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EFFECTS OF HIGH DIETARY COPPER ON THE UTILIZATION OF NUTRIENTS AND ON BLOOD AND INTESTINAL VARIABLES OF STARTER PIGS

By

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ABSTRACT

EFFECTS OF HIGH DIETARY COPPER ON THE UTILIZATION OF NUTRIENTS AND ON BLOOD AND INTESTINAL VARIABLES OF STARTER PIGS

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Four experiments were conducted to study the effects of copper sulfate ($CuSO_4 \cdot 5H_2O$) supplementation in the diet of weanling pigs on blood and intestinal variables and nutrient utilization. The treatments were a basal corn-soybean meal-dried whey diet (B) and the basal diet plus 250 ppm Cu (B+Cu). The pigs were meal-fed twice a day.

Piglets were catheterized in the jugular vein (Experiment 1) or in both the jugular and portal veins (Experiment 2). Blood samples were taken before and at several intervals after a meal. Feeding B+Cu reduced portal ammonia and ammonia absorption, but there was only a slight tendency for reduction in peripheral ammonia. No treatment differences in portal or peripheral glucose and urea and peripheral insulin and somatomedin-C were detected.

In Experiment 3 the piglets received the experimental diets for four weeks. The pigs were then euthanatized and samples of intestinal tissue and intestinal contents were collected at several sites in the gut. Feeding B+Cu reduced ammonia and increased urea concentration in the contents of

the upper and medial jejunum and also reduced ammonia in the lower colon. Feeding B+Cu resulted in a clear tendency for reduced mitotic index of the crypt epithelial cells.

Nutrient balance trials were carried out during weeks 2, 3 and 4 after weaning (Experiment 4). Except for a slightly improved digestible and metabolizable energy values of B+Cu no treatment differences were detected on digestibility and retention of nitrogen, calcium, phosphorus and copper.

The decreased mitotic index (and possibly turnover rate) of the intestinal epithelium as a consequence of lower intestinal ammonia production may be involved in the growth response due to high dietary copper.

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LIST OF ABBREVIATIONS

C	degrees Celsius
Ca	calcium
Cu	copper
cm	centimeter
dl	deciliter
g	gram
h	hour
kg	kilogram
µg	microgram
mg	milligram
ml	milliliter
mM	millimolar
min	minute
N	nitrogen
No.	number
nm	nanometer
P	phosphorus
ppm	parts per million
SEM	standard error of the mean
SED	standard error of the difference of means
IU	international unit

INTRODUCTION

For over 30 years copper addition to swine feeds, at levels well above the nutritional requirement, has been known to improve the performance of growing pigs. Copper sulfate ($CuSO_4 \cdot 5H_2O$) supplying up to 250 ppm Cu in the feed is today widely used in the swine industry, especially in starter pigs diets. It has been reported that during the starter period the improvement in growth and feed efficiency averaged 22% and 8%, respectively, due to copper supplementation (Wallace, 1967). A summary of more recent information (1980-88) indicated benefits of 18% and 7%, respectively, in those traits when high copper diets were used.

Antimicrobial feed additives (antibiotics and antibacterials) are also commonly used to improve performance of swine. The response of starter pigs to some of the most effective additives (Hays and Muir, 1979; Zimmerman, 1985) is similar to that achieved by copper supplementation. Copper is, however, a less expensive supplement than the other antimicrobials. In addition, a synergistic effect in the performance of starter pigs has been consistently observed when copper is combined with other antimicrobials.

There is concern, however, that feeding of antimicrobials to animals promotes bacterial resistance and this may constitute a human health hazard (NRC, 1988). In the case of copper, there is concern that excessive amounts of this mineral returned to the land in the manure may be environmentally harmful (Cromwell et al., 1981). Thus, there is a possibility that the use of these products in feeds will be restricted in the future.

Several hypotheses have been proposed to explain how the antimicrobials improve animal performance. The importance of research to elucidate the mechanisms of action of antimicrobials is based on the fact that, once the mechanisms have been identified, alternative methods of growth improvement may be developed. Copper may have a mode of action different from other antimicrobials, which would explain the synergism of the combination of high copper levels and antibiotics or antibacterials.

The objective of this study was to determine the effects of high dietary copper on (a) absorption and efficiency of utilization of nutrients, (b) production of ammonia in the intestinal tract and absorption of ammonia, (c) turnover rate of the intestinal epithelial cells and (d) anabolic hormones. This consists of an attempt to contribute to the knowledge of mechanisms of action of antimicrobials in swine diets.

REVIEW OF LITERATURE

1. Metabolism of Copper and Copper Requirement of Pigs

1.1. General metabolism

Due to the presence of copper in enzymes that have basic biological roles, this mineral is an essential nutrient in all animals that have been studied.

Dietary copper is absorbed from the stomach and small intestine of mammals, a substantial absorptive activity being also demonstrated in the sheep large intestines (Davis and Mertz, 1987). In the small intestine, according to these authors, copper appears to be absorbed by two mechanisms, one saturable and the other unsaturable, suggesting active transport for the former and simple diffusion for the latter. As with other transport systems, low concentrations of dietary copper are predominantly transported via the saturable, active pathway, whereas the diffusion process comes into play at higher concentrations.

Homeostasis of copper in the organism is achieved by controlling the rate of absorption. Based on the available evidence, Cousins (1985) presented a model of operation of the homeostatic mechanism. When concentration of copper in the enterocyte is high, the promoter for the metallothionein gene is activated, and enhanced transcription of the gene

occurs. As the enhanced level of metallothionein mRNA is translated, cytoplasmic levels of thionein polypeptides are increased and they bind available copper. This mechanism prevents the transport of copper across the basolateral membrane, reducing its absorption. Biosynthesis of metallothionein is also induced by zinc, cadmium and mercury in the intestinal mucosal cell. Metallothionein is a non enzymatic protein (≈ 10,000 daltons) which contains seven binding sites for copper, zinc or cadmium.

Copper is transferred to the portal blood bound to albumin, at several positions in the molecule, and to free amino acids. According to O'Dell (1984) this pool of copper constitutes 10% or less of the total copper in plasma and this is the form taken up by the liver.

The hepatic uptake of copper from the blood is rapid and efficient (Owen, 1982). Upon entering the liver parenchymal cells, copper (and/or glucocorticoids and CAMP) is believed to regulate the transcription of the ceruloplasmin gene. To a lesser extent than zinc, copper also stimulates the expression of the metallothionein gene. Copper is stored in the liver mostly in the form of metallothionein. Copper secreted from the hepatocytes is principally in the form of ceruloplasmin. This form is firmly bound and accounts for the remainding 90% or more of plasma copper.

Ceruloplasmin is a glycoprotein (≈ 132,000 daltons) containing six copper atoms per molecule and 7-8%

carbohydrate. Frieden (1980) summarized the potential functions of ceruloplasmin into five categories:

a) oxidation of Fe^{2+} as it is released from hepatocytes and converted to Fe^{3+} transferrin in the regulation of hepatic iron mobilization;

b) transport of copper to tissue sites;

c) serum antioxidation, in which it acts as a scavenger of free radicals and superoxide ion;

d) endogenous modulation of the inflammatory response;

e) oxidase activity for aromatic amines.

Ceruloplasmin is considered the primary source of copper for extrahepatic tissues. Frieden (1980) proposed that ceruloplasmin Cu^{2+} is reduced at a cell membrane receptor, and Cu^{1+} is subsequently transferred to an intracellular acceptor. Alternatively, intact ceruloplasmin may be taken up by endocytosis, and Cu^{1+} may then be released by proteolysis or a mechanism that subsequently recycles the protein to the plasma membrane for release.

In all species studied, a high proportion of ingested copper appears in the feces. Most of this is unabsorbed copper, including that from cells sloughed off the intestinal tract, but active excretion also occurs via the bile and intestinal mucosa.

1.2. Metabolic functions of copper

Nutrition experiments have shown that dietary copper is required for a variety of functions, as reviewed by Owen (1982), including bone formation, proper cardiac function,

connective tissue development, myelination of spinal cord, keratinization and tissue pigmentation. As a component of metalloenzymes, copper functions in electron transfer and enzymatic binding of molecular oxygen. The great diversity of signs of copper deficiency in different species cannot be totally explained on the basis of the present knowledge of the element´s biochemical functions. Some of the more thoroughly studied copper metalloenzymes (O´Dell, 1984; Cousins, 1985; Davis and Mertz, 1987) are:

a) Ferroxidase function of ceruloplasmin. As described above, the mobilization of Fe-ferritin stores in liver requires the oxidation of iron. Serum iron tends to be low in copper deficiency and hypochromic anemia develops while intestinal mucosa and liver iron levels are higher than normal.

b) Superoxide dismutase. The cytosolic form of this enzyme contains zinc and copper and is present in the liver and blood cells. It catalyses the dismutation of the superoxide anion $O_2^{=}$ + $O_2^{=}$ + $2H^+$ --> H_2O_2 + O_2. This constitutes a defense mechanism when $O_2^{=}$ is produced in the phagocytic respiratory burst.

c) Lysyl oxidase. It is the key enzyme in the formation of the cross-links in collagen and elastin. Its deficit probably accounts for the lesions of copper deficiency that affect bone and connective tissues, such as aortic rupture and fragile bones. Aside from its role as a component of the

enzyme, copper exerts a control function in the synthesis and activation of lysyl oxidase.

d) Monoamine oxidase enzymes. The copper containing polyphenyl-oxidases catalyse the conversion of tyrosine to the pigment melanin. The lack of hair or wool pigmentation is a manifestation of copper deficiency in several animal species, but not in pigs.

e) Cytochrome c oxidase. The decreased activity of this final step in the respiratory chain in copper deficiency is believed to be responsible for impaired phospholipid synthesis, inhibition of myelin formation and / or demyelination. This would finally result in neonatal ataxia, to which lambs are particularly sensitive.

The activity of the C_{18}, -9 desaturase enzyme in subcutaneous adipose tissue is decreased by copper deficiency and reversed by supplementation. Copper deficiency also results in increased serum triglyceride, phospholipids and cholesterol. Reproductive failure due to fetal death and resorption in some species, but not in pigs, and a scouring condition of cattle are also associated with copper deficiency.

1.3. Interactions affecting copper absorption and metabolism.

Cousins (1985) summarized the dietary or other factors that affect copper absorption. Uptake of copper from the gastrointestinal tract is increased by L-amino acids, citrate, phosphate, gluconate and high dietary protein. D-

amino acid complexes are absorbed to a lesser degree than L-amino acid complexes. On the other hand, the presence of phytate, ascorbic acid, thiomolybdate, fiber, bile and zinc cause a reduction in copper uptake. The thiomolybdate - copper interaction is of practical importance for ruminants, whereas the interaction of ascorbic acid with copper may create practical problems in humans.

Zinc may compete with copper for a common transport system at the intestinal lumen level. The strong antagonism between copper and zinc, however, is more related to the fact that metallothionein induction in the enterocytes is more sensitive to zinc than to copper, and the affinity of this protein for copper is greater than for zinc. Zinc is, therefore, able to more effectively reduce copper absorption than the reverse. Particularly interesting for nutrition research is the fact that feeding high dietary zinc to gestating sows produced copper-deficient pigs, by decreasing the copper stores in the liver of the neonates (Hill et al., 1983).

