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THE EFFECTS OF TYROSINE, P-HYDROXYPHENYLACETIC ACID, P-CRESOL AND BACITRACIN METHYLENE DISALICYLATE ON THE GROWTH OF WEANLING PIGS

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By

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ABSTRACT

THE EFFECTS OF TYROSINE, P-HYDROXYPHENYLACETIC ACID, P-CRESOL AND BACITRACIN METHYLENE DISALICYLATE ON THE GROWTH OF WEANLING PIGS

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P-cresol is the predominant volatile phenolic metabolite excreted in the urine of weanling pigs, and studies have suggested that the intestinal production of this metabolite by bacteria maybe related to decreased rate of growth. Evidence indicate that a two step process, involving at least 2 bacterial species is responsible for p-cresol production. Tyrosine is first degraded to p-hydroxyphenylacetic acid (PHPAA) and the PHPAA is then decarboxylated to p-cresol. Three nutritional studies were conducted which involved the addition of 0.75% p-cresol (PC), 0.75% p-hydroxyphenylacetic acid (PHPAA) and 3.0% tyrosine (T) to the diet of weanling pigs. All studies were treated with or without bacitracin methylene disalicylate (BMD). In each study, 16 weanling pigs were assigned by weight, sex and litter to one of 4 treatment groups: (1) NA (no antibiotic); (2) A (antibiotic); (3) NA plus PC or PHPAA or T and (4) A plus PC or PHPAA or T for 28 days. Results of the PHPAA

study indicate that 0.75% PHPAA in the diet significantly increased (P<.05) urinary excretion of PHPAA (8.8, 9.6, 222.4 and 279.6 mg/total urine/24 hr). However, excess PHPAA did not affect performance and none of the pigs developed external pathologigal lesions. BMD significantly increased (P<.05) urinary excretion of PHPAA and decreased (P<.05) p-cresol excretion. There was significant negative correlation (P<.05) between body weight gain and urinary excretion of p-cresol. Results of the PC study indicate that 0.75% PC in the diet depressed the overall percent body weight gain (BWG) of weanling pigs (136.2, 137.4, 114.7 and 118.2%). Significant reduction (P<.05) of BWG did not occur until the third week of treatment. Factorial analysis showed significant reduction (P<.05) in average daily gain (ADG) with the addition of 0.75% PC in the diet (418, 405, 380 and 378g). Pigs receiving the excess PC had lower average daily feed intake (ADFI). Results of the tyrosine study showed that 3.0% tyrosine significantly increased (P<.05) urinary excretion of PHPAA, p-hydroxyphenyllactic acid (PHPLA) and p-cresol. However, at 3.0% tyrosine in the diet, pigs did not develop external pathological lesions. Absorption of excess tyrosine, as assessed by urinary excretion of PHPAA and PHPLA appeared to be influenced by the level of feed intake. Excess tyrosine in the diet significantly reduced the weekly percent BWG during the 3rd week of treatment. By the 4th week of treatment however, the pigs appeared to have recovered from the excess tyrosine.

To my parents, in gratitude for their love and support.

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A	Antibiotic
ADFI	Average daily feed intake
ADG	Average daily gain
A-PC	Antibiotic plus p-cresol
A-PHPAA	Antibiotic plus p-hydroxyphenylacetic acid
A-T	Antibiotic plus tyrosine
BMD	Bacitracin Methylene Disalicylate
BWG	Body weight gain
F/G	Feed per gain ratio
GLC	Gas liquid chromatography
IP	Intraperitoneal
NA	No antibiotic
NA-PC	No antibiotic plus p-cresol
NA-PHPAA	No antibiotic plus p-hydroxyphenylacetic acid
NA-T	No antibiotic plus tyrosine
PBA	Phenylbutyric acid
PC	P-cresol
PHPAA	P-hydroxyphenylacetic acid
PHPLA	P-hydroxyphenyllactic acid
T	Tyrosine
TMS	Trimethylsilylacetamide

INTRODUCTION

The anaerobic bacterial degradation of aromatic amino acids in the intestinal tract results in the production of several volatile phenolic and aromatic metabolites (Smith, 1966; Scheline, 1968; Bakke, 1969c; Yasuhara et al., 1979 and Yokoyama et al., 1974, 1979, 1982). Among the commonly detected bacterial metabolites in the gastrointestinal tract are 4-methylphenol (p-cresol), 3-methylindole (skatole), phenol, 4-ethylphenol and indole (Yoshihara, 1977, 1978, 1979, 1981, 1982). These metabolites are found in the feces and urine of most animals (Bakke, 1969a, 1969b; Anderson, 1975; Spoelstra, 1977, 1978; Yasuhara et al., 1979; Yoshihara, 1980, 1981; Martin, 1982; Yokoyama et al., 1982; Hoshika et al., 1983 and Ward et al, 1983) including humans (Herter, 1908; Armstrong et al., 1956; Mori et al., 1978; Cummings et al., 1979 and Curtis et al., 1976). When absorbed from the intestine, these microbial metabolites are detoxified by either glucuronide or sulfate conjugation and excreted via the urine (Williams, 1959; Smith, et al. 1966; Parke, 1968; Dirmikis and Darbre, 1974; Aarbakke et al., 1976; Bone et al., 1976 and Mori et al., 1978).

The breakdown of aromatic amino acids and the production of phenolic and aromatic metabolites by intestinal

microorganism are supported by several observations:

1) Volatile phenols, p-cresol and phenols are absent in the urine of germ-free animals (Bakke and Midveldt, 1970).

2) The oral treatment of humans with antibiotics and the supplementation of antibiotics in animal diets has decreased urinary excretions of p-cresol and phenols (Duran, 1973 and Yokoyama et al., 1982).

3) The urinary excretion of p-cresol and phenols is increased in humans with gastrointestinal disorders. The malabsorption that has resulted from the disorder has increased the exposure of the intestinal contents to the gut microflora (Duran, 1973).

The potential toxic effects of phenolic and aromatic metabolites have been recognized for a long time (Senator, 1868; Herter, 1907 and Dalgliesh et al., 1958). The formation and excretion of these toxic metabolites have received attention in medical and biomedical research (Volterra, 1942; Bernhart and Zilliken, 1959; Muting, 1965 and Duran, 1973). However, the prevailing notion has been that these metabolites are produced in the intestinal tract in too low concentrations to be of concern and that humans and animals can clear these toxic compounds from their system because of their efficient detoxification mechanisms. Questions have been raised however, as to whether a constant passage of fairly large amounts of these toxic compounds through the body is completely harmless (Duran, 1973).

Marked increases in the intestinal production of these bacterial metabolites have been detected under various disease states in humans (Sprince, 1962; Bryan, 1971; Tamm et al., 1971; Van der Heiden et al., 1971a, 1971b; Duran et al., 1973; Wadman et al., 1973; Chung et al., 1975; Bone et al., 1976; Mori et al., 1978 and Cummings et al., 1979). However, no direct pathogenesis resulting from the intestinal production of these bacterial metabolites has been demonstrated in any species except for the finding that ruminal skatole production is responsible for the tryptophan-induced acute pulmonary edema and emphysema in cattle (Carlson et al., 1972, 1976 and Yokoyama et al., 1975).

The intestinal microbial degradation of tyrosine, particularly the production of p-cresol and the possible adverse effects of p-cresol on the growth and performance of weanling pigs have been studied by Yokoyama et al. (1982). It was shown that p-cresol is the predominant volatile phenolic and aromatic metabolite excreted in the urine and feces of weanling pigs. The study indicated a high negative correlation between the percent body weight gain of weanling pigs receiving sub-therapeutic levels of antibiotics and the total excretion of p-cresol.

Based on these results, it was hypothesized that p-cresol could be responsible or partially responsible for the growth depression in weanling pigs and that antibiotics

exert their growth promoting effect by inhibiting the bacterial production of p-cresol in the intestinal tract. The studies indicate that urinary p-cresol excretion could be used to gauge the effectiveness of an antibiotic in promoting growth or it could be used as a diagnostic tool to indicate intestinal malabsorption.

The growth promoting effect of dietary antibiotics is well documented however, the exact mechanism of how antibiotics affect growth is not fully understood. Several theories have been proposed but none is conclusive enough to gain general acceptance.

Given the controversy surrounding the intensive use of antibiotics in livestock production, there is a need to establish the exact mechanism on how antibiotics promote growth. The study of phenolic and other aromatic metabolites, has added an alternative approach to the understanding of the working mechanism of dietary antibiotics.

The adverse effects of long-term exposure of weanling pigs to p-cresol are not known. There is very little information on the effects of a constant exposure to p-cresol below levels that would normally induce clinical toxicity. These problems were examined in view of the implication of intestinal microbial metabolites to efficient swine production.

In this investigation, three nutritional studies were

conducted involving the addition of p-cresol, p-hydroxyphenylacetic acid and tyrosine to the diet of weanling pigs. In each of the feeding studies, pigs were either provided with or without BMD dietary supplementation. The objectives of these studies were:

1. To demonstrate an etiologic relationship between the bacterial production of p-cresol in the intestinal tract and the depression of growth of weanling pigs.

2. To determine the effects of dietary supplementation of antibiotics on urinary excretion of volatile phenolic and aromatic bacterial metabolites and to determine if a relationship exists between the degree of production of these metabolites and the growth performance of weanling pigs.

3. To determine the effects of excess tyrosine, p-hydroxyphenylacetic acid and p-cresol on growth performance and the urinary excretion of phenolic and aromatic bacterial metabolites in weanling pigs.

LITERATURE REVIEW

THE GROWTH PROMOTING EFFECT OF ANTIBIOTICS

During the last 35 years, dietary supplementation of subtherapeutic levels of antibiotics has been shown to improve growth rate and efficiency of feed utilization in growing-finishing swine. The growth promoting effect of dietary antibiotic supplementation is well documented (Jukes and Williams, 1953; Stokstad, 1954; Francois, 1962; Hays and Muir, 1979; Langlois et al. 1978 and Visek, 1978). The widespread use of dietary antibiotics is one of the major reasons for the use and development of highly intensive confinement systems that are prevalent in the swine industry today.

Despite the considerable amount of research data on antibiotics, the mechanism of action by which antibiotics stimulate growth is not fully understood. Several theories have been suggested (Jukes and Williams, 1953; Francois, 1962; Luckey, 1963; Francois and Michel, 1968; Visek, 1978; Cromwell, 1983) and the mode of action is thought to be related to:

1) a metabolic effect
2) a nutritional effect
3) a disease-control effect

The metabolic effect theory implies that antibiotics directly influence the metabolic processes in the animal (e.g. nitrogen retention, fatty acid oxidation, protein synthesis, metabolic rate, etc.). The feeding of chlortetracycline to pigs was reported by Braude and Johnson (1953) to affect water and nitrogen excretion. Using rat liver homogenates, Brody et al. (1954) observed that tetracycline inhibited fatty acid oxidation of the mitochondria. In one investigation, tetracycline inhibited protein synthesis (Hash et al., 1964) while in another study, antibiotic supplementation altered digestibility of proteins and amino acids (Eggum et al., 1979). The biological value of protein and the net protein utilization by rats was increased significantly by the addition of antibiotics (Campbell et al., 1982). However, there is still inadequate evidence to state that antibiotics affect protein or amino acid utilization (Baker et al., 1982).

The objection to the metabolic effect theory is that the normal tissue concentrations that result from the feeding of low levels of antibiotics are not sufficient to account for the growth response that results from the subtherapeutic supplementation of antibiotics (Hays, 1969; Cromwell, 1983). According to Baker et al. (1982), the growth response caused by antibiotic supplementation is not anabolic in nature. Instead, the growth promoting effect generally results from an increase in voluntary feed intake.

The nutritional effect theory is supported by a substantial amount of research data. The theory contends that the intestinal bacterial population is involved in the growth promoting response caused by the feeding of antibiotics. This is a concept that has been accepted since the early days of antibiotic feeding. The most compelling evidence supporting this theory is the lack of improved growth response with antibiotic supplementation under germ-free conditions. Coates et al. (1963) and Freeman et al. (1975) showed no growth stimulation in chicks fed antibiotic-supplemented diets under germ-free conditions. Whitehair and Thompson (1956), using pigs isolated immediately after delivery by caesarian section and fed purified diets supplemented with chlortetracycline, also reported negative growth response.

The frequently suggested mechanism involving bacterial metabolism is that the shifts in bacterial population from the feeding of antibiotics may result in greater availability of nutrients to the host animal or that the bacterial destruction of essential nutrients is reduced. Another suggested mechanism is that with dietary antibiotics there is increased efficiency of absorption and utilization of nutrients because the wall of the gastrointestinal tract is thinner (Visek, 1978).

The disease-control theory also has extensive research support. This theory suggests that antibiotics suppress the

bacteria in the intestinal tract that are responsible for subclinical and non-specific disease which prevent the animal from performing to its maximum genetic potential. Some of the evidences that support this theory are that responses to antibiotics are greater under high disease levels than under low disease levels, responses are greater in young pigs, which are less tolerant to stress and disease than are older pigs and that responses are greater in a dirty than in a clean environment (Speer et al., 1950; Hawbaker et al., 1960; Hays, 1969; Jukes, 1971).

The difficulty of resolving the exact mechanism by which antibiotics exert their growth promoting effect arises from the number of variables which must be considered. These include the species involved, the individual, its nutrition, the stage of production cycle, the environmental conditions and the antibiotic itself (Francois, 1962 and Hays and Muir, 1979).

A cause and effect relationship is difficult to establish because neither a consistent change in bacterial population could be established nor a specific pathogen could be incriminated with antibiotic feeding (Francois, 1962 and Visek, 1978). Particular genera (i.e. anaerobic clostridial) and species (i.e. Clostridium perfringens, Clostridium welchii, Clostridium aerogenes, S. liquefaciens and S. fecium have been studied (Sieburth et al., 1951; Rosomer et al., 1952; Lev and Forbes, 1959) but the results

of these investigations have been too inconsistent and contradictory to imply their specific involvement (Jukes and Williams, 1953).

This problem has led to suggestions that the stimulation of growth caused by antibiotics may be due to intestinal bacterial metabolism (Visek, 1978 and Francois, 1962). Changes in metabolism have been demonstrated specifically in glucose fermentation, amino acid deamination and decarboxylation and urea hydrolysis (Francois et al., 1955; Melnykowycz and Johansson, 1955 and Visek et al. 1959). The alteration in bacterial metabolism could influence nutrient availability of energy, nitrogen, certain water-soluble and fat-soluble vitamins and have a favorable effect on mineral metabolism to the animal (Francois, 1962).

Previous studies have shown that the feeding of virginiamycin to pigs resulted in the reduction of ammonia, amine and lactic acid production in the intestinal tract (Henderickx et al., 1981 and Hedde, 1981). These metabolites represent potential losses of protein and energy which suggests that virginiamycin spares both energy and amino acids for the pigs.

Vervaeke et al., (1979) and Knoebel and Black (1952) have suggested that the mechanism involved in the growth-promoting effect of antibiotics is a nutrient sparing effect. Vervaeke et al. (1979) showed that dietary

antibiotics spared glucose by decreasing the bacterial production of organic acids in the intestinal tract, thereby making more net energy available for growth. However, the evidence for a protein sparing effect is contradictory and the amino acid sparing effect has not been thoroughly investigated (Francois, 1962).

Differences between germ-free and conventional animals have provided the basis for developing theories about mechanisms of the growth promoting effects of dietary antibiotics. Morphologic differences between germ-free and conventional animals are similar to those between antibiotic-fed and non-antibiotic fed animals reared in conventional environment (Gordon, 1959; Gordon et al., 1958 and DeSomer et al., 1965). Visek (1978) suggested that the factors responsible for these differences also affect the growth of young animals and the growth response due to antibiotics. The mass of the small intestine tissue is greater and the mucosal cell surface is reported to be 33% larger in conventional animals (Gordon and Bruckner-Kardos, 1961) than in germ-free animals. The mucosal cells are also replaced 30% to 40% faster in conventional animals. The replaced mucosal cells are extruded into the intestinal lumen where they are digested (Lesher et al., 1964 and Meslin, 1971). Investigators (Dintzis and Hastings, 1953; Kornberg and Davies, 1955; Levenson et al., 1959; Visek et al., 1959; Norman and Widstrom, 1964; Delluva et al., 1968) have reported that the

breakdown of urea and bile acids occur in the intestinal lumen of conventional animals but not in germ-free animals and that the products of hydrolysis are reduced or abolished by antibiotics.

Products of amino acid metabolism have been known to influence growth depression, and investigators have suggested that the reduction in the formation of these products may be related to the growth-stimulating effect of antibiotics. Francois and Michel (1955) have earlier pointed out the relationship between the ability of antibiotics to promote growth and their ability to inhibit the deamination of arginine by the intestinal flora of the pig in vitro. The use of spiramycin, which is an effective growth promoter in pigs and a strong inhibitor of arginine deamination, provided further support to this argument. According to Francois (1962), the relationship between growth stimulation and deaminase inhibition is not limited to antibiotics. Copper sulphate and organic arsenicals, whose growth-promoting effects are known, are also inhibitors of deaminases.

Visek (1978) and Francois and Michel (1968) have suggested that the reduction of bacterial ammonia production in the gastrointestinal tract may be related to the growth-promoting effects of antibiotics. Ammonia, a product of protein breakdown during the digestive process, is a recognized toxin in warm-bloodied animals (Phillips et al.,1952;

Visek, 1964 and Visek, 1972). Bacterial breakdown of proteins in the intestinal lumen is reported to be the main source of ammonia in the body (Phillips et al., 1952).

Reductions of intestinal free ammonia improved growth rates in chicks and rats (Visek, 1964). Visek (1962) proposed that the improvement in the growth performance of rats and chicks immunized with jackbean urease was due to the decrease in ureolytic activity in the gastrointestinal tract. The effect of antibiotics or urease inhibitors on blood ammonia levels in animals is well documented (Schenker et al., 1967; Visek et al., 1968; Chow and Pond, 1971; Visek and Milner, 1974; Hindfelt et al., 1977; Sener et al., 1978; Visek, 1978; Drummond et al., 1980 and Bartley et al., 1981). Kornegay et al. (1964) studied the effects of urease immunization along with blood and intestinal ureolysis on the growth performance of growing pigs. They observed an increase in average daily gain (ADG) in pigs treated intraperitoneally (ip) with 10 units of urease (modified Sumner units/lb of BW). The plasma ammonia levels decreased when pigs were injected with 0.5 units of urease. They also found that urease immunization was effective in lowering intestinal urease activity. Zuidema et al. (1962) reported that antibiotics either inhibit or remove bacterial production of ammonia.

Visek (1978) observed that ammonia increased the mass

of intestinal tissues, and when bacterial production of ammonia was suppressed by dietary antibiotic supplementation, the weight of the intestinal tissues decreased. Visek (1978) postulated that when the intestinal tract is thinner due to the decrease in ammonia production, the efficiency of absorption and the utilization of nutrients are increased. Braude et al. (1955) reported that antibiotics reduced the thickness of the intestinal wall, resulting in greater absorption of nutrients.

Another area of investigation on the mode of action of antibiotics has focused on the metabolism of primary bile acids by the gastrointestinal microorganisms. The end products of bacterial degradation of primary bile acids are toxic, with the mono-hydroxyl compound lithocholic acid being the most toxic (Palmer, 1972, 1976). Bacterial degradation of bile acids has detrimental effects because the hydrolytic products of bile acids can change micelle formation, lipid absorption, intestinal histology and calcium absorption (Visek, 1978). It was hypothesized that the feeding of antibiotics would decrease the microbial deconjugation and dehydroxylation of primary bile acids into toxic compounds. It had been shown in rats, mice and humans that antibiotics affected the patterns and composition of the bile. Madsen et al. (1978) reported that the 5-day feeding of chlortetracycline to rats changed the bile acid patterns and these changes lasted for over 30 days. In an earlier

study, Linstedt and Norman (1956) observed that the feeding of oxytetracycline and phthatysulfathiazole to rats increased the biological half-life of cholic acid from 2-3 days to 10-15 days. Recently, Tracy et al. (1986) examined the effects of carbadox, an antimicrobial agent, on the biological half-life of chenodeoxycholic acid, a primary bile acid, in the growing pig. The fluctuations of the plasma bile acid concentrations were also studied. Their data showed that the dietary addition of carbadox at 58 ppm significantly affected the clearance of chenodeoxycholic acid from the hepatic portal veins. The plasma concentrations of the bile acids were significantly higher in the carbadox-treated pigs. This implied that more of the bile acids were in the hydroxylated and conjugated forms and this allowed for more recycling of bile acids. They also showed that the rate of excretion of bile acids in the feces increased in the carbadox-fed pigs. In contrast to the observations of Linstedt and Norman (1956) in rats, Tracy et al. (1986) found that the biological half-life of chenodeoxycholic acid decreased in the carbadox-treated pigs. The investigation of Tracy et al. (1986) indicated that the bile acid metabolism of the growing pig was significantly affected by the supplementation of carbadox.

A theory on the mechanism of action of antibiotics which has received very little research attention is the possible involvement of intestinal bacterial metabolites

produced by the anaerobic degradation of aromatic amino acids. Previous studies have shown a relationship between the intestinal bacterial production of volatile phenolic and aromatic metabolites and the growth promoting effect induced by dietary antibiotic supplementation. Bernhart and Zilliken (1959) demonstrated that the weight depression caused by excess tyrosine in the diet of rats was inversely correlated to the increase in urinary volatile phenol excretion and that chlortetracycline prevented the reduction in weight.

The effects of antibiotics on the excretion of phenolic compounds in humans was examined by Rogers et al. (1955). They reported that the oral administration of terramycin, aureomycin, chloramphenicol and penicillin to humans decreased the urinary excretion of volatile phenolic compounds. The effect of the antibiotic supplementation was mainly confined to the urine fraction containing p-cresol and phenols. The phenolic content of the feces decreased with terramycin whereas the fecal excretion of tyrosine increased. Parenteral administration of penicillin had a negligible effect on the urinary excretion of p-cresol and phenols. Rogers et al. (1955) also found that dietary supplementation of antibiotics barely modified the urinary excretion of p-hydroxyphenylpropionic acid, p-hydroxyphenylacetic acid and p-hydroxybenzoic acid.

Yokoyama et al., (1982) showed that weanling pigs on

CSP (chlortetracycline sulfamethazine penicillin) suppemented diet excreted less urinary p-cresol than pigs fed the unsupplemented diets. The CSP-treated pigs grew faster and had higher percentage weight gains than pigs receiving no antibiotics. A high inverse correlation (r= -0.73) was found between percent body weight gains and urinary p-cresol excretion. The study suggested that p-cresol may be a factor involved in the growth depression of weanling pigs and that dietary antibiotic supplementation may have promoted growth by reducing the intestinal production of p-cresol.

In marked contrast to the observations of Rogers et al. (1955), Bernhart and Zilliken (1959) and Yokoyama et al. (1982), which showed reduction of phenolic compounds with antibiotic supplementation, Huang and McCay (1953) reported that the urinary excretion of indican, a bacterial metabolite of indole, increased in pigs receiving diets supplemented with terramycin. The concentration of indican in the urine and the daily excretion of indican in pigs fed diets supplemented with terramycin averaged 106 ppm and 11.8 mg, respectively, while those pigs receiving the basal diets had an average of 65 ppm and 9.3 mg, respectively. In another study, however, Wisman and Engel (1957) could not demonstrate a growth depressing effect of dietary indole supplementation in chickens.

Recently, Shoemaker and Visek (1980) investigated the

influence of chlortetracycline on the urinary excretion of p-hydroxyphenylacetic acid and p-hydroxyphenyllactic acid. They found that p-hydroxyphenylacetic acid was primarily a bacterial product and p-hydroxyphenyllactic acid resulted from host processes. Rats fed chlortetracycline grew faster than controls and excreted more p-hydroxyphenylacetic acid. Rats which showed no antibiotic growth-promoting effect excreted less p-hydroxyphenylacetic acid while excretion of p-hydroxyphenyllactic acid remained unchange. Shoemaker and Visek (1980) concluded that the p-hydroxyphenylacetic/p-hydroxyphenyllactic acid ratio reflected the antibiotic effect on the amino acid degradation in the gastrointestinal tract.

THE EFFECTS OF EXCESS TYROSINE AND OTHER AMINO ACIDS

It is well known that excess dietary tyrosine will result in the development of external and histopathological lesions in animals (Hueper and Martin, 1943; Schweizer, 1947; Alam et al., 1966; Bocter et al., 1968, 1970; Muramatsu et al., 1971, 1975, 1976; Selye, 1971; Ip and Harper, 1973; Datta and Ghosh, 1977; Yamamoto and Muramatsu, 1982). The external toxic syndrome is characterized by the occurrence of eye and foot lesions. These lesions are generally observed within five to eight days after initiation of intakes of high tyrosine diets. The toxic effects of feeding excess tyrosine is well documented in rats (Hueper and Martin, 1943; Schweizer, 1947; Sadhu, 1951; Alam et al., 1966 and Bocter et al., 1968, 1970). Several factors were reported to affect the severity of tyrosine toxicity. The tolerance of rats for high levels of tyrosine was improved as the protein content of the diet was increased (Sauberlich, 1961 and Harper et al.,1966). However, when the tyrosine content of the diet was 10% or more, the toxic signs developed in rats even though the diet was adequate in protein (Martin, 1943). L-tyrosine was found to be more toxic than the D-tyrosine, with the D-isomer being excreted more rapidly in the urine than the L-isomer (Martin, 1943).

Investigators have examined the involvement of vitamin

deficiencies in the development of toxic signs due to intakes of high levels of tyrosine. Riboflavin and nicotinic acid-deficient rats were more susceptible to tyrosine toxicity than those that received supplements of riboflavin, nicotinic acid and/or tryptophan (Niven et al. 1946 and McKean et al., 1968).

Excess tyrosine in the diet also reduced feed intake and depressed the growth rate of animals (Martin and Hueper, 1943; Bernhart and Zilliken, 1959; Muramatsu et al., 1971, 1972; Friedman and Gumbmann, 1984 and Yokoyama et al., 1985). Depression of feed intake is generally considered an effect of excessive intakes of individual amino acids in relation to the other amino acids (Harper et al., 1970 and Baker and Tanksley, 1977). The growth retardation caused by the amino acid imbalance becomes less severe as the protein content of the diet is increased adequately (Harper, 1959).

Age and sex of animals appear to be factors in tyrosine toxicity. Early studies (Hueper and Martin, 1943; Schweizer, 1947) have shown that adult rats were more resistant to tyrosine toxicity than young rats and that male rats were more severely affected by tyrosine toxicity than females of the same age.

The external pathological lesions are characteristic symptoms of tyrosine toxicity, but several studies have indicated other toxic effects due to excessive intakes of

tyrosine. Ip and Harper (1975) showed that protein synthesis in the muscle and brain of rats given excess tyrosine was markedly inhibited. Similar inhibitory effects on protein synthesis were observed by Dillehay et al. (1980) when high concentrations of phenylalanine were added to the cell and tissue cultures. Early investigators (Hueper and Martin, 1943; Sadhu, 1951 and Deb and Biswas, 1965) have reported histopathological changes in the skin, pancreas, kidney and testis of rats fed diets low in protein and high in tyrosine. Schweizer (1947) indicated that excess tyrosine produced necrotic changes in the liver of rats and exerted a nephrotoxic effect in rabbits and dogs. The production of hepatic and other organ lesions, or even death, by excess tyrosine have also been confirmed by many investigators in dogs, rabbits, rats, guinea pigs and chickens (Shambough et al., 1929 and Hill et al., 1945). The reasons for this toxicity are not known.

The effect of tyrosine and antibiotic supplementation on the growth of weanling pigs and on the urinary excretion of p-cresol, p-hydroxyphenylacetic and p-hydroxyphenyllactic acids was investigated by Yokoyama et al. (1985). Results of their study showed that the addition of 3% tyrosine in the diet reduced the feed intake and growth rate of weanling pigs. However, at 3% tyrosine in the diet, the pigs did not develop external pathological lesions that characterized the effects of excess tyrosine in rats. The supplementation of

the diet with antibiotics appeared to alleviate the reduction in feed intake and decreased growth rate caused by excess tyrosine. The urinary excretion of p-cresol, p-hydroxyphenylacetic acid and p-hydroxyphenyllactic acid increased when 3% tyrosine was added to the diet. This indicated an increase in the absorption and metabolism of tyrosine. They also reported that the absorption of excess tyrosine, as assessed by the urinary excretion of p-hydroxyphenylacetic acid and p-hydroxyphenyllactic acid, was influenced by the level of feed intake.

In a feeding study where rats were given diets containing 10% L-tyrosine, Bakke (1969b) reported that p-cresol accounted for the bulk of the simple phenols found in the urine, although smaller amounts of phenols, hydroquinone, 4-methylcatecol and 4-methylguaiacol were also present. He suggested that the formation of simple phenols could have considerable significance in regard to the adverse effects observed during long-term feeding of high tyrosine diets. The findings of Bakke (1969b) supported the earlier observation of Bernhart and Zilliken (1959) which showed increased urinary excretion of volatile phenols in rats fed 7% and 10% tyrosine. Using thin-layer and gas chromatographic analysis of hydrolyzed rat urines, Bakke (1969b), however, found that the amounts of volatile phenols recovered in his study far exceeded the values reported earlier by Bernhart and Zilliken (1959) (103 vs 12 mg p-cresol/24hr). The discrepancy

between the two results was probably due to the low acid concentration (0.5 N HCL) used by Bernhart and Zilliken (1959) for the conjugate hydrolysis as compared to the 3 N H_2SO_4 used by Bakke (1969b). Bernhart and Zilliken (1959) also used the acid hydrolysis, steam distillation and the Folin-Ciocalteu phenol reagents for analysis of the urinary volatile phenols while Bakke (1969b) employed the acid hydrolysis-GLC procedures. In contrast to the findings of Bakke (1969b) and Bernhart and Zilliken (1959), which showed increased urinary excretion of volatile phenols with excess tyrosine, Schmidt et al. (1958) did not see an increase in the urinary excretion of volatile phenols in rats fed high levels of tyrosine.

The effects of various amino acid supplementation on total urinary phenol excretion in rats fed 3% tyrosine was examined by Alam et al. (1967). They reported that, during the first 2-day collection period (days 3 and 4), rats fed a low protein diet with 3% tyrosine with or without amino acid supplements had higher urinary excretion of total phenols than rats fed diets with no tyrosine. However during the second collection period (days 12 and 13), the urinary total phenol excretion did not increase in rats fed 3% tyrosine supplemented with 1.2% L-threonine or 2.5% glycine. During this collection period, the total phenol excretion of the L-threonine supplemented group decreased. The urinary phenol excretion in the L-threonine treatment group

decreased despite having the highest tyrosine intake among all rats. In both collection periods, the total urinary phenol excretions increased in rats fed 3% tyrosine plus methionine. However, the amount of excretion decreased markedly during the last collection period (days 21 and 22).

