gain/Feed
T1[b]	1.54	1.07	0.62	3.25	2.64	0.83	5.43	4.06	0.75
T2	1.82	1.36	0.76	3.47	2.51	0.74	5.96	4.15	0.71
T3	1.21	0.55	0.14	3.09	2.35	0.78	4.81	3.67	0.77
SEM[c]	0.26	0.20	0.23	0.38	0.16	0.08	0.72	0.41	0.05

[a]Data are in kilograms and represent least squares means from three replicate trials with a total of 12 pigs per treatment.
[b]T1 = control, T2 = high VFA treatment, T3 = low VFA treatment
[c]SEM = average standard error of the mean

Microflora

Escherichia coli concentrations were not affected (P =.22) by the addition of VFA to the drinking water (Figure 1). However, a time (day) effect (P = .0001) was observed with *E. coli* concentrations decreasing in all treatments groups by 3 d postweaning and decreasing again 10 d postweaning. In addition, a tendency existed for a treatment by day interaction (P = .10), with concentrations of *E. coli* being lower by 14 d postweaning in T2 pigs compared to controls.

Recovery of the challenge organism, as indicated by beta hemolysis, was minimal. Detection of hemolytic *E. coli* accounted for less than 0.5% of the colonies examined. No differences (P = .71) between treatments were observed for hemolytic *E. coli*, nor were any day effects (P = .39) observed.

The addition of VFA to drinking water did not change (P = .37) concentrations of lactobacilli in the ileum of pigs compared to the control diet (Figure 2). However, lactobacilli concentrations were observed to decrease (P = .0001) in all treatment groups by 3 d postweaning. By 7 d postweaning, concentrations had increased (P = .0001), but

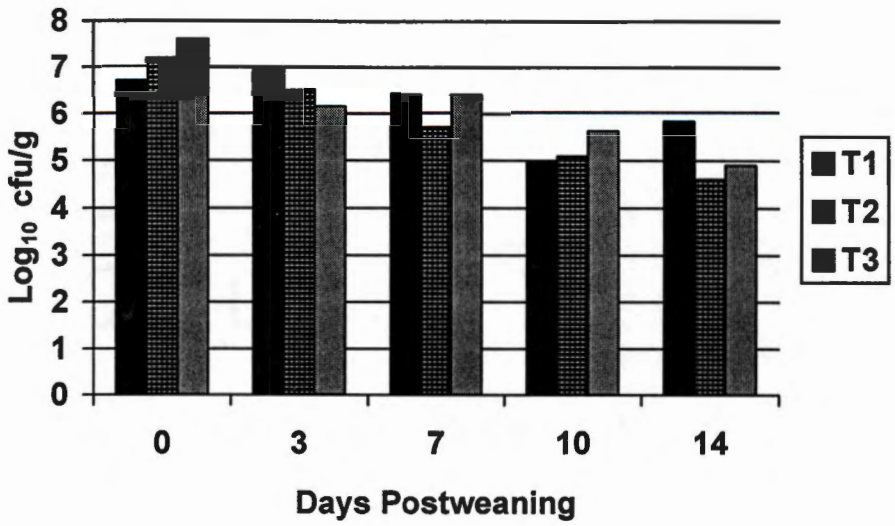

Figure 1: Effect of VFA inclusion in drinking water on ileal *E. coli* concentrations in weanling pigs. Data represent least squares means from three replicate trials with a total of 12 pigs per treatment.
Average SEM over all days = .61
T1=control, T2=high VFA, T3=low VFA

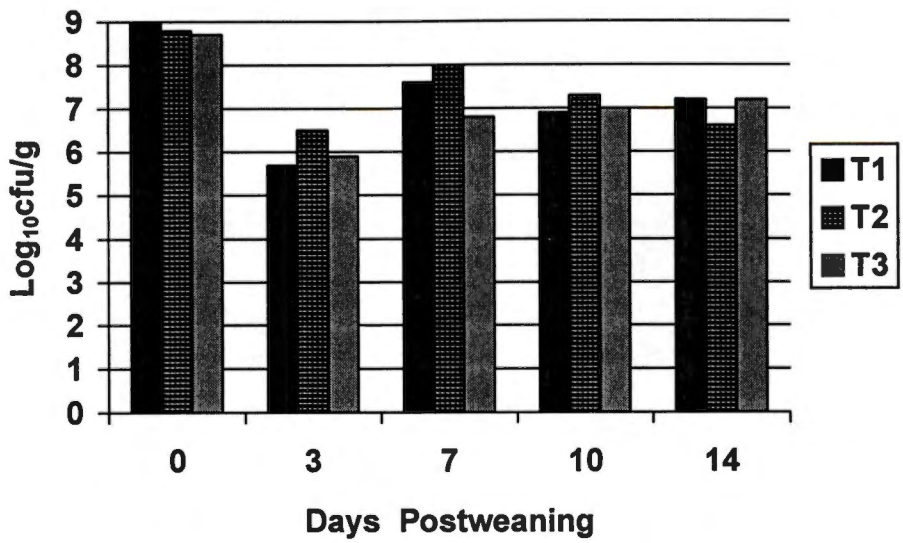

Figure 2: Effect of VFA inclusion in drinking water on ileal lactobacilli concentrations in weanling pigs. Data represent least squares means from three replicate trials with a total of 12 pigs per treatment.
Average SEM over all days = 0.22
T1=control, T2=high VFA, T3=low VFA

did not reach those observed preweaning.