Anemia is an expression of copper deficiency on iron metabolism. Iron uptake by the mucosal cells is not affected but the release of iron from enterocytes and hepatocytes to plasma is reduced in copper deficiency. Davis and Mertz (1987) described several cases in which copper/ceruloplasmin deficiency were not followed by anemia and suggested that other mechanisms are operational, in addition to the ferroxidase activity.

There is also influence of the endocrine system on copper metabolism. Briefly, the uptake of copper by the liver is stimulated by epinephrine. Synthesis of ceruloplasmin may be mediated by glucocorticoids, cAMP (through epinephrine and glucagon) and/or copper. Secretion of ceruplasmin, with change in plasma copper, is increased by glucocorticoids and to a lesser extent by estrogen and testosterone. Glucocorticoids also appear to increase biliary excretion of hepatic copper (Cousins, 1985). It is evident, thus, that stress and inflammatory conditions interact with copper.

1.4. Copper requirement of pigs

As for other nutrients, the dietary requirement of copper must be met to prevent deficiency. The accepted definition of deficiency is "a consistent and reproducible impairment of a biological function from normal to subnormal" (Mertz, 1981). The efforts to assess copper requirement of pigs have focused on the known metabolic functions that are depressed in copper deficiency and are responsive to this mineral. Given the variety of actions of copper in the organism and its involvement in several interactions, it could be expected that a series of minimum copper requirement exists depending on the interfering factors and on the criteria of adequacy employed.

Ullrey et al. (1960) observed that dietary levels of 6, 16 or 106 ppm copper fed to baby pigs did not affect performance variables or hematologic values. Levels of 16

ppm or higher increased the percent saturation of transferrin, albumin/globulin ratio and serum copper and iron concentrations. Based on these results, the NRC (1979) recommended 6 ppm copper as the dietary requirement of baby pigs and considered that the requirement would not be greater for later stages of growth.

Using purified diets based on glucose, casein, cellulose and fat, Okonkwo et al. (1979) defined more precisely the requirement of baby pigs for copper. Subnormal values for different response criteria were obtained when dietary copper was below the following concentrations: weight gains, 1.9 ppm; plasma and tissue copper concentration, plasma ceruloplasmin activity and bone strength, 2.8 ppm; bone histopathology, 3.2 ppm; copper balance, 4.9 ppm; hemoglobin concentration, 5.6 ppm. Brain superoxide dismutase activity was not affected by copper levels as low as 1.3 ppm, but erythrocyte superoxide dismutase activity was reduced when dietary copper was below 4.0 ppm. The level of 5.6 ppm copper was then selected as the requirement for this element in a purified diet for baby pigs. Later, Hill et al. (1983) determined that the copper requirement of baby pigs did not exceed 5 ppm, based on all the response criteria utilized (growth, serum and tissue copper, ceruloplasmin activity, hemoglobin concentration, aortic lysyl oxidase and hepatic and cardiac cytochrome *c* oxidase activities).

Compared to the above, the copper requirement of rats, poultry, sheep and cattle range between 3 and 10 ppm in the dry diet (Davis and Mertz, 1987). The authors also pointed out that the levels of copper in the most common feedstuffs vary from 4-8 ppm in the cereal grains to 15-30 ppm in the oilseed meals. Therefore these feeds themselves would be able to meet the copper requirement of animals in most situations.

Okonkwo et al. (1979) stated that it is important that a distinction be made between the minimum dietary requirement of copper for optimal metabolic functions (the nutritional requirement) and that level of copper which produces an antibiotic-like effect, resulting in increased performance (the pharmacologically effective dose).

2. Copper as Performance Improver in Pigs

Muir (1985) introduced a categorization of non-nutritive, exogenous substances which improve animal performance. These were referred to as "animal production improvers". Antimicrobial production improvers is one of the categories, including antibiotics, antibacterial agents and antifungal agents. This category, according to that author, consists of two classes: growth permittants and growth effectors. Growth permittants allow animals, presumably depressed in growth rate and feed efficiency by the action of intestinal microflora, to grow at their normal potential, while growth effectors improve growth and feed efficiency by controlling clinical and subclinical infectious diseases.

When included in swine diets at levels well above the dietary requirements, copper (usually as copper sulfate) has been shown for over 30 years to improve the performance of pigs (Braude, 1967; Braude, 1975; Edmonds et al., 1985). Based on present knowledge of the action of copper, it can be classified as an antibacterial growth permittant agent.

2.1. Effects of high dietary copper on performance

In 1945, Braude reported his observations that pigs had a peculiar craving for copper. First he noticed pigs licking copper rings placed in a new swine facility. Then he determined that the preference was specific to metallic copper in comparison to plates of other metals (aluminum, brass, magnesium, nickel and tin) to which the pigs had access. Other preference tests were conducted following these observations.

It was not until ten years later that the first report was published claiming that the addition of copper sulfate to the diet, corresponding to 250 ppm Cu, improved weight gain of growing pigs (Barber et al., 1955 cited by Braude, 1967). Subsequent studies confirmed that the improvement in performance of pigs was obtained with copper supplementation (Braude, 1967).

This finding prompted the interest of researchers in many countries, probably because at that time antibiotics were already being used as production improvers and copper sulfate had the potential to produce comparable results at a lower cost. Braude (1967) summarized the results of all

trials reported in the literature up to 1965. This summary included 83 tests worldwide in which the performance of pigs fed diets containing 250 ppm supplemental copper or unsupplemented diets was compared.

Wallace (1967) presented a review of studies conducted in the United States with pigs receiving high dietary copper (50 to 375 ppm Cu). He summarized published reports and data supplied by various Agricultural Experiment Stations and commercial feed companies. These consisted of 43 comparisons involving starter pigs, 57 comparisons with growing pigs and 54 comparisons with growing-finishing pigs. Braude (1975) compiled the results of 119 experiments reported during the period 1965-75 in which a direct comparison of performance of pigs receiving none or 250 ppm supplemental copper was possible. He presented separately the results of a large coordinated test carried out in Poland involving 3200 pigs per treatment.

A summary of the above mentioned reviews is presented in Table 1. To calculate the average percent response due to copper supplementation, the authors weighted the means for the number of pigs participating in each test.

The data presented in Table 1 bring out some points that deserve further consideration: (a) there was a wide variation among individual trials in the range of response to high dietary copper; (b) copper supplementation is much more effective in the starter period than in later growing and finishing periods; and (c) responses of similar

Table 1. Influence of high dietary copper on swine performance.

Reference	No. pigs per treat.	% improvement from feeding high copper	
		weight gain	feed conversion
Braude (1967)			
83 experiments	1215	8.1	5.4
Braude (1975)			
119 experiments	2630	9.1	7.4
Coordinated experiment	3206	5.0	3.9
Wallace (1967)			
43 starter pig trials	640	22.1	8.3
57 growing pig trials	1307	6.5	2.3
54 growing-finishing trials	1030	3.6	1.1

magnitude are observed with antibiotics and antibacterials as additives.

In the summary by Braude (1975), for example, the results of individual trials ranged from -15.6 to +37.1% for weight gain and from -13.2 to +24.1% for feed conversion due to copper supplementation. There were attempts to assess the causes of the variation in response to high dietary copper, but no factors have been conclusively identified. Level of dietary protein has not been found to influence the growth response to copper supplementation (Bunch et al., 1961; Lucas et al., 1962; Edmonds et al., 1985). The composition of the basal diet or the protein source of the diet were also unrelated to the response to copper. Comparisons of barley-fish meal vs corn-soybean meal diets (Lucas et al., 1962), canola meal vs soybean meal as protein source (Omole and Bowland, 1974) or corn-soybean meal vs corn-soybean meal - whey diets (Edmonds et al., 1985) showed that high dietary copper resulted in improved performance in all cases. In the latter report there was an interaction of level of dried whey x level of copper on pig performance (greater response to copper in the absence of whey) but the authors still found an additional effect of copper when diets containing dried whey were fed. Furthermore, when diets of the same composition, with or without supplemental copper, were fed to growing-finishing pigs in 8 stations (NCR - 42, 1974) a copper x station interaction was detected for weight gain and feed conversion. Five stations observed increased gains

in pigs fed copper, two showed reduced gains and one reported no difference, indicating that environmental or genetic differences affected the response to high dietary copper.

Because weanling pigs appeared to benefit most from high dietary copper (Table 1), more recent reports published in the United States involving this category of swine were compiled and the results are presented in Table 2. Comparisons were made between treatments or groups of treatments in which copper was the only antimicrobial additive and similar diets containing no copper. In all reports supplementary level of 250 ppm Cu was used, except one (Kornegay et al., 1986) where the level was 200 ppm Cu. The trials lasted 3 to 6 weeks after weaning, which occurred at 4 weeks in most cases. The average response to copper was similar to that of earlier studies with starter pigs, as shown in Table 1.

Although copper sulfate pentahydrate constitutes the standard form of copper supplementation, copper oxide, copper chloride and copper carbonate are also effective (Wallace, 1967); however, copper sulfide did not show any improvement in performance (Cromwell et al., 1978).

2.2. Effects of copper in comparison to and in combination with other antimicrobials.

Hays and Muir (1979) presented summaries of the growth response of pigs in the starter and in the grower stages to different antimicrobials. A comparison of these results and

Table 2. Influence of high dietary copper on performance of starter pigs. Summary of period 1975-88.

Reference	No. pigs per treat.	% change from feeding high copper: weight gain	% change from feeding high copper: feed conversion
Mahan (1980)	44	16.9	4.9
Stahly et al.(1980)	46	24.5	6.0
	43	16.6	5.2
	36	12.8	1.0
Cromwell et al.(1984)	48	24.4	11.0
	20	33.2	10.8
	20	38.8	20.5
Thulin et al.(1984)	80	0.0	1.3
Edmonds and Baker(1985)	12	32.0	-1.1
	16	-5.5	-10.0
Edmonds et al.(1985)	25	7.4	13.6
	15	46.5	13.8
	20	50.4	25.1
Kornegay et al.(1986)	32	13.8	9.7
Menten et al. (1987)	24	16.3	10.2
	18	17.6	11.8
Walker and Danielson (1988)	32	16.7	2.9
Weighted means		17.9	7.0

those in Tables 1 and 2 shows that the response of starter pigs to high dietary copper is comparable to that achieved with some antimicrobials (combination tetracycline - sulfamethazine - penicillin and carbadox) and is usually superior to the other products. On the other hand, the tetracycline - sulfamethazine - penicillin combination (ASP) and carbadox appear to produce better response than high copper when fed to grower pigs, with comparable improvements observed for the other antimicrobials.

Direct comparisons of copper vs other antimicrobials were also reviewed (Braude, 1967; Wallace, 1967) and in general the response was more favorable to copper supplementation. Wallace (1967) pointed out that pigs receiving ASP showed a greater response in rate of gain (17 vs 12%), but copper was more effective for feed conversion. More recent studies (Mahan, 1980; Stahly et al., 1980; Edmonds et al., 1985) indicated that the response of starter pigs to ASP, carbadox, chlortetracycline or virginiamycin was similar to high copper supplementation and that copper was of greater benefit than tylosin and sulfamethazine.