There is evidence suggesting that the rate of brain catecholamine synthesis is influenced by the tyrosine concentration in the brain (Gibson and Wurtman, 1978; Sved et al., 1979). Catecholamine neurotransmitters are produced from tyrosine in the sympathetic nerve terminals and in the adrenal gland (Stryer, 1981). Leibowitz and co-workers (1979, 1980) reported that, in the rat, the feeding response was inhibited by the increased activity of the catecholaminergic neurons of the hypothalamus. The data suggested that there was a depression in feed intake when the concentration of catecholamines in the hypothalamus was increased and that the reduction in feed intake could be alleviated by limiting the tyrosine availability to the brain.

Reeves and O'Dell (1984) studied the effects of dietary tyrosine levels on food intake in zinc-deficient rats. Their data showed that low levels of dietary tyrosine significantly increased feed intake in zinc-deficient rats and significantly decreased the concentration of tyrosine in the serum and anterior hypothalamus. Reduced levels of tyrosine in the hypothalamus led to decreases in the concentrations

of norepinephrine and dopamine in the anterior hypothalamus. However in another study, Badawy and Williams (1982) reported that rat brain catecholamine synthesis was enhanced by small doses of tyrosine but was inhibited when given at 50mg/kg body weight and above. They concluded that large doses of tyrosine caused substrate inhibition of tyrosine hydroxylase activity. Tyrosine hydroxylase catalyzes the hydroxylation of tyrosine to dopa (3,4-dihydroxyphenylalanine) (Stryer, 1981). This is the first step in the synthesis of catecholamine neurotransmitters and is a rate limiting reaction. The findings of Badawy and Williams (1982) confirmed the observation made earlier by Wurtman and Fernstrom (1976). Both studies suggested that high levels of tyrosine caused product inhibition of tyrosine hydroxylase activity, thus affecting the first and rate limiting step in the synthesis of catecholamines.

There are metabolic disorders in the pathway for the endogenous degradation of tyrosine and phenylalanine to acetoacetic acid. One of these is alcaptonuria, an inherited metabolic disorder, which is caused by the absence of homogentisate oxidase (Stryer, 1981; Lehninger, 1982). Homogentisate oxidase cleaves the aromatic ring of homogentisic acid to yield 4-maleylacetoacetic acid. In people with defective homogentisate oxidase, the homogentisic acid accumulates in the body fluids and is excreted in the urine. The urine turns black on standing as the homogentisic acid

is oxidized by atmospheric oxygen and polymerized to a melanin-like substance (Stryer, 1981 and Lehninger, 1982). Apart from the concern for the excretion of black urine, it is believed that people with this genetic disorder suffer no significant impairment of health (Lehninger, 1982).

Urinary excretion of homogentisic acid occurred regularly when high levels of tyrosine were supplemented in the diet (Dalgliesh, 1955). Early work by Abbot and Salmon (1943) also indicated that homogentisic acid was excreted in large amounts after feeding excess tyrosine. However, Bernhart and Zilliken (1959) found that only small amounts of homogentisic acid were excreted in the urine of rats fed high levels of tyrosine compared to the urinary excretion of total phenols and phenolic acid fractions. The assumption has always been that the dark color of the urine in rats fed excess tyrosine was due to homogentisic acid (Schweizer, 1947). Bernhart and Zilliken (1959) noted that the amount of color present in the rat urine was closely correlated with the volatile phenol content of the urine. Alam et al. (1966) reported that after three weeks of adding threonine and glycine to diets containing 3% tyrosine, there was a substantial increase in the urinary excretion of homogentisic acid. This finding suggested that the breakdown of tyrosine was increased by the addition of these amino acids.

It has been shown that phenolic acids derived from tyrosine inhibit the biosynthesis of sterols. Using rat

livers, Ranganathan and Ramarsarma (1973) observed that only those compounds with phenolic ring structures and carboxyl groups were inhibitory to the incorporation of (2-14C) mevalonate and non-saponifiable lipids in vitro. P-hydroxycinnamate was the most powerful inhibitor among the compounds tested. Organic acids without the aromatic ring structure were not inhibitory. They concluded that the presence of the aromatic ring and the carboxyl group were necessary structural requirements for the inhibition of cholesterol synthesis.

Bocter and Harper (1968) observed that rats fed a low protein diet containing 5% tyrosine developed external pathological lesions within a few days. When p-hydroxyphenylpyruvic acid was substituted for tyrosine, signs of toxicity did not develop within 2 weeks. Rats force-fed the high tyrosine diet showed only temporary improvement in weight gains which indicated that the low feed intake was an effect rather than the cause of tyrosine toxicity. They demonstrated that the depression in growth rate of rats fed excess tyrosine was not due to the reduction in feed intake as would have occurred with an amino acid imbalance. They concluded that the intermediates of the main pathway of tyrosine degradation such as p-hydroxyphenylpyruvic and homogentisic acid were not responsible for the development of the tyrosine-induced external pathological lesions.

However, Yanaka and Okumura (1982), using 8-day old

male chicks in a 10-day feeding study, reported that the growth retardation caused by high levels of tyrosine was due to the combined effects of the depression in feed intake and reduced efficiency of feed utilization. They found that the addition of 5% tyrosine to the basal diets significantly reduced the body weight gain, gain/feed ratio, metabolizable energy, nitrogen and energy retention rates at all levels of feed intakes (160, 140, 120, 100 and 80 grams per 10 days). These findings supported the observation made earlier by Okumura et al. (1980) who also found a positive correlation between body weight gain and feed intake in chicks fed high tyrosine diets. Chicks fed excess tyrosine had retarded growth rate, decreased feed intakes and feed/gain ratios and had developed external pathological lesions (Okumura et al., 1980). In contrast, early investigators (Hill et al., 1944) reported that day-old chicks grew at a normal rate and showed no evidence of toxic symptoms when fed chick starter ration containing 0.5% to 3.0% L-tyrosine.

The growth and metabolism of rats fed a low protein diet containing various levels of tyrosine and p-hydroxyphenylpyruvic acid (p-HPP) were studied by Yamamoto and Muramatsu (1982). They found that the growth rate and feed intake of rats fed a low protein diet containing 3% or more tyrosine were depressed, and rats developed external lesions of the foot and the eye. In rats fed the low protein diet containing graded levels of p-HPP, the growth rate and feed

intake were more depressed than the rats fed the same level of tyrosine, but the rats did not develop external lesions. Their findings supported the observations made by Bocter et al. (1968) and Ip et al. (1973) that there was a correlation between the free tyrosine concentration in the tissues and the development of eye and paw lesions which are characteristic signs of tyrosine toxicity. Yamamoto and Muramatsu (1982) also concluded that p-HPP was not associated with the development of the external pathological lesions. This finding was also in agreement with the previous observation made by Bocter and Harper (1968). In another study, Alam et al. (1967) reported a close relationship between plasma tyrosine concentration and the occurrence of pathologic lesions in rats fed the 3% tyrosine diet. Other investigators (Sauberlich, 1961; Muramatsu et al., 1972 and Ip and Harper, 1973) also found elevated levels of tyrosine in the plasma of rats consuming high tyrosine diets. The addition of glycine, methionine, tryptophan, threonine or the mixture of leucine, isoleucine and valine as well as the injection of cortisol which prevented the development of external pathological lesions, also lowered the plasma tyrosine concentration (Alam et al., 1967). Ip and Harper (1973) later demonstrated that the extraordinary accumulation of plasma tyrosine in rats consuming high levels of tyrosine and the development of toxic lesions were prevented by the supplementation of dietary protein.

Modifications to the diet have been made in an attempt to alleviate the growth depression and tissue-destroying effects of excess tyrosine. Tyrosine toxicity was counteracted by increasing the level of protein or free amino acids in the diet (Martin and Hueper, 1943; Schweizer, 1947; Alam et al. 1967; Muramatsu et al., 1971, 1972, 1975; Selye, 1971; Ip and Harper, 1973; Keichiro et al., 1975; Datta and Ghosh, 1977; Friedman and Gumbmann, 1984). Muramatsu et al. (1975) alleviated the growth retardation and the development of eye and paw lesions that occur in rats ingesting a 10% casein diet plus 5% tyrosine by the addition of 15% or 40% casein to the diet. In another study, Muramatsu et al. (1971) almost completely eliminated the toxic signs of excess tyrosine in rats by elevating the protein content of the diet to 25% or 40%. Other investigators have shown that the addition of 0.5%-1.25% L-threonine (Alam et al., 1966; Datta and Ghosh, 1977), 0.66% L-methionine plus 0.9% L-threonine or 1.25% L-threonine plus 0.2% L-tryptophan (Muramatsu et al., 1976) improved the growth rate of rats fed excess tyrosine to within the growth rate of the control group. The depression of weight gains in mice caused by the addition of D-tyrosine to amino acid or casein diets was significantly reduced by increasing the L-phenylalanine content of the amino acid diets and the protein content of the casein diets (Friedman and Gumbmann, 1984). Yanaka and Okumura (1981) showed that the supplementation of arginine and glycine alleviated the growth retardation of chicks

which were fed diets containing high levels of tyrosine.

In a more recent study, Yanaka and Okumura (1982) reported that the addition of 5% tyrosine to the basal diet decreased the free plasma concentration of arginine, glycine and threonine at all levels of feed intake (160, 140, 100 and 80 g/10 days) in 8-day old chicks. They suggested that the reduced concentrations of plasma free arginine, glycine and threonine in chicks fed excess tyrosine could result in deficiencies of these amino acids.

In their review of the adverse effects of excessive intakes of amino acids, Benevenga and Steele (1984) suggested that the first and second limiting amino acids are protective against the toxic effects of excess tyrosine. According to these investigators, the addition of these limiting amino acids could cause a substantial reduction in the circulating level of tyrosine and a reduction in the urinary excretion of phenols. Alam et al. (1967) indicated that the addition of glycine, methionine, tryptophan or the mixture of leucine, isoleucine and valine prevented the development of external pathological lesions in rats fed 3% tyrosine. Threonine supplementation alleviated both the growth-depressing effect and the occurrence of external lesions caused by the 3% tyrosine diet.

The effects of supplementing 7% or 10% tyrosine to complete diets based on lactose, sucrose and sucrose plus

chlortetracycline on the growth rate and on the urinary excretion of phenols in rats were examined by Bernhart and Zilliken (1959). The lactose-fed group had the lowest reduction in weight gains when rats were fed 7% tyrosine. At 10% tyrosine in the diet, only the lactose-fed rats gained weight. The gain in weight was correlated with lower urinary excretion of volatile phenols suggesting that lactose depressed or lowered the production of volatile phenols. At all levels of added tyrosine, the urinary excretion of volatile phenols was lowest in the lactose-fed group followed by the sucrose plus chlortetracycline group. The sucrose plus tyrosine group which had the highest reduction in weight gain also had the highest urinary excretion of volatile phenols. Bernhart and Zilliken (1959) suggested that the formation of volatile phenols by the intestinal microorganisms was a factor in the weight reduction caused by the high levels of dietary tyrosine. In the same study, Bernhart and Zilliken (1959) noticed the adaptation of rats to increased tyrosine intake which was indicated by the decrease in the urinary phenol excretion. The adaptation to excess tyrosine was correlated with the increase in growth rate. Other investigators (Alam et al., 1966; Bocter et al., 1970) also observed that the growth depression and the severity of lesions lessened over the two week periods of their studies which suggested that the rats adapted to the intake of high level of tyrosine.

Muramatsu et al. (1975) showed that the excretion of total phenols and volatile phenols was not directly related to the development of tyrosine toxicity. They found that the urinary excretion of total phenols in rats fed the 5% tyrosine diet supplemented with extra casein, wheat and corn gluten was higher than those of rats fed the 10% casein plus 5% tyrosine. The rats with the higher urinary excretion of total phenols did not develop any signs of tyrosine toxicity.

Most of the effective treatments that alleviated tyrosine toxicity have involved the supplementation of amino acids or the increase in protein contents of the diets. However, treatment with hormones has also been reported to influence tyrosine toxicity. Glucagon injection minimized the growth retardation and depression in feed intake and alleviated the external lesions in rats fed 3% or more tyrosine (Ip and Harper, 1973). The typical features of dietary tyrosine intoxication in rats can be prevented not only by glucocorticoids but also by catatoxic steroids devoid of glucocorticoid potency (Selye, 1971). Bocter (1967) found that thiouracil alleviated while thyroxine increased the toxic effects of excess tyrosine. Cortisol treatment was also effective in diminishing the toxic signs (Alam et al., 1967). Lin and Knox (1957) and Kenny and Flora (1961) reported that hydrocortisone injection resulted in more than a fourfold increase in tyrosine transaminase activity.

Since tyrosine transaminase catalyzes the initial step in tyrosine degradation, the studies implied that tyrosine breakdown is facilitated by the injection of hydrocortisone. Munro et al. (1963, 1965) indicated that the dietary supplementation of some amino acids in rats increased the production of corticosterone. Based on these observations, Alam et al. (1967) suggested that the amino acids that alleviated tyrosine toxicity may have exerted their effect by increasing the tyrosine transaminase activity through increased production of corticosterone. The increased activity of tyrosine transaminase lowered the plasma tyrosine concentration by enhancing the breakdown of tyrosine.

Muramatsu et al. (1971) studied the adverse effects of excess levels of 18 individual L-amino acids on growing rats. Rats were fed with a 10% casein diet containing 5% of individual amino acids for three weeks, and the growth depression of varying degrees was observed. Their data showed that L-tyrosine, L-phenylalanine and L-methionine produced the most severe growth depression.

Among the other amino acids, excess tryptophan (5% or more) is generally considered to be one of the more toxic amino acids based on growth and feed intake measurements of rats fed low-protein diets (Harper et al., 1970; Muramatsu et al. 1971, 1972). However, Penga et al. (1973) noted that when 5% tryptophan was fed to rats, the amount of tryptophan in the diet was about 33 fold the requirement for growth

compared to that of methionine which was only 8 times the requirement for growth. The data suggested that relative to requirement for growth, tryptophan is less toxic than methionine and maybe less toxic than previously indicated.

During the last 15 years, there had been considerable research on the role of tryptophan in the development of pulmonary emphysema and edema in ruminants. Carlson et al. (1968, 1972) and Johnson and Dyer, (1966) reported that, after intraruminal doses of tryptophan, cattle developed an acute pulmonary edema and emphysema which usually resulted in death. Subsequent studies showed high levels of skatole in the rumen and plasma of affected animals. The high concentration of skatole was induced by the administration of L-tryptophan (Yokoyama et al., 1975). It was theorized that skatole produced in the rumen from tryptophan is absorbed into the bloodstream and affects the lungs of animals (Carlson and Dickinson, 1978). The mode of action of skatole on the lungs is not known.

Among the amino acids required for protein synthesis, methionine is considered to be the most toxic (Benevenga and Steele, 1974). Excess methionine (3 or 4 times estimated requirements) in animals fed low protein diets caused marked suppression in growth and voluntary feed intake. Benevenga et al. (1976) and Peng et al., (1981) reported that the spleens of rats fed excess methionine were enlarged twofold and darkened and had twice the iron concentration of control

spleens. The kidney weights of these rats increased by 40% and liver weights increased by 30%. The elevated iron concentration in the spleen of rats fed excess methionine is thought to be caused by the increased turnover and destruction of the red blood cells (Cohen and Berg, 1956).

In their study of amino acid toxicity, Peng et al. (1973) found that rats fed a low protein diet with 5% L-histidine lost about 15% of their initial body weight within 4 days. Of the amino acids investigated (threonine, lysine, phenylalanine, leucine, histidine, methionine and tryptophan) only the weight loss observed with 5% tryptophan and methionine was more severe than the weight depression caused by excess histidine. Like tyrosine and tryptophan, the degree of growth depression with excess histidine was also affected by protein quantity and quality (Muramatsu et al., 1971, 1973).

Most of the research work on phenylalanine toxicity was conducted with the objective of understanding the genetic disorder phenylketonuria (PKU). Classical PKU is due to complete absence of phenylalanine hydroxylase, although hyperphenylalaninemia can result from the absence of dihydrobiopterin synthetase or dihydropteridine reductase (Benevenga, 1981). According to Kaufman (1977), the cause of PKU is the high level of phenylalanine. He suggested that the high blood levels of phenylalanine in individuals with PKU prevent the uptake of some amino acids in the brain which

affects protein synthesis and eventually affects the development of the brain. Studies have shown that brain protein synthesis was depressed when neonatal rats were injected with phenylalanine (Harper et al., 1973). The brains of individuals with PKU also have reduced lipid associated with myelin. People affected with PKU become extremely retarded soon after birth if they do not receive low phenylalanine-tyrosine diet. Treatment of individuals with PKU generally involves the consumption of a low phenylalanine-tyrosine diet for the rest of their lives.

THE EFFECTS OF P-CRESOL AND RELATED PHENOLIC COMPOUNDS

The bacterial degradation of tyrosine, particularly the production of p-cresol and the possible adverse effects of this metabolite on the growth and development of weanling pigs were examined at Michigan State University by Yokoyama et al. (1982). They investigated the effects of dietary antibiotic supplementation on the urinary and fecal excretion of volatile phenolic and aromatic metabolites. They also looked at the effects of antibiotics and bacterial metabolites on the growth performance of young pigs. Their data showed that:

1) Dietary antibiotic supplementation substantially decreased urinary p-cresol excretion but did not affect urinary phenol or 4-ethylphenol excretion.

2) Total fecal and urinary p-cresol excretion was inversely correlated to percent body weight gain (r=0.79).

3) Expressing the p-cresol excretion in terms of metabolic body size (Kg $B.W.^{3/4}$), resulted in highly significant treatment differences.

4) Dietary antibiotic supplementation did not affect the percent composition and concentrations of fecal bacterial metabolites.

P-cresol (4-methylphenol) is produced in the intestinal

tract of animals by the microbial degradation of tyrosine (Scheline, 1968; Yokoyama and Carlson, 1979). P-cresol is known to be produced by Clostridium difficile directly from tyrosine (Elsden et al., 1976). In another study, cell-extracts of Clostridium difficile were reported to form p-cresol by decarboxylation of p-hydroxyphenylacetic acid (D'Ari and Barker, 1985). Other investigators have suggested that the degradation of tyrosine to p-cresol by intestinal microorganisms takes place through the deamination of tyrosine to phloretic acid, followed by decarboxylation to 4-ethylphenol, oxidation to 4-hydroxyphenylacetic acid and decarboxylation of 4-hydroxyphenylacetic acid to p-cresol (Williams, 1959 and Parke, 1968). However, there is evidence to indicate that the predominant pathway for the intestinal production of p-cresol from tyrosine appears to be a two-step process that involves the metabolism of at least two bacterial species (Figure 1). One of these species is responsible for the degradation of tyrosine to p-hydroxyphenylacetic acid (PHPAA) and the other is responsible for the decarboxylation of PHPAA to p-cresol (Scheline, 1968; Bakke, 1969c; Spoelstra, 1978 and Yokoyama et al., 1979). The elimination or reduction of one of these bacterial species by dietary antibiotics could prevent or reduce p-cresol production.

P-cresol has been detected in the urine and feces of pigs (Spoelstra, 1977; Yasuhara and Fawa, 1979; Yokoyama et

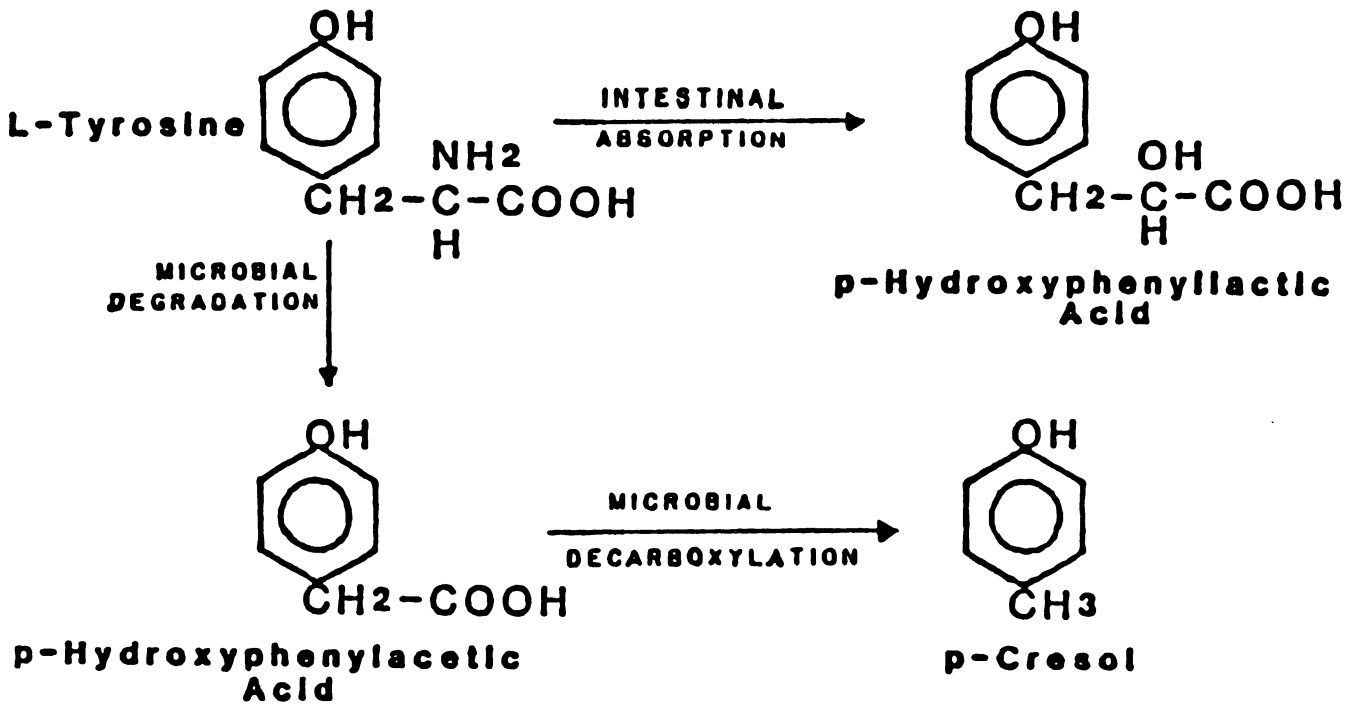


Figure 1. Pathway of tyrosine metabolism

al., 1982; Ward and Yokoyama, 1983). Studies by Yoshihara (1977, 1978) have indicated that in the pig, the major site of p-cresol production is the colon, but the metabolite is also detected in the contents of the stomach and the small intestine. P-cresol is absorbed from the skin or from the intestinal tract (Huber, 1977).

The decarboxylation of PHPAA to p-cresol has previously been demonstrated in incubations of rat cecal contents (Scheline, 1968 and Bakke, 1969c) and pig feces (Spoeltra, 1978). Recently, Ward and Yokoyama (1983) isolated the bacterium from pig feces and have characterized the bacterium in pure culture. The bacterium, assigned to the genus Lactobacillus, is a gram positive, obligate anaerobe, non-sporeforming, non-motile, ovoid rod which produces p-cresol from PHPAA. The bacterium does not use tyrosine directly to produce p-cresol.

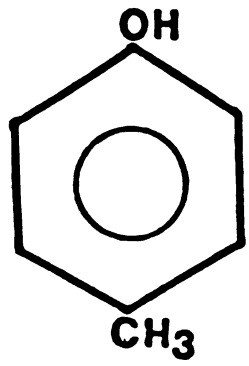
Studies have shown that p-cresol is the predominant volatile and phenolic metabolite excreted in the urine of weanling pigs (Spoelstra, 1978 and Yokoyama et al., 1982). The concentrations of urinary p-cresol detected in the weanling pig indicate that weanling pigs are exposed to higher levels of p-cresol than normal adult humans and that these levels more nearly approximate levels excreted by children suffering from coeliac disease (Duran et al., 1973). Dirmikis and Darbre (1974) reported 58.8 mg p-cresol/liter of urine in normal adult humans, and Bone et al. (1976) found

an average of 51.8 mg p-cresol/day. These levels of urinary p-cresol excretions were similar to those observed by Yokoyama et al. (1982) for weanling pigs on CSP (110 ppm chlortetracycline, 110 ppm sulfamethazine and 55 ppm penicillin) diets. The conclusion drawn from these comparisons is that the weanling pig is exposed to higher levels of p-cresol than normal adult humans, possibly due to the active bacterial production of p-cresol in the pig's intestinal tract.

P-cresol is a type B toxic compound (Figure 2), and the oral LD_{50} in rats is 1.8g/kg body weight (Deichmann and Witherup, 1944). In humans, 8 grams of a cresol isomer (ortho, meta, para) mixture will result in rapid circulatory collapse and death (Merck, 1976). Signs of acute toxicity include, eye and muscle twitching, subnormal temperature, increased salivation, dyspnea, incoordination, loss of appetite, sitting on the hind legs, convulsion and coma (Deichmann and Witherup, 1944; Merck, 1976).

Following seven weeks of daily subcutaneous injections of p-cresol, Matsumoto et al. (1963) observed that rats developed a convulsive sensitivity to loud, high frequency sounds. The use of p-cresol in rats by Matsumoto et al. (1983) has provided an alternative model for the study of seizure mechanisms in human epilepsy.

The effects of d-amphetamine and temperature on p-cresol and pentylenetetrazol-induced convulsions in rats



4-METHYLPHENOL

$CH_3C_6H_4OH$

- molecular weight: 108.14, boiling point: 202° C,
- melting point: 32-34° C, density: 1.034
- crystalline mass, phenol-like odor
- soluble in alcohol, ether and chloroform
- type B toxic compound, oral LD in rats: 1.8g/kg BW
- ip dose (100 mg/Kg BW) decreased BWG in rats
- single ip dose (100 mg/kg BW) in weanling pigs produced eye and muscle twitching, increased salivation, convulsion, incoordination, dyspnea, loss of appetite
- single oral dose (100 mg/Kg BW) did not produce toxic effects in weanling pigs

Figure 2. Structure, physical, chemical characteristics and toxic effects of p-cresol

were investigated by Yehuda and Carraso (1977). Rats received daily ip injections of p-cresol (0.1 mg/kg) for seven weeks. The effects of p-cresol gave rise to running, jumping and characteristic movements which Yehuda and Carraso (1977) described as an "amok" syndrome. When the daily injections of p-cresol were terminated, these behavioral effects lasted for another four weeks. Contrary to other observations, they found that the p-cresol treated rats gained weight compared to a matched untreated control group. The site of action of p-cresol and other phenol derivatives is not known. However, Yehuda and Carraso (1977) suggested that the potential site of action may be at the neurotransmitter level. They argued that, since phenol derivatives are highly toxic compounds, they could exert a common cortical action.

P-cresol is the chief contributor of malodor associated with swine waste (Spoelstra, 1978), and dust-borne phenols and indoles including p-cresol, have been extracted from dust sedimentation of finishing and piglet houses (Hartung and Rokicki, 1984). Sensory evaluation of the two pig buildings showed that the odor intensity in the finishing pig house was distinctly higher than in the piglet house. They found that the decrease in odor intensity in the piglet house coincided with the decrease in the concentration of p-cresol. This finding is supported by the observation that urinary p-cresol excretions tended to increase with age

(Yokoyama et al., 1982).

Although the toxic effects of an acute p-cresol exposure have been documented (Deichmann and Witherup, 1944; Merck, 1976), p-cresol has never been linked to a specific disease. However, volatile phenols have been implicated in the development of large bowel and bladder cancer (Bryan, 1971; Bone et al., 1976). Van der Heiden et al. (1971a, 1971b) found highly increased urinary excretions of phenylalanine and tyrosine metabolites in patients with severely impaired intestinal amino acid absorption and in patients with cystic fibrosis and coeliac disease. Microbial metabolites of tryptophan have been studied as possible agents involved in schizophrenia (Sprince, 1962), rheumatoid arthritis (Nakoneczna et al., 1969), colon and bladder cancer (Bryan, 1971 and Chung et al., 1975) and hepatic coma (Walshe et al., 1958).

Butylated hydroxytoluene (BHT), a compound with similar chemical structure to p-cresol, has been shown to cause lung injury and reduced body weight gains in mice (Marino and Mitchell, 1972; Hirai et al., 1977; Omaye et al., 1977; Malkinson, 1979; Hirose et al., 1981 and Mizutani et al., 1982). Other phenols which are analogs of BHT, like 2,6-di-tert-butylphenol, 4-tert-butyl-2-6-diisopropylphenol and 2,4,6-tri-tert-butylphenols were reported by Takahashi and Hiraga (1978, 1980) to induce hemorrhage in rats.

Mizutani et al. (1982) investigated the pulmonary toxicity of BHT and 23 other related alkylphenols and their structural requirements for toxic potency in mice. Of all the compounds examined, BHT, 2-tert-butyl-4-methylphenol and 2-tert-butyl-4-6-dimethylphenol (single ip dose, 500 mg/kg) caused significant reduction in body weight gains and produced a proliferation of alveolar cells and an accompanying increase in lung weights. It had been earlier established by histological observations that an increase in lung weight is a reliable indicator of lung damage caused by BHT (Witschi and Sahed, 1974; Sahed and Witschi, 1975). Of the other alkylphenols studied by Mizutani et al. (1982), 4-methylphenol (p-cresol); 2,4-dimethylphenol and 2,4,6-trimethylphenol caused significant reduction in body weights, but these compounds did not increase lung weights. Mizutani et al. (1982) observed that the structural requirements essential for the toxic effect was a phenolic ring structure with a methyl group in the 4th position and the presence of ortho-alkyl groups. The ortho-alkyl component provided a hindering effect on the hydroxyl group. Besides the position ortho to the hydroxyl group, Mizutani et al. (1982) indicated that at least one tert-butyl component was necessary to produce the pulmonary toxic effect. They observed that BHT, 2-tert-butyl-4-methylphenol and 2-tert-butyl-4-6-dimethylphenol exhibited strong pulmonary toxic effects whereas p-cresol, without the ortho-tert-butyl component, did not show pulmonary toxic effects. When the

methyl group in the 4th position was removed from the BHT and from the two other toxic alkylphenols, it resulted in complete loss of pulmonary toxic potency.

The exact mechanism of how BHT damaged the lungs is not known. Mizutani et al. (1982) hypothesized that the toxic phenols undergo metabolic oxidation to form p-quinone methides or metabolites that covalently bind to constituents of the pulmonary tissues and produce tissue lesions.