In all treatment groups, ileal streptococci concentrations were observed to decrease (P = .0001) by 3 d postweaning (Figure 3). An increase (P =.0001) in streptococci levels was observed by 7 d postweaning, but concentrations never reached those prior to weaning. Furthermore, streptococci concentrations were not influenced (P = .61) by the addition of VFA to the drinking water of weanling pigs.

Short-chain Fatty Acids

Ileal concentrations of VFA were not influenced by the addition of VFA to the drinking water (Table 3). Acetate levels for all treatment groups decreased (P =.0001) from day 0 to day 7 postweaning, with day 7 concentrations being the lowest throughout the study (Figure 4). Concentrations increased following day 7 postweaning; however, the highest concentration reached postweaning was approximately one-half of the preweaning concentration. For all treatments, propionate concentrations decreased (P = .0001) nearly 6-fold by day 3 postweaning, with concentrations remaining constant for the remainder of the study (Figure 5). Furthermore, butyrate concentrations decreased (P = .0001)

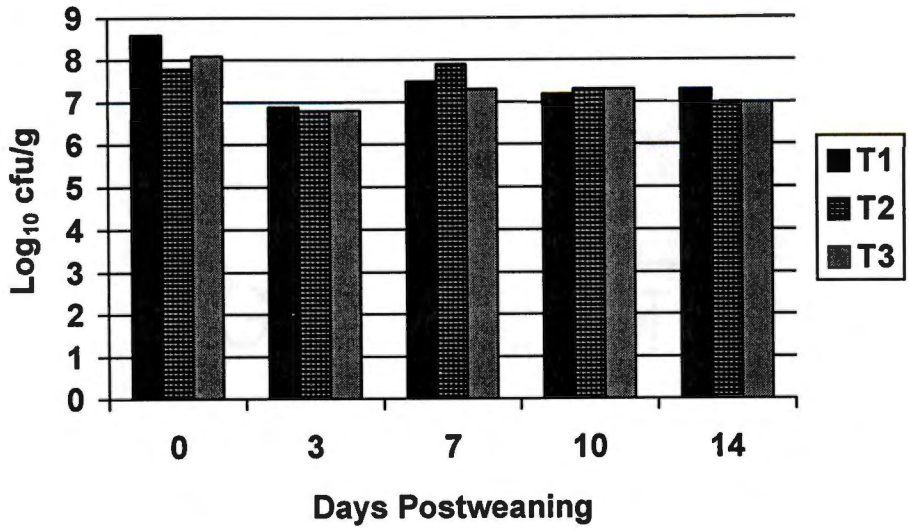

Figure 3: Effect of VFA inclusion in drinking water on ileal streptococci concentrations in weanling pigs. Data represent least squares means from three replicate trials with a total of 12 pigs per treatment.
Average SEM over all days = .20
T1=control, T2=high VFA, T3=low VFA

Table 3

Effect of VFA inclusion in drinking water on ileal VFA concentrations.[a]

	Acetate [b]			Propionate [b]			Butyrate [b]			Total [b]		
Days	T1[c]	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3
0	79.31	66.74	73.9	12.15	12.07	12.38	9.69	9.07	7.94	106.2	91.31	97.23
3	34.32	27.11	32.68	2.87	2.31	2.43	0.91	0.78	1.39	39.81	31.41	37.51
7	28.21	24.71	31.47	3.03	1.79	2.94	0.97	0.79	1.05	33.77	28.78	37.29
10	32.11	41.61	38.98	2.83	3.67	1.74	1.13	2.46	1.36	37.96	49.46	43.99
14	36.19	35.35	33.19	2.01	1.75	1.58	1.43	1.62	0.69	41.51	40.40	37.30
SEM[d]		5.64			1.12			1.10			7.31	

[a]Data are in mmoles/l and represent least squares means from three replicate trials with a total of 12 pigs per treatment

[b]Day effect, P = .0001

[c]T1 = control. T2 = high VFA treatment. T3 = low VFA treatment.