Another interesting fact is that high dietary copper promotes an additional response in pigs fed antibiotic-supplemented diets. Wallace (1967) summarized data showing that the combination of copper and chlortetracycline was more effective in improving pig performance than either individual additive. The synergistic effect of other antimicrobials and copper on starter pigs has also been

demonstrated for tylosin (Beames and Lloyd, 1965; Edmonds et al., 1985), ASP (Mahan, 1980; Edmonds et al., 1985), carbadox (Mahan, 1980), chlortetracycline and virginiamycin (Stahly et al., 1980). However, there is no evidence for such a synergism in growing-finishing pigs (Barber et al., 1978; Lima et al., 1981).

2.3. Systemic effects of high dietary copper

When copper is fed at growth permittant levels to pigs, the homeostatic regulation at the small intestine level is overcome by the copper load and a greater absorption through the hepatic portal vein occurs. In consequence of this increased absorption, systemic effects occur and some of them are reported in the literature.

a. Accumulation of copper in the liver

Cromwell et al. (1981) summarized the results of 18 experiments in which the liver copper concentration of pigs fed 250 ppm Cu during the growing-finishing period was determined after slaughter. They reported that livers of pigs fed normal diets (6 ppm supplemental copper) contained 15 - 30 ppm Cu on a dry-matter basis; high dietary copper resulted in a 10 to 15 - fold increase in liver copper, which averaged 244 ppm. Increases in liver copper of a greater magnitude than these were reported after feeding 250 ppm Cu diets to starter pigs for 3 to 4 weeks (Edmonds et al., 1985; Shurson, 1986). Liver iron concentrations have

been found to be depressed when 250 ppm Cu was fed (Prince et al., 1979; Lima et al., 1981; Shurson, 1986).

Based on a series of studies, Cromwell et al. (1981) reported that sulfide, either as sodium sulfide or ferrous sulfide, proved to be effective in preventing copper accumulation in the livers of pigs fed 250 ppm Cu. A 2:1 ratio of sulfide:copper reduced liver copper to a level that approached that of control pigs, without interfering with the improvement in performance. The results obtained by Prince et al. (1979) provided some evidence that sulfide reduced absorption of copper, rather than increased mobilization of copper from the livers. The depression in liver iron observed when high levels of copper are fed to pigs was reversed by the addition of sulfide (Lima et al., 1981). These authors suggested that the reduced response to supplemental copper often observed with finishing pigs may be associated with the copper buildup in liver tissue. There is very little effect of high copper diets on muscle tissue concentration of this mineral (Lillie et al., 1977).

b. Effects on blood variables

Plasma copper concentrations were only slightly increased by feeding 250 ppm Cu diets to pigs (Lillie et al., 1977; Roof and Mahan, 1982; Zhang et al., 1985; Shurson, 1986), showing that copper is efficiently taken up from portal blood by the liver.

The effects of diets with 250 ppm Cu on hemoglobin and hematocrit values have been studied (Gipp et al., 1973; NCR-

42, 1974; Lillie et al., 1977; Shurson, 1986) but the results were inconsistent, with small differences being detected. Comparing results of trials in which several levels of supplemental copper were fed to pigs, Wallace (1967) showed that hemoglobin values were seriously reduced when 375 ppm Cu or above was used. Anemia constitutes one of the signs of copper toxicity (NRC, 1979).

Plasma ceruloplasmin activity was not significantly affected by high copper feeding (Gipp et al., 1973; Zhang et al., 1985; Shurson, 1986). However Zhang et al. (1985) reported an increased plasma glutathione peroxidase activity when pigs were fed diets with 250 ppm Cu. The biological significance of this finding is not clear.

c. Effects on lipid metabolism

Taylor and Thomke (1964) reported that pigs receiving high dietary copper had softer subcutaneous adipose tissue, with more unsaturated fatty acids, than the control pigs. It is now well established that high copper feeding causes an increase in the proportion of palmitoleic, oleic and linoleic acids in the backfat, concomitant with a decrease in palmitic and stearic acids (Moore et al., 1968; Elliot and Bowland, 1969; Amer and Elliot, 1973a,b; Ho et al., 1975). Increases of 4 to 10% unsaturated fatty acids, as a proportion of total fatty acids, have been detected. There was also a 5 to 10 C reduction in the melting point of the subcutaneous adipose tissue.

After a series of studies, Ho et al. (1975) determined that high dietary copper induced a greater activity of the enzymes palmitoyl CoA, stearoyl CoA and oleoyl CoA desaturases in adipose and hepatic microsomes of pigs. They hypothesized that copper was functioning as an integral part of the enzymes and discarded the hypothesis that copper would function as an activating ion for desaturases.

In studies with rats and mice, insulin has been implicated in the regulation of acyl-CoA desaturase because (a) it restores to normal the low activity observed in diabetic rats (Gellhorn and Benjamin, 1964), (b) it increases the activity in normal rats (Inkpen et al., 1969) and (c) obese mice, which are hyperglycemic and hyperinsulinemic, possess an elevated activity (Enser, 1975). There are no reports, however, about a possible influence of insulin on desaturases in pigs.

2.4. Actions of copper in the gastrointestinal tract

a. Effects on digestive enzymes

Kirchgessner et al. (1976) determined that cupric ions (and nickel ions to some extent) caused an increase in the activity of pepsin, in in vitro studies. They proposed that this was due to the formation of a complex between pepsin and cations resulting in stabilization of pepsin with a reduction in its partial autolysis.

b. Effects on efficiency of nutrients utilization

The information available on how copper supplementation in the feed affects the utilization of energy, nitrogen and other nutrients is limited and inconclusive. Kirchgessner and Giessler (1961) conducted 2 trials with 15 kg and 30 kg pigs, respectively, receiving control or 250 ppm Cu diets (4 pigs per treatment). High dietary copper resulted in greater nitrogen (N) digestibility and retention, as a percent of intake. Braude (1967) also reported that a high copper diet resulted in improved N digestibility, % N retained and N retained/absorbed ratio. In that trial 4 pigs per treatment were used over 4 collection periods.

Castell and Bowland (1968), using three 25 kg pigs per treatment, reported that the 250 ppm Cu diet resulted in improved N digestibility, but did not affect N retention. Also, no differences were found in digestibility and retention of energy. Young et al. (1970) did not detect any change in N digestibility due to copper supplementation of corn-soybean meal diets, but there was a significant increase in % N retained. There was no effect on energy utilization. They used four pigs per treatment and the trial was conducted for three collection periods.

Shurson (1986) conducted two balance trials, one with four 7 kg pigs per treatment and the other with six 20 kg pigs per treatment, receiving corn-soybean diets supplemented or not supplemented with 250 ppm Cu. In the first trial, high dietary copper resulted in increased N

digestibility and % N retained with no change in the N retained/absorbed ratio. Pigs fed the high copper diet had a higher digestibility and retention of energy and also a higher phosphorus, copper and zinc retention. In the second trial, N digestibility was not affected by feeding high copper, but the % N retained was increased. There were no changes in energy utilization measurements.

The results of the studies above are not repeatable from trial to trial. An increase in N digestibility and % N retention due to high copper diets could be interpreted as more protein being enzymatically hydrolysed and the resulting amino acids absorbed and utilized by the animal. However, Young et al. (1970) and Shurson (1986) obtained a higher % N retention with no change in N digestibility, which suggests that, when pigs receive high dietary copper, a greater fraction of the N compounds absorbed consists of intact amino acids (assuming that no changes on the efficiency of amino acids utilization occur). This is complicated by the fact that Castell and Bowland (1968) found an increased N digestibility with no change in % N retention.

c. Effects on microbial population

Early in the studies on the use of copper as a performance improver in pigs, Sollman (1957) recognized that the bacteriostatic properties of copper in regard to the gut microflora could be related to the effects on performance, similar to antibiotics. Further studies demonstrated that

the effects of high dietary copper and antibiotics on the gut microflora may be similar or different, depending on the organisms and antibiotics considered.

Hawbaker et al. (1961) and Bunch et al. (1961) evaluated in a series of experiments the effects of feeding copper and/or antibiotics to pigs on fecal counts of coliforms, lactobacilli, total aerobes, total anaerobes, staphylococci, streptococci and molds and yeasts. Although the results of different trials did not show an absolute agreement, in general, feeding 250 ppm Cu (as $CuSO_4$) reduced the counts of aerobes, anaerobes, lactobacilli and streptococci in feces. Also, coliform and mold and yeast counts tended to be increased. The presence of oleandomycin or oxytetracycline in the feed caused a comparable increase in coliform and mold and yeast numbers (Hawbaker et al., 1961), but the antibiotic chlortetracycline caused a reduction in those groups of microorganisms (Bunch et al., 1961). When oleandomycin or oxytetracycline were fed in combination with copper, the effects of copper on the microorganisms appeared to be enhanced. Similarly, the improved performance achieved by using the additives tended to be enhanced when copper and either antibiotic were combined.

Miller et al. (1969) reported that growing-finishing pigs fed diets containing either 250 ppm Cu or a combination of oxytetracycline and neomycin sulfate had a reduced total fecal bacterial count in relation to controls. The magnitude

of the reduction, however, was much greater when copper was present in the diet. Fuller et al. (1960) observed a reduction in the numbers of streptococci and a shift in the proportion of 3 lactobacilli species when pigs were fed high copper diets. Smith and Jones (1963) did not observe any differences in fecal flora counts when pigs received high dietary copper.

Varel et al. (1987) collected rectal samples of fecal material from pigs fed either 125 ppm Cu, chlortetracycline-sulfamethazine-penicillin combination or unsupplemented diets during the growing-finishing period. Total viable bacteria counts, averaged over 3 collection periods, were not different among treatments, with a decrease over time being observed. However, the overall proportion of total organisms that were ureolytic was different. Fecal samples from pigs fed the basal diet contained 27% ureolytic organisms compared to only 10% when copper or the antibiotic combination was fed. *Streptococcus spp.* were the predominant urease-producing organisms isolated from pigs fed all diets, and a marked decrease in streptococci was observed when copper or the antibiotics was fed.