Hirose et al. (1981) studied the chronic toxicity of butylated hydroxytoluene in rats. Wistar rats were maintained on diets containing 0.25% or 1.0% BHT for 104 weeks. Both sexes of treated rats had reduced body weight gains, spleen weights and white blood cell counts compared to the untreated control group. Only the treated male rats showed reduction in serum triglycerides. Other pneumotoxicants, such as paraquat and monocrotaline, have also been known to depress growth in rats (Gillis et al., 1978 and Roth et al., 1979).

More recently, Sakai et al. (1982) demonstrated that indole compounds at low concentrations uncoupled oxidative phosphorylation in the isolated rat liver. At higher concentrations, indole inhibited electron transport. The infusion of indole caused a significant reduction of the adenylate energy charge in the rat liver. ADP was not efficiently phosphorylated in the liver mitochondria of the

indole perfused rats. In another study (Shen, 1983a), tyrosine and its acid metabolites such as tyramine, p-hydroxyphenylpyruvic, p-hydroxyphenyllactic and p-hydroxyphenylacetic acids were found to be potent noncompetitive inhibitors of liver dihydropteridine reductase. The enzyme is responsible for generating tetrahydrobiopterin, an essential cofactor for the hydroxylation of phenylalanine, tyrosine and tryptophan. In previous studies, Shen and associates (1982) reported that potent inhibitors of dihydropteridine reductase require a para-phenolic nucleus in their structures. The inhibition of dihydropteridine reductase activity could reduce the production of the biopterin cofactor and could consequently limit the synthesis of neurotransmitters.

Short term studies with mice showed that intraperitoneal (ip) injection of p-cresol (100 mg/kg body weight) significantly decreased percent body weight gain but unlike BHT, p-cresol did not increase lung weights (Mizutani et al., 1982). A single ip dose of p-cresol (100 mg/kg body weight) produced toxic effects like convulsions, muscle twitching, incoordination, dyspnea, loss of appetite and sitting on hind legs in weanling pigs (Lumanta et al., 1983). The pigs at this IP dosage level were able to recover from the toxic effects 3 hours after dosing however, the impaired appetite lasted for a day. In another investigation, Yokoyama et al. (1983) reported that single oral administration of p-cresol at 100 mg/kg BW did not produce

any toxic effects in weanling pigs.

Driezen and Spies (1948) and Hegna (1977) showed that p-cresol has bactericidal and fungicidal properties. Other investigators (DeGreef and Van Sumere, 1966; Van Sumere et al., 1975; Davidson and Branen, 1981; Jung and Fahey, 1983a, 1983b) reported that phenolic monomers (ferulic, caffeic, gallic and syringic acids) inhibited aerobic bacteria and several mammalian enzyme systems in vitro. According to Jenkins et al. (1957), the addition of alkyl groups to the benzene ring of phenolic compounds increased their antibacterial activity while increased hydroxylation decreased their antibacterial effects.

Skatole, a microbial by-product of tryptophan, is known to have a bacteriostatic effect on gram-negative bacteria. Tittsler et al. (1935) indicated that the species of the genera Salmonella and Shigella are more sensitive to skatole than the Escherichia and Aerobacter groups. Skatole was also observed to inhibit the growth of Lactobacillus acidophilus (Driezen and Spies, 1948). Eadie and Oxford (1954) reported that because of the lipophilic properties of skatole, it had a disintegrative effect on certain rumen ciliate protozoa.

Yokoyama and Carlson (1979) have indicated that because of their bacteriostatic properties, p-cresol and other bacterial metabolites like skatole, could play an important

role in determining the character of the intestinal microbial population. The production of p-cresol and skatole by the bacteria could be a protective mechanism against other bacteria in the intestinal tract.

Besides BHT, other phenolic compounds were also found to have detrimental effects in livestock and poultry when added to the diet. Jung and Fahey (1983a) reported that the inclusion of phenolic monomers (p-coumaric, ferulic, protocatehuic and salicylic acids and vanillin) in the diets reduced the feed intake in rats. They also observed a linear trend toward decreased feed intake with increasing concentrations of p-coumaric and ferulic acids in the diet. Phenolic monomers are derived from the shikimic acid pathway that occurs in vascular plants. In their review of the nutritional implications of phenolic monomers, Jung and Fahey (1983b) indicated that p-coumaric and ferulic acids are the two most common phenolic monomers in the diets of animals.

Another phenolic monomer which has a detrimental effect when added to the diet is gallic acid (3,4,5 trihydroxybenzoic acid), a naturally occurring polyphenol which is a major hydrolytic product of tannic acid. Relative to tannic acid, the addition of 1% gallic acid resulted in a 30% growth reduction in chickens (Kratzer et al., 1975). In rats, the palatability of the diets was depressed when gallic acid was included in the diet and the growth rates

were reduced when gallic acid was supplemented at 5% of the diet (Joslyn and Glick, 1969). However, Rayudu et al. (1970) found no effect of gallic acid on the growth rate of chicks when gallic acid was added at 1% of the diet.

In his investigation of rats, Glick (1981) reported that the suppressive effect of gallic acid on food intake was not mediated entirely through taste aversion or through other gastrointestinal factors, since a continuous daily infusion of a gallic solution (10 ml, 2%) resulted in a significant reduction in feed intake. It was indicated that the catechol moiety of gallic acid might be responsible for its suppressive effect on feed intake because the administration of its 4-O methyl derivative was significantly less effective in reducing feed intake. Glick (1981) also observed that the effect of gallic acid diminished with time, suggesting adaptation to the consumption of gallic acid.

Among the phenolic compounds, the nutritional effects of tannin are the most documented (Jung and Fahey, 1983b). Tannins have been shown to inhibit the growth of molds and the pre-harvest seed germination and provide resistance to bird damage in grain sorghums (Harris and Burns, 1970; McGrath et al., 1982). While these traits of tannins are useful, the high level of tannins has also adversely affected the utilization of grain sorghums in swine diets. The use of grain sorghum with high-tannin content in growing-finishing swine resulted in reduced feed efficiency

(Thrasher et al., 1975; Cousins et al., 1981 and Noland et al., 1981). When fed to growing-finishing pigs, diets containing high-tannin grain sorghum had lower energy, crude protein and dry matter digestibilities than diets containing low tannin grains (Myer et al., 1986). Chang and Fuller (1964) examined the effects of the tannin content of grain sorghums on the feeding value of sorghum for growing chicks. They found that the growth depression of growing chicks was directly related to the level of tannin found in the different varieties of grain sorghums used in the experiment. According to Jung and Fahey (1983b), phenolics not only bind with enzymes but they also form nutritionally unavailable complex with dietary proteins. Damaty and Hudson (1979) observed that gossypol, a phenolic compound in cotton seeds, reduced the amounts of hydrolyzable lysine, serine, threonine and methionine by binding with these amino acids during processing.

MATERIALS AND METHODS

Three nutritional studies were conducted involving the addition of p-cresol, p-hydroxyphenylacetic acid (PHPAA) and L-tyrosine to the diets of weanling pigs. Due to the limited information on the effects of PHPAA and because of our previous research work on p-cresol and L-tyrosine, the PHPAA feeding study was conducted first. This was followed by the p-cresol and L-tyrosine nutritional studies. Total urinary p-cresol excretions were determined in all three feeding trials while urinary excretions of p-hydroxyphenylacetic and p-hydroxyphenyllactic acids were analyzed in the PHPAA and L-tyrosine studies.

EXPERIMENT 1

Animals and Diets

Sixteen crossbred pigs, with average initial body weight of 10.9 kg, were assigned by weight, sex and litter to one of the following treatment groups:

1) No antibiotic (NA)
2) Antibiotic (A)
3) No antibiotic plus PHPAA (NA-PHPAA)
4) Antibiotic plus PHPAA (A-PHPAA)

The MSU starter ration (Table 1) was used as the basal diet. BMD (Bacitracin Methylene Disalicylate) soluble concentrate (containing 324 grams/lb Bacitracin MD, A.L. Laboratories, Inc., Englewood Cliffs, NJ) was supplemented at 110 ppm to the A and A-PHPAA diets. P-hydroxyphenylacetic acid (Sigma Chem. Co., St. Louis, MO) was added at 0.75% to the NA-PHPAA and A-PHPAA diets.

The experiment was designed such that the amount of p-cresol produced and absorbed from the addition of 0.75% PHPAA in the diets did not affect feed intake nor induce clinical toxicity. Based on the average initial body weight of 10.9 kg and on the following assumptions: daily feed intake at 5% of body weight, 80% absorption of PHPAA, 80% absorption of p-cresol and 50% conversion of PHPAA to p-cresol; it was estimated that pigs were exposed to about 320 mg of p-cresol per day from the addition of 0.75% PHPAA. A preliminary study showed that young pigs receiving no antibiotic supplementation can excrete up to 150 mg p-cresol/day via the urine. The total exposure of pigs to p-cresol resulting from the addition of 0.75% PHPAA to the diet plus the endogenous production of p-cresol, would be about 470 mg p-cresol per day. It was decided that, at this level of p-cresol exposure, feed intake would not be affected nor clinical toxicity be induced. We initially thought of increasing the p-cresol exposure by adding more PHPAA in the diet but we finally decided on the 0.75% level because we

TABLE 1. Diet composition (PHPAA study)

Ingredients	Treatment			
	NA	A	NA-PHPAA	A-PHPAA
Ground shelled corn	68.50	68.50	67.75	67.75
Soybean oil meal	17.50	17.50	17.50	17.50
Dried whey	10.00	10.00	10.00	10.00
Limestone	1.00	1.00	1.00	1.00
Dicalcium phosphate	1.50	1.50	1.50	1.50
Vitamin-trace mineral premix[a]	0.50	0.50	0.50	0.50
Selenium-vitamin E premix[b]	0.50	0.50	0.50	0.50
L-Lysine monohydrochloride	0.25	0.25	0.25	0.25
Sodium chloride	0.25	0.25	0.25	0.25
p-Hydroxyphenylacetic acid	0.00	0.00	0.75	0.75
BMD[c]	0.00	--	0.00	--
	100.00	100.00	100.00	100.00

[a] Provides the following per kg of diet: vitamin A acetate, 3300 IU; vit D3, 660 IU; menadione bisulfite, 2.2 mg; riboflavin, 3.3 mg; niacin, 17.6 mg; d-pantothenic acid, 13.2 mg; choline, 110 mg; vit B12 18.8 ug; zinc, 75.0 mg; iron, 59.4 mg; manganese, 37.4 mg; copper, 9.9 mg and iodine, 2.5 mg.

[b] Provides the following per kg of diet: selenium, 0.1 mg and vitamin E, 16.5 IU

[c] BMD (bacitracin methylene disalicylate soluble concentrate, 324g/lb bacitracin) added at 110 ppm

had no idea of the long term effects of excess PHPAA.

The treatment groups were blocked by litter because of concerns that genetics may have an effect on the level of response of some pigs to p-cresol exposure. Depending on litter size, each treatment group had at least one pig of the same sex from the same litter. There were two barrows and two gilts in each treatment group.

The pigs were fed twice daily with at least 2.5% of their body weight (BW) per feeding. The individual feeding cups were inspected four times daily to make sure that feed was always available. There was a one week adjustment period before the start of the 28-day feeding study. During the adjustment period, all the pigs were fed with the no antibiotic diets (NA). Feed consumption and refusals of individual pigs were recorded daily and feed/gain ratios were calculated. The pigs were individually maintained and fed in elevated stainless steel pens (45 x 90 x 76 cm) with screened floors and were housed in an environmentally-controlled room (27 C). There was free access to water through automatic waterers installed in individual pens. The pigs were weighed before the start of the experiment (0 time) and then reweighed at 7-day intervals throughout the 28-day feeding study.

Urine Collection

After collecting their weights, the pigs were transferred to individual metabolism cages (55 x 70 x 76 cm) for a 24-hour total urine collection. During the collection period, the pigs were removed twice daily from the metabolism cages for about 10-20 minutes and fed in their individual feeding pens (45 x 90 x 76 cm). By closely watching the pigs during these feeding periods, no urine losses were incurred during the collection. Urine was strained through 4 layers of cheesecloth and collected into 1000 ml Erlenmeyer flasks. To prevent microbial glucuronidase activity from fecal contamination, urine was collected in flasks containing 10 ml of 0.1 % Thimerosal (ethylmercurithiosalicylate, Sigma Chem. Co., St. Louis, MO) solution. Total urine volume was measured and recorded for the 24-hour collection. About 150 ml of urine sample was retained and stored in glass bottle at -20 C until analyzed. After the 24-hour urine collection, the pigs were transferred back to their individual stainless steel cages, where they were maintained and fed until the next urine collection. The individual steel cages were thoroughly cleaned while the pigs were in their respective metabolism cages. There were a total of 5 urine collections in the 28-day feeding study (0 time to 4th week).

Analytical Procedures

Total p-cresol Determination

P-cresol was extracted by taking 5 ml of urine sample into a large screw cap vial and acidifying it with 5 ml of 4 N HCL solution. The combined urine sample and HCl solution were hydrolyzed for one hour in a 100 C water bath. One ml of internal standard (p-methoxyphenol, 2 mg/ml ethanol, Sigma Chem. Co., St. Louis, MO) was added after cooling down the hydrolyzed sample,. To this total volume, 10 ml of ethyl ether was added after which the screw cap was shut very tightly. The vial was shaken vigorously and the solution was allowed to settle. Using disposable 9-inch pipettes, ether extracts were transferred to gas chromatograph vials. Two microliters of the ether extract was analyzed directly by gas liguid chromatography.

Identification and quantification of p-cresol in the urine sample was done with a Hewlett-Packard 5840A gas-liquid chromatograph (Hewlett-Packard Co., Farmington Hills, MI) equipped with an autoinjector and a hydrogen flame ionization detector. A stainless steel column (180 cm x .3125 cm) packed with 21% Carbowax 4000 and WAW-DMCS (60/80 mesh) (Anspec Company, Ann Arbor, MI) effectively separated the p-cresol without derivatization. Column temperature was isothermal at 180 C with injection (INJ), flame ionization

detector (FID) and thermal conductivity detector (TCD) temperatures set at 250 C, 250 C and 200 C, respectively. Chart speed was fixed at 1.00 in/min and attenuation was set at 8. Helium flow rate was at 50 ml/min. P-cresol was identified by its retention time, and its concentration was determined with the aid of a microprocessor and standard solution of p-cresol (1 mg/ml ethanol, Aldrich Chemical Co., Milwaukee, WI). The percent recovery of the internal standard was calculated for each urine sample. This allowed for the efficiency of the ether extraction to be corrected for each p-cresol determination.

Since we intended to study the ability of the weanling pig to detoxify p-cresol, which is absorbed and excreted via the urine, the amounts of free and conjugated p-cresol was examined. The amount of bound p-cresol was calculated by difference (total p-cresol less free p-cresol).

Free p-cresol Determination

Free p-cresol was analyzed by taking 5 ml of urine sample into a large screw cap vial and adjusting its pH by adding 5 ml of 4 N HCL. One ml of internal standard (p-methoxyphenol, 2 mg/ml ethanol) was added followed by 10 ml of ethyl ether. The vial was vigorously shaken, and then the solution was allowed to settle. Ether extract was transferred to the GC vial using a 9-inch disposable

pippete. Two microliters of the ether extract was directly analyzed by GLC. The percent recovery of the internal standard was calculated in each urine sample. This procedure allowed for the efficiency of the ether extraction to be corrected in each determination free p-cresol.

The following calculations were used in determining p-cresol concentrations (mg/total urine/24hr):

$$\frac{\text{amt of PC standard (ug/2ul)}}{\text{(A)}} = \frac{\text{area of PC standard}}{\text{area of PC in urine sample}}$$

(A) = amt of p-cresol in sample (ug/2ul or mg/ml)

$$\frac{\text{(A)}}{\text{(B)}} = \frac{\text{2 ml ether}}{\text{10 ml ether}}$$

(B) = amt of PC (mg) per 10 ml ether or
amt of PC (mg) per 5 ml urine sample

$$\frac{\text{amt of PMP standard (ug/2ul)}}{\text{(C)}} = \frac{\text{area of PMP standard}}{\text{area of PMP in urine sample}}$$

(C) = amt of PMP in urine sample (ug/2ul or mg/ml)

$$\frac{\text{(C)}}{\text{(D)}} = \frac{\text{2 ml ether}}{\text{10 ml ether}}$$

(D) = amt of PMP (mg) per 10 ml ether or
amt of PMP (mg) per 5 ml urine sample

$$\frac{\text{(D)}}{\text{amt of PMP internal standard}} \times 100 = \text{(E)}$$

(E) = % recovery of PMP in urine sample

$$\frac{(B)}{(E)} = (F)$$

(F) = corrected amt of PC (mg) per 5 ml urine sample

$$\frac{(F)}{(G)} = \frac{\text{urine volume (5 ml)}}{\text{total urine volume in 24 hours}}$$

(G) = amt of PC in mg per total urine per 24 hours

P-HPAA and P-HPLA Determination

Due to their non-volatility, p-hydroxyphenylacetic acid (PHPAA) and p-hydroxyphenyllactic acid (PHPLA) were derivatized by N,O-bis-(trimethylsilyl)-acetamide (TMS process) before GLC analysis. Five ml of urine sample were placed into a large screw cap vial and acidified with 5 ml of 4N HCL. The combined urine sample and HCL solution were hydrolyzed for 1 hour in a 100 C water bath. When the hydrolyzed sample cooled down, 1 ml of internal standard (4-phenylbutyric acid, 2 mg/ml ethanol, Aldrich Chem. Co., Inc., Milwaukee, WI) was added followed by 10 ml of ethyl ether. After adding the ethyl ether, the screw cap was shut very tightly, and then the vial was shaken vigorously and the solution allowed to settle. Two ml of ether extract was placed into a small screw cap vial and then evaporated to dryness under nitrogen at 60 C water bath. Chloroform (0.5

ml) was added to the residue and swirled. Using a micro syringe, 0.02 ml N-N-Dimethylforamide (Aldrich Chem. Co., Inc., Milwaukee, WI) was added followed by 0.2 ml N,O-bis-(trimethylsilyl)-acetate (Pierce Chem. Co., Rockford, IL). The sealed screw cap vial was incubated in a 60 C water bath for 30 minutes. After cooling, the solution was transferred to a GC vial, and two microliters were directly analyzed by GLC. Before the urinary excretions of PHPAA and PHPLA were analyzed by GLC, standard curves were determined using increasing concentrations of PHPAA, PHPLA and 4-phenylbutyric acid. The straight line in the standard curves showed the range and capacity of the GLC in detecting the different concentrations of PHPAA, PHPAA and 4-phenylbutyric acid.

The determination and quantification of PHPAA and PHPLA were done with a Hewlett-Packard 5840A gas-liquid chromatograph. Urine samples were analyzed using a 180 cm x 0.3125 cm stainless steel column packed with Carbowax 5000 (stationary phase OV-210 , chromasorb WHP 100/120 mesh, Alltech Associates, Inc., Deerfield, IL). Column temperature was set at 135 C while INJ, FID and TCD temperatures were fixed at 250, 250 and 200 C, respectively. Chart speed was at 0.70 in/min while attenuation was set at 8. Helium flow rate was at 50 ml/min. Metabolites were identified by their retention times, and their concentrations were determined with the aid of a microprocessor and standard solutions of PHPAA (Sigma Chem Co., St. Louis, MO), PHPLA (Sigma Chem.

Co., St. Louis, MO) and 4-phenylbutyric acid (Aldrich Chem. Co., Inc., Milwaukee, WI).

The following calculations were used in determining PHPAA and PHPLA concentrations (mg/total urine/24hr):

$$\frac{\text{amt of 4PBA standard (ug/2ul)}}{\text{(A)}} = \frac{\text{area of 4PBA standard}}{\text{area of 4PBA in urine sample}}$$

(A) = amt of 4PBA in urine sample (ug/2ul or mg/ml)

$$\frac{\text{(A)}}{\text{(B)}} = \frac{\text{2 ml (TMS volume)}}{\text{0.72 ml (TMS volume used)}}$$

(B) = amt of 4PBA (mg) per 0.72 ml of TMS volume

$$\frac{\text{(B)}}{\text{(C)}} = \frac{\text{2 ml ether}}{\text{10 ml ether}}$$

(C) = amt of 4PBA (mg) per 10 ml ether

$$\frac{\text{(C)}}{\text{amt of 4PBA standard (mg/ml)}} \times 100 = \text{(D)}$$

(D) = % recovery of 4PBA in urine sample

$$\frac{\text{amt of PHPAA standard (ug/2ul)}}{\text{(E)}} = \frac{\text{area of PHPAA standard}}{\text{area of PHPAA in urine sample}}$$

(E) = amt of PHPAA in urine sample (ug/2ul or mg/ml)

$$\frac{(E)}{(F)} = \frac{2 \text{ ml (TMS volume)}}{0.72 \text{ ml (TMS volume used)}}$$

(F) = amt of PHPAA per 0.72 ml of TMS volume

$$\frac{(F)}{(G)} = \frac{2 \text{ ml ether}}{10 \text{ ml ether}}$$

(G) = amt of PHPAA per 10 ml ether or
amt of PHPAA per 5 ml urine sample

$$\frac{(G)}{(D)} = (H)$$

(H) = corrected amt of PHPAA (mg) per 5 ml urine

$$\frac{(H)}{(I)} = \frac{\text{urine sample volume (5ml)}}{\text{total urine volume per 24 hours}}$$

(I) = amt of PHPAA in mg per total urine 24 hours

Data were arranged such that analysis could be done after 1st, 2nd, 3rd and 4th week of treatment. Overall means (1-4 wk) were also analyzed at the end of the study. Data collected were subjected to one way analysis of variance. The Student's T test was used to identify significant treatment differences. The difference between two group means was tested using the pooled analysis of variance.

The data was also analyzed using a 2x2 factorial design to show the main effects of PHPAA (NA-PHPAA + A-PHPAA vs NA

+ A) and BMD (A + A-PHPAA vs NA + NA-PHPAA) and their interactions.

The overall percent BWG for each pig (1-4wk) was regressed on the urinary p-cresol excretion (mean 1-4wk). Regression analysis were done on the NA + A, NA-PHPAA + A-PHPAA and NA + A + NA-PHPAA + A-PHPAA treatment groups.

EXPERIMENT 2

The second nutritional study involved the addition of p-cresol to the diet of weanling pigs. Sixteen crossbred pigs, with average initial weight of 9.5 kg were assigned by weight, sex and litter to one of four treatment groups:

1) No antibiotic (NA)
2) Antibiotic (A)
3) No antibiotic plus p-cresol (NA-PC)
4) Antibiotic plus p-cresol (A-PC)

The MSU starter ration (Table 2) was used as the basal diet in the feeding study. BMD soluble concentrate (containing 324 g/lb Bacitracin MD, A.L. Laboratories, Inc., Englewood Cliffs, NJ) was supplemented to the A and A-PC diets at 110 ppm. P-cresol (Aldrich Chem. Co., Inc, Milwaukee, WI) was added at 0.75% of the NA-PC and A-PC diets.

It was estimated that the total amount of p-cresol that

TABLE 2. Diet composition (p-cresol study)

Ingredients	Treatments			
	NA	A	NA-PC	A-PC
Ground shelled corn	68.50	68.50	67.75	67.75
Soybean oil meal	17.50	17.50	17.50	17.50
Dried whey	10.00	10.00	10.00	10.00
Limestone	1.00	1.00	1.00	1.00
Dicalcium phosphate	1.50	1.50	1.50	1.50
Vitamin-trace mineral premix[a]	0.50	0.50	0.50	0.50
Selenium-vitamin E premix[b]	0.50	0.50	0.50	0.50
L-Lysine monohydrochloride	0.25	0.25	0.25	0.25
Sodium chloride	0.25	0.25	0.25	0.25
P-cresol (99%)	0.00	0.00	0.75	0.75
BMD[c]	0.00	--	0.00	--
	100.00	100.00	100.00	100.00

[a] Provides the following per kg of diet: vitamin A acetate, 3300 IU; vit D3, 660 IU; menadione bisulfite, 2.2 mg; riboflavin, 3.3 mg; niacin, 17.6 mg; d-pantothenic acid, 13.2 mg; choline, 110 mg; vit B12, 18.8 ug; zinc, 75.0 mg; iron, 59.4 mg; manganese, 37.4 mg; copper, 9.9 mg and iodine, 2.5 mg.

[b] Provides the following per kg of diet: selenium, 0.1 mg and vitamin E, 16.5 IU

[c] BMD (bacitracin methylene disalicylate soluble concentrate, 324g/lb bacitracin) added at 110 ppm

would be absorbed from the addition of 0.75% p-cresol in the diet and the amount of p-cresol produced endogenously would not affect feed intake nor would it induce clinical toxicity. Based on the 9.5 kg average initial weight, 5% of body weight daily intake and on the assumption that 80% of the p-cresol added would be absorbed, it was calculated that the addition of 0.75% p-cresol would give a p-cresol exposure of approximately 2.85 grams per day.

The experimental protocol, schedules and urine collection procedures were similar to those described in Experiment 1. The procedures used to determine and quantify the total urinary p-cresol and free p-cresol excretions, as well as the statistical and regression analyses used, were the same as described in Experiment 1. Regression analyses were done on the NA + A, NA-PC + A-PC and NA + A + NA-PC + A-PC treatment groups.

EXPERIMENT 3

The third nutritional study involved the addition of 3% L-tyrosine to the diet of weanling pigs. Sixteen crossbred pigs, initially weighing 9.3 kg on the average were assigned by weight, sex and litter to one of four treatment groups:

1) No antibiotic (NA)
2) Antibiotic (A)

3) No antibiotic plus tyrosine (NA-T)

4) Antibiotic plus tyrosine (A-T)

The MSU starter ration (Table 3) was used as the basal ration in the feeding study. BMD concentrate (containing 324 grams/lb Bacitracin MD, A.L. Laboratories, Inc., Englewood Cliffs, NJ) was supplemented to the A and A-T diets at 110 ppm. L-tyrosine (Sigma Chem. Co., St. Louis, MO) was added at 3% of the NA-T and A-T diets.

Two feeding studies where 3% tyrosine was supplemented in the diets of weanling pigs were previously conducted (Yokoyama et al., 1985). Results from the two studies were not clear and some data were conflicting. Experiment 3, also supplementing 3% tyrosine in the diet, was conducted to compare results with the two previous tyrosine studies.

The analytical procedures, analyses and determination of p-cresol, free p-cresol, PHPAA and PHPLA were similar to those described in Experiments 1 and 2.

The statistical and regression analyses were also the same to those described in Experiment 1. Regression analyses were done on the NA + A, NA-T + A-T and NA + A + NA-T + A-T treatment groups.

TABLE 3. Diet composition (Tyrosine study)

Ingredients	Treatments			
	NA	A	NA-T	A-T
Ground shelled corn	68.50	68.50	65.50	65.50
Soybean oil meal	17.50	17.50	17.50	17.50
Dried whey	10.00	10.00	10.00	10.00
Limestone	1.00	1.00	1.00	1.00
Dicalcium phosphate	1.50	1.50	1.50	1.50
Vitamin-trace mineral premix[a]	0.50	0.50	0.50	0.50
Selenium-vitamin E premix[b]	0.50	0.50	0.50	0.50
L-Lysine monohydrochloride	0.25	0.25	0.25	0.25
Sodium chloride	0.25	0.25	0.25	0.25
L-Tyrosine	0.00	0.00	3.00	3.00
BMD[c]	0.00	--	0.00	--
	100.00	100.00	100.00	100.00

[a] Provides the following per kg of diet: vitamin A acetate, 3300 IU; vit D3, 660 IU; menadione bisulfite, 2.2 mg; riboflavin, 3.3 mg; niacin, 17.6 mg; d-pantothenic acid, 13.2 mg; choline, 110 mg; vit B12 18.8 ug; zinc, 75.0 mg; iron, 59.4 mg; manganese, 37.4 mg; copper, 9.9 mg and iodine, 2.5 mg.

[b] Provides the following per kg of diet: selenium, 0.1 mg and vitamin E, 16.5 IU

[c] BMD (bacitracin methylene disalicylate soluble concentrate, 324g/lb bacitracin) added at 110 ppm

RESULTS

EXPERIMENT 1

The effects of PHPAA and BMD on the urinary excretion of PHPAA are shown in Table 4. Mean urinary PHPAA excretion (mg/total urine/24 hr) for the NA, A, NA-PHPAA and A-PHPAA treatment groups averaged 8.74, 9.58, 222.37 and 279.57, respectively, over the 28-day feeding period. Pigs receiving the BMD plus PHPAA diets (A-PHPAA) had significantly higher ($P<.05$) mean urinary PHPAA excretion than those pigs receiving PHPAA without the antibiotic supplementation (NA-PHPAA) (279.57 vs 222.37 mg/24hr). Excess PHPAA in the diets significantly increased ($P<.05$) the mean urinary PHPAA excretion of pigs in the NA-PHPAA and A-PHPAA treatment groups compared to pigs receiving the NA and A dietary treatments (222.37 and 279.57 vs 8.74 and 9.58 mg/24hr). Significant treatment differences ($P>.05$) were also observed after the 1st, 2nd, 3rd and 4th week of the feeding study. PHPAA was detected in the urine of pigs in the NA and A treatment groups but levels were low during the entire feeding study. The difference in mean urinary PHPAA excretion (1-4wk) between the NA and A treatment groups was not significant. However, pigs in the A treatment group had slightly higher PHPAA excretion than those receiving the NA treatment. Urinary PHPAA excretion over time is shown in

TABLE 4. Effects of PHPAA and BMD on mean urinary PHPAA excretion after 1st, 2nd, 3rd and 4th week of treatment.

	Treatments				
WEEK	NA	A	NA-PHPAA	A-PHPAA	MSE[a]
	mg/total urine/24hr				
0	2.96	6.10	7.41	3.61	12
	b	b	c	c	
1	10.28	5.96	152.83	274.03	8308
	b	b	c	c	
2	6.02	9.26	277.70	287.00	3224
	b	b	c	c	
3	9.98	11.71	215.20	271.33	2568
	b	b	c	c	
4	8.83	11.39	243.77	285.91	2284
	b	b	c	d	
Mean (1-4)	8.78	9.58	222.37	279.57	3852

[a] Mean square error

b, c, d Values in the same row having the same or no superscript were not significantly different (P<.05)

Figure 3. Figures 4 and 5 show the PHPAA and 4-phenylbutyric acid standard curves.

