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Steven Victor Radecki

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MS degree in Anim. Sci.

E. R. Miller

Major professor

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Fumaric and Citric Acids as Feed Additives in Starter Pig Diets: Effect on Performance, Nutrient Balance, and Fecal Microflora.

By

Steven Victor Radecki

A THESIS

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ABSTRACT

Fumaric and Citric Acids as Feed Additives in Starter Pig Diets: Effect on Performance, Nutrient Balance, and Fecal Microflora.

By

Steven Victor Radecki

The growth lag generally encountered at the time of weaning has long been of economical concern to the commercial swine producer. Antibiotic supplementation to the diet has proven to reduce this lag in performance. Other compounds have produced a similar response in performance. Two of these are citric acid and fumaric acid. To evaluate the efficacy of adding these organic acids to practical diets of young, growing swine, growth performance, nutrient balance, as well as fecal microflora, were evaluated. Comparisons were made between antibiotic supplemented diets and diets containing organic acids. Fumaric acid supplementation improved pig performance early in the starter period. This performance equalled that of pigs fed diets containing antibiotics. Fumaric acid may improve the retention of N and Zn. Ca and P retention was unaffected by dietary addition of antibiotics or fumaric acid. The E.coli population did not change, and the total anaerobic counts increased when diets contained fumaric acid. Citric acid had no beneficial effect on growth performance.

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I. INTRODUCTION

In the pig, dietary, environmental and social changes introduced at the time of weaning generally cause a growth lag. This lag is of economical importance to the commercial pork producer. In an effort to minimize this growth lag, antibiotics as well as other feed additives have been supplemented to diets fed to these animals. Antibiotics, however, are currently under much scrutiny in the eyes of the public and regulatory agencies, and may, in the future, be banned from animal feeds. This truly would be a detriment to livestock producers.

In an effort to develop substitutes for antibiotics, other additives have been found to produce similar beneficial performance responses. Organic acids may be one class of these additives.

Researchers have shown improved growth performance similar to that observed by antibiotic supplementation when adding either fumaric or citric acids to the diets of weanling pigs (Kirchgessner and Roth, 1978, 1982; Falkowski and Aherne, 1984; Henry et al., 1985, Edmonds et al., 1985; Giesting and Easter, 1985).

The purpose of the following study was to further evaluate the effect of supplemention of weanling pig diets with either fumaric or citric acid.

II. REVIEW OF LITERATURE

A. Biochemical importance of fumaric and citric acids

Citric and fumaric acids are closly linked in the biochemistry of living animals. Both acids are integral components of the energy-producing citric acid cycle. Table 1 depicts some of the more important characteristics of these two acids.

1. Fumaric acid

Fumaric acid appears in the final stage of the citric acid cycle as a metabolite from the oxidation of succinate. It is one of the two products of phenylalanine and tyrosine metabolism.. Another pathway generating fumarate is that which produces purines. Finally, fumarate and aspartate link the urea cycle to the citric acid cycle (Smith, 1983).

The addition of this compound to the diet, therefore, may lead to increased ATP production by increasing the body's available fumarate. This then may help explain the improvement in feed efficiency generally seen in these type diets. However, at the relatively low level of supplementation, this mode of action seems unlikely to be of major impact.

2. Citric acid

Citrate is the first intermediary in the citric acid

TABLE 1. CHARACTERISTICS OF FUMARIC AND CITRIC ACIDS[1].

	Fumaric acid	Citric acid
Physical appearance	white crystalline	white crystalline
Molecular weight	116 daltons	192 daltons
Melting point	286 C	153 C
pk1	3.00	3.128
pk2	4.523	4.761
pk3		6.396

[1] Merck Index, Windholz, ed. (1976).

cycle. Citrate synthetase catalyzes the reaction involving oxaloacetate and acetyl CoA. This enzyme is allosterically inhibited by ATP. Therefore, if cellular energy were limiting and citric acid was in excess, ATP production may be enhanced.

B. Organic acids in animal feeds

1. Feed preservation

Organic acids, especially propionic and acetic acids, have been used as animal feed preservatives. Forsyth (1975) used 0.97 or 1.32% propionate or a mixture of propionic-acetic acid (80:20) to preserve high moisture corn fed to swine. The acid treatments proved to be adequate for mold inhibition in this type of feedstuff. Average daily gain, efficiency of gain, and feed intake were all similar to those values for pigs fed the control diet.

Propionic acid at 1.5% of the diet dry matter adequately suppressed mold growth and heating of high moisture corn (Young et al.,1970). Gain of pigs fed acid-preserved corn was similar or better than that of pigs receiving dry corn. When propionic acid was added to dry corn, gain and feed efficiency were significantly improved as compared to the dry corn without propionate (Young et al., 1970).

Acid-preservation of corn partially hydrolyzed the starch molecules, and subsequently, the free glucose of the feed was higher (Bayley et al.,1974). However, the

available energy, as well as the nitrogen utilization of the corn was variable.

2. Feed additive

Fumaric and citric acids have recently received considerable attention as growth promotants in swine diets. The research results, however, are highly variable. Factors that contribute to this variablity include: animal weight, age at weaning, other diet ingredients, and rearing environment.

In seven-day old weaned pigs, Kornegay et al. (1976) added 0.0 or 1.0% citric acid to two different diets, one a commercial sow milk replacer, and one an experimental dried skimmed milk diet. No benefit from citric acid was evident. Henry et al. (1985) also fed organic acids to very young, weaned pigs (3.1 kg, 10 days of age). The complex diet, containing either 0.0% organic acids, 1.5% fumaric acid, or 3.0% citric acid, was pelleted and fed free choice. Citric acid, in this trial, significantly increased average daily gain, and increased feed intake when compared to the other two diets. Fumaric acid, on the other hand, tended to depress growth when compared to the control. In a palatability study by this same group, all three diets were made available to each pen of pigs. The non-acidified diet appeared to be the diet of preference. The authors concluded that the improved growth response generally seen in acidified diets is not due to enhanced palatability, as had been suggested.

In the 5 kg pig, Kirchgessner and Roth (1978) investigated the growth stimulating effects of fumaric acid when included in the diet at 0.0, 1.5, 2.0, or 2.5%. Dietary levels of 1.5 or 2.0% significantly increased gain, feed intake, and improved the feed to gain ratio. Only slight changes in feed efficiency were observed at the 2.5% supplemental level.

Falkovski and Aherne (1984), working with 4 week old weanling pigs and complex diets, compared the growth response from additions of either citric or fumaric acids at 0.0, 1.0 or 2.0% of the diet. Although no significant response was seen, organic acids at 2.0% of the diet tended to decrease intake. Average daily gain tended to be improved by acid supplementation, however this was not significant. Feed efficiency, on the other hand, was improved significantly by organic acid supplementation, and a greater response was noted for fumaric as compared to citric acid.

In a 28 day trial involving 7.5 kg weanling pigs, Giesting and Easter (1985) supplemented diets with 2.0% propionic, fumaric, or citric acid. Feed efficiency was significantly improved when these acids were added to the diet. Average daily gain tended to be improved and feed intake appeared to be depressed by fumaric or citric acid supplementation. The findings in this study were quite variable. When fumaric acid was added in higher amounts (0.0, 1.0, 2.0, 3.0, or 4.0%), a significant

linear response was seen in feed efficiency as well as average daily gain. Diets in these two trials were simple corn-soybean meal fortified with vitamins and minerals.

No significant effect on growth performance was observed by either fumaric or citric acids when added to starter pig diets at levels of 0.0, 1.5, or 3.0% over a four week period (Radecki et al., 1986). A significant improvement in feed efficiency, however, was noted during the first week of the trial by pigs receiving 1.5% fumaric acid.

Kirchgessner and Roth (1978) also reported the effects of fumaric acid addition to diets of fattening hogs (18 to 90 kg). With diet levels of 0.0, 0.6, 1.2, 1.8, or 2.4%, both feed efficiency and daily gain were significantly improved as compared to controls when more than 0.6% acid was supplied. Acid supplementation did not affect carcass traits. A level of 1.5 to 2.0% was determined optimal by these authors.

In 47.5 kg finishing pigs, Giesting and Easter (1985) found no beneficial performance response to 1.5 or 3.0% fumaric acid supplementation.