The studies reported above were concerned with microbial populations in the feces. Data on the effect of copper on the microflora of the small intestines were not found in the literature. It is likely that changes in the microflora occur along the intestinal tract, as the substrates and other conditions change. In fact, in studies

cited by Vervaeke et al. (1979), it was found that the antibiotic virginiamycin caused a decrease in lactobacilli counts along the gastrointestinal tract of piglets and in different bacterial groups of slaughter pigs. According to Vervaeke et al. (1979), the dominant flora in the small intestine of pigs consists of coliforms, streptococci and lactobacilli. Regardless of the change in total microbial population, it is probably the change in the proportion of specific populations, as shown above, or changes in the metabolism of intestinal bacteria, as suggested by Visek (1978), that are the nutritionally important effects of antimicrobial feed additives.

d. Effects on urease activity and ammonia concentrations

Young et al. (1970) measured urease activity in the supernatant fraction of different feeds. When the feed had a high urease activity (containing raw soybeans), the presence of 125 or 250 ppm Cu reduced the activity of the enzyme, as measured by ammonia produced. Varel et al. (1987) reported that fecal samples of pigs fed diets containing 125 ppm Cu had a lower urease activity than control pigs. Chlortetracycline - sulfamethazine - penicillin combination, however, did not cause a reduction in urease activity in the fecal samples. On the other hand, neither copper nor the antibiotic combination depressed the ammonia concentration in relation to pigs fed the control diet. Shurson (1986) did not detect differences in ammonia or urea concentration in

the cecal contents of starter pigs fed control or 250 ppm Cu diets.

e. Effects on intestinal weight and intestinal mucosa

The weight of the small or large intestines of pigs was not affected by feeding high levels of copper, as reported by Shurson (1986). This is in contrast to what has been found with other antimicrobial agents in swine diets, which generally reduced the weight of the small intestines (Braude et al., 1955; Taylor and Harrington, 1955; Yen et al., 1986). Shurson (1986) also did not find differences in the intestinal thickness or depth of the submucosa along the small intestines of pigs due to copper supplementation of the diet. Nevertheless, the copper-fed pigs gained weight faster (23%) and more efficiently (10%) than the controls. Feeding antibiotics to pigs, however, has been found to reduce intestinal wall thickness (Braude et al., 1955, Taylor and Harrington, 1955). This same effect was observed in chicks (Pepper et al., 1953; Eyssen and De Sommer, 1963). Working with chickens, March et al. (1960) observed that antibiotics in the feed caused a decrease in the thickness of the intestinal wall even in the absence of any growth stimulating effect.

Comparisons of germ-free versus conventional pigs in the study of Shurson (1986) revealed that intestinal weights were reduced and intestinal thickness and submucosa depth tended to be reduced in germ-free pigs. This is in

agreement with the results of other studies with germ-free pigs and other animals.

f. Effect on glucose absorption

Although not conclusive, there was an indication that high dietary copper promoted increased glucose absorption, measured by portal plasma glucose concentration 3.5 hours after a meal (Shurson, 1986). Glucose level in the peripheral plasma tended to reflect that increase.

3. Proposed Mechanisms of Action of Copper and Other Antimicrobials as Performance Improvers

The data reported in the literature concerned with the mode of action of copper in improving performance of pigs are not sufficient to allow the formulation of hypotheses that explain the mechanisms involved. Because of the "antibiotic-like" response observed when high copper diets are fed to swine, the more extensive evidence available on how antibiotics and antibacterials may function as performance improvers will be discussed in conjunction with the findings with copper.

An analogy with animals in germ-free condition is also necessary to the interpretation of the changes observed when low levels of antimicrobial agents are fed. An important concept, accepted since the early days of antibiotic feed use, is that the growth response is due to actions on the microbial flora. In fact, the support for this is derived from comparisons of the effects of antimicrobials in the

feeds of conventional and germ-free animals, which pointed to the lack of improved growth under germ-free conditions. In a review by Pleasants (1968), chickens and pigs were classified as species that show equal or superior growth of germ-free animals compared to conventionally-reared animals. Antibiotics such as penicillin (Forbes and Park, 1959; Coates et al., 1963) and chlortetracycline (Freeman et al., 1975) failed to improve growth of germ-free chicks. Eyssen and De Somer (1967) inoculated germ-free chicks with microorganisms common to the intestinal tract and the growth to 14 days was reduced; addition of 100 ppm virginiamycin to the feed restored growth to that of the germ-free chicks.

Experiments with pigs (Whitehair and Thompson, 1956) isolated immediately after delivery by caesarean section and fed purified diets supplemented with chlortetracycline also did not result in a growth response. Feeding a diet containing 250 ppm Cu to germ-free weanling pigs, Shurson (1986) observed depressed performance, but this was attributed to a moderate copper toxicity caused by the supplemental copper.

The mechanisms of action that have been postulated (Hays, 1969; Visek, 1978) for the performance improvement by antimicrobials include: a) a nutrient sparing effect in which the antimicrobials may reduce the dietary requirement for certain nutrients by stimulating the growth of desirable organisms that synthesize vitamins or amino acids, by depressing the organisms that compete with the host animal

for nutrients, by increasing the availability of nutrients via chelation mechanisms, or by improving the absorptive capacity of the intestinal tract; b) a protective effect in which antimicrobials reduce the production of growth depressing toxins; c) a disease control effect, through suppression of microorganisms responsible for mild but unrecognized infections, and d) a metabolic effect, in that the antimicrobials directly affect the rate or pattern of the metabolic processes in the animal.

There is not a general acceptance of the evidence presented in support for each proposed mechanism of action. Some of them are interrelated and it is very likely that more than one effect takes place simultaneously, each contributing a fraction of the response, when antimicrobials improve performance of animals. Because antimicrobials may have a narrow or broad spectrum of antibacterial activity, their action on the gut microflora may promote growth by different mechanisms. The synergistic effect observed for copper and some antimicrobials in weanling pigs, described in an earlier section, may be an indication of different mechanisms of action of the additives combined, as suggested by Cromwell et al. (1981). The evidence that more strongly support each proposed mechanism of action is discussed below.

3.1. Sparing of amino acids and energy

In a review on the mode of action of antimicrobials, Hays (1969) indicated that the nutrient-sparing effect had

considerable research support. Some of the evidence reported in the review is:

a) the response to antibiotics was generally greater if the antibiotics were included in inadequate diets;

b) the level of protein required by pigs for maximum performance was less in the presence of dietary supplements of antibiotics;

c) animals fed antibiotics had an increased rate of glucose absorption;

d) antimicrobials in the feed enhanced the intestinal growth of some yeasts and coliforms other than *Escherichia coli*, which are able to synthesize essential nutrients for the host;

e) antimicrobials reduced the number of lactobacilli which were believed to compete with the host for nutrients;

f) the thinner intestinal wall observed in antibiotic-fed animals was associated with improved potential for absorption. The evidence for the role played by the microflora in this effect is based on the fact that feeding chicks the intestinal contents of infected chicks resulted in a thickening of the intestinal wall.

More recently, Yen and Killefer (1986) demonstrated that feeding diets supplemented with 55 ppm carbadox to gilts resulted in greater net portal glucose absorption. Shurson (1986) also indicated that high dietary copper promoted increased glucose absorption in starter pigs.

Visek (1978) summarized some differences between germ-free and conventional animals. It is evident that antimicrobial-fed animals tend to approach germ-free animals in many characteristics. For example, the potential for absorption of sugars and amino acids is doubled, the weight of the small intestines, the thickness of the mucosa and the basal metabolic rate are reduced in germ-free animals. From the similarities of germ-free and antimicrobial-fed animals in these features it can be inferred that the action of the additives on the gut microflora could be responsible for a more efficient use of nutrients resulting in the improved performance in relation to conventional animals.

The attractive hypothesis that the improved growth of animals receiving antimicrobials in the feed would, at least in part, result from the reduced intestinal weight and reduced basal metabolic rate was tested by Yen et al. (1986). They fed diets containing 55 ppm of the antibacterial carbadox or .31% of the antibiotic neomycin to young gilts. The antimicrobial-fed animals had a greater weight gain and feed efficiency than the controls and the small intestine weight was reduced by 10%. However, they were unable to detect differences in the basal metabolic rate due to the additives.

Another approach was utilized by Vervaeke et al. (1979) to evaluate the influence of antimicrobials on the energy metabolism of pigs. They incubated intestinal contents in vitro using both batch and continuous techniques. Ileal

fermentation seemed to be essentially a lactic acid fermentation and, secondarily, a volatile fatty acid fermentation, consisting principally of acetic acid. Addition of 50 ppm of either virginiamycin or spiramycin to the media resulted in decreased organic acid production and in sparing a measurable quantity of glucose. While the counts of total bacteria and coliforms remained unchanged, the numbers of the lactic acid bacteria streptococci and lactobacilli were decreased after addition of the antimicrobials. The authors further calculated that the sparing of carbohydrates with antibiotics could be expressed as a higher availability of net energy required for growth: 2.68% for virginiamycin and 1.56% for spiramycin. Chlortetracycline caused a reduction in the volatile fatty acids produced in vitro, when the contents of the upper small intestines were the source of microbes (Shurson, 1986), but no change was observed in the non-volatile fatty acids.

Vervaeke et al. (1979) also showed that the pH of the in vitro incubations of intestinal contents was elevated by addition of virginiamycin or spiramycin. This was attributed to the lower production of organic acids. Although the authors did not notice, it might be important to consider the effect of intestinal pH on enzyme activity. Kidder and Manners (1978) presented data showing that the optimum pH for pancreatic and intestinal enzymes are generally higher relative to the pH actually found in the small intestines of

the pig. Therefore, an increased digestion of carbohydrates, proteins and lipids could account for some of the hypothesized sparing of nutrients by antimicrobials.

Dierick et al. (1986a,b) studied the influence of antimicrobial additives on nitrogen metabolism in pigs in vitro and in vivo, respectively. When ileal contents of pigs fed a dry milk diet were incubated with either virginiamycin (50 ppm), spiramycin (50 ppm), carbadox (50 ppm) or copper sulfate (200 ppm Cu) there was virtually no degradation of amino acids, compared to about 20% degradation in the control. Dierick et al. (1986a) explained the sparing of amino acids by the antimicrobials as being due to less bacterial degradation, less incorporation into bacterial protein and protection of pancreatic enzymes against bacterial attack. In vivo experiments demonstrated that virginiamycin improved utilization of amino acids (Dierick et al., 1986b). Growing pigs fed skimmed milk-corn starch based diets had greater ileal digestibility of most amino acids (principally glycine, methionine, valine and lysine) with supplementation of 50 ppm virginiamycin. In a perfusion experiment through an isolated loop of the small intestine, the addition of 50 ppm virginiamycin to the amino acid solution resulted in a 9% improved absorption of amino acids (especially aspartic acid, threonine, serine, glutamic acid, glycine and alanine). The reason for enhanced nutrient absorption was not a thinner intestinal wall, as the antibiotic was not present in the diet. Based on indications

in the literature, the authors suggested that antimicrobials could enhance the activity of enzymes involved in the active transport of nutrients through the mucosa.

3.2. Depression in the formation of toxins

Ammonia has long been believed to be a substance toxic to most living cells (Dintzis and Hastings, 1953; Visek, 1972). Visek (1978) reviewed the literature on the decreased ammonia production in the gastrointestinal tract by feeding antimicrobials to several animal species. Although in some early reports the dosage of antimicrobials in the diet was very high (Dintzis and Hastings, 1953; Kornberg and Davies, 1955; Silen et al., 1955), later studies also demonstrated that growth-permittant levels of antimicrobials depressed ammonia in the gut.

According to Visek (1978) the involvement of the gut microflora on the generation of ammonia from nitrogenous substances was also early recognized. Evidences for this are the facts that in germ-free animals there is virtually no urea hydrolysis and the ammonia concentration in the cecum is about 10% of that in conventional animals. Animals fed antimicrobials tend to have characteristics similar to those of germ-free animals. Warren and Newton (1959) reported that portal blood ammonia concentrations of germ-free guinea pigs were about 25% of those in conventional animals and were reduced in conventional animals by oral intake of antibiotics.