The 2x2 factorial analysis of PHPAA and BMD on urinary PHPAA excretion is shown in Table 4a. PHPAA (NA-PHPAA + A-PHPAA vs NA + A), at 0.75% of the diet significantly increased ($P<.01$) urinary PHPAA excretion. BMD (A + A-PHPAA vs NA + NA-PHPAA) did not significantly affect PHPAA excretion ($P<.05$). The P value of BMD (1-4wk) was 0.06.

Table 5 shows the effects of PHPAA and BMD on the urinary p-cresol excretion over the entire feeding study. Mean (1-4wk) urinary p-cresol excretion (mg/total urine/24hr) averaged 193.50, 97.32, 192.91 and 143.80 for the NA, A, NA-PHPAA and A-PHPAA treatment groups, respectively. Pigs receiving the A-PHPAA diet excreted significantly less p-cresol ($P<.05$) than those pigs given the NA-PHPAA diet (143.80 vs 192.91 mg/24hr). Urinary p-cresol excretion of the BMD-supplemented control group without the PHPAA (A) was significantly lower ($P<.05$) than the no-antibiotic treatment group (NA) (97.32 vs 193.50 mg/24hr). BMD reduced the urinary p-cresol excretion of the A treatment group by 49.7 percent over the NA group. Pigs receiving the A and A-PHPAA diets excreted less urinary p-cresol than those receiving diets without the antibiotic supplementation (NA and NA-PHPAA). These treatment differences were observed after the first week of the study but statistically significant differences did not occur until after the third

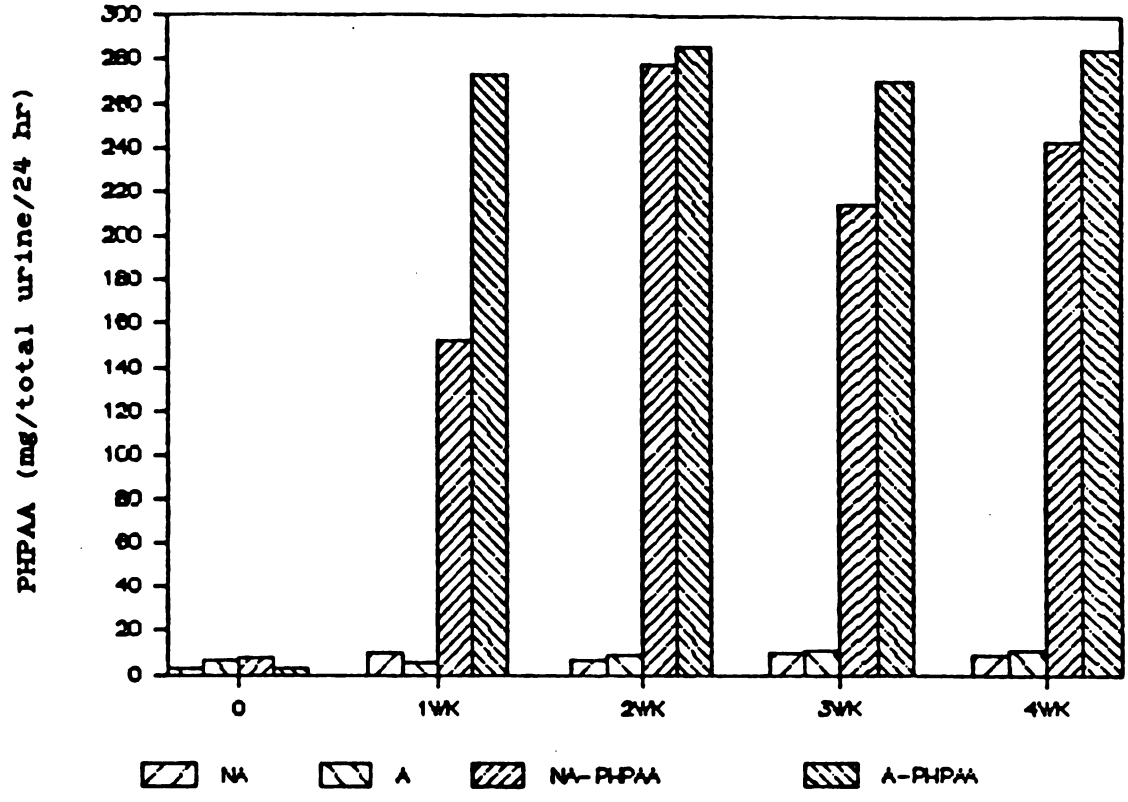


Figure 3. Total urinary PHPAA excretion after 1st, 2nd, 3rd and 4th week of treatment PHPAA study

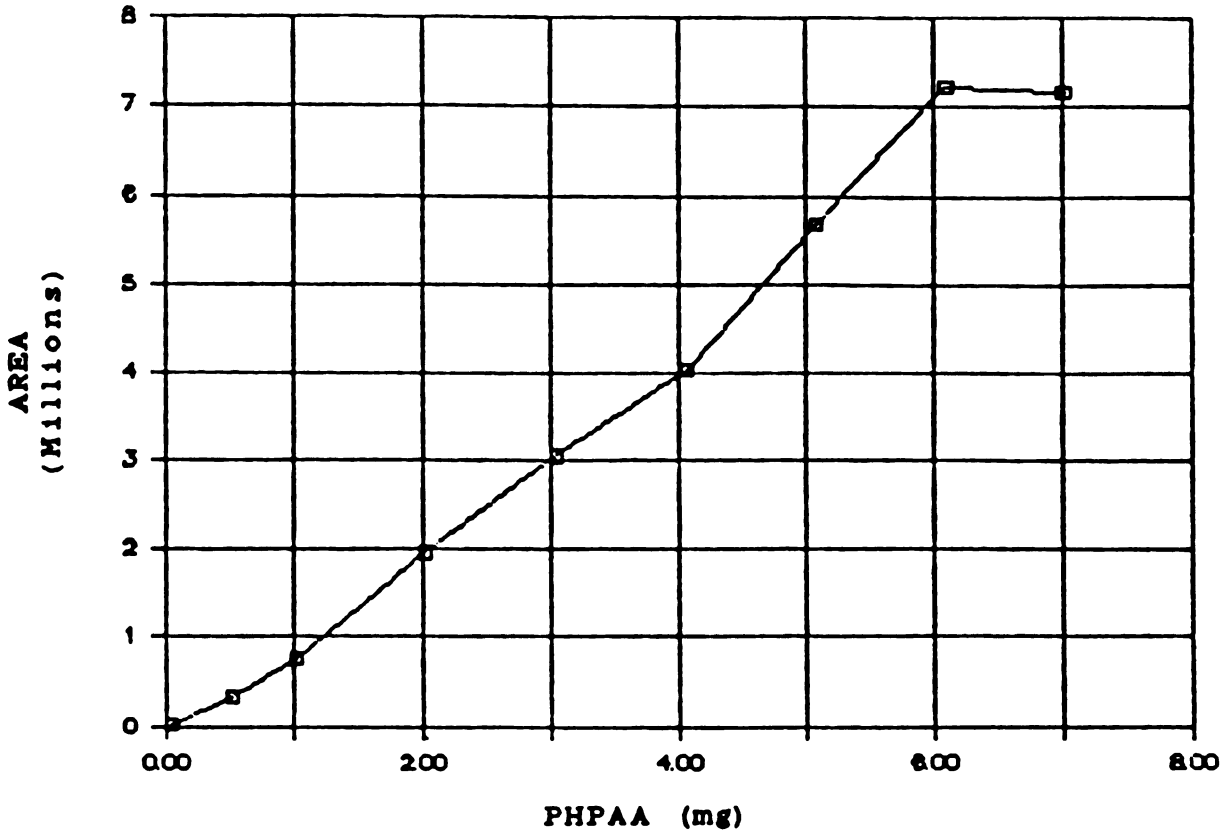


Figure 4. PHPAA standard curve

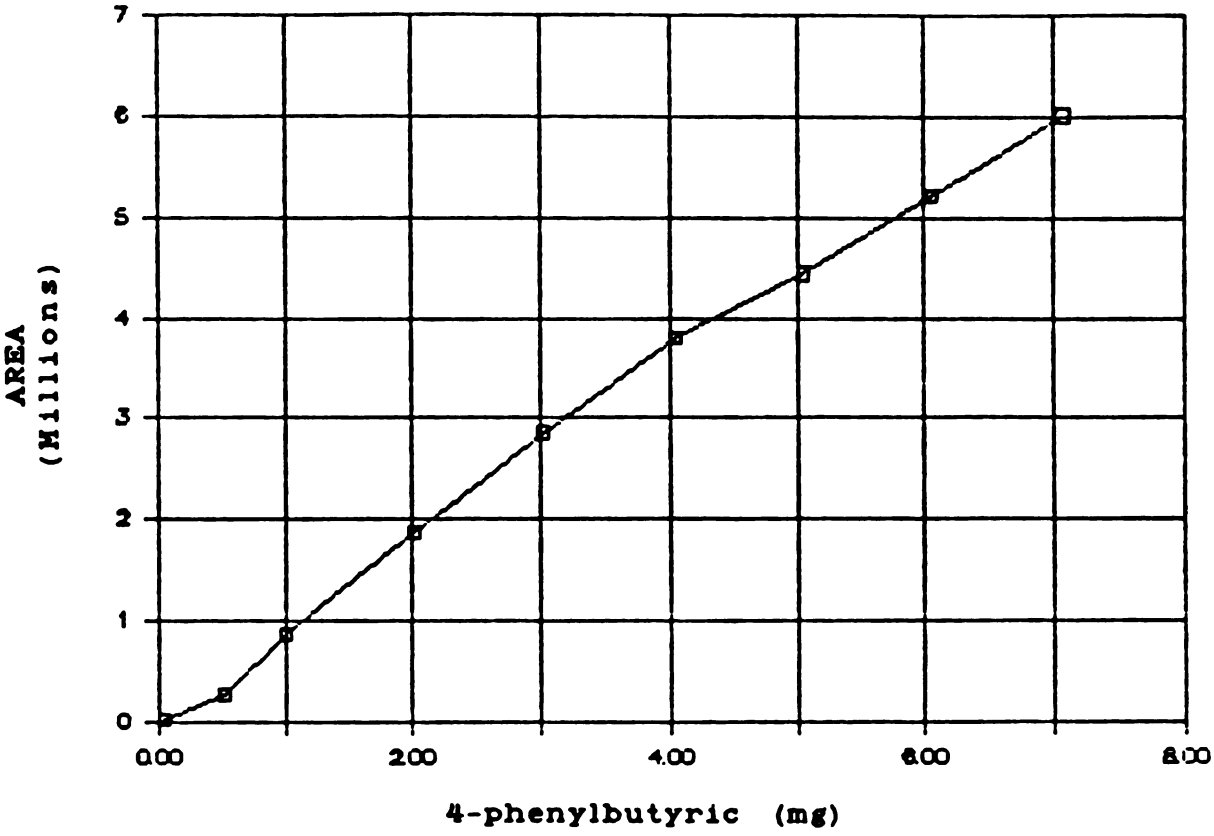


Figure 5. 4-phenylbutyric acid standard curve

TABLE 4a. Factorial analysis of PHPAA and BMD on mean urinary PHPAA excretion after 1st, 2nd, 3rd and 4th week of treatment.

	P values			MSE
WEEK	BMD	PHPAA	BMD x PHPAA	
1	NS	0.01	NS	8308
2	NS	0.01	NS	3224
3	NS	0.01	NS	2568
4	NS	0.01	NS	2284
Mean (1-4)	NS	0.01	NS	3852

MSE = Mean square error
NS = Not significant (P<.05)

TABLE 5. Effects of PHPAA and BMD on mean urinary p-cresol excretion after 1st, 2nd, 3rd and 4th week of treatment.

	Treatments				
WEEK	NA	A	NA-PHPAA	A-PHPAA	MSE[a]
	mg/total urine/24hr				
0	72.69	58.57	73.19	67.81	851
1	152.90	94.47	136.75	129.17	2909
2	226.24	108.27	183.98	125.30	13960
	b	c	b	b	
3	165.78	93.01	211.34	151.70	2809
	b	c	b	b	
4	229.27	93.53	239.57	169.04	3943
	b	c	b	d	
Mean (1-4)	193.50	97.32	192.91	143.80	5520

[a] Mean square error

[b, c, d] Values in the same row having the same or no superscript were not significantly different (P<.05)

week of treatment. Except for those in the A treatment group, urinary p-cresol excretion tended to increase with time with an increase (1-4wk) of 50% and 75% under the NA and NA-PHPAA treatment groups, respectively. Pigs receiving the A-PHPAA treatment increased their urinary p-cresol excretion by only 11% while that of the A treatment group decreased by 1.0%. Figure 6. shows the urinary p-cresol excretion over the 28-day feeding period.

Shown in Table 5a is the factorial analysis of PHPAA and BMD on urinary p-cresol excretion. BMD significantly reduced ($P<.01$) urinary p-cresol excretion (1-4wk). Significant reduction was observed during the 3rd and 4th week of treatment. PHPAA at 0.75% of the diet did not affect urinary p-cresol excretion.

The effects of PHPAA and BMD on total urinary p-cresol excretion per kg body weight are shown in Table 6. Unlike the total urinary p-cresol excretion (mg/total urine/24hr) which tended to increase with age, the urinary p-cresol excretion per unit body size appeared to level off in all treatment groups during the 3rd and 4th week of treatment. When calculated from the 1st to the 4th week of the feeding study, the percent change in urinary p-cresol excretion per kg B.W. averaged -13%, -21%, -1% and -29% for the NA, A, NA-PHPAA and A-PHPAA treatment groups, respectively. Urinary p-cresol excretion per kg B.W. decreased in all treatment groups, but there was substantial reduction in the BMD

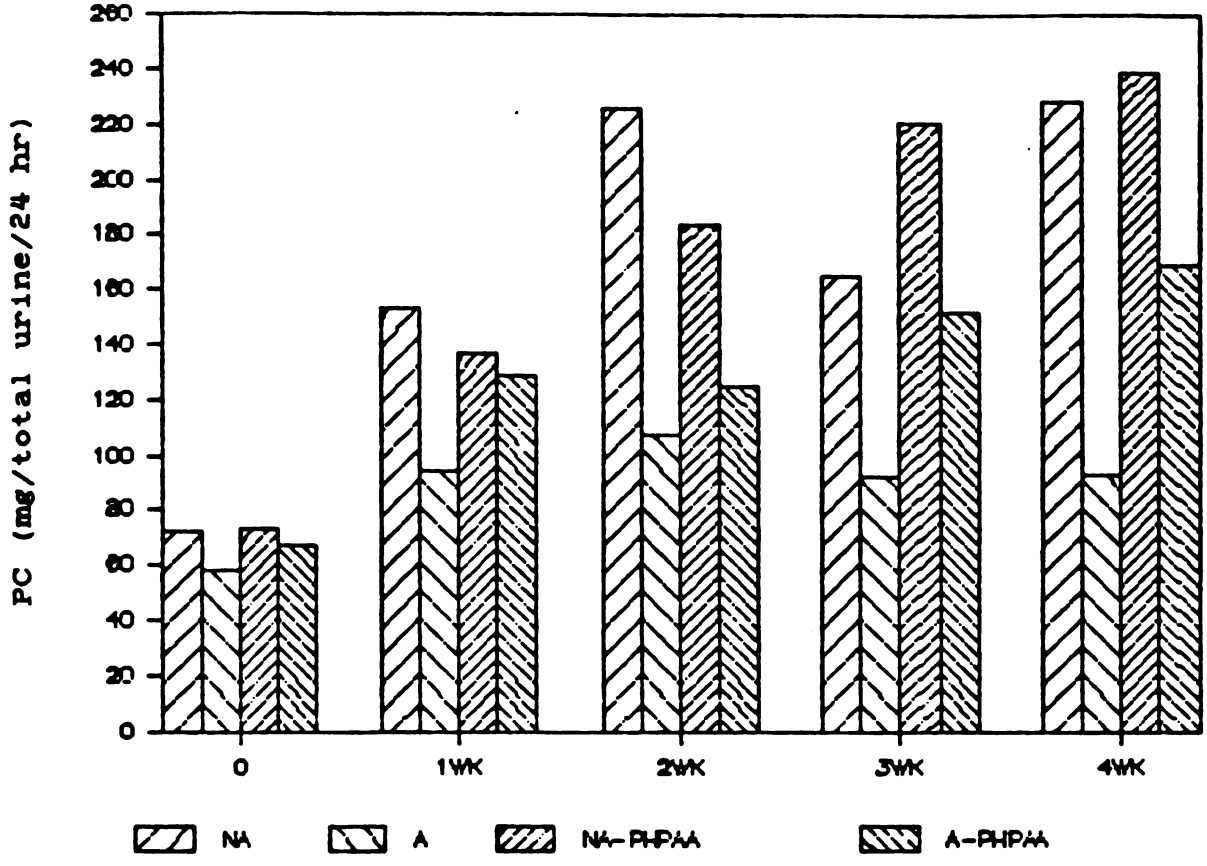


Figure 6. Total urinary p-cresol excretion after 1st, 2nd, 3rd and 4th week of treatment
PHPAA study

TABLE 5a. Factorial analysis of PHPAA and BMD on mean urinary p-cresol excretion after 1st, 2nd, 3rd and 4th week of treatment.

	P values			MSE
WEEK	BMD	PHPAA	BMD x PHPAA	
1	NS	NS	NS	2909
2	NS	NS	NS	13960
3	0.02	NS	NS	2809
4	0.01	NS	NS	3943
Mean (1-4)	0.01	NS	NS	5520

MSE = Mean square error
NS = Not significant (P<.05)

TABLE 6. Effects of PHPAA and BMD on urinary p-cresol excretion per kg body weight after 1st, 2nd, 3rd and 4th week of treatment.

	Treatments				
WEEK	NA	A	NA-PHPAA	A-PHPAA	MSE[a]
	mg/kg BW				
0	6.45	5.86	6.73	6.31	8.30
1	10.53	7.04	10.34	9.51	11.38
2	12.59 bd	6.36 c	11.22 b	7.06 d	35.24
3	7.82 bd	4.63 c	10.42 b	6.80 d	4.04
4	9.12	3.91	10.20	6.80	5.57
Mean (1-4)	10.01 b	5.48 c	10.54 b	7.60 d	12.87

[a] Mean square error

b, c, d Values in the same row having the same or no superscript were not significantly different ($P<.05$)

supplemented groups (A and A-PHPAA) compared to those that did not receive the antibiotic (NA and NA-PHPAA).

Mean p-cresol excretion per kg B.W. (1-4wk) averaged 9.30, 5.56, 9.78 and 7.34 mg/kg B.W. for the NA, A, NA-PHPAA and A-PHPAA treatment groups, respectively. Pigs on the BMD supplemented treatment group (A) excreted significantly less ($P<.05$) p-cresol per unit B.W. than pigs in the NA treatment group over the 28-day experimental period (5.56 vs 9.30 mg/kg B.W.). The difference between the A-PHPAA and NA-PHPAA treatment groups was also significant ($P<.05$). BMD reduced the urinary p-cresol excretion per kg B.W. in the A and A-PHPAA treatment groups by 40% and 25%, respectively. The factorial analysis is shown in Table 6a. BMD significantly reduced ($P<.05$) p-cresol excretion per kg body weight. PHPAA at 0.75% of the diet did not affect urinary p-cresol excretion per kg body weight.

Urinary free p-cresol excretions determined after the 1st, 2nd, 3rd and 4th week of treatment are shown in Table 7. Mean (1-4wk) free p-cresol excretions (mg/total urine/24hr) averaged 6.41, 10.53, 9.63 and 11.88 for the NA, A, NA-PHPAA and A-PHPAA treatment groups, respectively. None of the observed treatment differences was significant ($P<.05$) although the urinary excretion of free p-cresol tended to increase with time in all treatment groups.

Table 7a shows the factorial analysis of PHPAA and BMD

TABLE 6a. Factorial analysis of PHPAA and BMD on urinary p-cresol excretion per kg body weight after 1st, 2nd, 3rd and 4th week of treatment.

	P values			MSE
WEEK	BMD	PHPAA	BMD x PHPAA	
1	NS	NS	NS	11.37
2	NS	NS	NS	35.24
3	0.01	0.02	NS	4.04
4	0.01	NS	NS	5.57
Mean (1-4)	0.01	NS	NS	12.87

MSE = Mean square error
NS = Not significant (P<.05)

TABLE 7. Effects of PHPAA and BMD on mean urinary free p-cresol excretion after 1st, 2nd, 3rd and 4th week of treatment.

	Treatments				
WEEK	NA	A	NA-PHPAA	A-PHPAA	MSE[a]
	mg/total urine/24hr				
0	0.94	3.13	0.60	1.67	2.3
1	3.27	3.73	3.78	4.89	1.4
2	6.22	5.40	8.62	12.48	25.5
3	6.95	20.03	13.42	13.67	97.4
4	9.19	12.97	12.71	16.49	109.2
Mean (1-4)	6.41	10.53	9.63	11.88	68.0

[a] Mean square error
Treatment differences were not significant (P<.05)

on free p-cresol excretion. Neither PHPAA nor BMD significantly affected urinary free p-cresol excretion.

Table 8 shows the effects of PHPAA and BMD on conjugated PC/free PC ratio over the entire feeding study. Mean conjugated PC/free PC ratios averaged 36.96, 16.55, 23.07 and 16.36 for the NA, A, NA-PHPAA and A-PHPAA treatment groups, respectively. Treatment groups which were not supplemented with BMD (NA and NA-PHPAA) had significantly higher ($P<.05$) conjugated PC/free PC ratios than those supplemented with BMD (A and A-PHPAA). The ratio (1-4wk) of pigs receiving the NA dietary treatment was 123% higher than those given the BMD-supplemented diet (A) while the ratio of pigs in the NA-PHPAA treatment group was 41% more than those under the A-PHPAA dietary treatment. Although not significant, the conjugated PC/free PC ratio of pigs in the NA treatment group was 60% higher than those under the NA-PHPAA dietary treatment.

In all treatment groups, the conjugated PC/free PC ratio tended to decrease until the 3rd week of treatment. Except for the A-PHPAA treatment group, the ratio in all treatment groups slightly increased during the 4th week of treatment. The factorial analysis is shown in Table 8a. BMD significantly reduced ($P<.05$) conjugated PC/free PC ratio. PHPAA at 0.75% of the diet did not significantly affect the conjugated PC/free PC ratio.

TABLE 7a. Factorial analysis of PHPAA and BMD on mean urinary free p-cresol excretion after 1st, 2nd, 3rd and 4th week of treatment.

	P values			MSE
WEEK	BMD	PHPAA	BMD x PHPAA	
1	NS	NS	NS	1.4
2	NS	NS	NS	25.5
3	NS	NS	NS	97.4
4	NS	NS	NS	109.2
Mean (1-4)	NS	NS	NS	68.0

MSE = Mean square error
NS = Not significant (P<.05)

TABLE 8. Effects of PHPAA and BMD on conjugated p-cresol/ free p-cresol ratio after 1st, 2nd, 3rd and 4th week of treatment.

	Treatments				
WEEK	NA	A	NA-PHPAA	A-PHPAA	MSE[a]
	ratio				
0	60.41	23.67	70.42	46.37	574.5
1	51.71	24.03	35.89	24.63	374.5
2	49.26	17.69	22.52	14.38	825.1
3	22.87	9.67	15.96	13.99	69.0
4	24.00	14.82	17.90	12.46	92.4
Mean (1-4)	36.96[b]	16.55[c]	23.07[d]	16.36[c]	349.2

[a] Mean square error

[b, c, d] Values in the same row having the same or no superscript were not significantly different (P<.05)

TABLE 8a. Factorial analysis of PHPAA and BMD on conjugated p-cresol/free p-cresol ratio after 1st, 2nd, 3rd and 4th week of treatment.

	P values			MSE
WEEK	BMD	PHPAA	BMD x PHPAA	
1	NS	NS	NS	374.5
2	NS	NS	NS	825.1
3	NS	NS	NS	69.0
4	NS	NS	NS	92.4
Mean (1-4)	0.01	NS	NS	349.2

MSE = Mean square error
NS = Not significant (P<.05)

Table 9 shows the effects of PHPAA and BMD on overall percent BWG. Cumulative percent BWG at the end of the study averaged 114.90, 130.20, 116.60 and 131.10% for the NA, A, NA-PHPAA and A-PHPAA treatment groups, respectively. After the 4th week of treatment, the percent BWG of pigs receiving the A-PHPAA dietary treatment were 12.4% higher than those pigs receiving the NA-PHPAA diets (131.10% vs 116.60%). However, these differences were not significant (P<.05). When compared to the no antibiotic treatment group (NA), the percent BWG of the antibiotic-supplemented control group (A) increased by 13.3% during the 28-day feeding study (130.20% vs 114.90%), but these treatment differences were not significant (P<.05). Factorial analysis of PHPAA and BMD on overall percent BWG is shown in Table 9a. BMD and PHPAA at 0.75% of the diet did not significantly affect overall percent BWG. Figure 7 shows the percent cumulative BWG over time.

Table 10 and Figure 8 show the weekly percent BWG after 1st, 2nd, 3rd and 4th week of treatment. Although none of the treatment differences was significant (P<.05), the percent weekly BWG of the antibiotic-supplemented control group (A) was higher than the NA treatment group during the 1st week and 2nd week of treatment. During these periods, the weekly percent BWG were 31.86% vs 21.85% and 23.00% vs 21.15% respectively for the A and NA treatment groups. However, by the 3rd week and 4th week of treatment, BMD

TABLE 9. Effects of PHPAA and BMD on cumulative % BWG after 1st, 2nd, 3rd and 4th week of treatment.

	Treatments				
WEEK	NA	A	NA-PHPAA	A-PHPAA	MSE[a]
	cumulative % BWG				
1	21.85	31.86	20.75	25.50	75.1
2	47.50	62.20	49.30	59.30	156.3
3	81.70	97.90	83.80	97.50	268.8
4	114.90	130.20	116.60	131.10	505.0

[a] Mean square error
Treatment differences were not significant (P<.05)

TABLE 9a. Factorial analysis of PHPAA and BMD on cumulative percent BWG after 1st, 2nd, 3rd and 4th week of treatment.

	P values			MSE
WEEK	BMD	PHPAA	BMD x PHPAA	
1	NS	NS	NS	75.1
2	NS	NS	NS	156.3
3	NS	NS	NS	268.8
4	NS	NS	NS	505.0

MSE = Mean square error
NS = Not significant (P<.05)

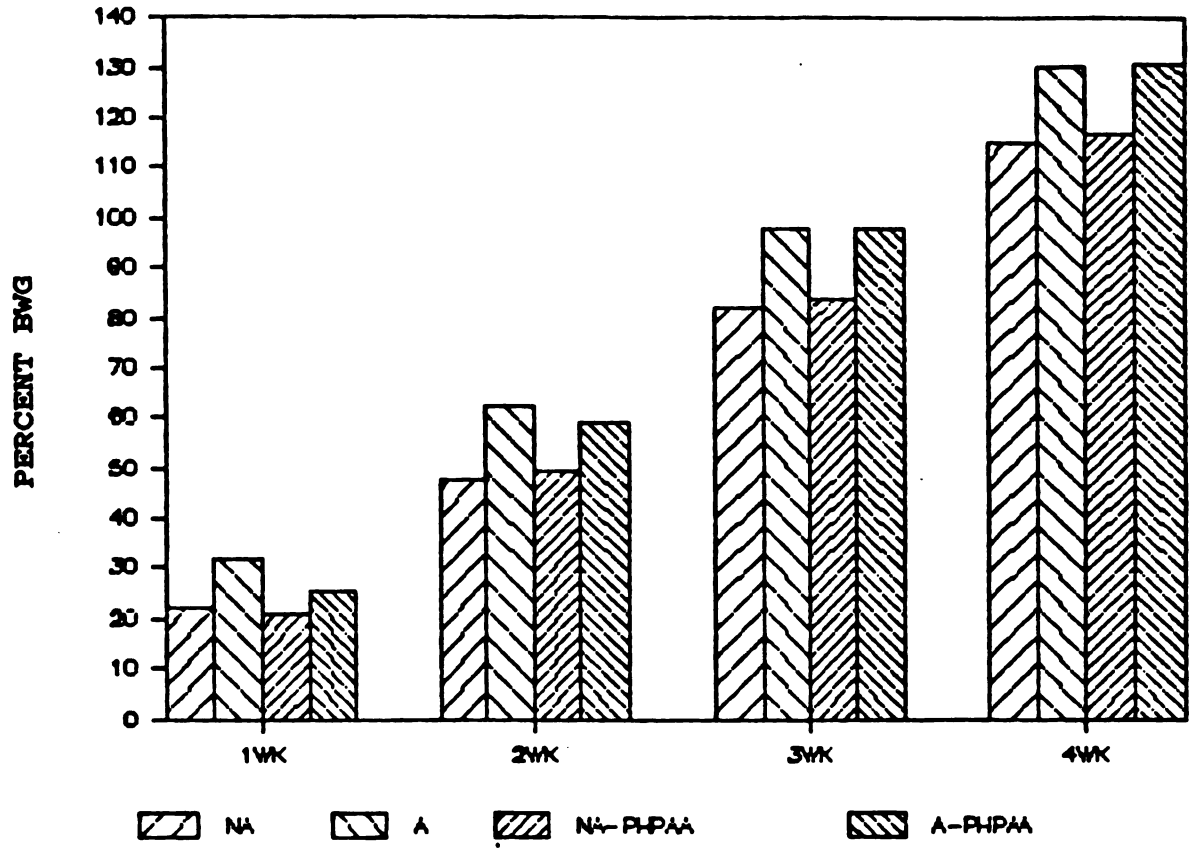


Figure 7. Cumulative percent body weight gain after 1st, 2nd, 3rd and 4th week of treatment PHPAA study

TABLE 10. Effects of PHPAA and BMD on weekly percent body weight gain after 1st, 2nd, 3rd week of treatment.