When the diet contained antibiotics, either a tylosin-sulfur premix, or a chlortetracycline/ sulfamethazine/penicillin premix, in addition to the organic acids, no additional improvement in performance parameters was seen (Kirchgessner and Roth, 1982; Edmonds et al., 1985). From this evidence it may be suggested that these two feed

additives (antibiotics and organic acids) share, at least partially, in their mode of action. If this were not true, we might expect to see an additive effect of antibiotics with organic acids.

C. Mode of Action.

1. Nutrient digestibility

Various modes of action of fumaric and citric acids in affecting growth performance have been investigated. The first of these mechanisms involves protein digestion and weanling pig development.

As the pig ages, its digestive capacity gradually increases (Manners, 1976). The key to protein digestion in the stomach is the activation of pepsinogen to pepsin. This change requires a low stomach pH of near 2.0. In the stomach, this acidification is mediated by the secretion of hydrochloric acid. Stomach pH of the very young pig is determined by the diet. In the neonate the stomach pH will be approximatly equal to diet pH (5.5), whereas in the adult, stomach pH is approximatly 2.0 (Manners, 1976). Lower pH values, and thus increased pepsin activity, may not be achieved until the pig reaches 7 or 8 weeks of age. This is due to at least two different factors. First, milk diets are very active acid buffers, and hence neutralize the hydrochloric acid that is secreted. Cereal-based diets, on the other hand have less acid-buffering action. A second factor involves the secretion of hydro-

chloric acid. When betazole hydrochloride was infused into the jugular vein of young pigs at a rate of 3 mg/hour for 2 hours to induce maximal acid secretion, Cranwell (1985) reported a significant positive correlation between maximal acid output and stomach weight of these pigs. Also, the inclusion of solid food in the diet increased acid secretion in the stomach. Therefore, as the pig ages, and is subjected to a cereal-based diet, as at weaning, acid secretion may be enhanced.

The lack of hydrochloric acid secretion, or it's ability to acidify the stomach sufficiently, delays the activation of pepsinogen, and hence plant protein digestion. However, the secretion of pepsinogen itself may also be a contributing factor to poor plant protein utilization encountered at weaning. Lewis et al. (1957) suggested that at 3 to 4 weeks of age, the pig may not have sufficient pepsin activity. Cranwell (1985) also concluded that the capacity of the stomach to secrete pepsin is very low in pigs 3 to 4 weeks of age, and thereafter increases rapidly. Furthermore, maximal levels of enzyme activity may not be reached until 7 weeks of age (Hartman et al., 1961; Manners, 1970). Therefore, the diets encountered at weaning, i.e., cereal based diets, may induce the adaptation of the enzyme mixture of the gastrointestinal tract to more adequatly digest this diet (Lindemann et al., 1986; Cranwell, 1985).

From this information, it can be concluded that the

newly weaned pig is probably unable to generate enough acid through hydrochloric acid secretion to fully activate the pepsinogen present. Hence, acidification of the diet, by organic acids, may aid in protein digestion by activating the pepsinogen available. This theory appears to be substantiated by the most consistent improvement in growth performance seen in pigs, feed efficiency. Nearly every researcher in this field has shown at least trends for improved feed conversion by organic acid supplementation (Kirchgessner and Roth, 1978; Falkowski and Aherne, 1984; Edmonds et al., 1985; Giesting and Easter, 1985; Radecki et al.,1986). Both citric and fumaric acids significantly reduced diet pH (Falkowski and Aherne, 1984; Edmonds et al., 1985).

Furthermore, Kirchgessner and Roth (1980), found that 1.0 and 2.0% fumaric acid supplementation significantly improved digestibilities of all crude nutrients (dry matter, crude protein, nitrogen free extract and crude ash). Digestible energy, as well as metabolizable energy, were also significantly higher. Nitrogen balance was improved by 5.7%. These authors concluded that fumaric acid's effect on the underdeveloped gastrointestinal tract was the key factor in the improvements observed. A tendency for improved protein digestion was also observed by Falkowski and Aherne (1984).

However, when 2.0% fumaric acid was added to either a high protein (20% crude protein) or control (16% crude

protein) diet and fed to 10 kg pigs, no protein x fumaric acid interaction was evident, suggesting that protein utilization is not enhanced by diet acidification (Giesting and Easter, 1985). The authors also suggested that the 16% crude protein diet itself may indeed have been deficient in crude protein, since a significant growth response was seen for both the high protein diet and fumaric acid supplementation.

Further evidence supporting the effect of diet acidification on protein utilization is the lack of response in older pigs, as previously reported (Giesting and Easter, 1985).

Since diet digestibility is also important in diet utilization, a simple diet, consisting primarily of corn and soybean meal, may require more acidification to show the same response as that seen from more complex diets (Giesting and Easter, 1985).

In contrast to the theory presented above, diet acidification by hydrochloric acid, phosphoric acid or 3.0% fumaric acid, produced variable results with respect to pig performance (Giesting et al.,1986). In this study, it appeared that when diet pH was depressed below 4.0, growth performance was also depressed.

2. Microbial control

Diet pH may also play a role in the control of microbes detrimental to the host animal.

In the gut of the young pig, pathogenic bacteria are

most likely always present. These organisms, under certain conditions, become more prevalant and thus produce a disease situation commonly resulting in diarrhea. The stress of weaning, be it the new environment, establishment of a new social order or the change in diet, frequently causes diarrhea in these animals. It would follow, then, that the conditions in the gut have changed to allow the rapid proliferation of the pathogenic bacteria previously mentioned.

During the diarrhea period, the population of both faculatives (E.coli) and anaerobes increases (McAllister et al., 1979). Coliform bacteria are normal entities in the gastrointestinal tract (March, 1979), however, it would appear, that when scouring occurs, there is also a dramatic shift in the coliform population. Prior to weaning, the dominant strain of E.coli is nonhemolytic (McAllister, 1979). However, within 2 to 7 days post-weaning, scouring pigs experience a dramatic increase in the number of hemolytic E.coli, to a point where this strain of E.coli forms the majority of the intestinal flora (Kenworthy et al., 1963; McAllister et al., 1979; Ducluzeau, 1985). These workers concluded that the intestinal environment created by weaning favors E.coli multiplication. High E.coli counts (10 to 10 cfu/gram) were also reported in weanling pigs by Barrow et al. (1979). However, counts were similar in both healthy and scouring pigs. These results would further support the findings of

McAllister (1979) that there may be a shift within the E.coli population. On the other hand, Barrow et al. (1979) concluded that the E.coli did not cause scouring, noting that antibacterials virtually eliminated the E.coli population, but diarrhea was still evident. Weaning did indeed increase the number of E.coli. Fuller et al.(1960) observed a satisfactory level of growth stimulation when supplementing pigs with penicillin and chlortetracycline, but no effect was noted in the number of bacteria present in the gut. Again, it would seem possible that the qualitative population may have shifted.

After weaning, the population of faculatives and strict anaerobes decline (McAllister, 1979; Ducluzeau, 1985). The gut pH value, and subsequently the coliform counts, were lower in weaned pigs that received a creep feed during the suckling period than in pigs not having access to the solid feed (Hampson et al., 1985). In the same study, the gastric contents tended to be more acidic, and fewer viable coliform organisms were noted in weaned pigs as compared to unweaned pigs.

As discussed previously, acid secretion in the unweaned young pig is probably insufficient to reduce stomach pH to the level generally seen in the mature animal. Furthermore, the secretion of hydrochloric acid, the major stomach acidifier, is stimulated by the ingestion of solid food. Therefore, when suckling pigs are given access to creep feed, hydrochloric acid secretion

may subsequently increase, and hence inhibit microbial proliferation. Manners (1976) further supports this hypothesis. He suggests that in the piglet, the relatively high pH of the stomach allows bacteria to multiply fairly freely. As the pH of the stomach contents drops, as in the mature animal, bacterial multiplication is greatly deterred (Manners, 1976). Consequently, if the diet, which is the major controlling factor of stomach pH in the very young pig, is acidified by some means, bacterial multiplication may be inhibited.

Through this inhibition, performance may be enhanced via improved feed utilization. Indeed, Scipioni et al. (1978), have shown that both fumaric and citric acids will decrease the number of coliforms and total anaerobic bacteria in the intestinal tract of pigs.