Dintzis and Hastings (1953) and Visek et al. (1959) demonstrated that the reduction in urease activity by antibiotics was due to effects on the microflora rather than to any direct effect on the enzyme. Copper, however, is an inhibitor of sulfhydryl enzymes such as urease (Visek, 1978). It has been shown that growth-permittant levels of copper inhibit urease activity in the feed (Young et al., 1970) and the feces (Varel et al., 1987) of swine. Urease present in the alimentary tract of animals is a product of plants and microbes. Ammonia is formed by the action of urease on urea, by deamination of amino acids and by other enzymes acting on nitrogenous substrates (Visek, 1972). Dierick et al. (1986b) determined ammonia and urea as a percentage of non-protein nitrogen in the intestinal contents of swine supplemented or not with antibiotics. They concluded that the greatest part of ammonia was derived from deamination of amino acids and not from ureolysis.

In swine, the production of ammonia was reduced by virginiamycin and spiramycin during incubations of ileal contents but not cecal contents (Vervaeke et al., 1979). These in vitro results were confirmed by Dierick et al. (1986a) who additionally showed that carbadox or copper in the incubation medium caused a 50% reduction in ammonia production. In vivo data from Dierick et al. (1986b) indicated that feeding market weight pigs with virginiamycin (20 ppm) reduced the ammonia in the small and large intestine contents by 18% and 15%, respectively. Spiramycin

(20 ppm) caused 17% and 35% reductions, respectively. On the other hand, Shurson (1986) did not find any effect of feeding 250 ppm Cu on ammonia concentration in the contents of the cecum of starter pigs and Varel et al. (1987) did not detect changes in the feces of market pigs fed 125 ppm Cu or a chlortetracycline (110 ppm) - sulfamethazine (110 ppm) - penicillin (55 ppm) combination. Yen and Killefer (1986) also did not observe change in the net portal absorption of ammonia in gilts following supplementation of the diets with 55 ppm carbadox.

The causal relationship between the reduction in ammonia formation in the intestinal tract and the improved performance as a result of antimicrobial feeding of animals has two main lines of evidence. First, rats and chicks immunized against urease had less urease activity in the intestinal tract and grew faster than the nonimmunized controls (Dang and Visek, 1960). In swine, however, immunization against urease reduced the enzyme activity in the gut contents but growth increases were not observed (Kornegay et al., 1964). Second, rats fed a cationic exchange resin that binds and removes ammonia had an improved weight gain (Holtzman and Visek, 1965). Visek (1978) reported that the intestinal mucosa of immunized rats had less protein and RNA and higher ratios of DNA/protein and DNA/RNA than that of controls. The small intestines had a lower wet weight and total protein. These effects were attributed to the decreased ammonia with the immunization.

Injection of urease in rats and chicks had no effect on growth rate and feed efficiency (Wagner et al., 1963).

Prior et al. (1974) studied the metabolic responses to ammonia in cells isolated from the small intestine of chickens. Ammonia in concentrations (up to 10 mM) normally found in the intestinal contents caused a generalized stimulation of glycolysis and of the tricarboxylic acid cycle. The authors concluded that ammonia shortens the normal metabolic life span of cells incubated in vitro and suggested that this may require a more rapid turnover of intestinal epithelial cells in vivo. In the review by Visek (1978) it was reported that germ-free animals have lower mitotic indices and a 30-40% lower turnover rate in the small intestine mucosa.

A decrease in the turnover rate of intestinal mucosal cells is the basis of an interesting hypothesis to explain, at least in part, the improved performance obtained with growth-permittants. Webster (1980) studied the contribution of the major body tissues to protein synthesis in young rats. His data suggested that, although the intestinal tissue contains less than 10% of total body protein, approximately 50% of the protein synthesis in the body occurs in intestinal tissue. Thus, a small decrease in protein synthesis by intestinal tissue would be expected to greatly lower the animal's protein and energy maintenance requirements. The spared nutrients and energy should then be available for body growth.

According to Visek (1984) the clearance of ammonia from the blood is normally so efficient that concentration in the peripheral venous blood remains very stable. Ammonia is used by mammalian tissues to form nitrogen containing substances like purines, pyrimidines and non-essential amino acids but most of it is converted to urea (Lewis, 1972). Although there is an energy cost for the functioning of the urea cycle in the liver (6 moles of ATP for each mole of urea), the reduction of the ammonia load when antimicrobials are fed probably is not energetically important for the animal. Holtzman and Visek (1965) calculated that the energy required to detoxify ammonia was less than 1% of the apparent energy spared by rats given chlortetracycline-supplemented diets.

There are other potentially toxic substances formed in the intestinal tract of animals as a result of microflora activity. These include amines, products of bile acid hydrolysis and p-cresol. The decreased production of these metabolites with antimicrobial feeding may also be involved with the improvement in performance. Drasar and Hill (1974) documented the toxic effects of various amines. Chlortetracycline (Larson and Hill, 1960) and virginiamycin or spiramycin (Dierick et al., 1986b) in swine feeds caused a decrease in amines concentration in the contents of the ileum and of the whole digestive tract.

Bile acid hydrolysis is absent in the intestinal tract of germ-free animals (Norman and Widstrom, 1964) and chicks

were adversely affected when received .2% dietary lithocholic acid, one of the products of bacterial hydrolysis of bile acids (Leveille et al., 1962). Recently, Feighner and Dashkevicz (1987) presented the first evidence that growth promoting levels of antibiotics in the feed caused a decrease in bile acid hydrolysis. In their study, avoparcin, bacitracin, efrotomycin, lincomycin, penicillin and virginiamycin improved the rate of weight gain and feed efficiency of chicks and decreased cholyltaurine hydrolase activity in ileal homogenates relative to those of nonmedicated control birds.

The phenolic compound p-cresol is a product of bacterial degradation of tyrosine in the intestinal tract. Yokoyama et al. (1982) reported a negative correlation between growth of weanling pigs on different antibiotics-supplemented diets and urinary p-cresol excretion. Lumanta et al. (1988) reported that feeding 0.75% p-cresol to weanling pigs depressed growth and the antibiotic bacitracin reduced urinary p-cresol excretion in piglets fed normal diets.

3.3. Control of diseases

Numerous studies support the hypothesis that the major benefits derived from the inclusion of antimicrobials as routine feed additives result from the suppression or control of subclinical or nonspecific diseases (Hays, 1969). Hays (1969) noted that the degree of response to antibiotics was inversely related to the well-being of the animals. For

example, the response to antibiotics (chlortetracycline in one case, spiramycin in the other) was doubled when pigs were housed in contaminated environments in comparison to that in clean environments. It was also shown that low doses of antibiotics in the feed helped in controlling experimentally induced hemorrhagic dysentery. Treatment with tylosin was effective in controlling the outbreak, but treatment followed by prophylactic administration of the antibiotic was more effective in restoring performance to normal.

The finding that copper supplementation, but not chlortetracycline or virginiamycin supplementation, tended to increase postweaning survival of pigs (Stahly et al., 1980) may also be related with the disease-control effect. Another evidence for this hypothesis is that antibiotics are effective in improving conception rate, litter size and in reducing the incidence of mastitis-metritis-agalactia syndrome in swine (Hays and Muir, 1979).

3.4. Metabolic effects

The metabolic effects resulting from feeding antimicrobials to animals, similarly to those described previously for copper, are not likely to be significant to the growth promotion obtained with these additives. However, according to Hays (1969), metabolic changes may be due to a disease-control effect, because the rate of metabolism may be influenced by systemic infections. That author reported literature data in which chlortetracycline affected water

and nitrogen excretion in pigs, suggesting influence on the metabolic rate, and tetracycline inhibited fatty acid oxidation by the mitochondria from rat liver.

The lack of support for this hypothesis can be further illustrated by the facts that antibiotics that are not absorbed or have limited absorption (Muir, 1985) provide similar magnitude of response in poultry and swine (Hays and Muir, 1979) as those that are absorbed.

MATERIALS AND METHODS

1. Experiment 1

This experiment was conducted to determine the effect of high dietary levels of copper on the concentration of glucose, ammonia and urea in the peripheral plasma of weanling pigs.

1.1. Experimental design and treatments

Twelve crossbred piglets were weaned at 24 days of age with an average weight of 7.4 kg. The pigs were not creep-fed during the nursing period. After weaning they were immediately transferred to cages in an environmentally controlled room, with temperature not lower than 28 C. For two days the piglets were maintained in pairs and had free access to feed and water. The unmedicated corn-soybean meal-dried whey experimental diet was offered during that period. After that, they were penned individually in elevated, mesh wire-bottomed, stainless steel pens (45 x 90 cm) and started receiving the dietary treatments. Each pig was randomly assigned to one treatment with the restrictions that litter of origin and sex were equalized between treatments. The treatments consisted of a basal diet and a similar diet in which 0.1% copper sulfate ($CuSO_4 \cdot 5H_2O$) replaced an equal

amount of corn (Table 3), to provide 250 ppm supplemental copper.

The initial weight of the piglets averaged 7.0 kg. All piglets received the same amount of feed, corresponding to about 3.5% of body weight per day. The daily ration was divided in 2 meals at 800 and 1800 hours. The feed was mixed with an equal amount of water to encourage rapid consumption. Additional water was added to the feeder to allow the pigs to clean the feed cup thoroughly.

On day 4 after initiation of the treatments, the pigs did not receive the morning meal and catheters were surgically implanted in the jugular vein. The catheters were made with polyvinyl tubes about 60 cm long, with an inner diameter of 1.5 mm. A 5 cm tip of silastic tubing was adapted to the polyvinyl tube. The catheters were treated twice a day for 2 days with TDMAC-heparin (Polysciences, Inc, Warrington, PA) that adheres to plastic surfaces and has antithrombogenic properties. After the last treatment with TDMAC-heparin, a tubing adapter was placed in each catheter followed by ethylene oxide gas sterilization.

Anesthesia was induced with intramuscular injection of acepromazine + atropine sulfate and was maintained with intravenous injection of sodium pentobarbital. In each pig a catheter was inserted in the jugular vein, directed towards the heart, and the vein was ligated in its cranial side. The catheters were passed under the subcutaneous adipose tissue and exteriorized dorsally. The external part of the catheter

Table 3. Composition of the experimental diets. Experiment 1 and 2.

Ingredients, %	Treatments	
	Basal	Basal + Cu
Corn, ground (IFN 4-02-935)	48.8	48.7
Soybean meal, 44 (IFN 5-04-604)	27.0	27.0
Whey, dried (IFN 4-01-182)	20.0	20.0
L-Lysine-HCl (IFN 5-08-022)	.15	.15
Monodicalcium phosphate (IFN 6-01-080)	1.20	1.20
Calcium carbonate (IFN 6-01-632)	.90	.90
Salt (IFN 6-04-152)	.20	.20
Vit.-Mineral premix[a]	.75	.75
Vit.E-Se premix[b]	1.00	1.00
$CuSO_4 \cdot 5H_2O$ (IFN 6-01-719)	-	.10[c]
Calculated lysine, %	1.20	1.20
Analysed values		
Exp.1: Ca, %	1.03	.97
P, %	.71	.67
Cu, ppm	18	260
Exp.2: Ca, %	1.12	1.12
P, %	.74	.73
Cu, ppm	20	275

[a] Supplying the following amounts per kg of diet: vit.A, 4950 IU; vit.D_3, 990 IU; menadione sodium bisulfite complex, 3.3 mg; riboflavin, 5.0 mg; d-pantothenic acid, 20 mg; niacin 27 mg; vit. B_{12}, 30 µg; choline chloride, 190 mg; zinc, 112 mg; iron, 90 mg; manganese, 5.6 mg; copper, 15 mg; iodine, 0.75 mg.