[d]SEM = average standard error of the mean over all days

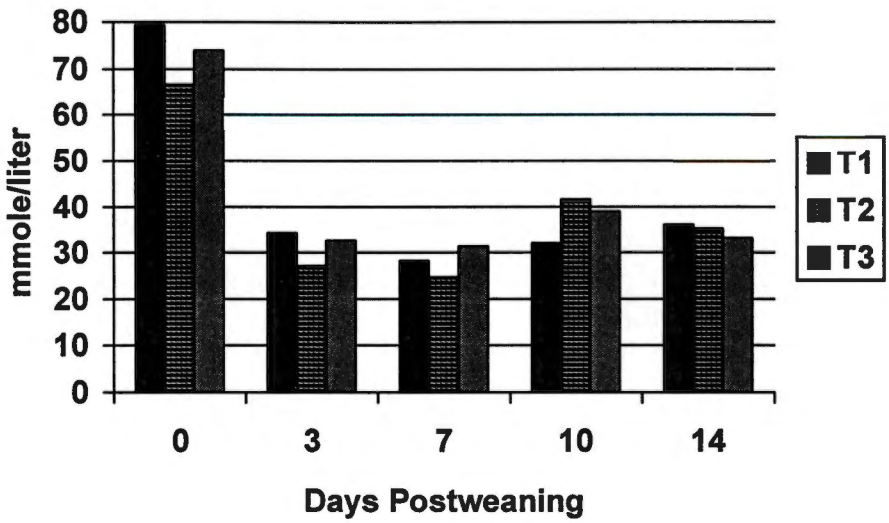

Figure 4: Effect of VFA inclusion in drinking water on ileal acetate concentrations in weanling pigs. Data are in mmoles/l and represent least squares means from three replicate trials with a total of 12 pigs per treatment. Average SEM over all days = 3.29
T1 = control. T2 = high VFA treatment. T3 = low VFA treatment

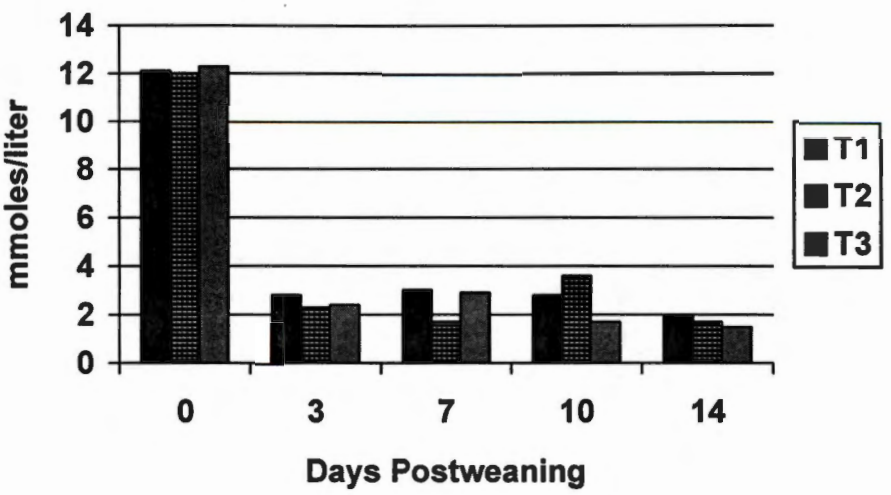

Figure 5: Effect of VFA inclusion in drinking water on ileal propionate concentrations in weanling pigs. Data are in mmoles/l and represent least squares means from three replicate trials with a total of 12 pigs per treatment. Average SEM over all days = 0.68
T1 = control. T2 = high VFA treatment. T3 = low VFA treatment

8-fold by day 3 postweaning and never increased thereafter (Figure 6). Total VFA (acetate, propionate, butyrate, isobutyrate, valarate, and isovalarate) were not affected (P = .75) by treatment. Concentrations were also observed to decrease (P = .0001) by day 3 postweaning, with concentrations remaining constant for the remainder of the study(Figure 7).

pH and Dry Matter

Treatment had no effect on ileal pH (P = .75) or DM (P = .81). However, ileal pH was observed to increase (P =.0001) by day 3 postweaning for all treatment groups and remained constant thereafter (Table 4). A time (day) effect (P = .0001) was observed for ileal DM with a decrease occurring by day 3 postweaning. Furthermore, ileal DM was noted to increase (P = .0001) by day 14 postweaning, with percent DM being greater postweaning compared to preweaning.