	Treatments				
WEEK	NA	A	NA-PHPAA	A-PHPAA	MSE[a]
	weekly % BWG				
1	21.85	31.86	20.75	25.38	75.6
2	21.15	23.00	23.58	26.90	11.9
3	23.20	21.90	23.35	23.98	20.7
4	18.43	16.23	17.88	16.75	21.2
Mean (1-4)	21.15	23.24	21.23	23.25	40.4

[a] Mean square error
Treatment differences were not significant (P<.05)

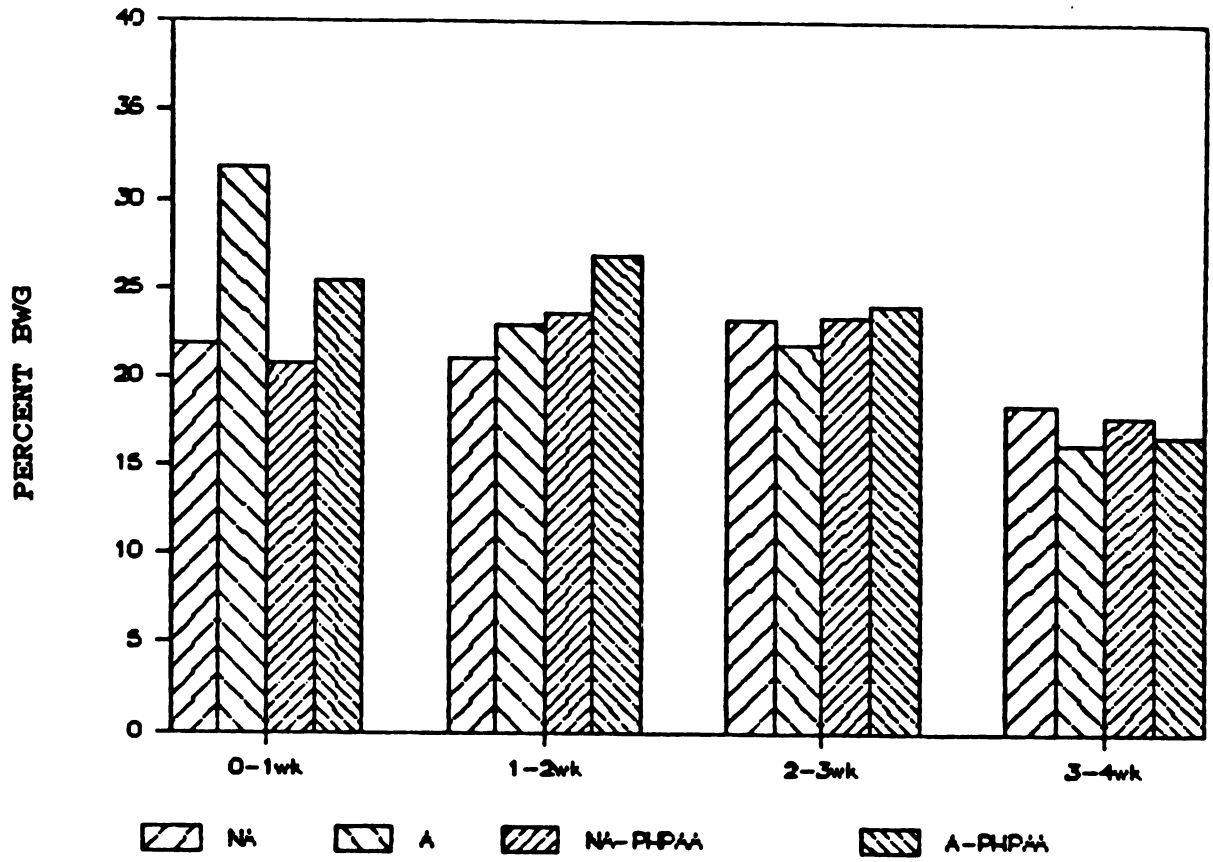


Figure 8. Weekly percent body weight gain after 1st, 2nd, 3rd and 4th week of treatment
PHPAA study

appeared to lose its growth-promoting effect. By the end of the 3rd and 4th week of treatment, the weekly percent BWG of pigs receiving the NA diet were slightly better than the antibiotic-supplemented group (A).

The effect of BMD on the weekly percent BWG was the same in the PHPAA-supplemented groups (NA-PHPAA and A-PHPAA). During the 1st week of the feeding study, the weekly percent BWG of the pigs receiving the A-PHPAA diet was 22% higher than those of pigs that were given the NA-PHPAA diets (25.38% vs 20.75%). By the 2nd week of treatment, the weekly percent BWG of the A-PHPAA treatment group was only 14% higher compared to pigs receiving the no-antibiotic plus PHPAA diets (26.90% vs 23.58%). However, by the end of the 3rd and 4th week of treatment, the weekly percent BWG of pigs receiving the NA-PHPAA diets was about the same or slightly higher than those in the A-PHPAA treatment group. The BMD used in this study appeared to lose its growth-promoting effect by the 3rd week of treatment. Shown in Table 10a is the factorial analysis of PHPAA and BMD on weekly percent BWG. There were no significant effects of PHPAA and BMD on weekly percent BWG.

The other performance parameters of weanling pigs receiving diets supplemented with PHPAA and BMD are shown in Table 11. Average daily feed intake (ADFI) of the A and A-PHPAA treatment groups was higher than those in the NA and NA-PHPAA groups (1025 and 1033 grams vs 978 and 983 grams),

TABLE 10a. Factorial analysis of PHPAA and BMD on weekly percent BWG after 1st, 2nd, 3rd and 4th week of treatment.

	P values			MSE
WEEK	BMD	PHPAA	BMD x PHPAA	
1	NS	NS	NS	75.6
2	NS	NS	NS	11.9
3	NS	NS	NS	20.7
4	NS	NS	NS	21.2
Mean (1-4)	NS	NS	NS	40.4

MSE = Mean square error
NS = Not significant (P<.05)

TABLE 11. Performance of weanling pigs receiving PHPAA and BMD supplemented diets.

	Treatments				
	NA	A	NA-PHPAA	A-PHPAA	MSE[a]
Number of pigs	4	4	4	4	
Initial weight, kg	11.65	10.20	10.88	10.95	
Final weight, kg	24.85	23.23	23.55	25.00	
Ave daily feed intake, g	978	1025	983	1033	.0005
Ave daily gain, g	455	449	437	485	.0003
Overall % BWG	114.90	130.20	116.60	131.10	505
Feed/gain ratio	2.16	2.29	2.26	2.16	.028

[a] Mean square error
Treatment differences were not significant (P<.05)

but none of these treatment differences was significant (P<.05). The ADFI of pigs in the antibiotic supplemented control group (A) increased by 4.8% over the NA treatment group while the ADFI of pigs that received the antibiotic plus PHPAA (A-PHPAA) was 5.1% higher than the pigs receiving the NA-PHPAA dietary treatment. Average daily gain (ADG) (1-4wk) were 455, 449, 437 and 485 grams while feed/gain (F/G) ratios averaged 2.16, 2.29, 2.26 and 2.16 for the NA, A, NA-PHPAA and A-PHPAA treatment groups, respectively. Pigs receiving the BMD-supplemented diets (A and A-PHPAA) tended to grow at a faster rate compared to pigs that did not receive the BMD-supplemented diets (NA and NA-PHPAA) but none of the parameters on pig performance was significantly different among the treatment groups.

Urinary PHPLA excretion over the entire PHPAA study is shown in Table 12. Mean urinary PHPLA excretion (mg/total urine/24hr) averaged (1-4wk) 4.92, 2.27, 1.61 and 3.18 mg for the NA, A, NA-PHPAA and A-PHPAA treatment groups, respectively. Levels of PHPLA in the urine were consistently low in all treatment groups for the duration of the 28-day feeding study. None of the treatment differences was significant. Factorial analysis of PHPAA and BMD on urinary excretion of PHPLA is shown in Table 12a. Neither BMD nor PHPAA significantly affected PHPLA excretion. However, the factorial analysis showed a significant interaction (P<.04) between PHPAA and BMD. Figure 9 shows the PHPLA standard

TABLE 12. Effects of PHPAA and BMD on mean urinary PHPLA excretion after 1st, 2nd, 3rd and 4th week of treatment.

	Treatments				
WEEK	NA	A	NA-PHPAA	A-PHPAA	MSE[a]
	mg/total urine/24hr				
0	3.01	1.25	2.34	3.10	4.8
1	2.92	4.78	0.85	1.44	5.3
2	2.94	2.57	0.36	2.97	7.0
3	3.60	0.00	1.78	4.74	9.9
4	10.23	1.74	3.43	3.56	39.8
Mean (1-4)	4.92	2.27	1.61	3.18	16.5

[a] Mean square error
Treatment differences were not significant (P<.05)

TABLE 12a. Factorial analysis of PHPAA and BMD on mean urinary PHPLA excretion after 1st, 2nd, 3rd and 4th week of treatment.

	P values			MSE
WEEK	BMD	PHPAA	BMD x PHPAA	
1	NS	0.03	NS	5.3
2	NS	NS	NS	7.0
3	NS	NS	0.05	9.9
4	NS	NS	NS	39.8
Mean (1-4)	NS	NS	0.04	16.5

MSE = Mean square error
NS = Not significant (P<.05)

curve.

The effects of PHPAA and BMD on PHPAA/PHPLA ratio after the 1st, 2nd, 3rd and 4th week of treatment are shown in Table 13. Mean PHPAA/PHPLA ratios (1-4wk) were 2.52, 1.09, 43.58 and 96.08 for the NA, A, NA-PHPAA and A-PHPAA treatment groups, respectively. Excess PHPAA in the diets significantly increased (P<.05) the PHPAA/PHPLA ratio of pigs in the NA-PHPAA and A-PHPAA treatment groups. The PHPAA/PHPLA ratio of pigs receiving the NA-PHPAA diet was 17 times higher than the ratio of pigs under the NA dietary treatment while the ratio of the A-PHPAA treatment group was 88 times higher than the antibiotic-supplemented treatment group (A). BMD significantly increased the PHPAA/PHPLA ratio of pigs receiving the A-PHPAA diet by 120% over those pigs given the NA-PHPAA diet (96.08 vs 43.58). The elevated PHPAA/PHPLA ratios of the pigs given the diets supplemented with PHPAA (NA-PHPAA and A-PHPAA) were observed during the 1st week of treatment. However, significant treatment differences (P<.05) were not noted until the 3rd week of treatment.

Shown in Table 13a is the factorial analysis of BMD and PHPAA. There was a significant increase (P<.01) in PHPAA/PHPLA ratio resulting from the addition of 0.75% PHPAA in the diet. BMD did not significantly affect (P<.05) the PHPAA/PHPLA ratio. BMD had a P value of 0.08. Figure 10 shows the PHPAA/PHPLA ratio over time.

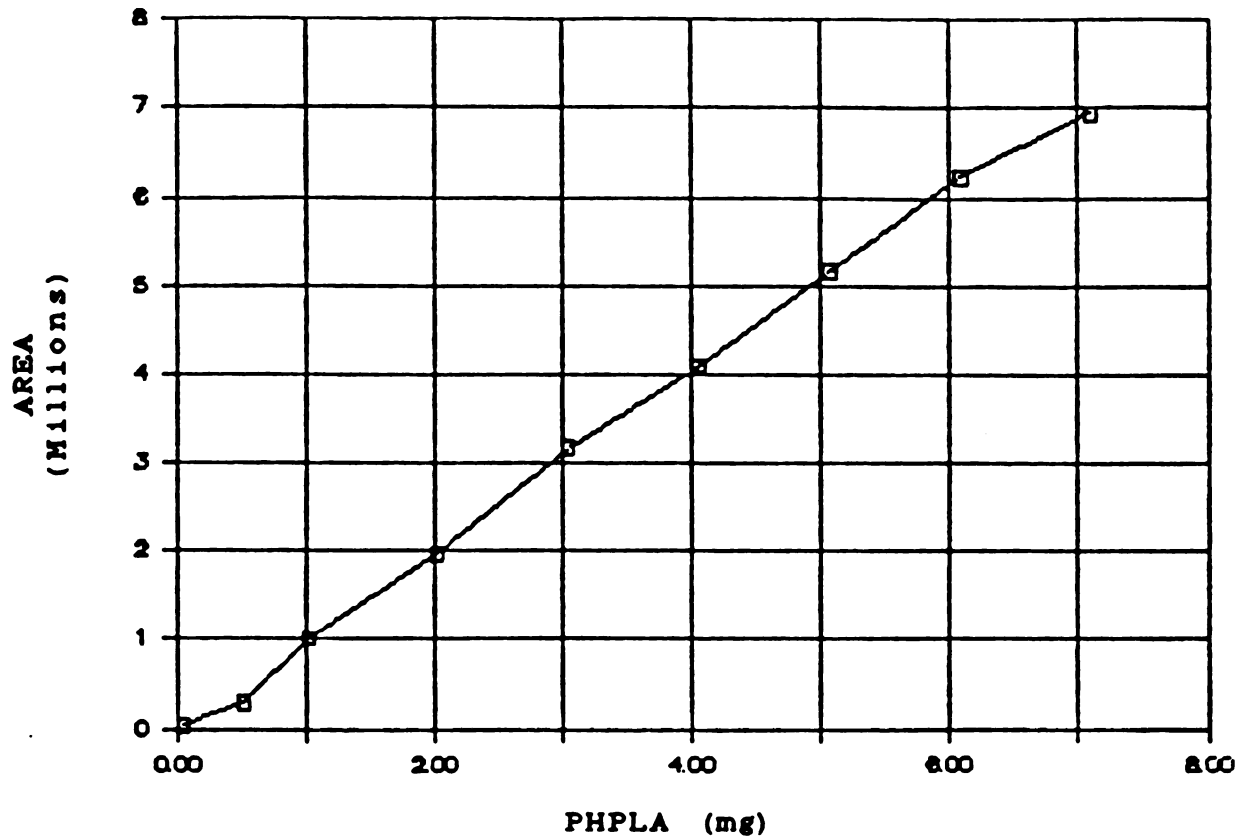


Figure 9. PHPLA standard curve

TABLE 13. Effects of PHPAA and BMD on PHPAA/PHPLA ratio after 1st, 2nd, 3rd and 4th week of treatment.

	Treatments				
WEEK	NA	A	NA-PHPAA	A-PHPAA	MSE[a]
	ratio				
1	5.36	2.69	36.77	124.66	4228
2	1.70 b	1.13 c	26.88 b	122.69 d	8068
3	2.18 b	0.00 b	7.51 c	36.95 c	217
4	0.84	0.54	77.15	100.02	1602
Mean (1-4)	2.52 b	1.09 b	43.58 c	96.08 d	3365

[a] Mean error square

b, c, d Values in the same row having the same or no superscript were not significantly different (P<.05)

TABLE 13a. Factorial analysis of PHPAA and BMD on PHPAA/ PHPLA ratio after 1st, 2nd, 3rd and 4th week of treatment.

	P values			MSE
WEEK	BMD	PHPAA	BMD x PHPAA	
1	NS	0.01	NS	4228
2	NS	NS	NS	8068
3	NS	0.01	0.05	217
4	NS	0.01	NS	1602
Mean (1-4)	NS	0.01	NS	3365

MSE = Mean square error
NS = Not significant (P<.05)

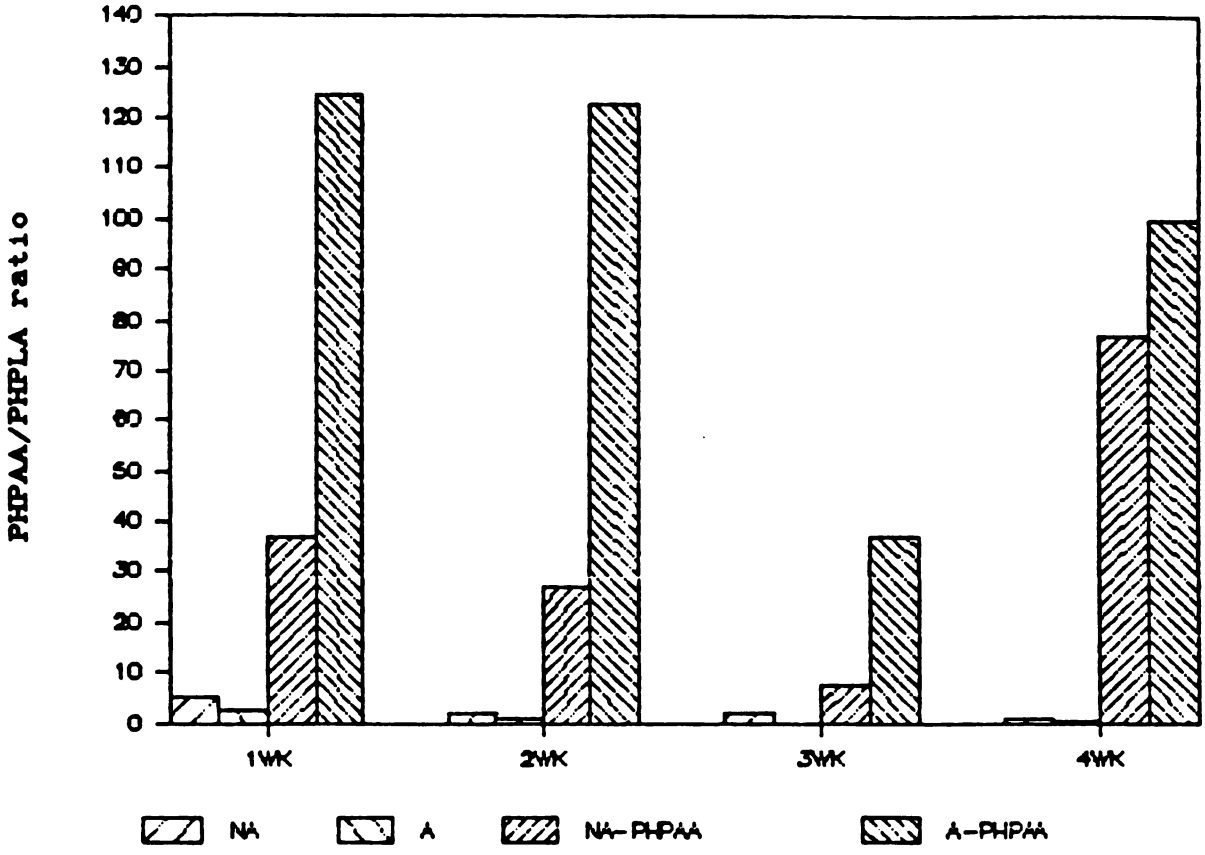


Figure 10. PHPAA/PHPLA ratio after 1st, 2nd, 3rd and 4th week of treatment
PHPAA study

Table 14 shows the effects of PHPAA and BMD on mean urine volume during the entire feeding study. Urine volume increased in all treatment groups as the pigs got older. During the 28-day PHPAA feeding study, urine volume increased by 53.9, 105.8, 23.4 and 5.7% (1-4wk) for the NA, A, NA-PHPAA and A-PHPAA treatment groups, respectively. Pigs in the NA and NA-PHPAA treatment groups had higher urine volume than those under the A and A-PHPAA dietary treatments (523 and 497 ml/24hr vs 485 and 465 ml/24hr). However, these differences were not significant ($P<.05$). The factorial analysis of PHPAA and BMD is shown in Table 14a. The effects of PHPAA and BMD on urine volume were not significant.

A summary of the mean (1-4wk) urinary PHPAA, PHPLA, p-cresol and free p-cresol excretions, conjugated PC/free PC ratio, urine volume, PHPAA/PHPLA ratio, ADFI and overall percent BWG found in the PHPAA study is shown in Table 15.

EXPERIMENT 2

The effects of p-cresol and BMD on mean urinary p-cresol excretion is shown in Table 16. Mean (1-4wk) urinary p-cresol excretion (mg/total urine/24hr) averaged 83.78, 84.31, 865.30 and 960.80 for the NA, A, NA-PC and A-PC treatment groups, respectively. The addition of 0.75%

TABLE 14. Effects of PHPAA and BMD on urine volume after 1st, 2nd, 3rd and 4th week of treatment.

	Treatments				
WEEK	NA	A	NA-PHPAA	A-PHPAA	MSEa
	ml/24hr				
0	202	180	221	213	2492
1	397	330	401	438	19203
2	505	394	576	416	42013
3	579	538	514	543	26778
4	611	679	495	463	31930
Mean (1-4)	523	485	497	465	32292

a
Mean square error
Treatment differences were not significant (P<.05)

TABLE 14a. Factorial analysis of PHPAA and BMD on mean urine volume after 1st, 2nd, 3rd and 4th week of treatment.

	P values			MSE
WEEK	BMD	PHPAA	BMD x PHPAA	
1	NS	NS	NS	19203
2	NS	NS	NS	42013
3	NS	NS	NS	26778
4	NS	NS	NS	31930
Mean (1-4)	NS	NS	NS	32292

MSE = Mean square error
NS = Not significant (P<.05)

TABLE 15. Summary of mean urinary PHPAA, PHPLA, p-cresol, free p-cresol excretions, conjugated PC/free PC ratio, PHPAA/PHPLA ratio, ADFI and overall percent BWG (PHPAA study).

	Treatments			
	NA	A	NA-PHPAA	A-PHPAA
Number of animals	4	4	4	4
PHPAA (mg/24hr, 1-4wk)	8.78 [a]	9.58 [a]	222.37 [b]	279.57 [c]
PHPLA (mg/24hr, 1-4wk)	4.92 [a]	2.27 [a]	1.61 [b]	3.18 [c]
PHPAA/PHPLA ratio	2.52 [a]	1.09 [a]	43.58 [b]	96.08 [c]
PC (mg/24hr, 1-4wk)	193.55 [a]	97.32 [b]	192.91 [a]	143.80 [c]
PC (mg/kg body weight)	10.01 [a]	5.48 [b]	10.54 [a]	7.60 [c]
FREE PC (mg/24hr, 1-4wk)	6.41 [a]	10.53 [b]	9.63 [c]	11.88 [b]
Bound PC/Free PC ratio	36.96	16.55	23.07	16.36
ADFI, grams	978	1025	983	1033
Overall % BWG	114.90	130.20	116.60	131.10

a, b, c
Values in the same row having the same or no superscript were not significantly different ($P<0.5$)

TABLE 16. Effects of p-cresol and BMD on mean urinary p-cresol excretion after 1st, 2nd, 3rd and 4th week of treatment.

	Treatments				
WEEK	NA	A	NA-PC	A-PC	MSE[a]
	mg/total urine/24hr				
0	67.04	73.14	56.56	41.12	1041
	b	b	c	c	
1	71.17	56.11	846.74	710.96	11068
	b	b	c	c	
2	43.89	58.60	408.23	550.16	7366
	b	b	c	c	
3	76.83	75.98	1018.84	1158.84	70445
	b	b	c	c	
4	143.21	146.55	1187.37	1423.91	56176
	b	b	c	c	
Mean (1-4)	83.78	84.31	865.30	960.80	84463

[a] Mean square error

[b, c] Values in the same row having the same or no superscript were not significantly different (P<.05)

p-cresol in the diets significantly increased (P<.01) the urinary p-cresol excretions of pigs in the NA-PC and A-PC treatment groups compared to those under the NA and A dietary treatments. Elevated levels of the urinary p-cresol excretion were observed in the NA-PC and A-PC treatment groups during the 1st week of treatment, and the amount of p-cresol excretion in these groups continued to increase for the duration of the study.

There was no significant difference in the urinary excretion of p-cresol between the NA and A treatment groups (83.78 vs 84.31 mg/24hr) suggesting that the addition of BMD to the diet (A) did not decrease the endogenous production of p-cresol. The urinary p-cresol excretion in the A treatment group increased by 161.2% (1-4wk) compared to the NA treatment group which increased by 101.2 percent. The difference in urinary p-cresol excretion between the NA-PC and A-PC treatment groups was also not significant. Pigs receiving the A-PC diet had slightly higher urinary p-cresol excretion than pigs on the NA-PC dietary treatment (960.80 vs 865.30 mg/24hr). The BMD used in this study (A-PC) was not effective in decreasing the endogenous production of p-cresol. Pigs under the A-PC dietary treatment increased urinary p-cresol excretion by 100.2% (1-4wk) compared to the 40% increase in the NA-PC treatment group. Table 16a shows the factorial analysis of p-cresol (NA-PC + A-PC vs NA + A) and BMD (NA + NA-PC vs A + A-PC). The addition of 0.75%

TABLE 16a. Factorial analysis of p-cresol and BMD on mean urinary p-cresol excretion after 1st, 2nd, 3rd and 4th week of treatment.

	P values			MSE
WEEK	BMD	PC	BMD x PC	
1	NS	0.01	NS	11068
2	NS	0.01	NS	7366
3	NS	0.01	NS	70445
4	NS	0.01	NS	56176
Mean (1-4)	NS	0.01	NS	84463

MSE = Mean square error
NS = Not significant (P<.05)

p-cresol in the diet significantly increased (P<.01) urinary p-cresol excretion. There was no significant effect of BMD on p-cresol excretion. Figure 11 shows the urinary excretion of p-cresol during the 28-day feeding study.

The effects of p-cresol and BMD on urinary excretion of p-cresol per kg B.W. is shown in Table 17. Treatment groups supplemented with 0.75% p-cresol (NA-PC and A-PC) had significantly higher urinary p-cresol excretion per kg B.W. than pigs in the NA and A treatment groups (P<.05) during the entire feeding study. Mean (1-4wk) urinary p-cresol excretion per kg B.W. averaged 5.63, 5.88, 44.67 and 48.58 mg/kg B.W. for the NA, A, NA-PC and A-PC treatment groups, respectively. During the 28-day experimental period, the percent change in urinary p-cresol excretion per kg B.W. (1-4wk) for the NA, A, NA-PC and A-PC treatment groups averaged 12, 18, -20 and 9%, respectively. BMD did not reduce the urinary p-cresol excretion per kg B.W. in the A and A-PC treatment groups compared to those that received the NA and NA-PC dietary treatments. Table 17a shows the factorial analysis of p-cresol and BMD on p-cresol excretion per kg body weight. There was a significant increase in p-cresol excretion per kg body weight with the addition of 0.75% p-cresol in the diet. BMD did not have a significant effect.

Table 18 shows the effects of p-cresol and BMD on urinary free p-cresol excretion. Mean urinary free p-cresol excretion (mg/total urine/24hr) averaged (1-4wk) 20.93,

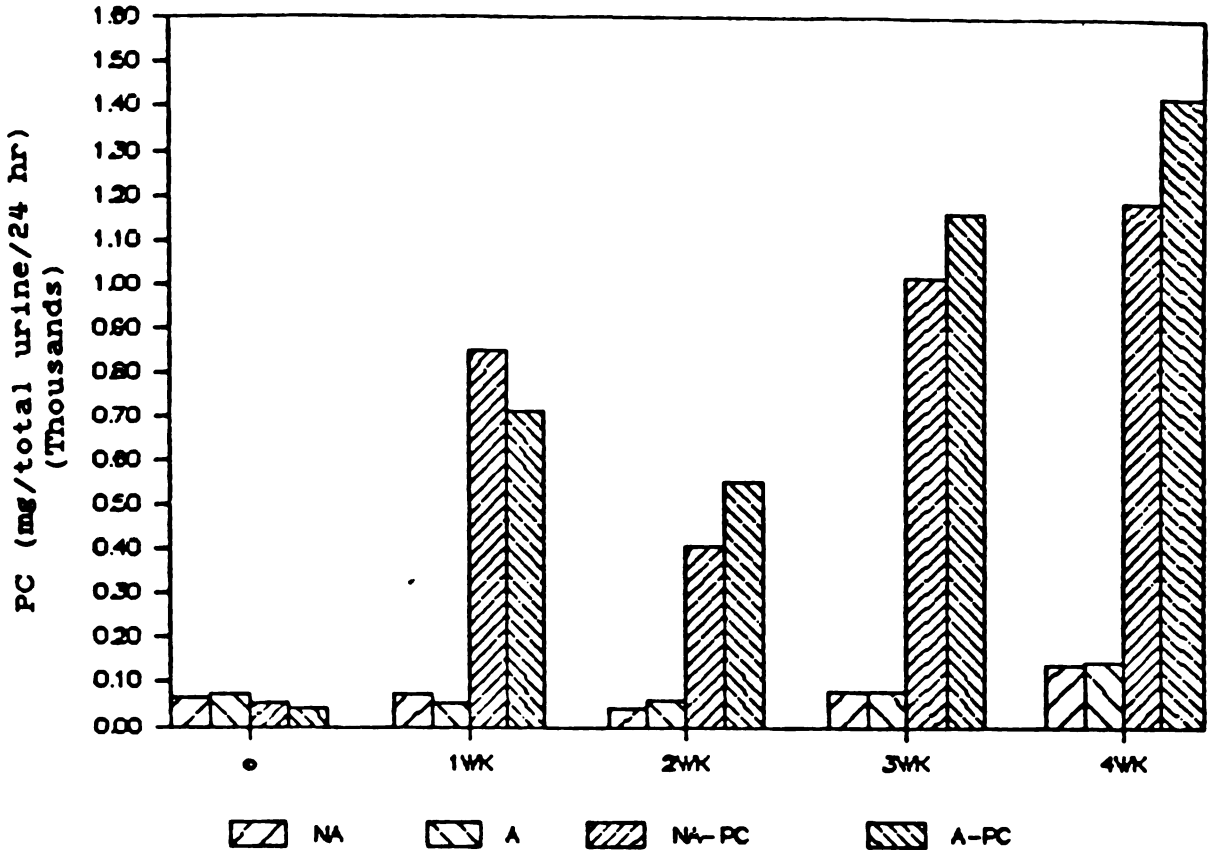


Figure 11. Total urinary p-cresol excretion after 1st, 2nd, 3rd and 4th week of treatment P-cresol study

TABLE 17. Effects of p-cresol and BMD on urinary p-cresol excretion per kg body weight after 1st, 2nd, 3rd and 4th week of treatment.

	Treatments				
WEEK	NA	A	NA-PC	A-PC	MSE[a]
	mg/kg BW				
0	7.32	8.30	5.68	4.27	8.31
	b	b	c	c	
1	6.17	5.00	71.55	64.12	86.54
	b	b	c	c	
2	3.11	4.58	28.96	39.67	37.84
	b	b	c	c	
3	4.68	4.65	58.70	65.27	128.56
	b	b	c	c	
4	6.90	6.90	57.44	69.59	146.74
	b	b	c	c	
Mean (1-4)	5.63	5.88	44.67	48.58	182.00

[a] Mean square error

[b, c] Values in the same row having the same or no superscript were not significantly different ($P<.05$)

TABLE 17a. Factorial analysis of p-cresol and BMD on urinary p-cresol excretion per kg body weight after 1st, 2nd, 3rd and 4th week of treatment.

	P values			MSE
WEEK	BMD	PC	BMD x PC	
1	NS	0.01	NS	86.54
2	NS	0.01	NS	37.84
3	NS	0.01	NS	128.56
4	NS	0.01	NS	146.74
Mean (1-4)	NS	0.01	NS	181.99

MSE = Mean square error
NS = Not significant (P<.05)

TABLE 18. Effects of p-cresol and BMD on mean urinary free p-cresol excretion after 1st, 2nd, 3rd and 4th week of treatment.