In contrast to these findings, earlier work with citric acid in swine diets, resulted in no change in coliform numbers (Mansson and Olsson, 1962). Clostridia perfringens and enterococci were both significantly reduced. With regards to the coliform bacteria, it can again be suggested that a shift in this population occured.

On the other hand, the bacterial population may change metabolically. Visek (1978) suggested that antibacterial agents modified the microflora or their products, and therefore changed the utilization of nutrients by the host animal. A number of researchers have investi-

gated this theory.

The intestinal microflora are able to deaminate or decarboxylate amino acids present in the gut, and hence negatively influence protein digestion. Twenty to thirty percent of the free amino acids were degraded by the microflora found in the ileal contents (Dierick et al., 1986a). Addition of virginiamycin, spiramycin, carbadox, or copper sulfate greatly reduced this activity (Dierick et al., 1986a).

E.coli bacteria are very capable of decarboxylating many amino acids, including lysine (Hedde, 1982; Dierick et al., 1986a; Yen et al., 1979). Since this amino acid is first limiting in most swine diets, this degradation is of major concern.

Nitrogen digestion and absorption in the small intestine, as well as nitrogen balance, are enhanced when antibacterials are fed to pigs (Dierick et al., 1986b), supporting the nutrient sparing effect of the supplements previously described. Furthermore, since the greatest response to these compounds is generally seen in young growing animals, when gain is mostly water and protein, it would follow that some aspect of protein utilization may be enhanced.

Energy utilization is enhanced in a similar manner. Carbohydrate metablism by bacteria is changed both quantitatively and qualitatively when antibiotics are added to the diet (Vervaeke et al., 1979). The modifications in

metabolism resulted in a higher percent of net energy available for digestion in the small intestine. These changes in metabolism may very well be enough to support the improvement in growth performance generally seen with supplementation of antibiotics.

If organic acids are also able to influence the intestinal microflora in this way, it may provide a mode of action for these additives as well.

3. Mineral absorption

Adequate amounts of minerals are utilized to complement the improved gain seen with 2.0% fumaric acid supplementation (Roth et al., 1982). No increase in requirement is encountered due to the increased performance of pigs. Fumaric acid has been shown to complex with various cations, and subsequently increase the apparent absorption of calcium, phosphorus, magnesium, and zinc (Kirchgessner and Roth, 1982). Calcium and phosphorus balance was also improved.

4. Enzyme activity

As previously discussed, protein and energy utilization or metabolism may be enhanced by organic acid supplementation. When a high energy, high protein diet was fed to rats, aspartate transferase, alanine transferase, and succinate dehydrogenase activities were increased (Tschierschvitz et al., 1982). The two amino transferases are important enzymes involved in the transfer of amino groups to carbon skeletons, resulting in various amino

acids, such as alanine or aspartate. Succinate dehydrogenase is involved in the citric acid cycle, making it an important enzyme in energy production and carbohydrate utilization. With this evidence, it would appear that fumaric acid supplementation may be involved in an improvement in the intermediary metabolism of both energy and protein (Kirchgessner and Roth, 1982).

From the research previously reviewed, it would seem evident that organic acid supplementation of swine diets may result in a variety of responses. The exact mode of action is yet to be determined.

III. MATERIALS AND METHODS

Four trials were conducted to study the effects of fumaric and citric acid supplementation to diets of weanling pigs. Trials 1 and 2 were used to determine the influence of these organic acids on various measures of animal performance. Trial 3 was used to determine the effect of fumaric acid on nutrient balance. In trial 4, effects of diet treatments on fecal microflora were studied.

A. Growth Performance Studies

Two 28 day starter trials were conducted to determine performance responses due to organic acid supplementation. Trial 1 involved fumaric acid supplementation, and in trial 2, citric acid was added to the diet. Other than this, the two trials were identical and will be discussed together.

A 2 x 3 factorial design was constructed to involve 2 levels of antibiotic premix (0.0 or 0.25%), and 3 levels of organic acids (0.0, 1.5, or 3.0%). The optimal levels of organic acid, as well as any interaction with the antibiotic, could then be determined. These diet supplements were added to a control diet at the expense of corn starch. Diet composition is shown in Table 2. The

TABLE 2. COMPOSITION OF DIETS FOR TRIALS 1 AND 2.

Ingredient (%)	Diet 1	2	3	4	5	6
Corn starch	4.00	2.50	1.00	3.75	2.25	0.75
Ground shelled corn	63.60	63.60	63.60	63.60	63.60	63.60
Soybean meal (44%)	28.00	28.00	28.00	28.00	28.00	28.00
Mono-dicalcium phosphate	1.80	1.80	1.80	1.80	1.80	1.80
Calcium carbonate	1.00	1.00	1.00	1.00	1.00	1.00
Salt	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin-trace mineral premix[a]	0.50	0.50	0.50	0.50	0.50	0.50
Vit. E-Se premix[b]	0.50	0.50	0.50	0.50	0.50	0.50
L-lysine hydrochloride premix[c]	0.20	0.20	0.20	0.20	0.20	0.20
Antibiotic premix[d]	0.00	0.00	0.00	0.25	0.25	0.25
Organic acid[e]	0.00	1.50	3.00	0.00	1.50	3.00
	100.00	100.00	100.00	100.00	100.00	100.00

[a] See table 3 for composition of vitamin-trace mineral premix.

[b] Supplying 0.1 mg of Se and 17 IU of vitamin E per kg of diet.

[c] Containing 78% L-lysine.

[d] Containing 4.4% chlortetracycline, 4.4% sulfamethazine and 2.2% penicillin.

[e] Fumaric acid supplied by Monsanto Chemical Co., St. Louis, MO. Citric acid supplied by Miles Laboratory, Elkhart IN.

composition of the vitamin-trace mineral premix used in all diets is listed in Table 3. Table 4 shows the calculated nutrient and energy density of the control starter diet.

Ninety-six crossbred pigs were allotted into 12 groups of 8 pigs each. Pens were balanced on initial weight, barrow to gilt ratio, and litter, to reduce the experimental error. Due to the wide weight ranges encountered at weaning, pigs were grouped into either a heavy (7.7 kg average) or a light (6.3 kg average) class to reduce experimental error. The six dietary treatments were then randomly assigned to one pen in each weight class.

At weekly intervals, individual pigs were weighed and pen feed consumption measured. Average daily gain, average daily feed intake for each pen, and pen feed efficiency values were then calculated. Unthrifty pigs, three from trial 1 and six from trial 2, were removed from the trial when deemed necessary to assure random treatment effects.

Pigs were housed in an environmentally controlled nursery. Pens were 1.2 x 2 meters in dimension. Pen layout was a two gutter gravity flow system, the gutters were covered by woven wire. A concrete sleeping area divided the wire floor areas and hot water heat was contained within the concrete. Each pen contained two automatic nipple waterers and one stainless steel feeder with five feeder spaces.

TABLE 3. COMPOSITION OF VITAMIN-TRACE MINERAL PREMIX.

Nutrient	Amount supplied per kg diet
Vitamin A	3300 IU
Vitamin D	660 IU
Menadione sodium bisulfite	2.2 mg
Riboflavin	3.3 mg
Niacin	18 mg
d-Pantothenic acid	13 mg
Choline	110 mg
Vitamin B12	20 ug
Zinc	75 mg
Manganese	34 mg
Iron	60 mg
Copper	10 mg
Iodine	0.5 mg

TABLE 4. CALCULATED ENERGY AND NUTRIENT DENSITY, TRIALS 1 AND 2.

Item	NRC[a]	Control diet
Metabolizable energy, kcal/kg	3160	3127
Crude protein, %	18.00	17.80
Lysine, %	0.79	1.10
Calcium, %	0.65	0.79
Phosphorus, %	0.55	0.72

[a] NRC (1979).

In order to determine the interactions between treatment and age (weeks), data was broken down into three time categories: week one, weeks 1 and 2, and finally, the entire four week period. Statistically analysis was done using a one way analysis of variance (Gill, 1978a,c).