GASTROINTESTINAL SITES

Microflora

Escherichia coli concentrations from the gastrointestinal sites, taken at 38 d-of-age, were not different between treatments (P = .24, Figure 8). However, *E. coli* concentrations were higher (P = .0001) in the cecum

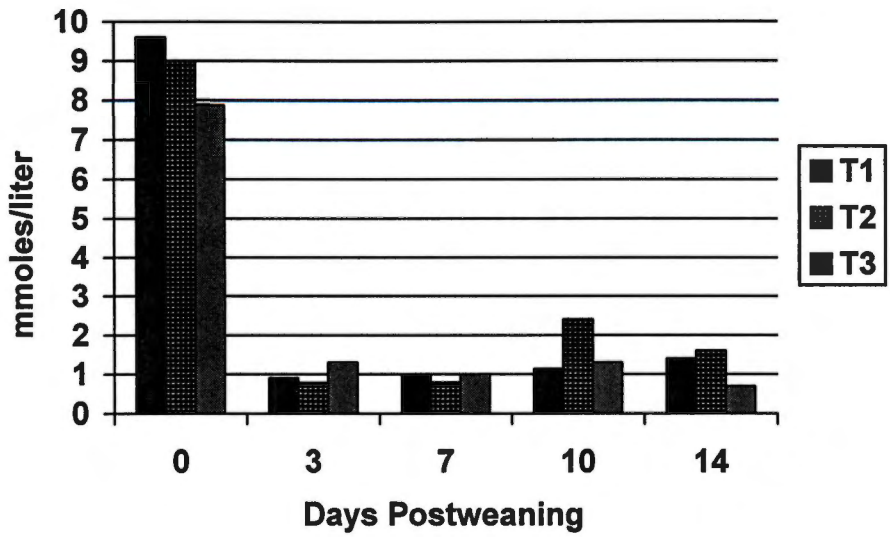

Figure 6: Effect of VFA inclusion in drinking water on ileal butyrate concentrations in weanling pigs. Data are in mmoles/l and represent least squares means from three replicate trials with a total of 12 pigs per treatment. Average SEM over all days = 0.71
T1 = control. T2 = high VFA treatment. T3 = low VFA treatment.

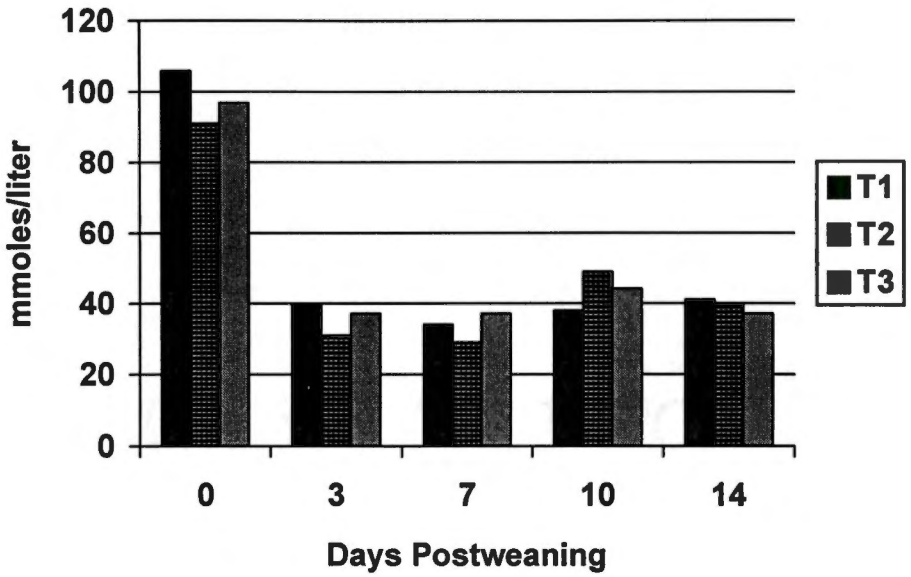

Figure 7: Effect of VFA inclusion in drinking water on total ileal VFA concentrations in weanling pigs. Data are in mmoles/l and represent least squares means from three replicate trials with a total of 12 pigs per treatment. Average SEM over all days = 4.25
T1 = control. T2 = high VFA treatment. T3 = low VFA treatment.

Table 4

Effect of VFA inclusion in drinking water on ileal pH and percentage of dry matter in weanling pigs[a]

Days Postweaning	pH[b]			Percentage of Dry Matter		
	T1[c]	T2	T3	T1	T2	T3
0	6.67	6.83	6.51	5.18	3.99	3.65
3	7.16	6.86	6.99	2.11	2.99	1.97
7	7.17	7.08	7.20	4.17	4.46	3.03
10	7.03	7.02	6.71	4.24	3.57	3.70
14	6.94	7.21	7.16	6.87	5.54	8.07
SEM[d]		.25			.89	

[a]Data represent least squares means from three replicate trials with a total of 12 pigs per treatment.
[b]Day effects, P = .0001
[c]T1 = control. T2 = high VFA treatment. T3 = low VFA treatment.
[d]SEM = average standard error of the mean over all days