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

[c] Supplying 250 mg Cu per kg of diet.

was kept inside a patch sutured to the skin. Sterile (autoclaved at 121 C for 30 minutes) 3.5% sodium citrate was the anticoagulant used to maintain the catheters patent. The catheters were sealed with injection caps. Each pig received three intramuscular injections of oxytetracycline, with 2 day intervals between injections. The catheters were flushed daily with the sodium citrate solution.

Blood samples were taken on day 12. Each sample consisted of 5 ml of blood. The first sample was taken immediately before the morning meal, followed by samplings at 1, 2, 3, and 4 h after the beginning of the meal. The blood from the pigs that consumed all of their allotted feed was collected. The blood was transferred to tubes containing sodium heparin as anticoagulant, kept in an ice bath and centrifuged at 4 C within 20 min. Plasma was harvested and maintained in an ice bath until determination of ammonia, which was carried out on the same day. The samples were subsequently frozen at -20 C until analysed for glucose and urea.

1.2. Laboratory determinations

Ammonia concentrations in the plasma samples were determined by an enzymatic method using a commercial kit (Procedure 170-UV, Sigma Diagnostics, St.Louis, MO). The method is based on reductive amination of 2-oxoglutarate, using glutamate dehydrogenase (GLDH) and reduced nicotinamide adenine dinucleotide (NADH) as follows:

$$\text{2-Oxoglutarate} + NH_3 + \text{NADH} \xrightarrow{\text{GLDH}} \text{Glutamate} + \text{NAD}$$

A decrease in absorbance at 340 nm, due to the oxidation of NADH, is proportional to the plasma ammonia concentration. A spectrophotometer (Model 2400, Beckman Instruments, Fullerton, CA) with 1 cm lightpath cuvettes was used to record the absorbances.

The concentration of glucose in the plasma was determined using the Glucose (Trinder) kit (Procedure 315, Sigma Diagnostics, St. Louis, MO). The enzymatic reactions involved in the assay were as follows:

$$\text{Glucose} + H_2O + O_2 \xrightarrow{\text{Glucose Oxidase}} \text{Gluconic acid} + H_2O_2$$

$$H_2O_2 + \text{4 - Aminoantipyrine} + \text{p - Hydrobenzene sulfonate} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine dye} + H_2O$$

Glucose is first oxidized to gluconic acid and hydrogen peroxide, in the reaction catalysed by glucose oxidase. The hydrogen peroxide formed reacts in the presence of peroxidase with 4 - aminoantipyrine and p - hydrobenzene sulfonate to form a quinoneimine dye, with an absorbance maximum at 505 nm. The intensity of the color produced is directly proportional to the glucose concentration in the sample. A spectrophotometer (Gilford Instrument Laboratories, Inc., Oberlin, OH) was used to record the absorbances.

Plasma urea nitrogen concentration was determined using a blood urea nitrogen kit (Procedure 535, Sigma Diagnostics, St. Louis, MO). The method is based on the

direct interaction of urea with diacetyl monoxime at 100 C as follows:

Diacetyl monoxime + Urea----->Pink chromogen + Hydroxylamine

Urea concentration is directly proportional to the intensity of the color produced, which was recorded in a spectrophotometer (Gilford Instrument Laboratories, Inc., Oberlin, OH) at 530 nm.

1.3. Statistical analysis

The experimental design in this trial was a split-plot with repeated measurements in time. The linear model that describes the observed responses is:

$Y_{ijk} = M + T_i + A(i)j + P_k + (TP)_{ik} + E(ijk)$,

where Y_{ijk} = observed variable;

M = overall mean;

T_i = treatment effect, i = 1,2;

$A(i)j$ = effect of animal j within treatment i, j=1...n;

P_k = period (sampling time) effect, k = 1...5;

$(TP)_{ik}$ = interaction of treatments and periods;

$E(ijk)$ = residual error.

Analysis of variance and comparisons of means were performed according to Gill (1986). Comparisons of treatment means within sampling time were made using the Student t test, and comparisons of post-prandial means with the pre-feeding mean were made using Dunnett's t statistic.

2. Experiment 2

The effects of high dietary copper on portal and peripheral plasma concentration of glucose, ammonia and urea and the absorption of these substances by weanling pigs were studied in this experiment. The plasma levels of insulin and somatomedin-C were also determined.

2.1. Experimental design and treatments

Eight castrated male crossbred piglets were weaned at 28 days of age with an average weight of 8.5 kg. The pigs received creep feed during the last week of the nursing period. They were transferred after weaning to the same experimental facilities described for Experiment 1. The management of the animals was similar to that previously described. The average weight of the piglets on trial was 8.1 kg. The experimental diets were the same as those presented in Table 3.

The surgical implantation of the catheters was performed on days 4 to 6 after the initiation of the treatments. The catheters were prepared as described for Experiment 1. Anesthesia was induced with acepromazine + atropine sulfate or ketamine and was maintained with halothane via an endotracheal tube. Each pig had one catheter implanted in the jugular vein, as described previously, and one catheter in the hepatic portal vein. This catheter was introduced in the cranial mesenteric vein and reached the portal vein. Both catheters were exteriorized at the same site and protected with a patch.

Sodium heparin (10 units/ml) in sterile saline was the anticoagulant used. Each pig received two intramuscular injections of oxytetracycline.

After recovery from the surgery and when feed intake was normal (day 10) repeated simultaneous samples of peripheral and portal blood were taken via the catheters. Following a 16 h fast, the first sample was taken 30 min before the morning meal (120 g of feed). Subsequent blood samples were collected 1/2, 1, 1 2/3, 2 1/3, 3, 4, 6 and 8 h after the meal. Overall, about 60 ml of blood were drawn from each pig. Peripheral blood was separated into two tubes, one containing sodium EDTA and the other containing sodium heparin as anticoagulant. Portal blood samples were transferred to heparinized tubes. All tubes were kept in the ice bath and centrifuged at 4 C within 20 min; plasma was harvested and returned to the ice bath.

Heparinized plasma samples were used for ammonia, glucose and urea determinations and EDTA plasma samples were used in the insulin and somatomedin-C assays. Insulin was determined based on indications (Yen and Killefer, 1986; Shurson, 1986) that antimicrobial feed additives could increase blood glucose levels via increased absorption. Somatomedin C was assayed because its synthesis in liver in response to growth hormone is believed to be mediated by insulin (Spencer, 1985). The growth hormone receptors in the liver are known to be regulated, at least in part, by insulin.

Heparinized plasma was frozen at -70 C overnight, assayed for ammonia the next day and refrozen at -20 C until the other analyses. EDTA plasma was frozen at -20 C until analysed.

2.2. Laboratory determinations

Plasma levels of ammonia, glucose and urea nitrogen were determined as described for Experiment 1. Concentrations of insulin in plasma were determined with a commercial solid-phase radioimmunoassay kit (Diagnostic Products Corporation, Los Angeles, CA). Somatomedin-C was determined in plasma samples taken before the meal and 30 min, 3 h and 6 h after the meal. The SM-C radioimmunoassay kit (Nichols Institute Diagnostics, San Juan Capistrano, CA) was used for plasma somatomedin-C determinations.

2.3. Statistical analysis

Statistical analyses were conducted for the variables: concentration of ammonia, glucose and urea in portal and peripheral plasma and porto-peripheral difference of these substances, concentration of insulin and somatomedin-C in peripheral plasma. The experimental design and the linear model of Experiment 1 apply in this case, except that there were 4 periods of sampling for somatomedin-C and 9 for the other variables.

3. Experiment 3

This experiment was conducted to determine the effects of feeding high levels of copper to starter pigs on ammonia

and urea concentration in the contents of the intestinal tract of the animals. Also, estimates of the mitotic index (percent of cells in mitotic division) in the crypts of Lieberkühn were obtained in several sites along the intestinal tract of the pigs.

3.1. Experimental design and treatments

Twelve castrated male crossbred pigs were weaned at 30 days of age with an average weight of 8.3 kg. They were transferred after weaning to the same facilities described for Experiment 1. The pigs were paired according to litter of origin and initial weight, and each pig was randomly assigned to one of two treatments. The treatments consisted of a basal corn-soybean meal-dried whey diet with or without 0.1% $CuSO_4 \cdot 5H_2O$ supplementation (Table 4). The pigs had free-access to their respective diets and water for 5 days after weaning. Following this period they were pair-fed in two meals a day, as described for Experiment 4. Live weight was recorded weekly.

After 4 weeks on trial, the pigs, averaging 14.0 kg live weight, were sacrificed and samples were collected. The pigs were euthanatized by intravenous administration of T-61 Euthanasia Solution (Hoechst, Somerville, NJ) 3.5 after being fed a 270 g meal. The intestines were clamped at several points to prevent the flow of digesta within the gut and then removed intact from the carcass. The jejunum was divided into 3 sections of approximately equal length and the colon was divided into spiral and transverse colon. Gut

Table 4. Composition of the basal diet. Experiment 3 and 4.

Ingredients, %	
Corn, ground (IFN 4-02-935)	55.5
Soybean meal, 44 (IFN 5-04-604)	30.0
Whey, dried (IFN 4-01-182)	10.0
L-Lysine . HCl (IFN 5-08-022)	.15
Monodicalcium phosphate (IFN 6-01-080)	1.30
Calcium carbonate (IFN 6-01-632)	1.00
Salt (IFN 6-04-152)	.30
Vit.-Mineral premix[a]	.75
Vit.E - Se premix[b]	1.00
Calculated lysine, %	1.20
Analysed values	
Weeks 1-3: Ca, %	1.08
P, %	0.70
Cu, ppm[c]	25
Week 4: Ca, %	1.02
P, %	0.66
Cu, ppm[c]	25

[a] Supplying the following amounts per kg of diet: vit.A, 4950 IU; vit.D_3, 990 IU; menadione sodium bisulfite complex, 3.3 mg; riboflavin, 5.0 mg; d-pantothenic acid, 20 mg; niacin 27 mg; vit. B_{12}, 30 µg; choline chloride, 190 mg; zinc, 112 mg; iron, 90 mg; manganese, 5.6 mg; copper, 15 mg; iodine, 0.75 mg.

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

[c] The copper sulfate supplemented diets contained 265 ppm Cu.

contents were collected from each of the jejunum sections (proximal, medial and distal, respectively), cecum (at the blind end) and from each of the colon sections. Intestinal tissue samples were collected from each of these sites.

The contents from each intestinal site were collected into two air-tight plastic bags. One of the bags was kept in the ice bath and assayed the same day for ammonia and the other was immediately frozen by immersion in acetone-dry ice and stored at -20 C until analysed for urea. The tissue samples were rinsed and stored in buffered formalin (4.0 g sodium phosphate, 6.5 g disodium phosphate in 1000 ml of 10% formaldehyde solution) for fixation.