B. Nutrient Balance

To determine the influence of fumaric acid supplementation in the diets of weanling pigs on nutrient utilization and balance, twelve weanling pigs, averaging 8.2 kg each, were assigned to 3 dietary treatments. These diets included a negative control, a starter diet containing antibiotics or 1.5% fumaric acid. This level of fumaric acid was used based on trial one, as well as previous studies by other researchers (Kirchgessner and Roth, 1978, 1982; Edmonds et al., 1985). To determine diet pH, 20 grams of diet were weighed into a 250 ml beaker. Forty ml of deionized water were added to form a slurry. This mixture was continuously mixed with a stir bar and electro-magnetic mixer. An electronic pH meter was then used to determine the diet pH. This procedure was repeated three times for each diet. Statistical analysis of diet pH was performed using a one-way analysis of variance (Gill, 1978a,c). See Table 5 for diet composition and pH. Treatment groups were balanced on litter, barrow to gilt ratio, and weight. A 10 day acclimation period was used to allow the pigs to adjust to the diets and feeding regime. Pigs were fed twice per day in individual stainless steel pens,

TABLE 5. COMPOSITION OF DIETS FOR NUTRIENT BALANCE TRIAL.

Ingredient (%)	Diet		
	Control	Antibiotic	Fumaric acid
Corn starch	4.00	3.75	2.50
Ground shelled corn	63.60	63.60	63.60
Soybean meal (44%)	28.00	28.00	28.00
Mono-dicalcium phosphate	1.80	1.80	1.80
Calcium carbonate	1.00	1.00	1.00
Salt	0.40	0.40	0.40
Vitamin-trace mineral premix [a]	0.50	0.50	0.50
Vitamin E-Se premix [b]	0.50	0.50	0.50
L-lysine hydrochloride premix [c]	0.20	0.20	0.20
Antibiotic premix [d]	0.00	0.25	0.00
Fumaric acid	0.00	0.00	1.50
	100.00	100.00	100.00
diet pH [e]	5.60	5.51	4.06

[a] See Table 3.

[b] Supplying 0.1 mg Se and 17 IU of Vitamin E per kg of diet.

[c] Containing 78% L-lysine.

[d] Containing 4.4% chlortetracycline, 4.4% sulfamethazine and 2.2% penicillin (Cyanamid, ASP-250).

[e] Acid effect, P <.001, (standard error of the means = 0.063).

and feces and urine from each pig was collected in stainless steel cages. Feed was individually weighed out in an amount equal to approximately 2% of the body weight at each feeding. An equal amount of water was mixed with this diet to form a slurry. This procedure enabled the pigs to consume the feed rapidly. After feeding, pigs were returned to the collection cages. This then minimized experimental error due to contamination. Prior to the collection period, all cages and feeding pens were cleaned and prepared for the collection of feces and urine. A three day collection period followed. To prevent microbial degradation of urinary compounds, 10 ml of a 1.0% solution of thimersol was added to the plastic containers used to collect the urine. Environmental temperature was maintained at 27°C. After the collection period, individual collections of feces were dried, weighed, and finely ground. Urine samples were weighed to determine the total amount excreted, then subsampled. All samples were packaged in airtight containers and refrigerated until analyses could be performed.

Analyses of samples included gross energy, N, Ca, P, and Zn. Gross energy determinations were made via adiabatic bomb calorimetry[1]. From this information metabolizable and digestible energy of the feed was calculated. Urine samples were freeze-dried on cotton balls

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[1] Parr Instrument Company, Moline, IL.

prior to analysis. The contribution of energy from the cotton was then subtracted from the overall energy. Feed and fecal samples were pelleted before analysis.

Determination of N in feed, feces, and urine was done by a semi-micro kjeldahl method (A.O.A.C., 1980). After digestion of the samples, a colorimetric analysis was used to determine the N concentration. Apparent protein digestibility, apparent biological value of the protein, and apparent net protein utilization were all calculated for each diet.

Analysis of both Ca and Zn was via atomic absorption spectrophotometry[1]. Phosphorus concentration was determined by colorimetric spectrophotometry (Gomori, 1942). Retention of these elements was then calculated.

Data were analyzed using a simple one-way analysis of variance (Gill, 1978a,c).

C. Microbial evaluation

This trial was conducted concurrently with the nutrient balance study previously described. Of the 12 pigs used in that study, two from each treatment were used to determine the effects of the three diets on the microflora in the feces.

A fresh fecal sample was collected at weaning, at 7 days postweaning, and finally at 2 weeks postweaning.

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[1] Model 951, Instrumentation Laboratory, Inc., Lexington, MA.

These samples were analyzed for both total anaerobic microbes and E.coli.

A 1:10 dilution of the fecal sample was made with distilled water. Using the anaerobic roll tube technique of Hungate (1970), 9.0 ml of a sterile dilution medium (Table 6) were pipetted into sterile test tubes. A 1.0 ml sample of the diluted fecal sample was then introduced into the medium. Serial dilutions were then made up to a dilution of 10^{-12}. From these dilutions, 1.0 ml was used to inoculate test tubes containing 9.0 ml of a sterile agar medium (Table 9), again under anaerobic conditions. Agar was maintained in a water bath at a temperature of 50°C. Immediately after introduction of the sample into the agar solution, the agar containing tubes were rolled in an ice bath to set the agar as well as to ensure an even coating of agar and sample on the walls of the test tube. These tubes were then allowed to cool for sufficient length of time to ensure proper setting of the agar. The roll tubes were then returned to the test tube racks, and placed in an incubation chamber with a temperature of 39°C. Two days later, the tubes were removed from the incubation chamber, and colonies of bacteria were counted and recorded.

Determination of the E.coli populations of the samples was done using a selective medium under aerobic conditions. Petri dishes were prepared by pouring enough of a hot agar solution (37g/l E C Medium[1] plus 15g/l agar)

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[1] Difco Laboratories, Detroit, MI.

TABLE 6. COMPOSITION OF THE DILUTION MEDIUM FOR TRIAL 4.

Compound	Amount, ml
Mineral #1[a]	52.50
Mineral #2[b]	52.50
Resazurin	0.70
Cysteine sodium sulfide	14.00
Sodium carbonate	35.00
Distilled water	545.30
	700.00

[a] See table 7.

[b] See table 8.

TABLE 7. COMPOSITION OF MINERAL # 1.

Compound	Amount
K_2HPO_4	6.00 g
Distilled Water	1000.00 ml

TABLE 8. COMPOSITION OF MINERAL #2.

Compound	Amount
KH_2PO_4	6.00 g
$(NH_4)_2SO_4$	6.00 g
NaCl	12.00 g
$MgSO_4 \cdot 7H_2O$	2.45 g
$CaCl_2 \cdot 2H_2O$	1.59 g
Distilled water	1000.00 ml

TABLE 9. COMPOSITION OF THE AGAR MEDIUM USED FOR THE DETERMINATION OF ANAEROBIC BACTERIAL COUNTS IN TRIAL 4.

Compound	Amount
Glucose	0.25 g
Cellobiose	0.25 g
Starch	0.25 g
Xylose	0.25 g
Maltose	0.25 g
Trypticase	2.00 g
Glycerol	1.25 ml
Hemin solution (.05%)	1.25 ml
Mineral #1[a]	75.00 ml
Mineral #2[b]	75.00 ml
Resazurin solution (.1%)	1.00 ml
Clarified rumen fluid	400.00 ml
Agar	20.00 g
Cysteine sodium sulfide (2.5%)	20.00 ml
Sodium carbonate (8.0%)	50.00 ml
Distilled water	353.25 ml
	1000.00 ml

[a] See table 7.

[b] See table 8.

into the dish to completely cover the bottom of the dish. These plates were then allowed to cool and set. Prepared dishes were stored under refrigeration until needed. Nine ml of a dilution medium (0.1% proteose peptone) was pipetted into clean test tubes and sterilized. Using the same 1:10 dilution sample previously mentioned, serial dilutions were again made up to 10^{-12}. A 0.2 ml sample was pipetted out of the dilution tubes and aseptically introduced onto the prepared petri dishes. Due to the sticky nature of the E.coli bacteria, an effort was made to ensure an even distibution of the microbes. This enabled a more accurate count to be made. Inoculated plates were then placed into an incubation chamber with a temperature of 45°C. Colony counts were made approximately one day later.

In order to assess the changes in population over time, a split-plot analysis was used to determine statistical differences in the data (Gill, 1978b,c).