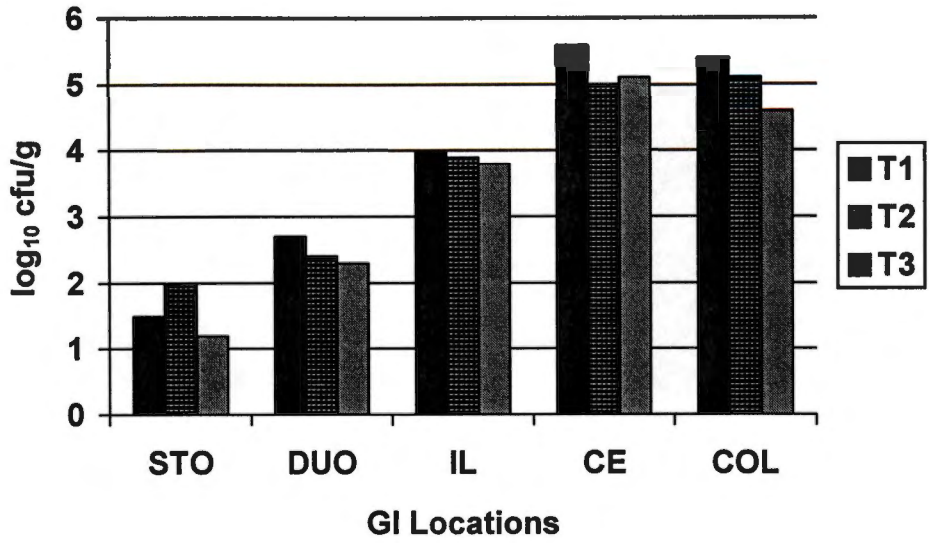

Figure 8: Effect of VFA inclusion in drinking water on stomach, duodenal, ileal, cecal, and spiral colon *E. coli* concentrations in weanling pigs. Data represent least squares means from three replicate trials with a total of 12 pigs per treatment.
Average SEM over all locations = 0.29
T1=control, T2=high VFA, T3=low VFA

and spiral colon than in the small intestine or stomach of pigs for all treatments. Ileal concentrations were higher (P = .0001) than those found in the stomach or duodenum for all treatments.

A location effect (P = .0001) was observed for lactobacilli concentrations, with concentrations increasing from the duodenum to the ileum and then increasing again from the ileum to the cecum for all treatments (Figure 9). No treatment effects were observed (P = .64) for lactobacilli concentrations.

No treatment differences were detected(P = .35) for streptococci concentrations throughout the GI tract (Figure 10). A location effect was noted (P = .0001) with concentrations increasing from the duodenum to the cecum, and ileal concentrations being higher than those in the duodenum. Cecal and spiral colon concentrations were greater than those in the ileum.

Short-chain Fatty Acids

Volatile fatty acid concentrations from various GI sites were not affected by treatment (Table 5). However, a significant location effect was noted (P = .0001) with acetate concentrations increasing from the duodenum to the

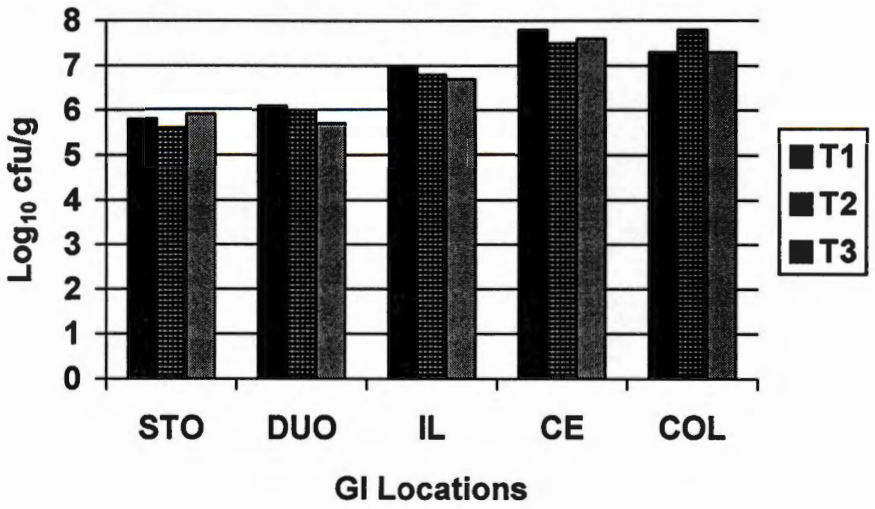

Figure 9: Effect of VFA inclusion in drinking water on stomach, duodenal, ileal, cecal, and spiral colon lactobacilli concentrations in weanling pigs. Data represent least squares means from three replicate trials with a total of 12 pigs per treatment.
Average SEM over all locations = 0.56
T1=control, T2=high VFA, T3=low VFA