	Treatments				
WEEK	NA	A	NA-PC	A-PC	MSE[a]
	mg/total urine/24hr				
0	3.99	4.85	2.55	2.79	8.6
	b	b	c	c	
1	8.78	4.69	56.78	63.56	460.9
	b	b	c	c	
2	10.50	8.91	47.66	65.68	240.7
	b	b	c	c	
3	32.05	15.07	63.90	68.06	990.1
	b	b	c	c	
4	32.41	20.20	78.33	73.59	795.9
	b	b	c	c	
Mean (1-4)	20.93	12.22	61.67	67.72	578.2

[a] Mean square error

[b, c] Values in the same row having the same or no superscript were not significantly different (P<.05)

12.22, 61.67 and 67.72 for the NA, A, NA-PC and A-PC treatment groups, respectively. Excess p-cresol in the diets significantly increased ($P<.05$) the urinary excretion of free p-cresol of pigs receiving the NA-PC and A-PC diets compared to pigs receiving the NA and A dietary treatments (61.67 and 67.72 vs 20.93 and 12.22 mg/24hr). Differences in the urinary free p-cresol excretion between those that received the NA-PC and A-PC dietary treatments and between the NA and A treatment groups were not significant. The substantially high level of urinary free p-cresol excretion in the NA-PC and A-PC treatment groups were observed during the 1st week of treatment and the trend continued until the end of the feeding period. Mean free p-cresol excretion of pigs receiving the A diet was 41.61% lower than those fed the NA diets (12.22% vs 20.93%) but this difference was not significant. In contrast, the urinary free p-cresol excretion in the A-PC treatment group was 9.8% higher than the NA-PC treatment group but this difference was also not significant. The factorial analysis of BMD and PC is shown in Table 18a. P-cresol at 0.75% of the diet significantly increased ($P<.01$) urinary excretion of free p-cresol. There was no significant effect of BMD on free p-cresol excretion. Figure 12 shows the mean urinary free p-cresol excretion over time.

The effects of p-cresol and BMD on conjugated PC/free PC ratio over the entire feeding study is shown in Table 19.

TABLE 18a. Factorial analysis of p-cresol and BMD on mean urinary free p-cresol excretion after 1st, 2nd, 3rd and 4th week of treatment.

	P values			MSE
WEEK	BMD	PC	BMD x PC	
1	NS	0.01	NS	460.9
2	NS	0.01	NS	240.7
3	NS	0.01	NS	990.1
4	NS	0.01	NS	795.9
Mean (1-4)	NS	0.01	NS	578.2

MSE = Mean square error
NS = Not significant (P<.05)

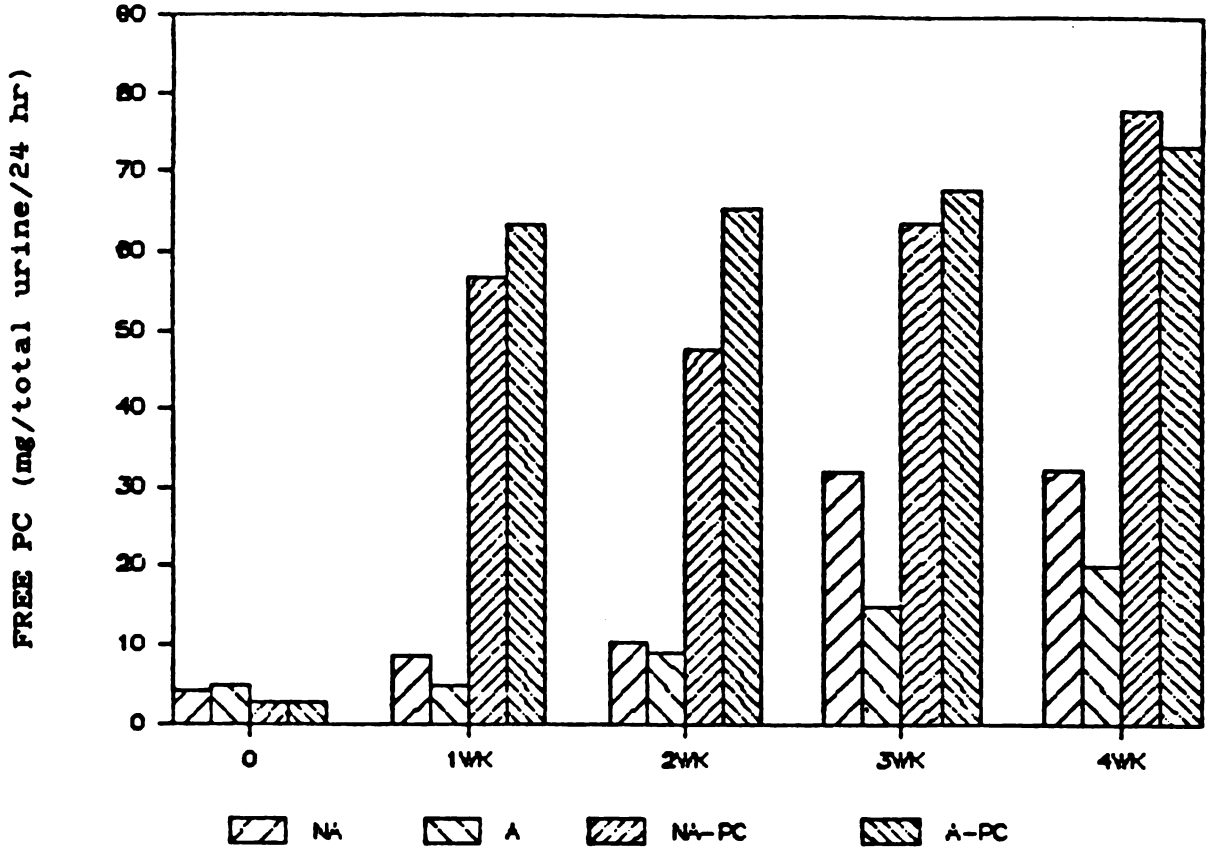


Figure 12. Urinary free p-cresol excretion after 1st, 2nd, 3rd and 4th week of treatment
P-cresol study

TABLE 19. Effects of p-cresol and BMD on conjugated PC/free p-cresol ratio after 1st, 2nd, 3rd and 4th week of treatment.

	Treatments				
WEEK	NA	A	NA-PC	A-PC	MSE[a]
	ratio				
0	63.05	68.28	54.02	38.22	892.9
1	15.89	14.71	14.23	15.32	99.6
2	6.91	6.64	7.95	7.75	10.6
3	6.76[b]	5.79[b]	15.53[b]	16.49[c]	15.4
4	8.83	8.60	15.29	19.28	34.1
Mean (1-4)	9.60[bd]	8.93[b]	13.25[cd]	14.71[c]	46.3

[a] Mean square error

[b, c, d] Values in the same row having the same or no superscript were not significantly different (P<.05)

Conjugated PC/free PC ratio (1-4wk) averaged 9.60, 8.93, 13.25 and 14.71 for the NA, A, NA-PC and A-PC treatment groups, respectively. Pigs receiving the p-cresol supplemented diets (NA-PC and A-PC) had higher conjugated PC/free PC ratio than those in the NA and A treatment groups. The difference between the A-PC and A treatment groups was significant ($P<.05$) however, that of the NA-PC and A-PC treatment groups was not statistically significant. The supplementation of BMD in the A and A-PC treatment groups did not have any significant effect on the conjugated PC/free PC ratios compared to those that did not receive the antibiotic (NA and NA-PC). During the four week experimental period, the percent change (1-4wk) in conjugated PC/free PC ratio averaged -44.43, -41.54, 7.45 and 25.85% for the NA, A, NA-PC and A-PC treatment groups, respectively. The biggest decrease in conjugated PC/free PC ratio were made by the NA and A treatment groups which did not receive the 0.75% p-cresol in the diet. The factorial analysis is shown in Table 19a. Addition of 0.75% in the diet significantly increased ($P<.01$) the conjugated PC/free PC ratio. BMD had no significant effect on the conjugated PC/free PC ratio.

Table 20 shows the effects of p-cresol and BMD on cumulative percent BWG after the 1st, 2nd, 3rd and 4th week of treatment. Overall percent BWG at the end of the study averaged 136.18, 137.37, 114.65 and 118.19% for the NA, A, NA-PC and A-PC treatment groups respectively. Pigs

TABLE 19a. Factorial analysis of p-cresol and BMD on conjugated PC/free p-cresol ratio after 1st, 2nd, 3rd and 4th week of treatment.

	P values			MSE
WEEK	BMD	PC	BMD x PC	
1	NS	NS	NS	99.6
2	NS	NS	NS	10.6
3	NS	0.01	NS	15.4
4	NS	0.01	NS	34.1
Mean (1-4)	NS	0.01	NS	46.3

MSE = Mean square error
NS = Not significant (P<.05)

TABLE 20. Effects of p-cresol and BMD on cumulative % BWG after 1st, 2nd, 3rd and 4th week of treatment.

	Treatments				
WEEK	NA	A	NA-PC	A-PC	MSE[a]
	cumulative % BWG				
1	22.85	24.91	19.71	20.66	22.6
2	53.15	51.11	46.60	46.97	176.0
3	87.51	89.12	77.64	81.70	491.7
4	136.18	137.37	114.65	118.19	949.0

[a] Mean square error
Treatment differences were not significant ($P<.05$)

receiving the NA-PC dietary treatment had 15.8% less overall BWG than those in the NA treatment group (114.65% vs 136.18%) while the BWG in the A-PC treatment group was 14% less than the A treatment group (118.19% vs 137.37%). Pigs receiving the excess p-cresol tended to have lower percent BWG than the control group. The reduction in percent BWG of the pigs in the NA-PC and A-PC treatment groups was apparent after the 1st week of treatment and the decline continued until the end of the feeding study. The cumulative percent BWG (4th wk) of the NA and A treatment groups were about the same (136.18% vs 137.37%) and that of the NA-PC and A-PC groups were also similar (114.65% vs 118.19%) suggesting that the BMD used in this study was not effective in promoting growth. The factorial analysis is shown in Table 20a. Neither BMD nor the supplementation of 0.75% p-cresol in the diet had significant effect on cumulative percent body weight gain. Figure 13 shows the cumulative percent BWG over 28-day feeding study.

The weekly percent BWG as affected by the addition of p-cresol and BMD is shown in Table 21. During the 1st, 2nd and 3rd week of treatment, the weekly percent BWG of pigs that received the NA-PC and A-PC diets were lower than those in the NA and A treatment groups, but the observed treatment differences were not significant. However, by the 4th week of treatment, the addition of p-cresol to the diets significantly decreased ($P<.05$) the weekly percent BWG of pigs in

TABLE 20a. Factorial analysis of p-cresol and BMD on cumulative percent BWG after 1st, 2nd, 3rd and 4th week of treatment.

	P values			MSE
WEEK	BMD	PC	BMD x PC	
1	NS	NS	NS	22.6
2	NS	NS	NS	176.0
3	NS	NS	NS	491.7
4	NS	NS	NS	949.0

MSE = Mean square error
NS = Not significant (P<.05)

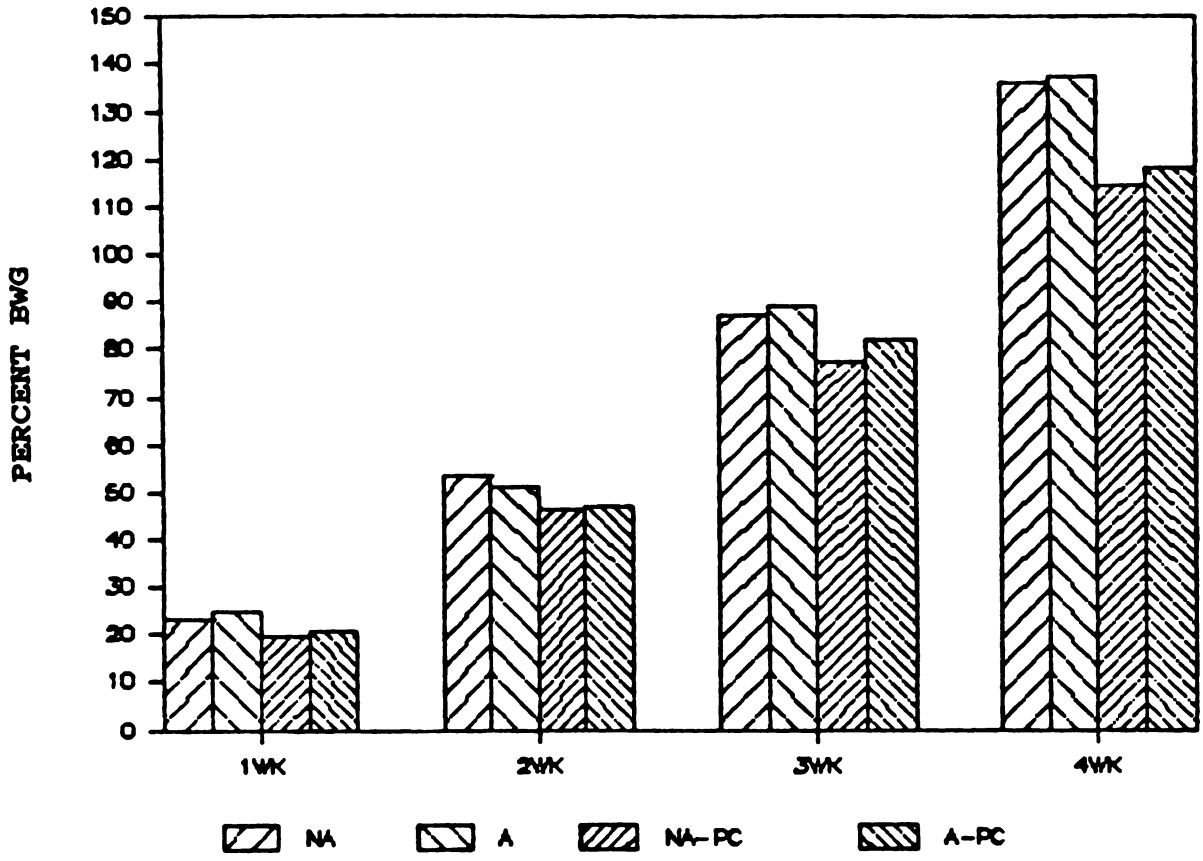


Figure 13. Cumulative percent body weight gain after 1st, 2nd, 3rd and 4th week of treatment P-cresol study

TABLE 21. Effects of p-cresol and BMD on weekly percent body weight gain after 1st, 2nd, 3rd and 4th week of treatment.

	Treatments				
WEEK	NA	A	NA-PC	A-PC	MSE[a]
	weekly % BWG				
1	22.85	24.91	19.71	20.66	22.55
2	24.52	20.85	22.34	21.96	74.60
3	22.31	24.71	20.98	23.52	23.26
4	25.65[b]	25.42[b]	20.80[c]	19.97[c]	8.74
Mean (1-4)	23.83[b]	23.97[b]	20.96[c]	21.53[bc]	27.90

[a] Mean square error

[b, c] Values in the same row having the same or no superscript were not significantly different (P<.05)

the NA-PC and A-PC treatment groups . Pigs receiving the NA-PC and A-PC diets had 20.80% and 19.97% weekly BWG while those under the NA and A dietary treatments had 25.65% and 25.42%, respectively. Figure 14 shows the weekly percent BWG over the 28-day feeding study. The factorial analysis is shown in Table 21a. Addition of 0.75% p-cresol significantly reduced ($P<.04$) weekly percent BWG. Significant reduction due to supplementation of p-cresol occurred during the 4th week of treatment. BMD did not have a significant effect on weekly percent BWG.

The other performance parameters of pigs receiving the p-cresol and BMD supplemented diets are shown in Table 22. ADFI averaged 818, 850, 810 and 790 grams while ADG were 418, 405, 380 and 378 grams for the NA, A, NA-PC and A-PC treatment groups, respectively. Pigs receiving the p-cresol supplemented diets (NA-PC and A-PC) tended to have lower ADFI than pigs in the NA and A treatment groups. However, these differences were not significant. None of the F/G ratios which averaged 1.95, 2.10, 2.12 and 2.09 for the NA, A, NA-PC and A-PC treatment groups, respectively, was significant.

BMD failed to improve the ADG of pigs in the A and A-PC treatment groups. Compared to the NA and NA-PC treatment groups which averaged 418 and 380 g ADG, respectively, pigs receiving the A and A-PC diets had 405 and 378 g ADG,

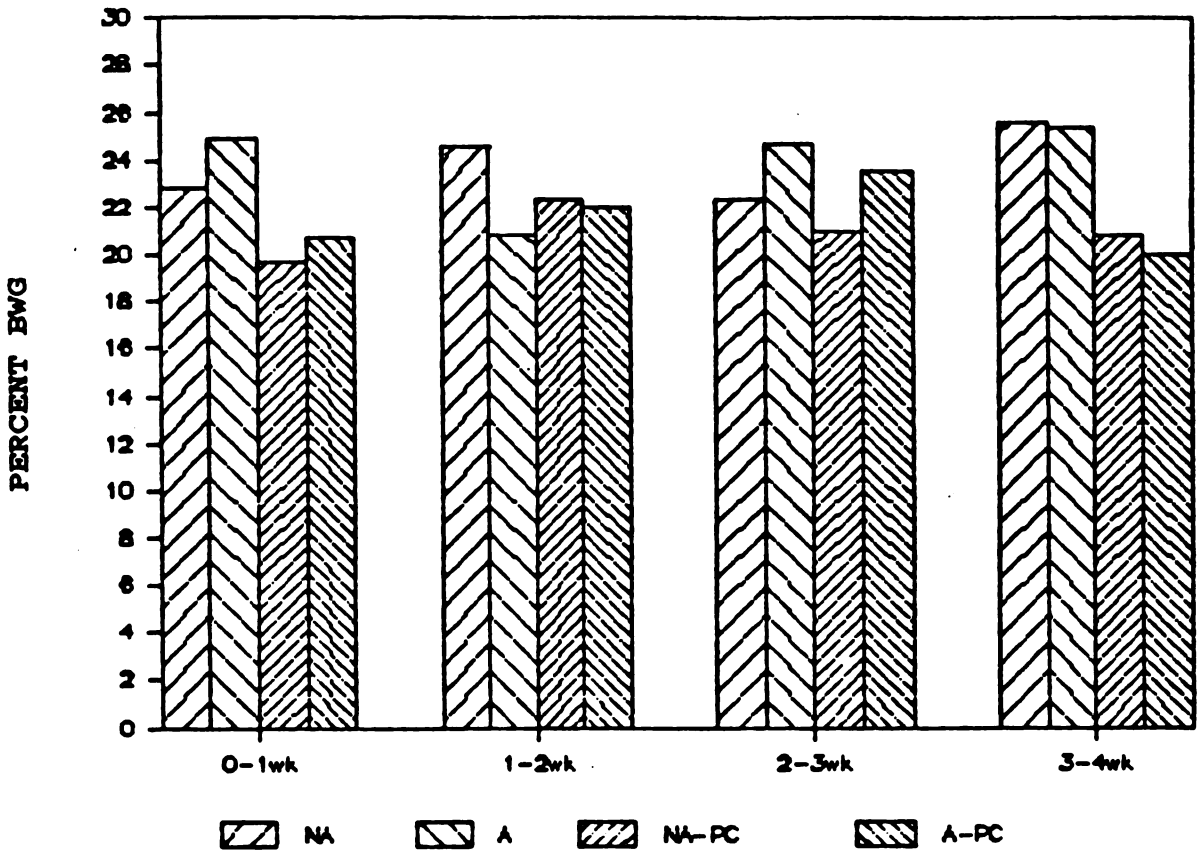


Figure 14. Weekly percent BWG after 1st, 2nd, 3rd and 4th week of treatment
P-cresol study

TABLE 21a. Factorial analysis of p-cresol and BMD on weekly percent body weight gain after 1st, 2nd, 3rd and 4th week of treatment.

	P values			MSE
WEEK	BMD	PC	BMD x PC	
1	NS	NS	NS	22.55
2	NS	NS	NS	74.60
3	NS	NS	NS	23.26
4	NS	0.01	NS	8.74
Mean (1-4)	NS	0.04	NS	27.90

MSE = Mean square error
NS = Not significant (P<.05)

TABLE 22. Performance of weanling pigs receiving p-cresol and BMD supplemented diets.

	Treatments				
	NA	A	NA-PC	A-PC	MSE[a]
Number of pigs	4	4	4	4	
Initial weight, kg	9.44	9.08	9.99	9.55	
Final weight, kg	21.55	20.83	21.09	20.45	
Ave daily feed intake, g	818	850	810	790	.007
Ave daily gain, g	418	405	380	378	.0008
Overall % BWG	136.18	137.37	114.65	118.19	949
Feed/gain ratio	1.95	2.10	2.12	2.09	.022

[a] Mean square error
Treatment differences were not significant (P<.05)

respectively. However, the addition of BMD did not improve the ADFI in the A-PC treatment group when compared to the ADFI of pigs that received the NA-PC dietary treatment (790 vs 810 grams).

Factorial analysis of p-cresol and BMD on ADG, ADFI and F/G ratio is shown in Table 22a. P-cresol at 0.75% of the diet significantly reduced (P<.04) ADG. BMD did not have a significant effect on ADG. Addition of p-cresol or BMD did not significantly affect ADFI and F/G ratio.

Table 23 shows the effects of p-cresol and BMD on urine volume after the 1st, 2nd, 3rd and 4th week of treatment. Urine volume increased in all treatment groups as the pig got older. Urine volume in the NA, A, NA-PC and A-PC treatment groups increased by 66, 69, 68 and 27% (1-4wk), respectively. The factorial analysis of PC and BMD on urine volume is shown in Table 23a. The addition of 0.75% p-cresol in the diet significantly reduced (P<.03) urine volume. BMD did not have an effect on urine volume. The summary of mean urinary p-cresol, free p-cresol excretions, ADFI and overall percent BWG in the 28-day feeding study is shown in Table 24.

TABLE 22a. Factorial analysis of p-cresol and BMD on ADG, ADFI and F/G ratio

	P values			MSE
	BMD	PC	BMD x PC	
ADG	NS	0.04	NS	0.0008
ADFI	NS	NS	NS	0.007
F/G	NS	NS	NS	0.022

MSE = Mean square error
NS = Not significant (P<.05)

TABLE 23. Effects of p-cresol and BMD on mean urine volume after 1st, 2nd, 3rd and 4th week of treatment.

	Treatments				
WEEK	NA	A	NA-PC	A-PC	MSE[a]
	ml/24hr				
0	98	138	123	111	3011
1	244	268	204	268	15548
2	389	359	271	276	9560
3	390	400	439	260	6426
4	406	453	344	341	13385
Mean (1-4)	357	369	314	286	13669

[a] Mean square error
Treatment differences were not significant (P<.05)

TABLE 23a. Factorial analysis of p-cresol and BMD on mean urine volume after 1st, 2nd, 3rd and 4th week of treatment.

	P values			MSE
WEEK	BMD	PC	BMD x PC	
1	NS	NS	NS	15548
2	NS	NS	NS	9560
3	0.05	NS	0.03	6426
4	NS	0.05	NS	13385
Mean (1-4)	NS	0.03	NS	13669

MSE = Mean square error
NS = Not significant (P<.05)

TABLE 24. Summary of mean urinary p-cresol and free p-cresol excretions, conjugated PC/free PC ratio, ADFI and overall percent BWG (p-cresol study).

	Treatments			
	NA	A	NA-PC	A-PC
Number of animals	4	4	4	4
PC (mg/24hr, 1-4wk)	83.78 a	84.31 a	865.30 b	960.80 b
PC (mg/kg body weight)	5.21 a	5.28 a	54.16 b	59.66 b
FREE PC (mg/24hr, 1-4wk)	20.93 a	12.22 a	61.67 b	67.72 b
Bound PC/Free PC ratio	9.60 ac	8.93 a	13.25 bc	14.71 b
ADFI, grams	818	850	810	790
Overall % BWG	136.18	137.37	114.65	118.19

a, b, c
Values in the same row having the same or no superscript were not significantly different (P<.05)

EXPERIMENT 3

The mean urinary PHPAA excretion over the 28-day tyrosine feeding study is shown in Table 25. Mean (1-4wk) urinary PHPAA excretion (mg/total urine/24hr) averaged 6.72, 7.50, 197.30 and 117.58 for the NA, A, NA-T and A-T treatment groups, respectively. The excess tyrosine in the diets significantly increased ($P<.05$) the mean (1-4wk) PHPAA excretion in the NA-T and A-T treatment groups when compared to the urinary PHPAA excretion of pigs in the A and A-T treatment groups. The high levels of PHPAA excreted in the NA-T and A-T groups were observed after the first week of treatment, but significant treatment differences did not occur until the end of the 2nd week. BMD reduced the urinary PHPAA excretion of pigs receiving the A-T diet by 40.41% when compared to those that received the NA-T diet, but the treatment difference was not significant. Compared to the NA treatment group, the addition of BMD did not have any significant effect on the urinary PHPAA excretion of pigs receiving the A dietary treatment (7.50 vs 6.72 mg/24hr). The factorial analysis of tyrosine (NA-T + A-T vs NA + A) and BMD (A + A-T vs NA + NA-T) on PHPAA excretion is shown in Table 25a. Addition of 3% tyrosine in the diet significantly increased ($P<0.1$) urinary PHPAA excretion. BMD did not affect PHPAA excretion. Figure 15 shows the urinary PHPAA excretion over time.

TABLE 25. Effects of tyrosine and BMD on mean urinary PHPAA excretion after 1st, 2nd, 3rd and 4th week of treatment.

	Treatments				
WEEK	NA	A	NA-T	A-T	MSE[a]
	mg/total urine/24hr				
0	1.59	2.74	3.93	2.54	4.4
1	6.63 b	3.23 b	196.68 c	38.52 d	14550
2	12.43 b	6.17 b	294.84 c	73.03 c	7032
3	3.49 b	10.15 b	216.00 c	134.00 c	6894
4	4.32 b	10.45 b	81.74 c	224.75 c	8054
Mean (1-4)	6.72 b	7.50 b	197.30 c	117.58 c	10192

[a] Mean square error

[b, c, d] Values in the same row having the same or no superscript were not significantly different (P<.05)

TABLE 25. Effects of tyrosine and BMD on mean urinary PHPAA excretion after 1st, 2nd, 3rd and 4th week of treatment.

	Treatments				
WEEK	NA	A	NA-T	A-T	MSE[a]
	mg/total urine/24hr				
0	1.59	2.74	3.93	2.54	4.4
1	6.63 b	3.23 b	196.68 c	38.52 d	14550
2	12.43 b	6.17 b	294.84 c	73.03 c	7032
3	3.49 b	10.15 b	216.00 c	134.00 c	6894
4	4.32 b	10.45 b	81.74 c	224.75 c	8054
Mean (1-4)	6.72 b	7.50 b	197.30 c	117.58 c	10192

[a] Mean square error

b, c, d Values in the same row having the same or no superscript were not significantly different (P<.05)

TABLE 25a. Factorial analysis of tyrosine and BMD on mean urinary PHPAA excretion after 1st, 2nd, 3rd and 4th week of treatment.

	P values			MSE
WEEK	BMD	T	BMD x T	
1	NS	NS	NS	14550
2	0.01	0.01	0.02	7032
3	NS	0.01	NS	6894
4	NS	0.01	NS	8054
Mean (1-4)	NS	0.01	NS	10192

MSE = Mean square error
NS = Not significant (P<.05)

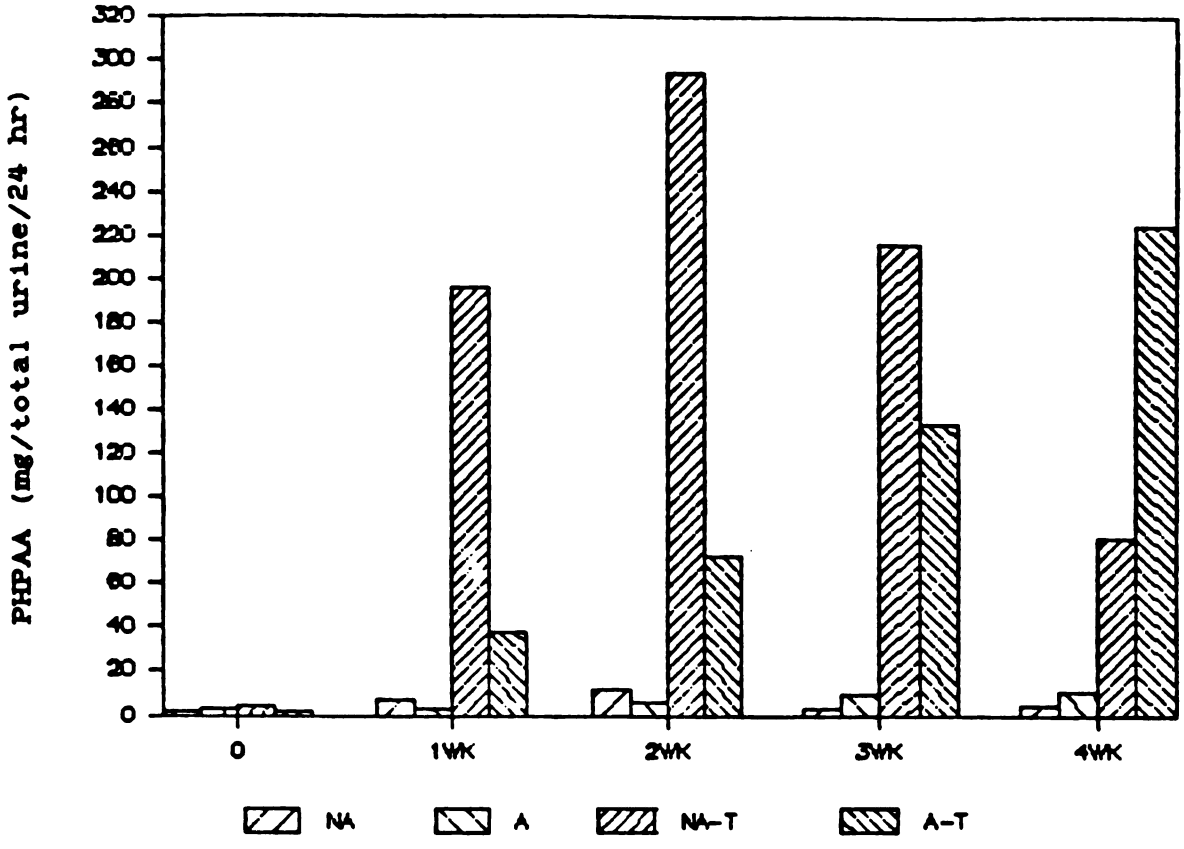


Figure 15. Total urinary PHPAA excretion after 1st, 2nd, 3rd and 4th week of treatment
Tyrosine study

Table 26 shows the effects of tyrosine and BMD on the urinary p-cresol excretion. Mean (1-4wk) urinary p-cresol excretion (mg/total urine/24hr) for the 28-day feeding study averaged 118.94, 93.89, 139.88 and 77.21 for the NA, A, NA-T and A-T treatment groups, respectively. Pigs fed the BMD plus tyrosine diet (A-T) excreted 44% less urinary p-cresol than those receiving the NA-T dietary treatment (77.21 vs 139.88 mg/24hr). The difference between the A-T and NA-T treatment groups was significant ($P<.05$). Pigs receiving the antibiotic-supplemented diet without tyrosine (A) also had lower mean urinary p-cresol excretion than those receiving the NA diets (93.89 vs 118.94 mg/24hr), but this treatment difference was not significant. Compared to the NA treatment group, the excess tyrosine in the diet significantly increased ($P<.05$) the p-cresol excretion in the NA-T treatment group (118.94 vs 139.88 mg/24hr). The opposite effect occurred in the A-T treatment group, where the addition of tyrosine in the diet (A-T), decreased the urinary excretion of p-cresol when compared to that of the A treatment group (77.21 vs 93.89 mg/24hr). The urinary excretion of p-cresol increased with time regardless of treatment. When calculated from the 1st to 4th week of treatment, the excretion of p-cresol increased by 140% and 156% under the NA and NA-T treatment groups and by 6% and 55% under the A and A-T treatment groups, respectively. The factorial analysis of tyrosine and BMD is shown in Table 26a. BMD

TABLE 26. Effects of tyrosine and BMD on mean urinary p-cresol excretion after 1st, 2nd, 3rd and 4th week of treatment.