IV. RESULTS AND DISCUSSION

A. Growth Performance Studies

1. Fumaric Acid

Overall effects of dietary treatment on growth performance are depicted in Table 10. During the first week of the trial, pigs receiving fumaric acid supplemented diets gained significantly ($P < .03$) faster as compared to pigs not consuming these diets. Addition of the antibiotic premix to the diet had no beneficial effect on average daily gain (ADG). No significant interaction between the two feed additives was evident. Although no significant differences were seen during the first two week period, it would appear that diet 2, which contained 1.5% fumaric acid with the antibiotic supplement, was able to promote growth to the same extent as diet 4, the antibiotic containing diet without acid supplementation. This becomes important if fumaric acid is to substitute for the antibiotic. For the overall four week period, diet 2 appeared to be able to promote the growth of pigs consuming this diet as compared to pigs consuming the control diet. Supplementation of antibiotic to the diet significantly ($P < .07$) ADG as compared to pigs not receiving this additive. There was no significant interaction between the two additives.

TABLE 10. EFFECT OF FUMARIC ACID AND/OR ANTIBIOTIC SUPPLEMENTATION ON PIG GROWTH PERFORMANCE.

	Dietary treatments									
	No antibiotic			With antibiotic				P - value		
Acid level, %	0.0	1.5	3.0	0.0	1.5	3.0				
Item							SE[a]	F	A	F x A
Number of replicate pens	2	2	2	2	2	2				
Number of pigs	16	15	15	16	15	16				
Ave. int. wt, kg	7.03	7.02	7.23	7.20	7.15	7.10	0.88	NS[b]	NS	NS
Ave. fin. wt, kg	12.50	13.65	12.50	15.50	14.20	13.90	1.75	NS	NS	NS
Average daily gain, g										
Week 1	44	86	77	16	95	64	17.30	.03	NS	NS
Weeks 1-2	79	152	107	145	134	129	24.00	NS	NS	NS
Weeks 1-4	195	236	188	295	252	242	32.40	NS	.07	NS
Average daily feed intake, g										
Week 1	166	229	179	191	186	175	30.30	NS	NS	NS
Weeks 1-2	229	297	247	290	203	268	41.30	NS	NS	NS
Weeks 1-4	379	424	377	515	476	458	62.20	NS	NS	NS
F/G										
Week 1	7.56	2.71	2.36	11.94	1.97	2.81	2.13	.02	NS	NS
Weeks 1-2	2.86	1.95	2.34	2.07	1.98	2.05	0.12	.01	.01	.03
Weeks 1-4	1.94	1.79	2.00	1.74	1.89	1.89	0.06	NS	NS	NS

[a] Standard error of the mean.

[b] Not Significant ($P < .10$).

Feed intake during week 1 was not significantly effected by diet. However, intake appeared to be increased when pigs were consuming the diet containing 1.5% fumaric acid without antibiotic supplementation. During the initial 2 weeks of trial 1, both additives appeared to stimulate feed intake as compared to the controls. Finally, for the overall 4 week trial, it appeared that pigs receiving diets containing the feed additives ate more of these diets than the control fed pigs. However, this most likely was a consequence of the apparent improved growth of these pigs, as opposed to a treatment effect.

Fumaric acid significantly improved ($P < .02$) the feed to gain ratio during the first week of the trial when compared to diets not containing this additive. Pigs consuming diets supplemented with the antibiotic showed no significant improvement in this performance measure as compared to the pigs consuming diets without this supplement. The response to fumaric acid continued through the second week, as the feed to gain ratio was still significantly improved ($P < .01$) as compared to non-acidified diets. The ratio was also significantly improve ($P < .01$) by antibiotic supplementation during this period. These two feed additives in combination were also able to significantly improve ($P < .03$) this ratio as compared to the control. No improvement was observed for the overall four week period.

The improved performance observed during the early stages of this trial in pigs receiving the fumaric acid supplemented diets, would seem to support the evidence that the young pig, at weaning, may be unable to sufficiently digest nutrients. The apparent lack of adequate levels of hydrochloric acid in the stomach (Manners, 1972; Caldwell, 1986) may limit the activation of pepsinogen, and hence limit protein utilization. Therefore, by lowering the diet pH with fumaric acid supplementation, the pig may be more capable of making the digestive adjustments necessary at the time of weaning and consequently minimize the growth lag generally encountered at this time.

2. Citric Acid

Overall effects of dietary treatment on growth performance is depicted in Table 11. No significant improvement in ADG was observed from any dietary treatment during any period, when compared to the control. There was a strong tendency ($P < .11$), however, for antibiotic supplementation to improve ADG for the four week period. Citric acid supplementation appeared to depress growth, especially in the second half of the trial.

In regards to feed intake, citric acid supplementation tended to depress intake during all periods, while intake appeared to be improved upon the addition of the antibiotic. Again, there was no significant interaction between the two feed additives.

Antibiotic supplementation significanly improved

TABLE 11. EFFECT OF CITRIC ACID AND/OR ANTIBIOTIC SUPPLEMENTATION ON PIG GROWTH PERFORMANCE.

	Dietary treatments									
	No antibiotic			With antibiotic				P - value		
Acid level, %	0.0	1.5	3.0	0.0	1.5	3.0				
Item							SE[a]	C	A	C x A
Number of replicate pens	2	2	2	2	2	2				
Number of pigs	15	15	15	15	16	14				
Ave. int. wt, kg	7.09	6.97	7.14	7.05	6.86	7.04	1.26	NS[b]	NS	NS
Ave. fin. wt, kg	15.20	14.12	14.36	16.00	15.60	16.80	2.27	NS	NS	NS
Average daily gain, g										
Week 1	120	121	116	171	166	171	47.90	NS	NS	NS
Weeks 1-2	147	95	113	197	145	184	32.80	NS	.08	NS
Weeks 1-4	290	254	259	322	311	349	39.00	NS	NS	NS
Average daily feed intake, g										
Week 1	302	295	264	331	300	277	26.80	NS	NS	NS
Weeks 1-2	324	286	265	349	308	322	51.10	NS	NS	NS
Weeks 1-4	583	526	494	621	617	637	74.70	NS	NS	NS
F/G										
Week 1	2.57	2.59	2.39	2.26	2.19	1.62	0.57	NS	NS	NS
Weeks 1-2	2.31	3.40	2.35	1.78	2.17	1.77	0.43	NS	.07	NS
Weeks 1-4	2.02	2.08	1.92	1.93	2.00	1.83	0.07	NS	NS	NS

[a] Standard error of the mean.

[b] Not Significant ($P < .10$).

($P < .07$) the feed to gain ratio during the first two weeks of the trial, as compared to diets without this supplement. Addition of citric acid to the diet did not benefit the feed to gain ratio during this period. For the entire four week period, both citric acid and antibiotic supplementation tended ($P < .12$, $P < .17$, respectivly) to improve the feed to gain ratio.

The lack of a positive response from this organic acid agrees with observations by some researchers (Kornegay et al., 1976; Edmonds et al., 1985), and contrasts those of others (Kirchgessner and Roth, 1982; Falkovski and Aherne, 1984; Henry et al., 1985; Giesting and Easter, 1985). The variablity of responses, therefore, is quite evident. Citric acid decreases diet pH less than fumaric acid (Edmonds et al., 1985) when supplied at the same level in the diet, and therefore the effect of acidification of the diet may indeed be less than that of the same level by fumaric acid. This may be further substantiated by the fact that a higher level of citric acid, 3.0% as opposed to 1.5% fumaric acid, appeared to be more effective than lesser levels. Henry et al. (1985), using levels of 3.0% citric acid in the diet, found citric acid to significantly increase average daily gain and feed intake. With lower levels (0.75%), Edmonds et al. (1985) observed variable performance responses.

Other factors may be involved with the inconsistency

of the research reports involving citric acid supplementation to diets of swine. These factors may include initial weight of weanling pigs as well as diet type and the presence of creep feed. If the mode of action of acidified diets is via increased nutrient utilization, the younger pig, not having been exposed to a creep feed, may encounter a greater beneficial performance response due to diet acidification than older pigs which had consumed a creep feed during suckling. The older pig would have made the necessary digestive enzymatic changes to cope with the diet fed during the starter period. The young pig would not have had this opportunity.

The pigs used in this trial were approximately the same weight as those used by Edmonds et al. (1985) but heavier than the 3.1 kg pigs used by Henry et al. (1985). This may also be one of the reasons the response to diet acidification was minimal.