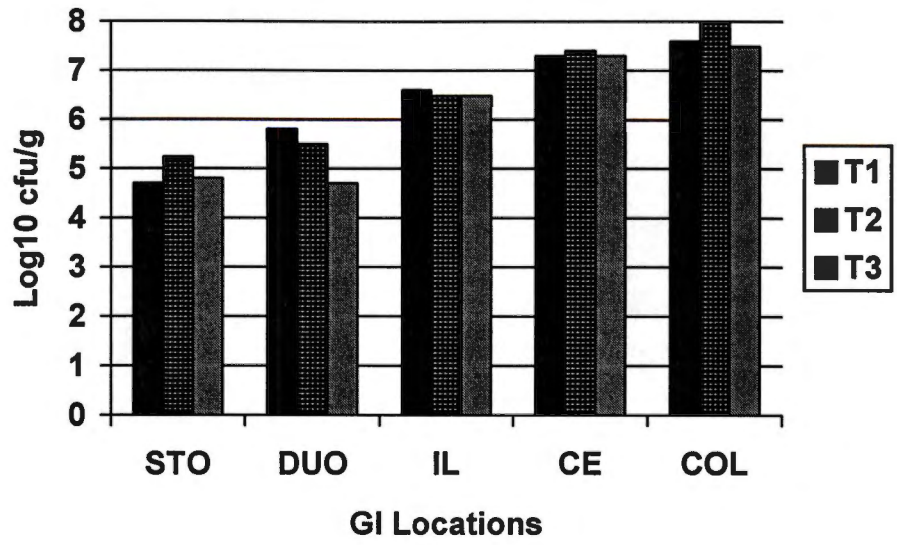

Figure 10: Effect of VFA inclusion in drinking water on stomach, duodenal, ileal, cecal, and spiral colon streptococci concentrations in weanling pigs. Data represent least squares means from three replicate trials with a total of 12 pigs per treatment.
Average SEM over all locations = 0.42
T1=control, T2=high VFA, T3=low VFA

Table 5

Effect of VFA inclusion in drinking water on gastrointestinal VFA concentrations in weanling pigs.[a]

	Acetate[b]			Propionate[b]			Butyrate[b]			Total[b]		
Loc[c]	T1[d]	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3
STO	4.74	8.34	11.87	ND	ND	ND	ND	0.37	ND	4.14	9.13	13.18
DUO	4.52	7.27	7.25	ND	ND	ND	ND	ND	ND	5.42	7.91	8.31
IL	27.25	23.18	29.26	ND	0.34	ND	0.89	0.77	1.42	29.37	25.69	32.17
CE	69.68	60.59	71.46	28.28	25.91	31.74	14.18	12.14	10.15	116.1	101.4	115.8
COL	37.96	35.22	32.35	14.08	12.74	14.11	6.72	5.96	4.96	61.81	56.49	52.75
SEM[e]		6.02			4.96			1.70			8.92	

[a]Data taken at day 17 postweaning are in mmoles/l and represent least squares means from three replicate trials with a total of 12 pigs per treatment

[b]Location effect, P = .0001

[c]Loc = location

[d]T1 = control. T2 = high level VFA treatment. T3 = low level VFA treatment.

[e]SEM = average standard error of the mean over all locations

ileum and from the ileum to the cecum. Concentrations in the spiral colon (P = .0001) were higher than the levels in the ileum but lower than the levels found in the cecum. Concentrations of propionate in the cecum were significantly higher (P = .0001) than at any other GI site; however, spiral colon concentrations were higher (P = .0001) than those in the stomach, duodenum, or ileum. Propionate levels were not different (P > .11) for the stomach, duodenum, and ileum. Furthermore, there were no differences (P > .11) in butyrate concentrations for the stomach, duodenum, or ileum. A location effect (P = .0001) was observed with concentrations increasing from the ileum to the cecum, but a decrease was noted from the cecum to the spiral colon. Spiral colon concentrations of butyrate were higher (P = .0001) than those in the stomach, duodenum, or ileum. In addition, total VFA concentrations were not affected (P = .53) by treatment; however, a location effect (P = .0001) was noted with concentrations increasing from the duodenum to the ileum and again from the ileum to the cecum. Concentrations decreased (P = .0001) from the cecum to the spiral colon, but concentrations in the spiral colon were higher than those in the stomach, duodenum, or ileum. Cecal

concentrations were the highest (P = .0001) among all sites with levels approaching concentrations found in the rumen of bovines.

pH and Dry Matter

Treatment had no effect on pH at the various GI sites (Table 6). A location effect (P = .0001) was observed with pH increasing from the stomach to the duodenum. Furthermore, pH was greater (P = .0001) in the spiral colon than in the duodenum; however, duodenal, ileal, and cecal pH were not different (P > .05), and spiral colon pH was not different(P > .05) from the ileum or cecum. As expected, DM content increased (P = .0001) from the duodenum to the spiral colon, with DM of stomach content being similar to that of the spiral colon.