3.2. Laboratory procedures

To determine ammonia nitrogen concentration, the fresh intestinal contents were diluted about 1:10, mixed well, centrifuged at 5,000 x g at 4 C for 15 min and the supernatant was harvested for the assay. The procedure of Chaney and Marbach (1962) was utilized, with some modifications. This procedure consists of a catalysed conversion of ammonia to indophenol, producing a stable blue color. Briefly, to 0.4 ml of the sample were added 2.5 ml of solution 1 (10.0 g phenol and 50 mg sodium nitroprusside/l) and 2.5 ml of solution 2 (5.0 g sodium hydroxide and 420 mg sodium hypochlorite/l). Absorbance was recorded after 30 min at room temperature, using a spectrophotometer (Gilford Instrument Laboratories, Inc., Oberlin, OH) at 625 nm.

Ammonium sulfate was used as standard and results were calculated as μg ammonia N/g fresh material.

To determine urea nitrogen concentration, the frozen samples were thawed, diluted about 1:4, mixed well and centrifuged at 7,000 x g at 4 C for 15 min. The assay was carried out using the blood urea nitrogen kit described for Experiment 1. The jejunum samples were processed without deproteinization, and the cecum and colon samples were deproteinized following the procedures of the kit. The results were calculated as μg urea N/g fresh material.

The intestinal tissue specimens were processed for histologic examination. They were trimmed, imbedded in paraffin and 8 μm sections were mounted on glass slides and stained with eosin and hematoxylin. The specimens were cut transversely to the intestinal wall, so that the crypts were sectioned longitudinally. The slides were examined under the microscope at 450X magnification. First, an area of the specimens where most of the crypts could be viewed along all their lengths was selected at lower magnification (100X). Then, at least 600 enterocytes of adjacent crypts were counted in the selected area. The mitotic index, a measurement of intestinal epithelium proliferation, was calculated as the percentage of crypt enterocytes undergoing mitotic division.

3.3. Statistical analysis

The experimental design in this trial was split-plot with repeated measurements in space (sites along the

intestinal tract). The linear model for this type of experiment is:

$Y_{ijk} = M + T_i + A(i)_j + S_k + (TS)_{ik} + E(ijk)$, where

Y_{ijk} = observed variable;

M = overall mean;

T_i = effect of treatment i, i = 1,2;

$A(i)_j$ = effect of animal within treatment, j=1,...,6;

S_k = effect of sites, k=1,...,6;

$(TS)_{ij}$ = effect of treatment x site interaction;

$E(ijk)$ = residual error.

Analysis of variance was conducted according to Gill (1986) and comparisons of treatments within sites were made using the Student t test.

4. Experiment 4

A balance trial over three collection periods was conducted to determine the effects of high dietary copper on the utilization of energy, crude protein, calcium, phosphorus and copper by starter pigs.

4.1. Experimental design and animals

The same animals and treatments used in Experiment 3 were used in the balance trial. To ensure that the composition of the experimental diets differed only in the copper content, only one batch of the basal diet was mixed in a small mixer. Half of the batch was removed and copper sulfate was added to the other half to supply 250 ppm Cu and mixed again. Beginning on day 6 after weaning, the pigs were

placed individually in stainless steel collection cages (55 x 70 x 76 cm). At this time a meal-feeding program started and was maintained until the end of the experiment. The two experimental diets were pair-fed in two meals a day in amounts averaging 3.9, 3.8 and 3.8% of body weight during the three collection periods, respectively. The pigs were weighed weekly, and weight gain and feed efficiency were calculated.

At each feeding time the pigs were removed from the collection cages and placed in a separate feeding pen to prevent feed contamination of the feces and urine. To encourage rapid consumption of the diets, which were prepared with finely ground corn, they were mixed with an equal amount of water immediately before being offered. Additional water was added to allow the pigs to clean the feed cups thoroughly. The pigs were then transferred back to their respective collection cages. Any spills or refusals of feed were collected in an aluminum tray placed under the feeding pen, air-dried and deducted from the allowance. No feces or urine were lost at any feeding period.

After a 5-day adaptation period, the cages were cleaned and total collections of feces and urine were made during 3 days. Following the first collection period, feces and urine were collected during two more 3-day periods, after the pigs were adapted for 4 days to the new feeding level. The amount of diet fed was maintained constant for each pair of pigs

during the collection period and for at least three meals (about 36 hours) prior to the collection period.

Within the cages, feces were collected on a fine wire screen beneath the stainless steel slotted floor. Urine was drained from a stainless steel tray located beneath the wire screen into plastic containers. Approximately 50 ml of 6N hydrochloric acid were added to the urine containers, before starting the collections, to prevent microbial degradation. Feces were collected daily and stored frozen in plastic bags.

At the end of each collection period, urine was filtered through glass wool, the volume recorded and a 100 ml aliquot was stored in an air tight plastic bottle at 4 C for subsequent laboratory analyses. The daily fecal samples were pooled, dried in an oven at 60 C for 2 days, weighed and ground using a Wiley mill for subsequent analyses.

4.2. Laboratory procedures

Feed, feces and urine samples were subjected, in duplicate, to the following determinations: gross energy, nitrogen, calcium, phosphorus and copper. Gross energy was determined using an adiabatic bomb calorimeter (Parr Instrument Co., Moline, IL). Prior to the energy measurements, about .7 g of feed or feces were pelleted and 5 ml aliquots of urine were placed on cotton balls of known weight and freeze dried.

The samples for nitrogen determinations were digested using the semi-micro Kjeldahl method (AOAC, 1984) and the

nitrogen was quantified in an auto-analyser (Technicon II, Tarrytown, NY). About .35 g and .28 g of feed and feces, respectively, and .8 ml of urine were used in the digestion and diluted to 100 ml in a volumetric flask.

Samples for determination of minerals were prepared by wet digestion in Phillips beakers on a hot plate with nitric acid and perchloric acid. About 1.0 g and .3 g of feed and feces, respectively, and 10 ml of urine were sampled for digestion. Calcium, after diluting with $SrCl_2$ to prevent matrix interference, and copper were determined by flame atomic absorption spectrometry (Model 951, Instrumentation Laboratory, Inc., Lexington, MA), after appropriate dilutions of the digested samples. Phosphorus was determined colorimetrically according to Gomorri (1942), the absorbance being recorded in a spectrophotometer (Gilford Instrument Laboratories, Inc., Oberlin, OH).

The laboratory determinations were used to estimate the following variables:

1. Digestible energy % (DE %) = $\frac{\text{GE Intake - Fecal GE}}{\text{GE Intake}} \times 100$

2. Metabolizable energy % (ME %) = $\frac{\text{GE Int.-Fec.GE-Urin.GE}}{\text{GE Intake}} \times 100$

3. Nitrogen digestibility % (ND %) = $\frac{\text{N Intake-Fecal N}}{\text{N Intake}} \times 100$

4. Nitrogen retention % (NR %) = $\frac{\text{N Int.-Fec.N-Urin. N}}{\text{N Intake}} \times 100$

5. NR/ND % = $\frac{\text{NR\%}}{\text{ND\%}} \times 100$

6. Mineral digestibility % = $\frac{\text{Mineral Int.-Fecal Mineral}}{\text{Mineral Intake}} \times 100$

7. Mineral retention % = $\frac{\text{Min.Int.} - \text{Fec.Min.} - \text{Urin.Min.}}{\text{Mineral Intake}} \times 100$

where GE is gross energy and Mineral is either calcium, phosphorus or copper. The calculations were based on the values obtained for each animal on each 3-day collection period.

4.3. Statistical analysis

This experiment was performed in a randomized complete block design, with each pair of pigs as a block. The linear model for this type of experiment is ,

$Y_{ij} = M + T_i + B_j + E_{(ij)}$,

where Y_{ij} = observed variable;

M = overall mean;

T_i = fixed effect of treatment i, i=1,2;

B_j = fixed effect of block j, j=1...6;

$E_{(ij)}$ = residual error.

Analysis of variance was conducted according to Gill (1978). When the blocking procedure was not efficient, the sum of squares and degrees of freedom for the effects of blocks and residual error were pooled and a pooled error mean square was calculated.

RESULTS AND DISCUSSION

1. Experiments 1 and 2

The results and discussion of Experiments 1 and 2 are presented in this section because both experiments had similar designs and blood determinations were concerned with common variables.

1.1. General observations

Some of the piglets in Experiment 1 did not return to normal levels of feed intake after the surgery, probably due to the development of infection subsequent to the surgery. The pigs that did not consume the allotted feed on the day of the blood sampling were deleted from the experiment. Thus, blood samples were collected from three pigs on the basal diet treatment and from four pigs on the high copper treatment. In Experiment 2 the jugular catheter of one of the pigs and the portal catheters of two pigs lost the function before the day when the blood samples were drawn. Thus, peripheral blood samples were collected from seven pigs and portal samples from six pigs.

It must be taken into consideration that the surgeries in these studies were carried out a few days after the weaning of the piglets at about 4 weeks of age. This may have an effect on infection and loss of function of the

catheters because the production of antibodies is small until 4-5 weeks of age (Pond and Houpt, 1978) and may be decreased as a consequence of weaning. In most of the reports in the literature in which blood vessels were catheterized, the pigs weighed above 30 kg. The main reason for using weanling pigs in this study was because the response to copper supplementation has been shown to be greater following the weaning of piglets.

1.2. Glucose

The peripheral plasma glucose concentrations of the pigs fed the two diets in Experiment 1 are shown in Figure 1. No significant changes ($P>.10$) were observed by feeding the experimental diet supplemented with 250 ppm Cu, at any of the sampling times, up to 4 h after the meal. Compared to the fasting level (concentration before the meal), the plasma glucose concentrations of the pigs on both treatments were increased ($P<.05$) following the meal, but the increase did not reach significance ($P>.05$) at 2 h after the meal for the pigs fed the basal diet. No treatment x sampling time interaction was detected.