	Treatments				
WEEK	NA	A	NA-T	A-T	MSE[a]
	mg/total urine/24hr				
0	91.40	69.44	86.85	63.65	835
1	67.90	76.19	82.82	82.05	2691
2	100.33[b]	130.52[b]	120.36[b]	37.42[c]	1418
3	144.89	88.27	144.43	62.55	2285
4	162.63	80.58	211.92	126.83	3996
Mean (1-4)	118.94[bc]	93.89[bd]	139.88[c]	77.21[d]	3446

[a] Mean square error

[b, c, d] Values in the same row having the same or no superscript were not significantly different (P<.05)

TABLE 26a. Factorial analysis of tyrosine and BMD on mean urinary p-cresol excretion after 1st, 2nd, 3rd and 4th week of treatment.

	P values			MSE
WEEK	BMD	T	BMD x T	
1	NS	NS	NS	2691
2	NS	NS	0.01	1418
3	0.01	NS	NS	2285
4	0.02	NS	NS	3996
Mean (1-4)	0.01	NS	NS	3446

MSE = Mean square error
NS = Not significant (P<.05)

significantly reduced (P<.05) urinary p-cresol excretion. Addition of 3% tyrosine did not have a significant effect on p-cresol excretion. Figure 16 shows the urinary excretion of total p-cresol over the 28-day feeding study.

The effects of tyrosine and BMD on urinary p-cresol excretion per kg B.W. is shown in Table 27. Mean p-cresol excretion per unit B.W. averaged 7.14, 6.26, 8.04 and 4.82 for the NA, A, NA-T and A-T treatment groups respectively. BMD significantly reduced the urinary p-cresol excretion per kg B.W. of pigs in the A-T treatment group compared to those in the NA-T treatment group. The urinary excretion of p-cresol per kg B.W. of pigs receiving the A dietary treatment was lower than those in the NA treatment group however, the difference was not significant. The percent change in urinary p-cresol excretion per kg B.W., when calculated from the 1st to the 4th collection period averaged, 8, -49, 24 and -30% for the NA, A, NA-PC and A-PC treatment groups, respectively. Unlike the data in Table 26 which tended to show increase p-cresol excretion (mg/total urine/24 hr) with time, the urinary excretion of p-cresol when expressed on per kg B.W. basis appeared to level off over time in the NA and A treatment groups. There was a slight increase in the urinary p-cresol excretion per kg B.W. over time in the tyrosine-supplemented treatment groups (NA-T and A-T). The factorial analysis is shown in Table 27a. BMD significantly reduced (P<.01) p-cresol excretion per kg B.W. but the

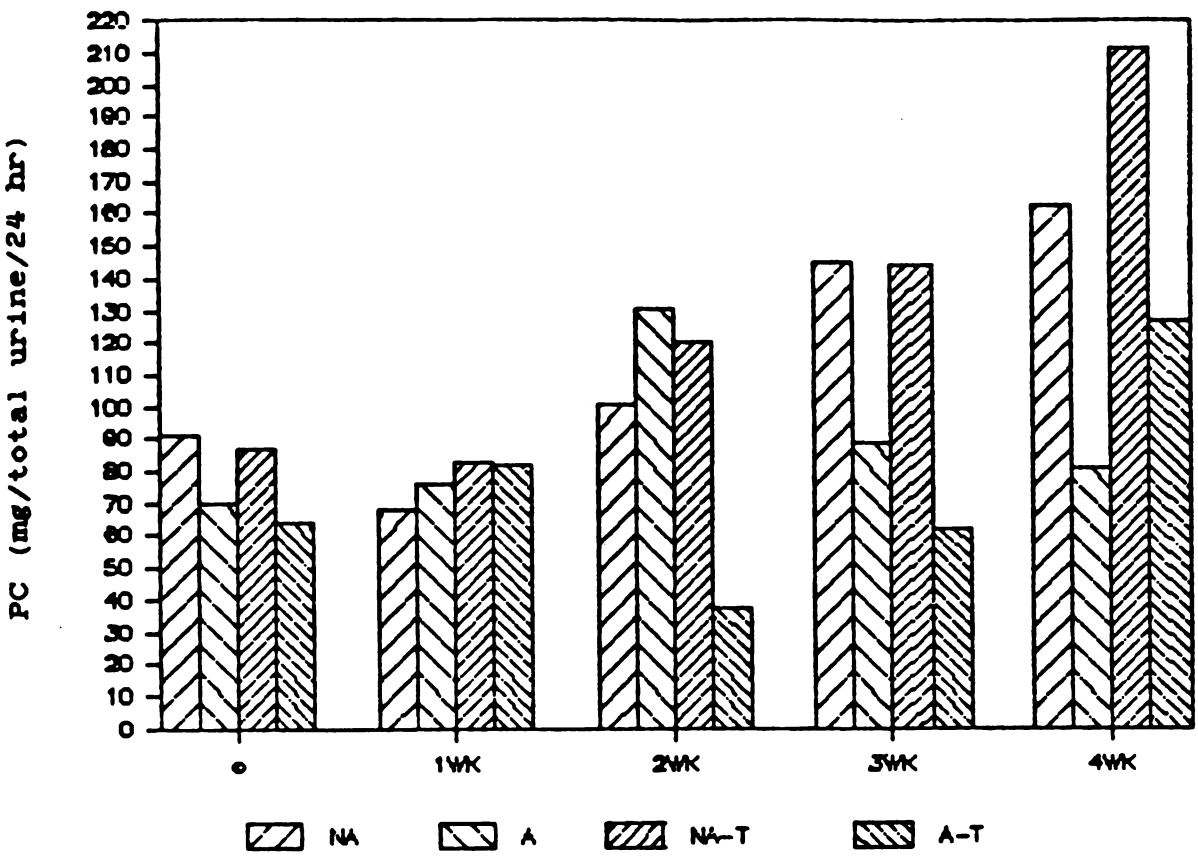


Figure 16. Total urinary p-cresol excretion after 1st, 2nd, 3rd and 4th week of treatment Tyrosine study

TABLE 27. Effects of tyrosine and BMD on urinary p-cresol excretion per kg body weight after 1st, 2nd, 3rd and 4th week of treatment.

	Treatments				
WEEK	NA	A	NA-T	A-T	MSE[a]
	mg/kg BW				
0	10.14	7.94	9.03	6.56	8.42
1	6.44 b	6.87 c	7.26 bd	7.87 e	23.61
2	6.78 b	9.75 c	7.69 bd	2.69 ce	4.38
3	8.45 b	4.91 c	8.21 b	3.20 bc	6.01
4	6.92 bc	3.53 bd	8.99 c	5.54 d	5.15
Mean (1-4)	7.14	6.26	8.04	4.82	10.68

[a] Mean square error

b, c, d, e Values in the same row having the same or no superscript were not significantly different (P<.05)

TABLE 27a. Factorial analysis of tyrosine and BMD on urinary p-cresol excretion per kg body weight after 1st, 2nd, 3rd and 4th week of treatment.

	P values			MSE
WEEK	BMD	T	BMD x T	
1	NS	NS	NS	23.61
2	NS	0.01	0.01	4.37
3	0.01	NS	NS	6.01
4	0.01	NS	NS	5.11
Mean (1-4)	0.01	NS	NS	10.68

MSE = Mean square error
NS = Not significant (P<.05)

effect of 3% tyrosine in the diet was not significant.

Mean (1-4wk) urinary free p-cresol excretion (mg/total urine/24hr) averaged 9.73, 11.50, 9.63 and 5.25 for the NA, A, NA-T and A-T treatment groups, respectively (Table 28). No treatment differences were significant although the urinary free p-cresol excretion tended to increase in all groups for the duration of the study. The factorial analysis (Table 28a) showed no significant effects due to the addition of BMD or 3% tyrosine.

Table 29 shows the effects of tyrosine and BMD on conjugated PC/free PC ratio over the 28-day feeding study. The conjugated PC/free PC ratio for the NA, A, NA-T and A-T treatment groups averaged 15.65, 19.65, 16.26 and 15.25 (1-4wk), respectively. None of the treatment differences was significant. During the four week feeding study, the conjugated PC/free PC ratio decreased by -53.3, -47.63, -39.59 and -27.87% for the NA, A, NA-T and A-T treatment groups, respectively (1-4wk). The factorial analysis (Table 29a) also showed no significant effects due to BMD or 3% tyrosine.

The effects of tyrosine and BMD on cumulative percent BWG after the 1st, 2nd, 3rd and 4th week of treatment is shown in Table 30. Percent BWG at the end of the study were 149.61, 148.74, 144.57 and 143.93 for the NA, A, NA-T and A-T treatment groups, respectively. There was a decrease in

TABLE 28. Effects of tyrosine and BMD on mean urinary free p-cresol excretion after 1st, 2nd, 3rd and 4th week of treatment.

	Treatments				
WEEK	NA	A	NA-T	A-T	MSE[a]
	mg/total urine/24hr				
0	4.15	3.08	3.44	2.53	1.9
1	6.15	4.24	4.96	3.55	13.3
2	4.37	6.82	5.28	1.89	11.1
	b	c	b	c	
3	7.69	3.94	8.58	4.27	6.2
4	20.72	30.99	19.70	11.29	654.1
Mean (1-4)	9.73	11.50	9.63	5.25	195.2

[a] Mean square error

[b, c] Values in the same row having the same or no superscript were not significantly different (P<.05)

TABLE 28a. Factorial analysis of tyrosine and BMD on mean urinary free p-cresol excretion after 1st, 2nd, 3rd and 4th week of treatment.

	P values			MSE
WEEK	BMD	T	BMD x T	
1	NS	NS	NS	13.3
2	NS	NS	NS	11.1
3	0.01	NS	NS	6.2
4	NS	NS	NS	654.1
Mean (1-4)	NS	NS	NS	195.2

MSE = Mean square error
NS = Not significant (P<.05)

TABLE 29. Effects of tyrosine and BMD on conjugated PC/free PC ratio after 1st, 2nd, 3rd and 4th week of treatment.

	Treatments				
WEEK	NA	A	NA-T	A-T	MSE[a]
	ratio				
0	23.24	24.48	26.07	26.03	52.2
1	15.44	23.39	16.29	20.85	95.6
2	21.52	26.46	22.02	13.05	89.7
3	18.44	19.00	16.92	12.06	67.2
4	7.21	12.25	9.84	15.04	49.3
Mean (1-4)	15.65	19.65	16.26	15.25	84.5

[a] Mean square error.
Treatment differences were not significant (P<.05)

TABLE 29a. Factorial analysis of tyrosine and BMD on conjugated PC/free PC ratio after 1st, 2nd, 3rd and 4th week of treatment.

	P values			MSE
WEEK	BMD	T	BMD x T	
1	NS	NS	NS	95.6
2	NS	NS	NS	89.7
3	NS	NS	NS	68.2
4	NS	NS	NS	49.3
Mean (1-4)	NS	NS	NS	84.5

MSE = Mean square error
NS = Not significant (P<.05)

TABLE 30. Effects of tyrosine and BMD on cumulative % BWG after 1st, 2nd, 3rd and 4th week of treatment.

	Treatments				
WEEK	NA	A	NA-T	A-T	MSE[a]
	cumulative % BWG				
1	16.89	21.17	19.95	18.48	45.1
2	51.86	51.70	64.95	66.13	198.0
3	88.62	90.69	89.93	85.44	291.9
4	149.61	148.74	144.57	143.93	794.2

[a] Mean square error
Treatment differences were not significant (P<.05)

the percent BWG by the 4th week in the tyrosine fed groups (NA-T and A-T) when compared to the NA and A treatment groups (144.57 and 143.93% vs 149.61 and 148.74%), but these differences were not significant. The use of BMD in this study did not improve the overall percent BWG of pigs in the A and A-T groups. The factorial analysis is shown in Table 30a. BMD or 3% tyrosine did not have significant effects on cumulative percent BWG. The cumulative percent BWG over time is shown in Figure 17.

The weekly percent BWG of pigs fed the tyrosine and antibiotic supplemented-diets is shown in Table 31. Excess tyrosine in the diets (NA-T and A-T) did not affect the weekly percent BWG of pigs during the first two weeks of treatment. However, the addition of 3% tyrosine significantly reduced the weekly percent BWG during the 3rd week of treatment. Pigs receiving the NA-T and A-T dietary treatments had 15.44% and 11.68% weekly BWG while those under the NA and A treatment diets had 24.24% and 25.65% respectively. By the 3-4wk of treatment, pigs in the NA-T and A-T treatment groups appeared to have recovered and had the same weekly percent BWG as those pigs in the NA and A treatment groups. BMD was not effective in improving the percent weekly BWG at any point of the study. The factorial analysis in Table 31a also showed no significant effect on weekly percent BWG with the addition of 3% tyrosine or BMD. Figure 18 shows the effect of tyrosine and BMD on the weekly

TABLE 30a. Factorial analysis of tyrosine and BMD on cumulative percent BWG after 1st, 2nd, 3rd and 4th week of treatment.

	P values			MSE
WEEK	BMD	T	BMD x T	
1	NS	NS	NS	45.1
2	NS	NS	NS	198.0
3	NS	NS	NS	291.9
4	NS	NS	NS	794.2

MSE = Mean square error
NS = Not significant (P<.05)

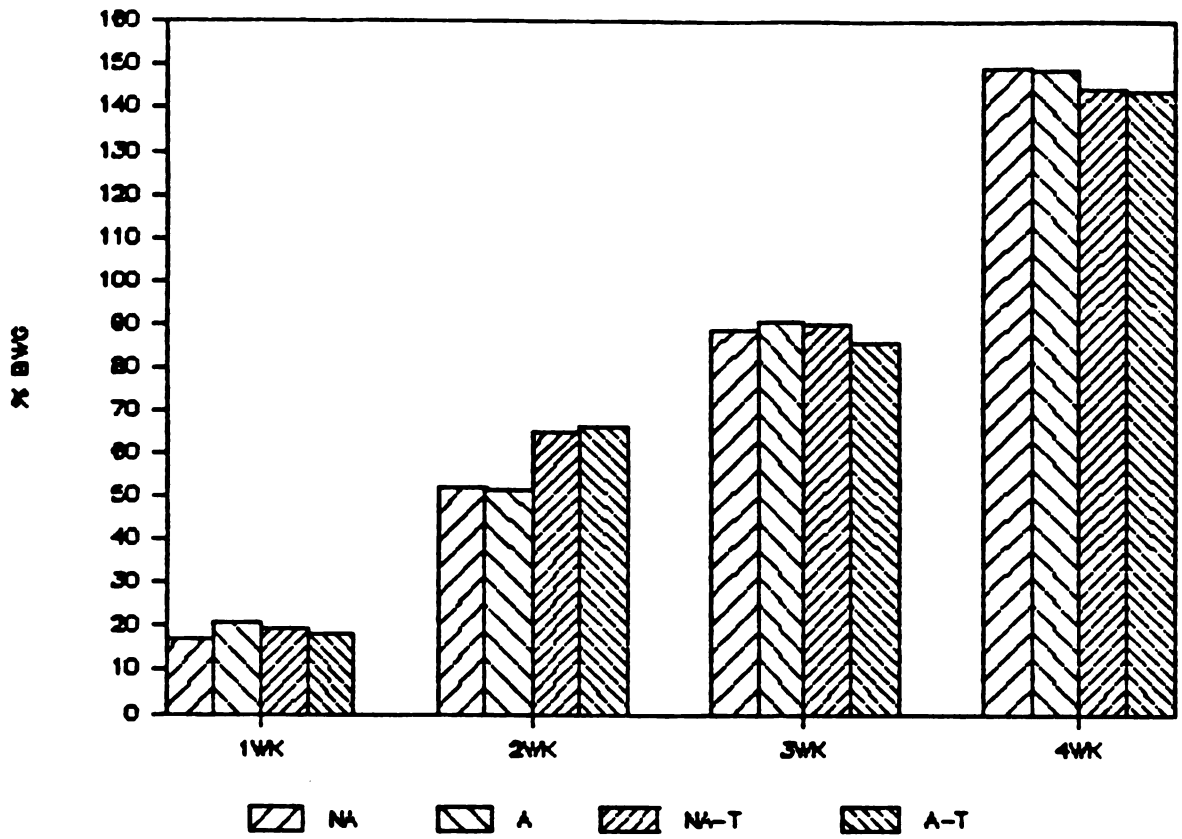


Figure 17. Cumulative percent body weight gain Tyrosine study

TABLE 31. Effects of tyrosine and BMD on weekly percent body weight gain after 1st, 2nd, 3rd and 4th week of treatment.

	Treatments				
WEEK	NA	A	NA-T	A-T	MSE[a]
	weekly % BWG				
1	16.89	21.17	19.95	18.48	45.05
2	29.99	25.05	37.44	40.54	86.81
	b	b	c	c	
3	24.24	25.65	15.44	11.68	35.75
4	32.08	30.04	29.23	31.58	69.78
Mean (1-4)	25.79	25.46	25.51	25.57	112.15

[a] Mean square error

[b, c] Values in the same row having the same or no superscript were not significantly different

TABLE 31a. Factorial analysis of tyrosine and BMD on weekly percent body weight gain after 1st, 2nd, 3rd and 4th week of treatment.

	P values			MSE
WEEK	BMD	T	BMD x T	
1	NS	NS	NS	45.05
2	NS	0.02	NS	86.81
3	NS	0.01	NS	35.75
4	NS	NS	NS	69.78
Mean (1-4)	NS	NS	NS	112.15

MSE = Mean square error
NS = Not significant (P<.05)

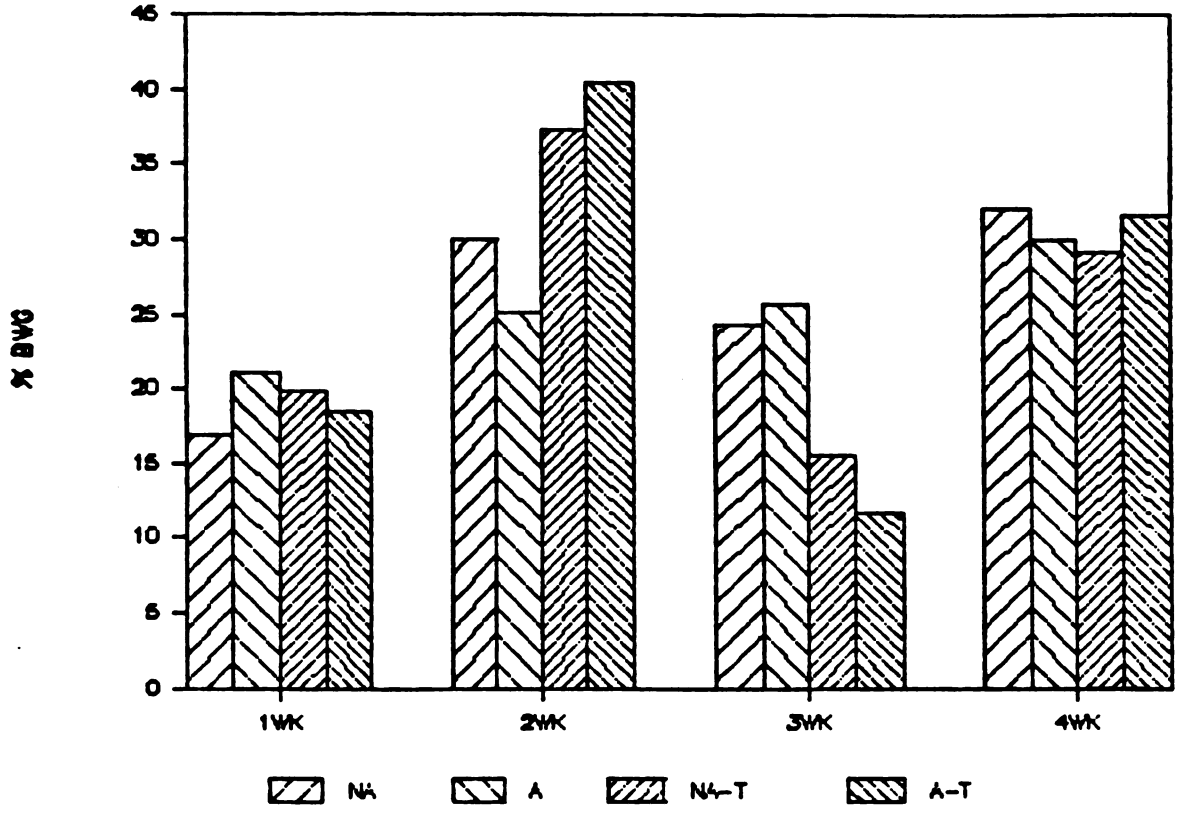


Figure 18. Weekly percent body weight gain
Tyrosine study

percent BWG.

Table 32 shows the effects of tyrosine and BMD on other performance parameters during the 28-day feeding study. No significant differences were observed between treatments for either ADFI, ADG or F/G ratios. In contrast to the results obtained in the PHPAA study, the pigs on the BMD-supplemented diets (A and A-T) had lower ADFI and ADG than those receiving the NA and NA-T diets. The ADFI of pigs averaged 939 and 920 grams in the A and A-T treatment groups while those in the NA and NA-T treatment groups had 978 and 996 grams, respectively. Pigs receiving the NA and NA-T dietary treatments had 448 and 465 grams ADG compared to the pigs in the A and A-T treatment groups which had 459 and 465 grams, respectively.

Table 33 shows the effects of tyrosine and BMD on urinary PHPLA excretion. Mean urinary PHPLA excretion (mg/total urine/24hr) after 1st, 2nd, 3rd and 4th week of treatment were 16.50, 8.92, 110.06 and 315.49 mg for the NA, A, NA-T and A-T treatment groups, respectively. Excess tyrosine in the diets (NA-T and A-T) significantly increased ($P<.05$) the mean (1-4wk) urinary PHPLA excretion compared to those in the NA and A treatment groups. The difference between the NA-T and A-T treatment groups was also significant ($P<.05$) with the A-T group having 187% more PHPLA excretion than those in the NA-T treatment group (315.49 vs

TABLE 32. Performance of weanling pigs receiving tyrosine and BMD supplemented diets.

	Treatments				
	NA	A	NA-T	A-T	MSE[a]
Number of pigs	4	4	4	4	
Initial weight, kg	9.33	9.18	9.59	9.47	
Final weight, kg	22.60	22.18	23.38	22.88	
Ave daily feed intake, g	978	939	996	920	24801
Ave daily gain, g	459	448	475	465	8639
Overall % BWG	149.61	148.74	144.57	143.93	794
Feed/gain ratio	2.14	2.10	2.11	2.01	0.03

[a] Mean error square
Treatment differences were not significant (P<.05)

TABLE 33. Effects of tyrosine and BMD on mean urinary PHPLA excretion after 1st, 2nd, 3rd and 4th week of treatment.

	Treatments				
WEEK	NA	A	NA-T	A-T	MSE[a]
	mg/total urine/24hr				
0	3.46	7.39	17.22	16.37	63
1	34.65	5.78	104.39	102.28	7699
	b	b	c	c	
2	8.44	5.30	121.39	125.72	1487
	b	b	c	d	
3	14.50	16.21	198.80	666.41	29586
4	8.40	8.40	15.67	367.55	68739
	b	b	c	d	
Mean (1-4)	16.50	8.92	110.06	315.49	36489

[a] Mean square error

b, c, d Values in the same row having the same or no superscript were not significantly different (P<.05)

110.06 mg/24hr). Urinary excretion of PHPLA between the NA and A treatment groups were not significant. Elevated levels of PHPLA in the urine were noticed after the 1st week of treatment, but significant treatment differences was not observed until the 2nd week of the study. The factorial analysis is shown in Table 33a. The addition of 3% tyrosine and BMD significantly increased PHPLA excretion.

The effects of excess tyrosine and the addition of BMD on PHPAA/PHPLA ratio over the 28-day feeding study are shown in Table 34. Mean PHPAA/PHPLA ratio (1-4wk) averaged 0.77, 1.02, 3.74 and 2.02 for the NA, A, NA-T and A-T treatment groups, respectively. Excess tyrosine in the diet significantly increased the PHPAA/PHPLA ratio ($P<.05$) in the NA-T treatment group compared to pigs receiving the NA diets (3.74 vs 0.77). Differences in PHPAA/PHPLA ratio between NA-T and A-T treatment groups were not significant although the ratio of pigs under the A-T dietary treatment was 46% lower than those receiving the NA-T diets. Differences between the NA, A and A-T treatment groups were not significant. The factorial analysis is shown in Table 34a. Addition of 3% tyrosine significantly increased PHPAA/PHPLA ratio but BMD did not affect PHPAA/PHPLA ratio.

The mean urine volume of pigs receiving tyrosine and BMD is shown in Table 35. Mean urine volume (ml/24hr) averaged 692, 493, 557 and 558 for the NA, A, NA-T and A-T

TABLE 33a. Factorial analysis of tyrosine and BMD on mean urinary PHPLA excretion after 1st, 2nd, 3rd and 4th week of treatment.

	P values			MSE
WEEK	BMD	T	BMD x T	
1	NS	NS	NS	7699
2	NS	0.01	NS	1487
3	0.01	0.01	0.01	29586
4	NS	0.01	0.02	68739
Mean (1-4)	0.04	0.01	0.02	36489

MSE = Mean square error
NS = Not significant (P<.05)

TABLE 34. Effects of tyrosine and BMD on PHPAA/PHPLA ratio after 1st, 2nd, 3rd and 4th week of treatment.

	Treatments				
WEEK	NA	A	NA-T	A-T	MSE[a]
	ratio				
1	0.58	0.61	4.31	2.05	9.70
2	1.54	1.22	3.03	0.68	1.26
3	0.32	0.70	1.24	0.21	0.28
4	0.62	1.53	6.38	5.16	24.44
Mean (1-4)	0.77[b]	1.02[b]	3.74[c]	2.02[bc]	9.16

[a] Mean error square

[b, c] Values in the same row having the same or no superscript were not significantly different (P<.05)

TABLE 34a. Factorial analysis of tyrosine and BMD on PHPAA/PHPLA ratio after 1st, 2nd, 3rd and 4th week of treatment.

	P values			MSE
WEEK	BMD	T	BMD x T	
1	NS	NS	NS	9.70
2	0.03	NS	NS	1.26
3	NS	NS	0.02	0.28
4	NS	NS	NS	24.44
Mean (1-4)	NS	0.01	NS	9.16

MSE = Mean square error
NS = Not significant (P<.05)

TABLE 35. Effects of tyrosine and BMD on mean urine volume.

	Treatments				
WEEK	NA	A	NA-T	A-T	MSE[a]
	ml/24hr				
0	251	86	231	161	4904
1	378	144	238	256	49403
2	753	468	508	506	51820
3	756	695	731	615	69915
4	880	665	753	854	77961
Mean (1-4)	692	493	557	558	95995

[a] Mean square error
Treatment differences were not significant (P<.05)

treatment groups, respectively. Although treatment differences were not significant, urine volume increased with time regardless of treatment. Urine volume increased (1-4wk) by 133% and 242% under the NA and A treatments and by 216% and 233% under the NA-T and A-T treatment groups, respectively. The percent increase in urine volume was higher in the BMD-supplemented groups (A and A-T) than the percent increase in the NA and NA-T treatment groups. However, these differences were not significant. The factorial analysis (Table 35a) also showed no significant effect on urine volume due to the addition of BMD or 3% tyrosine.

The effects of excess tyrosine and BMD on urinary PHPAA, PHPLA, p-cresol, free p-cresol excretions and on conjugated PC/free PC and PHPAA/PHPLA ratios, ADFI and on overall percent BWG are summarized in Table 36.

Table 37 shows the correlation between percent body weight gain and urinary excretion of p-cresol. The correlation probability is also shown in this table. When the NA and A treatment groups were combined, the correlation were -0.7635, -0.1413 and -0.7431 for the PHPAA, p-cresol and tyrosine feeding studies, respectively. When data from the three studies were pooled, the combined NA and A treatment groups (24 pigs) had a -0.5038 correlation. Combining all treatment groups across the three feeding studies (48 pigs), the correlation was -0.3432 with a probability of 0.0169.

TABLE 35a. Factorial analysis of tyrosine and BMD on mean urine volume after 1st, 2nd, 3rd and 4th week of treatment.

	P values			MSE
WEEK	BMD	T	BMD x T	
1	NS	NS	NS	49403
2	NS	NS	NS	51820
3	NS	NS	NS	69915
4	NS	NS	NS	77961
Mean (1-4)	NS	NS	NS	95994

MSE = Mean square error
NS = Not significant (P<.05)

TABLE 36. Summary of of mean urinary PHPAA, PHPLA, p-cresol, free p-cresol excretions, conjugated PC/free PC ratio, PHPAA/PHPLA ratio, ADFI and overall % BWG (Tyrosine study).