Table 6

Effect of VFA inclusion in drinking water on gastrointestinal pH and percentage of dry matter in weanling pigs[a]

	pH[b]			Percentage of Dry Matter[b]		
Loc[c]	T1[d]	T2	T3	T1	T2	T3
STO	3.41	3.24	3.42	17.3	18.52	19.02
DUO	5.95	5.91	5.73	4.52	6.31	5.11
IL	6.20	6.97	5.86	9.74	9.47	8.56
CE	5.94	6.35	6.12	12.31	11.81	11.62
COL	6.36	6.61	6.60	20.62	17.85	18.49
SEM[e]		0.37			2.08	

[a]Data represent least squares means from three replicate trials with a total of 12 pigs per treatment.
[b]Location effect, P = .0001
[c]Loc = location
[d]T1 = control. T2 = high VFA treatment. T3 = low VFA treatment.
[e]SEM = average standard error of the mean over all locations

5. DISCUSSION

Performance

The addition of VFA to drinking water of weanling pigs had no significant effect on water intake, feed intake, weight gain, or feed efficiency. These data are similar to the findings of Kornegay et al.(1976), who reported no beneficial effects from the addition of 1% citric acid to the diets of 7-d-old weaned pigs. In addition, Falkowski and Aherne (1984) reported that the inclusion of fumaric or citric acid to the diets did not significantly affect daily feed intake. In contrast to these findings, Kershaw et al.(1966) and Cole et al.(1968) reported that drinking water consisting of 0.8 to 1% lactic acid significantly improved growth rate and feed efficiency. The tendency for feed intake to be greater during the first week for T2 may be due to an increase in gastric emptying and intestinal motility, causing an increase in appetite. Malbert et al.(1994) reported that the infusion of VFA into the ileum increased gastric emptying and intestinal motility. The tendency for increased water intake would be a direct response to higher feed consumption. Similarities among intake following week 1 can possibly be explained by the adaptation of pigs to

their respective diet.

Microflora

Cole et al.(1968) reported that drinking water consisting of 0.8% lactic acid reduced the number of hemolytic as well as total *E. coli* in the duodenum and jejunum of weanling pigs. In addition, Mathew et al.(1991) found that in ileal cannulated pigs, the addition of 1% propionic acid in the feed decreased *E. coli* concentrations. In contrast to the previous findings, *E. coli* concentrations in this study were not affected by the addition of VFA to drinking water. The lack of response may be due to the addition of lower concentrations of VFA to drinking water as compared to the higher concentrations of organic acids used in earlier studies. Furthermore, the typical postweaning increase in total *E. coli* concentrations reported by Mathew et al. (1996) was not observed. A decrease in total *E. coli* concentrations by 3d postweaning, as observed in this study, is similar to the observations of McAllister et al.(1979). These findings may be due in part to the environment in which this study was conducted, with pigs having sole access to feed and no contact with other animals. In a conventional rearing system, as the one used by Mathew et

al.(1996) disease can spread through pig to pig contact or by contact with feces of other animals.

Minimal detection of hemolytic *E. coli* may have been due in part to the absence of pig to pig or pig to feces contact. However, because enterotoxigenic *E. coli* (ETEC) serogroups characteristically adhere to the gut mucosa as a prerequisite to virulence (Gaastra and De Graaf, 1982), an increase in hemolytic numbers may not have been detected by sampling of intestinal contents (Mathew et al., 1994). In addition, the low detection of hemolytic *E. coli* could also be due to poor colonization by the K88:H13 challenge organism.

As in previous studies (Mathew et al., 1994; 1996), significant postweaning decreases in ileal lactobacilli concentrations by 3 days after weaning were observed. The decrease in lactobacilli by 3 d postweaning coincides with the significant increase in ileal pH also observed at that time. These findings are in agreement with the observations of Drassar and Barrow (1985) who reported that lactobacilli thrive in acidic environments, but decrease as pH reaches more basic levels. Furthermore, the increase in lactobacilli concentrations by 7 d postweaning is in

agreement with Mathew et al.(1994; 1996).

As with lactobacilli, streptococci concentrations were observed to decrease by 3 d postweaning, followed by an increase by 7 d postweaning. In contrast, Mathew et al. (1996) reported that streptococci concentrations remained relatively constant throughout the study until 21 d postweaning, when concentrations increased approximately 10-fold. However, similar to Mathew et al.(1994; 1996), an increase in streptococci concentrations coincided with a decrease in total *E. coli*. It is possible that streptococci compete with *E. coli* for binding sites on the intestinal mucosa and help to resist colonization, as reported with lactobacilli (Blomberg et al., 1993).