B. Nutrient Balance

Diet analysis is shown in Table 12.

1. Energy Balance

Gross energy (GE) concentrations of the three diets used in trial 3 were significantly different ($P<.08$). The fumaric acid supplemented diet contained the lowest GE level. Consequently, the GE intake of the fumaric acid supplemented pigs appeared to be lower than the other 2 diet treatments. Urinary and fecal energy values were not statistically different for the 3 treatments. Therefore,

TABLE 12. DIET ANALYSIS FOR TRIAL 3.

Item	NRC[a]	Control	ASP-250	Fumarate
DE[b], kcal/g	3.50	3.52	3.66	3.45
ME[c], kcal/g	3.40	3.46	3.60	3.38
Crude protein, %	20.00	18.77	17.74	19.25
Calcium, %	0.80	0.89	0.98	0.87
Phosphorus, %	0.60	0.81	0.77	0.76
Zinc, ppm	100	101	113	116

[a] NRC (1979).
[b] Digestible energy.
[c] Metabolizable energy.

digestible energy (DE), metabolizable energy (ME) and nitrogen-corrected ME (MEN), were all significantly lower ($P<0.005$) for the fumaric acid diet. All values presented for ME and DE, however, meet or exceed the suggested caloric density of diets fed to the 5-10 kg pig (NRC, 1979).

Contrary to these findings, Kirchgessner and Roth (1980) found significant improvements in both ME and DE when feeding 1.0 or 2.0% fumaric acid to piglets. However, improvements were small, 1.8 and 2.5% respectively for ME, and 1.5 and 2.1% respectively for DE. Furthermore, the ratio of DE:GE and MEN:GE were not different in this trial, suggesting that the depression in DE and ME were more a consequence of the reduced intake of GE, and not of the dietary treatment. See Table 13 for a summary of the effects of the three treatments on energy balance.

2. Nitrogen Balance

Due to an apparently lower analyzed value of dietary crude protein in the antibiotic supplemented diet, dietary intake of nitrogen was lower for this diet than the other 2 diets ($P<0.01$). For crude protein levels of these diets, see Table 12. Fecal excretion of N was not different among treatments. Consequently, daily absorbed N was lower for the antibiotic diet ($P<0.005$, See Table 14). This most likely is a factor of the lower N intake as opposed to a treatment effect.

Although no significant differences were noted, daily

TABLE 13. EFFECTS OF DIETARY TREATMENTS ON ENERGY BALANCE.

Item[a]	Diet			SE[b]
	Control	Antibiotic	Fumaric acid	
GE[c], kcal/g	3.97[d,e]	4.15[d]	3.91[e]	0.10
Daily feed intake, g	313.00	300.00	300.00	--
Daily GE intake, kcal	1243.00	1245.00	1173.00	--
Daily fecal GE, kcal	142.00	148.00	139.00	10.34
DE[f], kcal/g	3.52[g]	3.66[h]	3.45[i]	0.03
DE/GE x 100%	88.70	88.20	88.20	0.68
Daily urine GE, kcal	19.80	20.20	20.80	2.80
ME[j], kcal/g	3.46[g]	3.60[h]	3.38[i]	0.32
Daily N balance[k], g	5.40	5.03	5.95	0.56
Caloric correction[l], kcal	36.60	34.10	40.30	3.79
MEN[m], kcal/g	3.34[g]	3.48[h]	3.24[i]	0.03
MEN/GE x 100%	84.10	83.90	82.90	0.71

[a] Values represent the average of 4 pigs.

[b] Standard error of the means.

[c] Gross energy.

[d,e] Means within rows with different superscripts differ (P<.08).

[f] Digestible energy.

[g,h,i] Means within rows with different superscripts differ (P<.005).

[j] Metabolizable energy.

[k] Daily N balance, see Table 14.

[l] Caloric correction = N balance(g) x 6.77 kcal/g N.

[m] Nitrogen-corrected ME.

TABLE 14. EFFECTS OF DIETARY TREATMENT ON NITROGEN BALANCE.

Item[a]	Diet			SE[b]
	Control	Antibiotic	Fumaric acid	
Daily N intake, g	9.39[c]	8.51[d]	9.25[c]	0.05
Daily fecal N, g	1.48	1.46	1.38	0.13
Daily absorbed N, g	7.91[e]	7.06[f]	7.88[e]	0.13
Apparent digestibility of N, %	84.20	82.90	85.10	1.11
Daily urinary N, g	2.51	2.07	1.94	0.49
Daily N balance, g	5.40	4.98	5.94	0.58
Net protein util.[g], %	57.35	59.20	64.22	5.69
Biological value of protein, %[h]	68.01	70.90	74.53	6.72

[a] Values represent means of 4 pigs.

[b] Standard errors of means.

[c,d] Means within rows with different superscripts differ (P<.01).

[e,f] Means within rows with different superscripts differ (P<.005).

[g] Net protein utilization = (N retained/ N intake) x 100%.

[h] Biological value = (N retained/ N absorbed) x 100%.

urinary excretion of N tended to be lowest for the acidified diets, suggesting the N in this diet was utilized by the pig more effectively than the N in the other diets. Daily N balance appeared to be improved, as did net protein utilization (NPU) and biological value (BV) of the protein when fumaric acid was included in the diet. Other researchers (Kirchgessner and Roth, 1980; Falkowski and Aherne, 1984) have also indicated improved protein utilization when young pigs received diets containing fumaric acid. Digestibility of the N was not affected by diet acidification, as had been suggested by Kirchgessner and Roth (1980) as well as Falkowski and Aherne (1984). It would appear, therefore, that in this trial, the gastrointestinal tract of pigs were developed sufficiently to enzymatically digest the protein supplied in the diet. The 10 day acclimation period prior to the collection period may have played an important role in masking any effect the acidification may have had on nutrient utilization. As was previously discussed, at the time of weaning, the pig may not be able to adequately secrete the enzymes and compounds necessary to effectively digest various nutrients (Hartman et al., 1961; Manners, 1976; Cranwell, 1985). By 2 weeks post-weaning, the pig has apparently enzymatically adapted to the diet imposed upon it at weaning. The 10 day acclimation period may have given the pigs time to adapt to the diets, and hence no benefit was seen from diet acidification. Further sup-

porting evidence can be found in Trial 1, where fumaric acid diets tended to improve performance only during the early stages of the trial. This may explain the contrasting results of other researchers previously cited. The same argument may be used to help explain the observations noted in the energy balance portion of this trial.

3. Mineral Balance

The effects of dietary treatments on mineral balance are shown in Table 15.

a. Calcium

As previously shown in Table 12, the calcium levels in the diets, especially the antibiotic supplemented diet, exceeded the requirements for 5 to 10 kg pigs (NRC, 1979). The increased intake of Ca by pigs consuming diet A appeared to result in higher urinary levels of Ca ($P<0.01$). The lack of an improvement in retention and balance, as reported by Kirchgessner and Roth (1980), may be accounted for, to some extent, by the apparent excess Ca present in the diet. Pigs fed these 3 diets were probably able to absorb more than adequate amounts of Ca. Any excess absorbed may be excreted in the urine.

b. Phosphorus

Daily intake of P exceeded the requirements outlined by NRC (1979)(see Table 12). The daily balance of phosphorus was greatest in pigs fed the control diet ($P<0.001$). Subsequently, the percent retention was also greatest for this group of pigs ($P<0.025$). This may be due

TABLE 15. EFFECTS OF DIETARY TREATMENT ON MINERAL BALANCE.

Mineral[a]	Diet			SE[b]
	Control	Antibiotic	Fumaric acid	
Calcium				
Daily intake, g	2.79	2.93	2.63	--
Daily fecal, g	0.73	0.84	0.79	0.105
Daily urine, g	0.01[c]	0.06[d]	0.01[c]	0.011
Daily balance, g	2.05	2.03	1.82	0.09
Retention, %	73.48	69.30	69.47	3.48
Phosphorus				
Daily intake, g	2.55	2.30	2.29	--
Daily fecal, g	0.50	0.55	0.53	0.024
Daily urine, g	0.06	0.03	0.02	0.017
Daily balance, g	1.99[e]	1.72[f]	1.74[f]	0.04
Retention, %	78.00[g]	74.80[h]	75.90[h]	0.944
Zinc				
Daily intake, mg	31.70	34.10	35.10	--
Daily fecal, mg	22.01[i]	25.10[j]	23.93[i,j]	1.39
Daily urine, mg	1.17	1.40	0.98	0.27
Daily balance, mg	8.52	7.60	10.19	1.33
Retention, %	26.88	22.29	29.03	4.10

[a] Values represent the average of 4 pigs.