	Treatments			
	NA	A	NA-T	A-T
Number of animals	4	4	4	4
PHPAA (mg/24hr, 1-4wk)	6.72 [a]	7.50 [a]	197.30 [b]	117.58 [b]
PHPLA (mg/24hr, 1-4wk)	16.50 [a]	8.92 [a]	110.06 [b]	315.49 [c]
PHPAA/PHPLA ratio	0.77 [a]	1.02 [a]	3.74 [b]	2.02 [ab]
PC (mg/24hr, 1-4wk)	118.94 [ab]	93.89 [ac]	139.88 [b]	77.21 [c]
PC (mg/kg body weight)	7.14 [ab]	6.26 [ac]	8.04 [b]	4.82 [c]
FREE PC (mg/24hr, 1-4wk)	9.7	11.50	9.63	5.25
Bound PC/Free PC ratio	15.65	19.65	16.26	15.25
ADFI, grams	978	939	996	920
Overall % BWG	149.61	148.74	144.57	143.93

a, b, c
Values in the same row having the same or no superscript were not significantly different (P<05)

Table 37. Correlation of percent body wight gain (1-4 wk) on urinary p-cresol excretion (1-4 wk).

Treatments	No. of pigs	Correlation	Probability
PHPAA study:			
NA + A	8	-0.7635	0.0275
NA-PHPAA + A-PHPAA	8	+0.3518	0.3928
All treatments	16	-0.2645	0.3222
P-cresol study:			
NA + A	8	-0.1413	0.7386
NA-PC + A-PC	8	-0.4558	0.2564
All treatments	16	-0.4072	0.1175
Tyrosine study:			
NA + A	8	-0.7431	0.0346
NA-TY + A-TY	8	-0.3278	0.4280
All treatments	16	-0.5172	0.0402
3 studies pooled:			
NA only	12	-0.5671	0.0545
A only	12	-0.5496	0.0641
NA + A	24	-0.5038	0.0121
NA-TRT + A-TRT	24	-0.4198	0.0411
All treatments	48	-0.3432	0.0169

DISCUSSION

EXPERIMENT 1

The results of the PHPAA feeding study indicate that the addition of 0.75% PHPAA to the diet significantly increased the urinary excretion of PHPAA, but the excess PHPAA did not adversely affect the performance of weanling pigs. No toxic signs or external lesions were observed in any of the pigs receiving 0.75% PHPAA. The overall percent BWG of pigs receiving the NA-PHPAA and A-PHPAA diets were 116.60% and 131.10%, respectively. These figures were comparable to the percent BWG of pigs under the NA and A dietary treatments which averaged 114.90% and 130.20%, respectively. BMD supplementation significantly increased the urinary excretion of PHPAA ($P<.05$). Previous studies have also indicated increased urinary PHPAA excretion with dietary antibiotic supplementation (Shoemaker et al. 1980; Rogers et al. 1955). The increase in urinary PHPAA excretion could be due to the inhibitory effect of BMD on the bacteria that decarboxylates PHPAA to p-cresol, thus causing a buildup of the PHPAA metabolite in the process. Yokoyama et al. (1985) reported that Bacitracin (BMD) was one of the more effective antibiotics in inhibiting the growth of the p-cresol producing bacteria.

Average daily feed intake (ADFI) tended to increase with the addition of BMD. The ADFI for the A, A-PHPAA, NA and NA-PHPAA treatment groups averaged 1025, 1033, 978 and 983 g, respectively. Baker (1982) indicated that the growth promoting effect of antibiotics generally results from an increase in voluntary feed intake. The high level of feed intake increased the available PHPAA, and this could have allowed the intestinal bacteria to decarboxylate more PHPAA. However, the addition of BMD in the diets significantly decreased the intestinal production of p-cresol in both the A and A-PHPAA treatment groups ($P<.05$). The level of urinary p-cresol excretion under the A dietary treatment was significantly lower than those in the A-PHPAA treatment group ($P<.05$) suggesting that the excess PHPAA might have either increased the production of p-cresol or that it may have affected the inhibitory effect of the BMD on the p-cresol producing bacteria. The high feed intake of pigs in the A-PHPAA treatment group could have also caused the elevated levels of urinary p-cresol excretion. Because of the increased feed intake, more PHPAA became available in the lower gut for conversion to p-cresol.

Compared to the NA treatment group, the addition of 0.75% PHPAA in the diet did not increase the bacterial production of p-cresol in pigs receiving the NA-PHPAA treatment (193.55 vs 192.91 mg/24hr). This suggests that there was enough available PHPAA in the NA group that was

decarboxylated to p-cresol. The level of p-cresol excretion in the NA treatment group shows the relatively high level of p-cresol production in weanling pigs. It is known that there is active bacterial production of p-cresol in the intestinal tract of weanling pigs. The average amount (1-4wk) of p-cresol excreted in the urine of weanling pigs receiving the NA diet (Table 5) is about three times the amount detected in normal adult humans (Bone et al., 1976; Dirmikis and Darbre, 1974). The amount of urinary p-cresol in weanling pigs nearly approximates the level found in children with certain intestinal disorders, such as celiac disease (Duran et al., 1973), or people consuming high protein diets (Cummings et al. 1979).

The PHPAA study also suggests that the intestinal production of p-cresol could be related to the growth depressing effect in weanling pigs. The NA and NA-PHPAA treatment groups, which had the highest urinary excretion of p-cresol (193.55 and 192.91 mg/24hr), also had the lowest overall percent BWG (114.90 and 116.60%) among the treatment groups. On the other hand, the BMD supplemented groups (A and A-PHPAA) had 97.32 and 143.80 mg/24hr of urinary p-cresol excretion while overall BWG averaged 130.20% and 131.10%, respectively. It appears from the study that the supplementation of BMD stimulated the growth of weanling pigs by reducing the bacterial production of p-cresol.

The total urinary p-cresol excretion (mg/total

urine/24hr) tended to increase as the animal got older and heavier during the 28-day feeding study. However, when expressed on the basis of unit body size (Table 6), the urinary p-cresol excretion was higher at the beginning of the study compared to the last two weeks of treatment. The data suggest that younger pigs have higher actual exposure to p-cresol than older pigs, although older pigs show high total urinary p-cresol excretion. When calculated from the first to the fourth week of treatment, the level of urinary p-cresol excretion/kg BW actually decreased in all treatment groups. The BMD used in the PHPAA study which tended to promote growth, significantly decreased the urinary p-cresol excretion/kg BW ($P<.05$). Yokoyama et al. (1982) reported that when the size factor was removed, by expressing p-cresol excretion on the basis of metabolic body size, a relatively constant level of exposure could be demonstrated.

Total urinary p-cresol excretion might not be a good indicator of intestinal p-cresol production in weanling pigs because without considering the size factor, the measurement does not indicate actual exposure. Heavier pigs which excrete high levels of total urinary p-cresol might not show signs of growth depression because their actual exposure to p-cresol relative to body size is low.

Increased feed intakes usually result in higher bacterial production of p-cresol because more unabsorbed tyrosine and more PHPAA are converted to p-cresol in the lower gut.

Dietary antibiotics either inhibit the degradation of tyrosine to PHPAA or prevent the decarboxylation of PHPAA to p-cresol resulting in low overall production of the metabolite. It appears from the study that the effect of dietary antibiotics is twofold. It tends to increase feed intake and it decreases the bacterial production of p-cresol. By increasing feed intake, dietary antibiotics increase weight gain and in the process lower the p-cresol exposure per unit body size. Dietary antibiotics exert its effect by reducing the intestinal production of p-cresol.

The conjugated p-cresol/free p-cresol ratios (Table 8) show the extent of detoxification by weanling pigs. The ratios are higher in treatment groups which were not supplemented with antibiotics (NA and NA-PHPAA). Based on the excreted form of p-cresol, the pig uses the uridine diphosphate-glucuronic acid derived from glucose in the uronic acid pathway to form the glucuronide conjugates of p-cresol (Spoelstra, 1978; Kao et al., 1979). In treatment groups which were not supplemented with antibiotics, the pigs apparently used more glucose and ATP for the detoxification and excretion of p-cresol. The reduction in intestinal production of p-cresol by dietary antibiotics may have saved the animal glucose and ATP which are necessary for the detoxification and excretion of the toxic bacterial metabolite. Vervaeke et al. (1979) have recently hypothesized that dietary antibiotics spare glucose by decreasing the bacterial

production of organic acids in the intestinal tract resulting in more net energy available for growth.

When data from the NA and A treatment groups were combined, a significant negative correlation (r= -0.7635) was obtained when the percent BWG of each pig was regressed on urinary p-cresol excretion. This is comparable to the negative correlation (r= -0.73) found by Yokoyama et al. (1982) when they gave 110 ppm chlortetracycline, 110 ppm sulfamethazine and 55 ppm penicillin (CSP), 40 ppm lincomycin hydrochloride (LI) or no antibiotic (NA) dietary treatments to weanling pigs.

However when the NA-PHPAA and A-PHPAA treatment groups were combined, the correlation of percent BWG on urinary p-cresol excretion was positive (r= +0.3518) although the correlation was not significant. There is nothing to compare these data with because no study was done in this area before. The positive correlation is unexpected and is in contrast to the consistent negative correlation observed between the percent BWG and the urinary p-cresol excretion. The positive correlation only occurred in the PHPAA supplemented treatment groups (NA-PHPAA and A-PHPAA).

Results seem to indicate that the effect of excess PHPAA on weanling pigs is different from that of p-cresol. When correlation was done separately on the NA-PHPAA and A-PHPAA treatment groups, r= +0.7081 and r= +0.6397 were

obtained, respectively. This was startling because the A-PHPAA treatment group had 131.10% overall BWG and 143.8 mg urinary p-cresol excretion while pigs receiving the NA-PHPAA dietary treatment had 116.60% BWG and 192.91 mg p-cresol excretion.

The effect of PHPAA on growth is not known. However, PHPAA being an acidic compound might have lowered the pH in the intestinal tract and it might have altered the microbial metabolism in the process. The effect of p-cresol might have been masked or overwhelmed by the high concentration of PHPAA. It is possible that the change in microbial metabolism caused by the excess PHPAA might have prevented the bacterial degradation of primary bile acids. The hydrolytic products of bile acids are known toxic compounds (Palmer, 1972, 1976), and they change the absorption of lipids and calcium, micelle formation and intestinal structure (Visek, 1978). The low intestinal pH caused by excess PHPAA may have decreased the microbial deconjugation and dehydroxylation of primary bile acids into toxic compounds. However, this possible effect of excess PHPAA on bile acids does not explain why the NA-PHPAA treatment group had 11% less overall BWG and excreted 34% more urinary p-cresol than the A-PHPAA treatment group. Since both NA-PHPAA and A-PHPAA were supplemented with 0.75% PHPAA, the improvement in BWG and the reduction of p-cresol production in the A-PHPAA treatment group could only be attributed to the addition of

BMD.

The urinary PHPAA excretion of pigs receiving the A-PHPAA dietary treatment was 25% higher than those under the NA-PHPAA treatment group (279.57 vs 222.37 mg). The elevated levels of PHPAA in the A-PHPAA treatment group could be explained by the inhibitory effect of BMD on the bacteria that converts PHPAA to p-cresol or it could be due to the high feed intake of pigs in the A-PHPAA treatment group.

EXPERIMENT 2

The results of the p-cresol study indicate that growth is depressed when weanling pigs are fed diets supplemented with 0.75% p-cresol for 28 days. Pigs receiving diets supplemented with p-cresol (NA-PC and A-PC combined) had 15% lower overall BWG than those receiving the diets with no p-cresol (116.4% vs 136.8%). Significant reduction in weekly percent BWG occurred during the 4th week of treatment, suggesting that weanling pigs tolerated the addition of 0.75% p-cresol for about three weeks before BWG is affected. P-cresol is a known toxic compound (Deichmann et al. 1944; Merck, 1976) however, the mechanism by which p-cresol adversely affects growth is not known.

The inability of the weanling pig to cope with the high level of exposure to p-cresol may result in some metabolic

stress which can be manifested in growth depression. The kind and form of metabolic stress is not known, but it is possible that the growth retardation could be initiated by a systemic pneumotoxic effect of p-cresol. Compounds like BHT, which is related to p-cresol in chemical structure, have been reported to cause reproducible lung injuries in rats (Marino and Mitchell, 1972; Hirai et al., 1977). Intraruminal and intravenous administration of skatole, a bacterial metabolite of tryptophan degradation, have produced lung lesions in cattle (Carlson et al., 1975). Preliminary studies at Michigan State University (MSU) showed that oral administration of 2-4 grams of p-cresol to weanling pigs weighing 9 kg on the average resulted in neurological symptoms and pulmonary distress. The necropsied pigs showed extensive lung damage, including edema and hemorrhages. There is an on-going gnotobiotic study at MSU, where treated pigs were inoculated with the bacteria that produces p-cresol and provided with the PHPAA substrate. Results of the study are still being analyzed.

There were suggestions that p-cresol exerts its growth-depressing effect by causing pneumotoxicity in weanling pigs. If the growth retarding effect is the only clinical manifestation of pneumotoxicity, then it is possible that the problem caused by the pig's high exposure to p-cresol could escape detection. Dietary antibiotic supplementation could alleviate the pneumotoxic effect by decreasing the

exposure to p-cresol. This could result in the reduction of metabolic stress that eventually affects growth.

The physiological significance of p-cresol to other intestinal microorganisms is becoming evident because the metabolite is known to have bactericidal and fungicidal properties (Driezen and Spies, 1948; Hegna, 1977). In this regard, p-cresol could be affecting the character of the intestinal microbial population. The mechanism by which p-cresol exerts its bactericidal effect is not known. However, skatole a phenolic compound produced by the bacterial degradation of tryptophan, is reported to have lipophilic properties that disintegrate membrane structures and causes cell immobility (Eadie and Oxford, 1954; Hogg and Elliot, 1951). It is possible that the addition of p-cresol in the diet and the inability of BMD to inhibit its endogenous production may have provided greater protection to the p-cresol producing bacteria and may have consequently improved its standing in the microbial population. A shift in intestinal bacterial population may have resulted from the accumulation of p-cresol, and this may have led to the development of a more tolerant or more resistant population to p-cresol. How this will eventually affect the host is not known.

Previous studies (Mizutani et al., 1982) have shown that single ip dose of p-cresol (100 mg/kg body weight) significantly decreased the percent BWG in rats. In

weanling pigs, single ip dose (100 mg/kg body weight) produced convulsions, muscle twitching, incoordination, dyspnea and loss of appetite. In another study, Yokoyama et al. (1983) reported that single oral dose of p-cresol (100 mg/kg body weight) given to weanling pigs averaging 5.7 kg body weight did not produce any signs of clinical toxicity. They found that the ADG of pigs receiving the single oral dose of p-cresol was reduced by 16% compared to the ADG of pigs in the control group, but the treatment difference was not significant. In this study, the addition of 0.75% p-cresol reduced the ADG of the NA-PC treatment group by 9.09% compared to those receiving the NA dietary treatment (380 vs 418 g). Pigs given the A-PC diets had 6.67% lower ADG compared to those that received the antibiotic (A) treatment (378 vs 405 g). The weanling pigs used in this study were heavier at the start of the experiment than the pigs used by Yokoyama et al. (1983). The pigs that received the single oral dose of p-cresol at 100 mg/kg BW had higher actual exposure to p-cresol at a given time than the pigs used in this study which received 0.75% p-cresol in their diet for 28 days. The present study suggests that weanling pigs weighing approximately 9-10 kg can tolerate 0.75% p-cresol in the diet for 28 days without external signs of clinical toxicity. Although not significant, the addition of 0.75% p-cresol in the diet reduced the percent BWG of weanling pigs at the end of the study.

The use of especially designed metabolism cages has limited the number of animals to sixteen in any given feeding experiment. With four treatment groups, only four animals could be assigned to each treatment at the maximum. Some of the insignificant differences could be due to the small numbers of animals used in the study. To detect significant differences, particularly in performance parameters, the study would require more animals than what were used in this study.

The weekly percent BWG of pigs were not affected by the excess p-cresol until the last week of treatment (Table 21). By the 4th week of treatment, pigs receiving the p-cresol supplemented diets (NA-PC and A-PC) had significantly lower percent BWG than those in the NA and A treatment groups (20.80% and 19.97% vs 25.65% and 25.42%, respectively). It appears from the study that weanling pigs weighing approximately 9.5 kg could tolerate 0.75% of p-cresol in the diet for about three weeks before a significant reduction in growth is observed.

The increase in urinary p-cresol excretion observed in all treatment groups (Table 16) suggests that the intestinal bacteria responsible for p-cresol production were either resistant or have developed resistance to the BMD used in the study. In this study, BMD was not effective in reducing the bacterial production of p-cresol (Table 16 and 17). Intestinal production of p-cresol was higher in the BMD

supplemented group (A) than the NA treatment group probably due to the slightly higher feed intake of the antibiotic (A) than the no antibiotic (NA) treatment group (850 vs 818g). The A-PC group also had higher p-cresol excretion than the NA-PC treatment group. The difference could be due to the higher absorption of the added p-cresol in the diet and not due to the increased endogenous production of p-cresol in the A-PC treatment group. This is so because feed intake was slightly lower in the A-PC than the NA-PC treatment groups (790 vs 810g). Schmidt et al. (1958) also reported an increase in the urinary excretion of volatile phenols in rats fed with diets supplemented with dietary antibiotics. In the antibiotic-supplemented treatment groups (A and A-PC), there was a decrease in urinary p-cresol excretion in the second week of treatment followed by an increase in the third and fourth week of treatment. Rogers (1955) also noticed a transient decrease, followed by an increase, in urinary volatile phenols with oral administration of antibiotics to humans. The reason for this fluctuation is not known.

The BMD used in the p-cresol study was the same antibiotic that was used in the PHPAA study. However, the p-cresol study was conducted four months after the PHPAA study. In contrast, the BMD in the PHPAA study was used immediately after the antibiotic was received from the supplier. Although the BMD was kept under room temperature

at all times, it is possible that after four months of storage the BMD has lost its biologic activity and thus had lost its capacity to promote growth.

Compared to the correlation (r= -0.7635) obtained in the PHPAA study and the correlation (r= -0.73) observed by Yokoyama et al. (1982) for the combined NA and A treatment groups, the negative correlation in the current study is relatively low (r= -0.1413). The reason for this is not known, but the difference could be due to the BMD used in the p-cresol study which was ineffective in reducing the intestinal production of p-cresol and consequently was not effective in promoting growth.

EXPERIMENT 3

The results of the tyrosine study indicate that feeding diets supplemented with 3% tyrosine to weanling pigs (ave wt 9.3 kg) for 28 days significantly increased urinary excretion of PHPAA and PHPLA. It appears from the study that the differences observed in the urinary excretion of PHPAA and PHPLA are related to the level of feed intake and the absorption of tyrosine. The amount of PHPLA excreted was substantially higher than the PHPAA in the A-T treatment group (315 mg PHPLA/24hr vs 117 mg PHPAA/24hr). Daily feed intake of pigs receiving the A-T was lower than those in the NA-T treatment group (920 vs 996 grams). The substantial

increase in the urinary PHPLA excretion of pigs in the A-T group indicates that much of the dietary tyrosine was absorbed and metabolized endogenously. The PHPLA urinary excretion was substantially less than the PHPAA urinary excretion in the NA-T treatment group (110 mg PHPLA/24hr vs 197 mg PHPAA/24hr) where the daily feed intake was higher compared to that of the A-T treatment group. This suggests that in the NA-T treatment group less tyrosine was being absorbed and metabolized endogenously. The result was that more unabsorbed tyrosine was made available in the intestinal tract for the bacteria to degrade to PHPAA. This explains why the urinary PHPAA excretion was higher than the urinary excretion of PHPLA in the NA-T treatment group.

The external lesions which are characteristic of tyrosine toxicity in rats, were not observed in any of the pigs receiving the 3% tyrosine diet. The absence of foot and eye lesions in pigs receiving the excess tyrosine could have been due to the well-balanced pig starter used in the study. In studies where rats developed the external lesions, low protein, high tyrosine diets were used. The development of external lesions in rats were either prevented or alleviated when the protein content of the feed was increased or when amino acids were added to the diets.

The addition of 3% tyrosine significantly increased the urinary p-cresol excretion of pigs in the NA-T treatment group compared to those receiving the NA dietary treatment

(139.88 vs 118.94 mg/24hr). However, the urinary p-cresol excretion of pigs in the A-T treatment group was less than the p-cresol excretion of pigs receiving only the antibiotic supplemented diet (A) (77.21 vs 93.89 mg/24hr). It appeared from the study that the observed differences in p-cresol excretion were also related to the amount of feed intake and the absorption of tyrosine. The pigs in the NA-T treatment group had higher feed intake, higher level of PHPAA and lower urinary PHPLA excretion. Urinary p-cresol excretion averaged 139.88 and 77.21 mg/24hr for the NA-T and A-T treatment groups, respectively. Thus, more PHPAA in the NA-T treatment group was made available for decarboxylation to p-cresol.

The overall percent BWG was lower in pigs receiving the tyrosine supplemented diets although these differences were not significant. However, the excess tyrosine significantly reduced the weekly percent body weight gain during the 3rd week of treatment. By the last week of the feeding study, the pigs in the NA-T and A-T treatment groups were able to recover, and their percent BWG were similar to pigs receiving the NA and A dietary treatments.

Table 30 shows that the BMD used in the tyrosine study neither improved the overall percent BWG nor increased the feed intake. Urinary p-cresol excretion in the antibiotic supplemented treatment group (A) was less than the NA treatment group (93.89 vs 118.94 mg/24hr) however, this was not

significant (Table 26). In this study, it appeared that p-cresol production was not sufficiently reduced by BMD such that the level of p-cresol remained high in the A-T treatment group. In the PHPAA study where the BMD used was effective in improving growth, the reduction in urinary p-cresol excretion was 50% compared to the 21% reduction found in this study.

It is also possible that the reduction in urinary p-cresol excretion in the antibiotic-supplemented treatment group (A) might not have been caused by the addition of BMD but rather by the low feed intake of pigs in the A treatment group. The ADFI averaged 939 g and 978 g for the A and NA treatment groups, respecively. The low feed intake might have increased the absorption of nutrients and might have decreased the amount of unabsorbed tyrosine. Consequently, this might have reduced the available PHPAA for decarboxylation to p-cresol. With the higher ADFI in the NA treatment group, the level of p-cresol was also relatively higher than those in the A treatment group.

In treatment groups supplemented with tyrosine (A-T and NA-T), the urinary p-cresol excretion was significantly reduced when BMD was added to the diet (77.21 vs 139.88 mg/24hr). Although there is considerable reduction in p-cresol excretion, the overall percent BWG of the pigs in both the A-T and NA-T treatment groups were the same (143.93% vs 144.57%).

The low p-cresol excretion in the A-T treatment group might have been due to the low ADFI and not due to the addition of BMD. The ADFI averaged 920 and 996 g for the A-T and NA-T treatment groups, respectively. The low ADFI of pigs under the A-T dietary treatment increased the absorption of nutrients. This can be seen by the increased urinary excretion of PHPLA which came from the metabolism of absorbed tyrosine. As a result, less tyrosine was converted to PHPAA and less p-cresol was produced by the bacteria. Although the amount of p-cresol was considerably lower in the A-T treatment group than in the NA-T treatment group, the amount of p-cresol exposure might still be high enough to adversely affect the BWG of weanling pigs.

The BMD that was supplemented in the tyrosine study was the same antibiotic that was used in the PHPAA and p-cresol studies. The tyrosine study was not conducted until 3 months after the p-cresol study or 7 months after the PHPAA study. The BMD used in both the tyrosine and p-cresol feeding studies was not effective in either promoting growth or in reducing the bacterial production of p-cresol. The BMD used in these trials may have lost its biologic activity during storage.

When the NA and A treatment groups were combined, a significant inverse correlation ($r = -0.7431$) was obtained between the percent BWG of each pig and the urinary p-cresol

excretion. The figure is comparable to the negative correlation observed in the PHPAA study (r= -0.7635) using the same treatment groups and is close to the correlation reported earlier by Yokoyama et al. (1982) (r= -0.73). However, when compared to the negative correlation found in the p-cresol study, the negative correlation in the tyrosine study is substantially higher (r= -0.7431). In both feeding studies the BMD used was not effective in promoting growth. The average initial weights of the pigs used in the two studies were almost the same. The pigs in the tyrosine studies had an average initial weight of 9.3 kg while those in the p-cresol study had an average weight of 9.5 kg. The difference between the two feeding studies was the level of feed intake. In the tyrosine study, the ADFI of pigs in the NA and A treatment groups averaged 978 and 939 g, respectively. The weanling pigs in the p-cresol study had 818 and 850 g ADFI for the NA and A treatment groups, respectively. The difference in the feed intakes could be due to the time the two studies were conducted. The p-cresol study was done in summer while the tyrosine study was conducted in fall. The heat during the summer might have reduced the ADFI of pigs in the p-cresol study. The low feed intake might have increased nutrient absorption and decreased the amount of unabsorbed tyrosine/PHPAA in the lower gut. This substantially decreased the substrate that could have been used by the bacteria to produce p-cresol. The reduced feed intake in the p-cresol study may have accounted for the low

negative correlation between BWG and urinary excretion of p-cresol. The PHPAA and tyrosine feeding studies had high negative correlations and in both cases the level of feed intakes were high.

CONCLUSIONS

1. The addition of 0.75% PHPAA to the diet significantly increased the urinary excretion of PHPAA in weanling pigs weighing approximately 10.9 kg, but the excess dietary PHPAA did not adversely affect the performance. None of the pigs receiving the excess PHPAA for 28 days developed external lesions.

2. BMD supplementation (110 ppm) significantly increased ($P<.05$) urinary excretion of PHPAA and tended to increase average daily feed intake.

3. BMD supplementation (110 ppm) significantly decreased ($P<.05$) urinary excretion of p-cresol.

4. There was significant negative correlation between body weight gain and urinary excretion of p-cresol.

5. The addition of 0.75% PHPAA to the diet did not significantly increase urinary excretion of p-cresol, indicating that enough PHPAA was available in the negative control group for decarboxylation to p-cresol.

6. Urinary excretion of p-cresol increased with age however, when expressed on the basis of unit body size, p-cresol excretion was higher in younger than older pigs.

7. The conjugated p-cresol/free p-cresol ratios were higher in treatment groups which were not supplemented with BMD, suggesting that the pigs in the non-antibiotic treatment groups were using more of the uridine diphosphate-glucuronic acid to form glucuronic conjugates of p-cresol.

8. Excess PHPAA in the diet did not affect urine volume which increased with age in all treatment groups.

9. The addition of 0.75% PHPAA to the diet significantly increased (<.05) PHPAA/PHPLA ratio. Urinary PHPLA excretion was consistently low throughout the duration of the PHPAA study.

10. BMD supplementation (110 ppm) significantly increased (P<.05) PHPAA/PHPLA ratio.

11. Results of the p-cresol study indicate that the addition of 0.75% p-cresol to the diet for 28 days depressed the growth of weanling pigs (ave wt 9.5 kg). Significant reduction (P<.05) in BWG did not occur until the 4th week of treatment.

12. The addition of 0.75% p-cresol significantly increased (P<.05) the urinary excretion of total and free p-cresol.

13. Weanling pigs receiving the excess p-cresol (0.75%) tended to have lower average daily feed intake (ADFI).

14. Results of the tyrosine study show that feeding diets supplemented with 3.0% tyrosine to weanling pigs (ave wt 9.5 kg) for 28 days significantly increased the urinary excretion of PHPAA and PHPLA. However, pigs given the 3.0% tyrosine did not develop external lesions.

15. The absorption of excess tyrosine as assessed by the urinary excretion of PHPAA and PHPLA appears to be influenced by the level of feed intake.

16. Excess tyrosine in the diet resulted in decreased overall growth rate. However, by the 4th week of treatment, the pigs appeared to have recovered from the excess tyrosine.

17. The addition of 3.0% tyrosine in the diet significantly increased ($P<.05$) PHPAA/PHPLA ratio.

18. The addition of 3.0% tyrosine in the diet did not significantly affect urinary excretion of p-cresol.

RECOMMENDATIONS

Results of this research suggest that future studies investigating the intestinal production of p-cresol take the following into consideration:

1. The addition of PHPAA and tyrosine should probably be avoided because they tend to confound and complicate the study. The positive correlation between BWG and the urinary excretion of p-cresol in treatment groups supplemented with PHPAA indicates that excess PHPAA exerts an effect different from that of p-cresol. The excess PHPAA may have overwhelmed or masked the effect of p-cresol. The addition of tyrosine may have created an amino acid imbalance that resulted in reduced voluntary feed intake. This complicates the study because decreased feed intake also reduces intestinal production of p-cresol.

2. Weanling pigs should be investigated for p-cresol production under actual feeding conditions, where pigs are fed standard rations ad lib with or without antibiotic supplementation. In this setting, p-cresol production simulates actual farm conditions and is not necessarily induced by the addition of appropriate intermediate precursors. This procedure was done by Yokoyama et al. (1982) but only using 15 pigs. The study should be replicated several

times.

3. Antibiotics should be tested in vitro to check their effectiveness in preventing the growth of the p-cresol producing bacteria before they are used in the study. This procedure could avoid the use of antibiotics which may have lost their biologic activity during storage. The procedure also allows a check on the consistency of the antibiotics before their use in succeeding studies.

4. Measuring p-cresol concentrations based on total urine volume is prone to a lot of problems. The accidental loss of urine samples due to animal movement in the metabolism cages, urine evaporation especially during hot weather which tends to abnormally increase p-cresol concentration, urine spillage during collection periods, fecal and other contamination of urine samples are only some of the problems. Since the concentration of p-cresol is based on total urine volume, a small loss of urine sample could change p-cresol measurements. Because spilled urine tends to dry up over time, one might not know that there was some loss of urine sample and might proceed with the p-cresol measurement. Other methods of p-cresol measurement should be tried and compared with the current procedure. One method that could be considered is measuring p-cresol based on per unit of a stable compound in the urine, e.g., based on per unit of creatinine in the urine.

5. Since it is determined that younger pigs have higher actual p-cresol exposure per unit body size than older and heavier pigs, it is advisable to use newly weaned pigs in the study.

6. If possible, weanling pigs from sows which were not exposed to antibiotics during pregnancy should be used in the study. This is to make sure that the effect on p-cresol production is due to the dietary antibiotic treatment and not due to the influence of residual antibiotics that may have accumulated in the pig through the sow's milk. If this is not possible, a two week adjustment period, where experimental animals are fed basal rations without dietary antibiotics, is recommended before the start of the study.

7. Since there is concern for possible genetic effect, it is advisable to block the study by litter.

8. To avoid the confounding effects of other antibiotics, one kind of antibiotic should be used per study. In cases where antibiotic combinations like CSP are used, it would be difficult to single out the particular antibiotic that is effective against the p-cresol producing bacteria. Antibiotic combinations could be used if the objective is to test the best possible mixture of antibiotics that would inhibit the intestinal production of p-cresol.

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