Short-chain Fatty Acids

Changes in fermentation acids following weaning indicate that major shifts are occurring in bacterial populations and/or fermentative pathways. The main objective of this study was to maintain ileal VFA concentrations throughout the weaning transition. However, ileal VFA concentrations were not affected by treatment, with total VFA levels decreasing following weaning. These shifts in VFA concentrations have been previously observed

(Mathew et al., 1993; 1994; 1996). In agreement with Mathew et al.(1993), concentrations of VFA were higher prior to weaning compared to any other time during the study. These findings may initially be due in part to decreased feed intake associated with weaning, and later due to rapid utilization of VFA by the growing pig. Acetate concentrations decreased in all treatment groups from days 0 to 7 postweaning, with an increase following day 7. Propionate concentrations decreased by day 3 postweaning, with concentrations remaining constant through day 14 postweaning. These findings are similar to those reported by Risley et al.(1991).

Barcroft et al.(1944) showed that some absorption of VFA takes place in the stomach and the upper small intestine. In addition, absorption appears to be mostly passive and increases linearly with corresponding decreases in pH or increases in concentration (Hollander et al., 1986). Therefore, the lack of response to additional VFA in the diet may be due in part to the extremely low pH of the stomach and to an increase in VFA absorption. Furthermore, predominant celluloytic species of bacteria are known to utilize VFA as an energy source, thus possibly decreasing

total VFA.

Ileal pH

It has been speculated that supplementation with organic acids may decrease intestinal pH; however, VFA addition to drinking water in this study had no effect on ileal pH. Burnell et al.(1988) and Risely et al.(1991) noted similar results with the addition of citric and fumaric acid. Additionally, pH was observed to increase by day 3 postweaning and remained constant through day 14. Smith and Jones (1963) and Mathew et al.(1994) also saw an increase in intestinal pH following weaning. As demonstrated in the current study, Mathew et al.(1994) postulated that the increase in pH could be due to the initial fasting period observed with many newly weaned pigs.

GASTROINTESTINAL SITES

Microflora

Increases in microflora concentrations from the stomach to the hindgut, as observed in this study, are similar to those observed in past research (Risely et al., 1992). One possible explanation for these observations is the differences in rates of passage between GI sites. Digesta flow is more rapid in the stomach and upper GI tract as

compared to more distal sites, thus flushing bacteria to the lower GI tract. In addition, slower flow rates, as found in the hindgut, allow more time for digestion, which results in bacterial proliferation. Furthermore, increased microflora concentrations may be attributed to higher pH which is typically found in the hindgut.

Short-chain Fatty Acids

The observed increases in VFA concentrations from the stomach to the cecum follow similar patterns to those reported for microflora. Increases in total VFA concentrations from the duodenum to the ileum and from the ileum to the cecum follow a similar pattern as previously reported (Risely et al., 1992). Shorter retention times in the stomach and upper GI tract may not allow sufficient time for microbial degradation of fermentable substrates, thus lowering the amount of VFA produced in those areas. Whereas in the hindgut, longer retention times allow for higher VFA production rates.

Unexpectedly, treatment had no effect on VFA concentrations at any of the sampled locations. However, due to rapid absorption and metabolism of VFA by epithelial tissue, the additional VFA may have been utilized by stomach

epithelium preventing the escape to adjacent locations. Volatile fatty acids not metabolized by epithelial tissue enters the portal vein, thus monitoring of portal vein VFA concentrations at the stomach, may have aided in determining where the additional VFA is being utilized.

Gastrointestinal pH

Typical patterns for pH were observed, with treatment having no effect. The observed increases in pH from the stomach to the spiral colon followed similar patterns to that of microflora as well as VFA. Previous work with organic acids indicated similar results with supplementation having no effect on intestinal pH (Burnell et al., 1988; Risely et al., 1991; Risely et al., 1992).

6. IMPLICATIONS

The addition of VFA to drinking water of weanling pigs had no significant effect on microflora, VFA, pH, or performance. The additional VFA may have provided supplemental energy for gastrointestinal maintenance and growth as well as energy for whole body function; however, specific benefits with regard to gastrointestinal health of young pigs were not observed. Based on these data, addition of VFA to weanling pig diets is not recommended for use in a production environment.

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VITA

Robert Jason Vickers was born August 15, 1972, and is the son of Robert T. and Bonnie S. Vickers. He received his elementary and secondary education in Bolivar, Tennessee, graduating from Bolivar Central High School in May of 1990. He then entered the University of Tennessee, Knoxville majoring in Animal Science and Wildlife and Fisheries. He married Tammy Lynn Waggoner of Jackson, TN on August 13, 1994. Jason graduated from the University of Tennessee in May, 1996 with two B.S. degrees, one in Animal Science and one in Wildlife and Fisheries. He returned in June of 1996 to the University of Tennessee to begin his graduate work. Jason received his Master of Science with a major in Animal Science in May 1998.