[b] Standard error of the means.

[c,d] Means within rows with different superscripts differ ($P<.01$).

[e,f] Means within rows with different superscripts differ ($P<.001$).

[g,h] Means within rows with different superscripts differ ($P<.025$).

[i,j] Means within rows with different superscripts differ ($P<.09$).

in part to the tendency for increased P intake in these pigs, as pigs receiving the other two diets demonstrated no statistical differences in these measurements. For a summary of results, see Table 15.

c. Zinc

Intake of Zn was apparently lowest for pigs receiving the control diet. This is related to the lower levels of Zn in this diet. However, diet levels were still sufficient to meet NRC (1979) requirements (See Table 12). Antibiotic supplementation increased the amount of Zn in the feces significantly as compared to the control. No statistical differences could be seen between the antibiotic supplemented diet and the acidified diet. Furthermore, no difference was observed for this measure between the control diet and the fumaric acid supplemented diets, even though the fumaric acid diet contained more Zn. Consequently, daily balance tended to be improved by acidification of the diet. This intake appeared to increase the percent Zn retained (See Table 15).

These results, combined with the results noted in the nitrogen balance study, where N retained appeared to be improved by diet acidification, may be connected. Zinc is an essential co-factor in many of the enzymes involved in protein synthesis. Consequently, it may be suggested that when protein synthesis is increased, the Zn requirement also increases. Thus, the retention of Zn would be increased. Van Campen and House (1974) showed that when rats

were given low protein diets, they also retained less Zn. These workers concluded that the amount of Zn that an animal can utilize may be dependent upon the level of dietary protein. McCall et al. (1961) observed that rats fed high levels of protein (30%) in the diet were more tolerant of Zn than rats receiving lower levels of protein (20%). This then would seem to suggest that these animals are able to utilize the extra Zn available. Pond et al. (1985) reported that when diets high in protein and zinc (165ppm) were fed to young pigs, plasma levels of Zn were increased. However, pigs fed diets low in protein did not experience the increased plasma Zn levels when 165 ppm of Zn was added to the diet. These authors concluded that protein deficiency may decrease the utilization of Zn by the pig. Finally, Tschierschvitz et al. (1982) found that fumaric acid supplementation of high energy, high protein rat diets increased the activities of aspartate and alanine transferases, important enzymes involved in non-essential amino acid synthesis. Further investigation of the protein level x zinc requirement interaction is needed.

C. Microbial Evaluation

1. Anaerobic Bacterial Counts

The increase in total anaerobes for the acidified diets was significantly greater than the increase seen with the antibiotic diet ($P < 0.005$) over the two week period (Table 16). Figure 1 graphically depicts the

TABLE 16. EFFECTS OF DIETARY TREATMENTS ON THE FECAL ANAEROBIC BACTERIA POPULATION (log number/gram feces).

Time	Dietary treatment		
	Control	Antibiotic	Fumaric acid
Initial[a,b,c]	9.561	9.390	8.975
7 days	9.415	9.065	9.440
14 days	10.135	9.600	10.020
% change[d]	+6.00	+2.20	+11.60

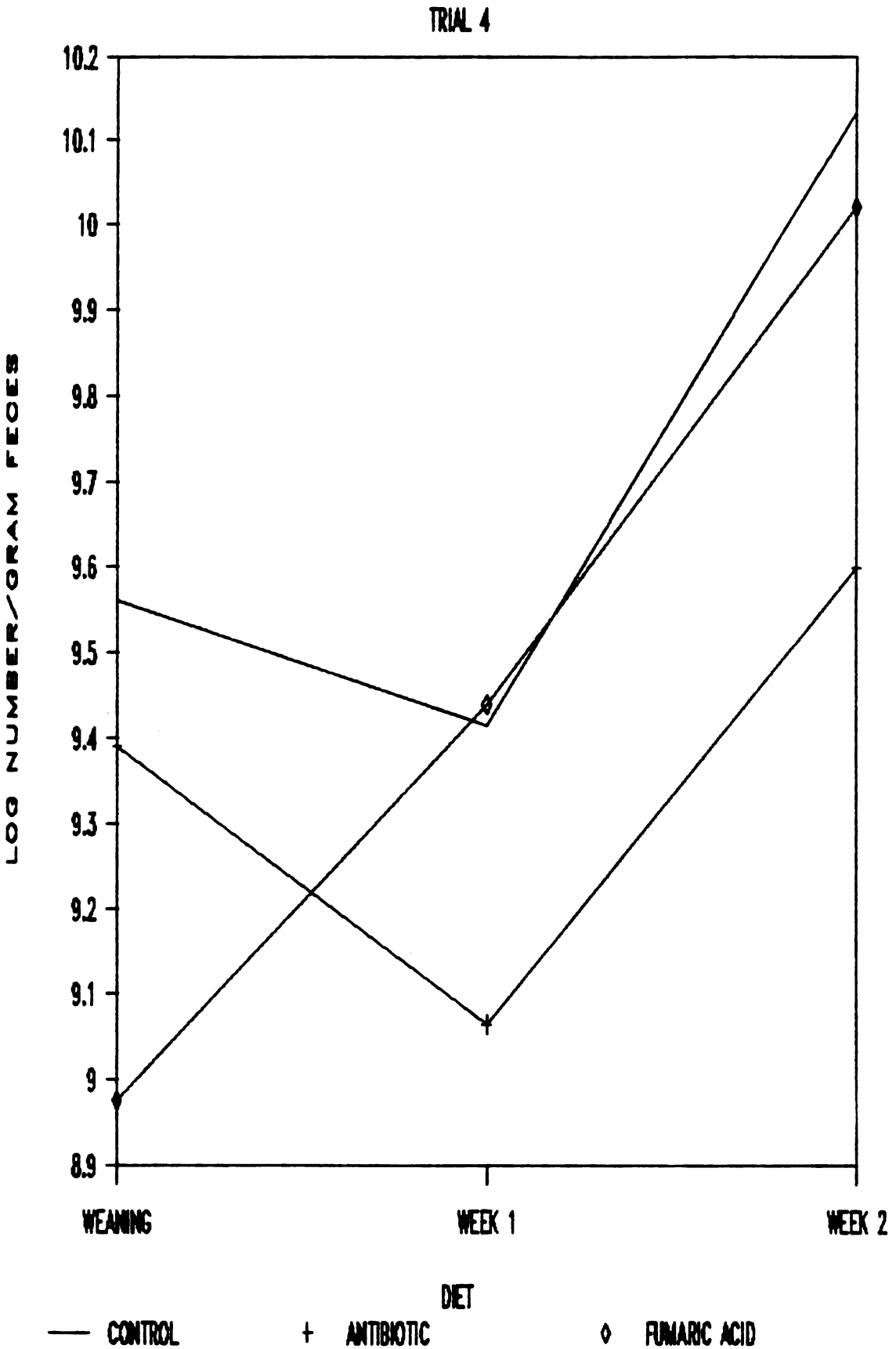
[a] All values represent means of 8 counts, 4/pig/treatment.

[b] Standard error of the means = 0.19.

[c] Note: The standard error of the difference between two means = 0.22. Data were so lowly correlated across weeks that the standard error of the differences of 2 means was essentially the same for differences among and within weeks.

[d] Change in populations over time for FA vs A was significant, $P < 0.005$.

FIGURE 1. TOTAL ANAEROBIC FECAL COUNTS



change in population over the two week period. The anaerobic microbial flora of the feces in the antibiotic supplemented diets remained mostly constant, while the acidified diet allowed increased proliferation of bacteria. The results for the acidified diet contrast with the findings of Scipioni et al. (1978), who found that the addition of 0.75% fumaric acid decreased the number of anaerobic bacteria in the intestinal tract of pigs. Citric acid also depressed the microflora of the small intestine (Mansson et al., 1962; Scipioni et al., 1978).

The increase in anaerobic bacteria encountered, however, may be of little consequence. Pigs in the trial showed no signs of diarrhea, or any other signs associated with increased microflora populations. Furthermore, this increase in the microflora population had no detrimental effects on the concurrent balance trial. It may be that the number of anaerobic bacteria did not reach a level where it would affect pig performance. Willingale and Briggs (1955) determined the normal range for fecal anaerobic bacterial counts in young pigs to be between 1.0×10^{8} and 9.9×10^{9} bacteria per gram of feces. For there to be any significant increase in total numbers, the fecal count would have to exceed this range. The counts for this trial were within this range.

2. E.coli

Acidification of the diet seemed to maintain E.coli

numbers in the feces, while the other two diets appeared to inhibit proliferation of this bacterium. Generally, at the time of weaning, E.coli numbers will increase (McAllister, 1979; Barrow et al., 1979). As shown in Table 17 and Figure 2, this was not the case in this study. Environmental cleanliness, along with the absence of direct fecal contact by the pigs, may be factors involved in these observations. Scipioni et al. (1978) found that fumaric acid decreased coliform numbers in the small intestine of pigs. However, Mansson and Olsson (1962) observed that 1.0% citric acid had no influence on the quantitative coliform population. During the diarrhea period, there is a dramatic increase in the ratio of hemolytic strains to nonhemolytic strains (Kenworthy, 1963; McAllister, 1979; Barrow et al., 1979). In the current study, no attempt was made to identify the various strains of E.coli present. As mentioned previously, pigs remained healthy throughout the trial, and it can be concluded that the microflora changes observed in this trial had little, if any, influence on pig performance.

TABLE 17. EFFECTS OF DIETARY TREATMENTS ON THE FECAL *E.COLI* POPULATION (log number/gram feces).

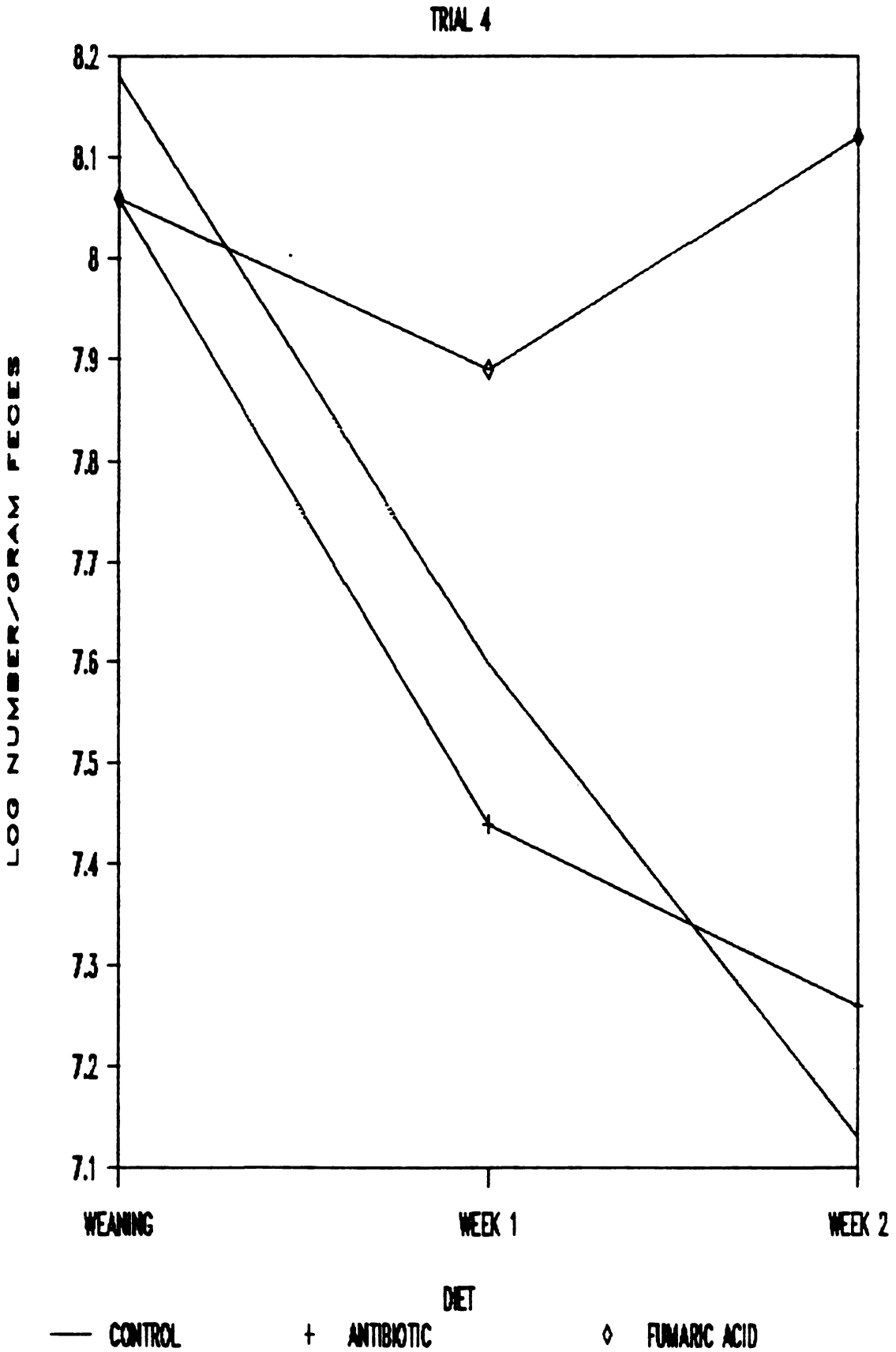
Time	Dietary treatment		
	Control	Antibiotic	Fumaric acid
[a,b,c]			
Initial	8.18	8.06	8.06
7 days	7.60	7.44	7.89
14 days	7.13	7.26	8.12
% change	-12.80	-10.00	0.00

[a] All values represent means of 8 counts, 4/pig/treatment.

[b] Standard error of the means = 0.50.

[c] Note: The standard error of the difference between two means = 0.625. Data were so lowly correlated across weeks that the standard error of the differences of 2 means was essentially the same for differences among and within weeks.

FIGURE 2. FECAL E.COLI COUNTS



V. CONCLUSIONS

1) Fumaric acid supplementation of the diet of weanling pigs significantly improved ($P < .03$) ADG during week 1 as compared to non-acidifed diets in a 4 week trial. Supplementation of antibiotics to the diet significantly ($P < .07$) improved ADG for the four week period, as compared to diets not containing this additive. Feed intake tended to be increased when diets contained either 1.5% fumaric acid or the antibiotic. During week 1 of the trial, the feed to gain ratio was significantly improved by fumaric acid supplementation, as compared to the other diets. This effect continued through the second week. The antibiotic supplement was also able to significantly improve this measure for the first 2 week period as compared to the control. However, for the entire 4 week period, no significant differences were observed for this performance measure.

2) Citric acid had no benefical influence on average daily gain, feed intake, or feed efficency during any phase of the 4 week trial. The antibiotic supplemented diets, however, were able to stimulate significant improvements in ADG and the feed to gain ratio as compared to diets not containing this additive.

3) 1.5% fumaric acid supplementation significantly lowered

diet gross energy, digestible energy, metabolizable energy, and metabolizable energy corrected for nitrogen, when compared to the control and antibiotic supplemented diets. These measures were greatest when diets contained the antibiotic supplement.

4) Nitrogen balance, net protein utilization, and the biological value of the protein appeared to be greatest when the diet contained 1.5% fumaric acid.

5) Retention of calcium was not affected by dietary treatment. Daily balance of phosphorus, as well as the percent retention of this mineral, were greatest for the control diet. Zinc retention appeared to be improved by the addition of 1.5% fumaric acid to the diet.

6) The population of fecal anaerobic bacteria significantly changed over a two week period, fumaric acid supplementation produced the greatest increase in numbers, and antibiotic supplementation the lowest increase. E.coli counts did not differ significantly. However, while pigs fed the control and antibiotic supplemented diets tended to have lower fecal E. coli populations over time, 1.5% fumaric acid addition to the diet did not lower fecal E.coli population over time.

VI. LIST OF REFERENCES

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