In Experiment 2 there were also no differences in peripheral plasma glucose between treatments ($P>.10$) at any sampling time (Figure 2). The pigs receiving the high dietary copper treatment tended to have lower glucose concentrations than pigs fed the basal diet up to 4 h after the meal and higher levels thereafter. This led to a treatment x time interaction ($P<.01$). Compared to the

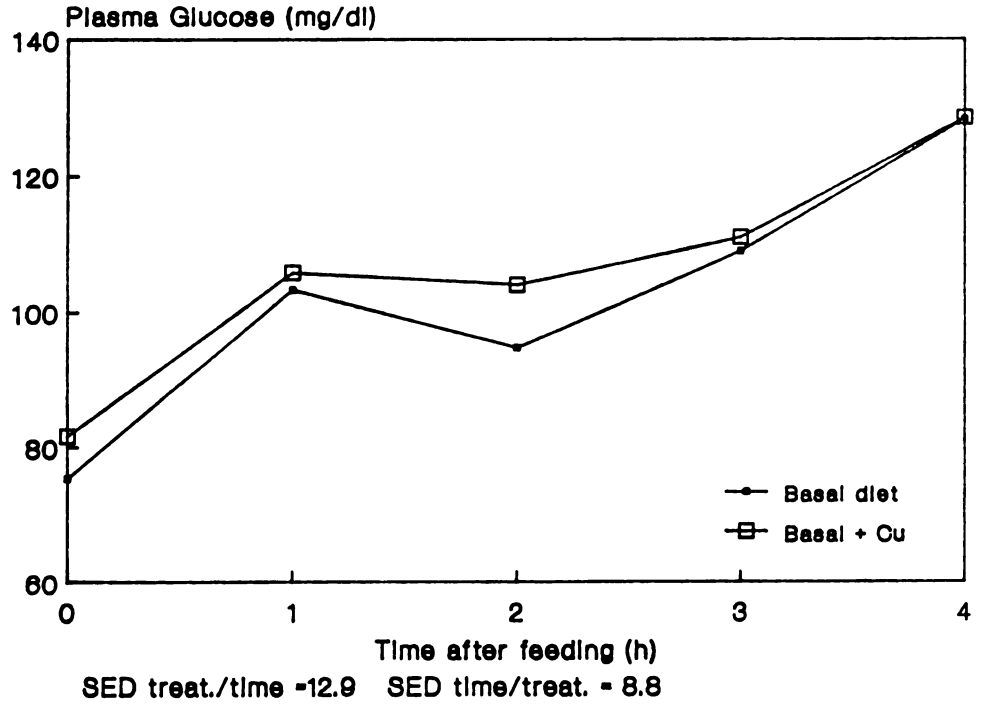

Figure 1. Glucose concentration in peripheral plasma, mg/dl. Experiment 1.

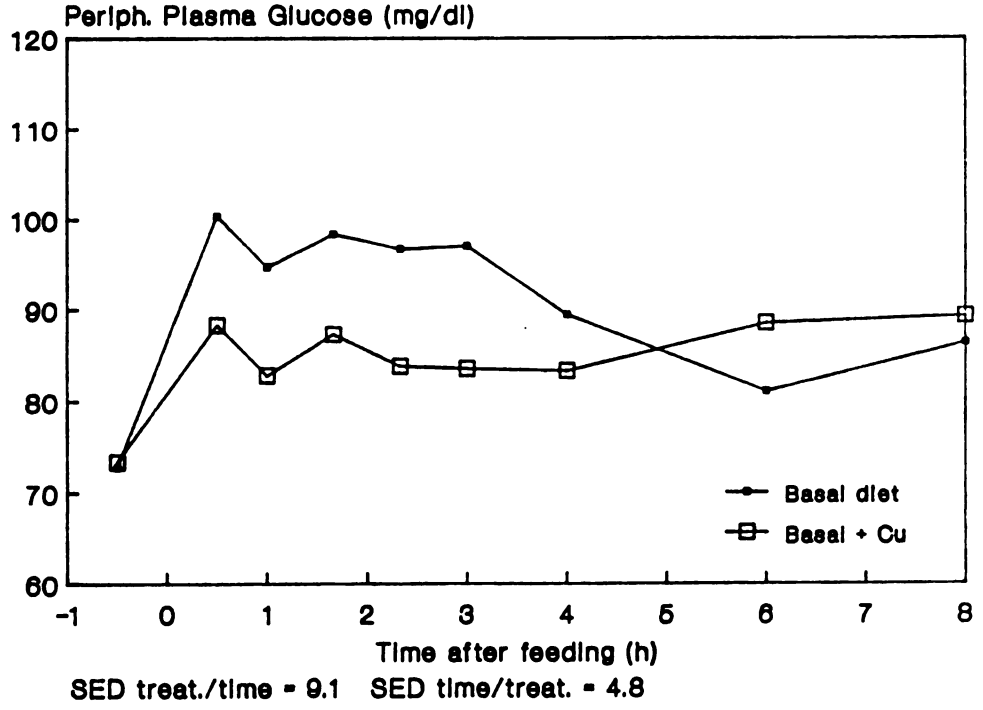

Figure 2. Glucose concentration in peripheral plasma, mg/dl. Experiment 2.

fasting glucose level, the peripheral plasma glucose values of the pigs on the control diet were higher ($P<.01$) up to 4 h after the meal but not after that. For the high copper treatment, on the other hand, glucose levels were higher ($P<.01$) than the fasting level at 1/2, 1 2/3, 6 and 8 h after the meal but not at the other sampling times.

The portal plasma glucose concentrations of the pigs fed the two diets were not different ($P>.10$) at any sampling time, although pigs fed the high copper diet tended again to have numerically lower values (Figure 3). Glucose concentrations were maintained above the basal levels ($P<.05$) up to 2 1/3 and 3 h after the meal for the high copper and the control treatments, respectively. Treatment x time interaction was not significant ($P>.10$). The porto-peripheral differences, as estimates of glucose absorption, of the two treatments are presented in Figure 4. The copper-fed pigs had a higher porto-peripheral glucose difference at 2 1/3 h after feeding ($P<.05$), although no treatment effect was detected ($P>.10$) at any other time. The post-prandial changes in porto-peripheral glucose differences relative to the fasting level were not significant ($P>.05$) for treatments. No treatments x time interaction was detected ($P>.10$).

The double-catheter technique utilized in Experiment 2 permits a chronological and qualitative study of absorption of nutrients or other metabolites. In addition, in order to quantitatively determine the absorption of a substance,

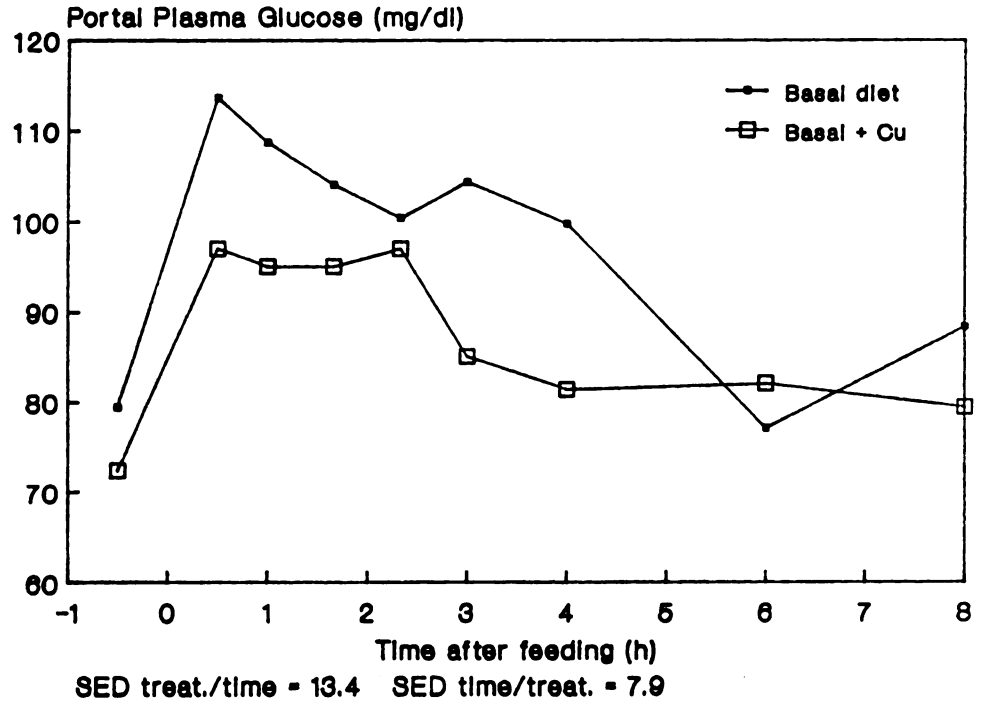

Figure 3. Glucose concentration in portal plasma, mg/dl. Experiment 2.

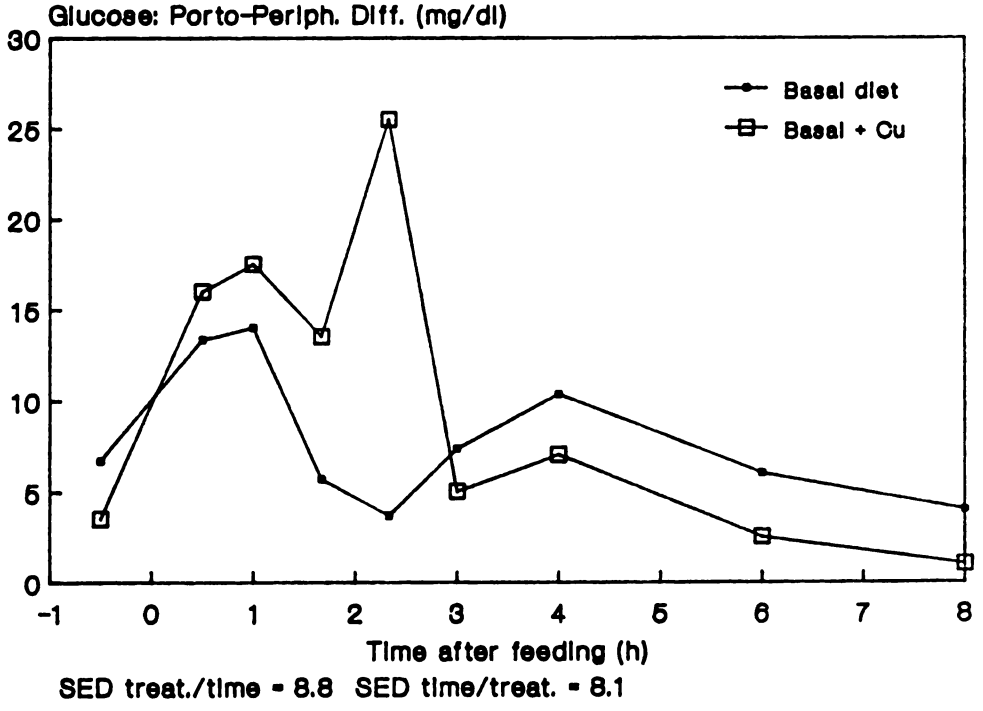

Figure 4. Porto-peripheral glucose difference, mg/dl. Experiment 2.

measurements of the blood flow rate in the portal vein would be necessary. By multiplying the porto-peripheral difference of the concentrations of the substance by the blood flow rate, the instantaneous absorption rate can be determined. Rérat et al. (1980) discussed the techniques utilized for portal blood flow rate measurements, which require complex instrumentation. For comparative purposes, however, the porto-peripheral difference alone is a good indication of absorption, principally, if the assumption that the flow rate is not affected by treatments is correct. In fact, Yen and Killefer (1986) did not detect differences in portal vein blood flow rate when gilts received carbadox-supplemented or control diets. Malmlof (1987) also assumed that the portal blood flow in growing pigs was not differently affected by treatments in a study involving high- and low-fiber diets.

The pattern of variation in portal or peripheral plasma glucose concentration during the post-prandial period observed in Experiment 2 could be expected based on work by Aumaitre et al. (1973). They sampled blood from the jugular and portal veins of pigs every 5 min for determination of reducing sugars over a period of 8 h after glucose or corn starch meals. They established theoretical curves for portal and peripheral glycemic levels, characterized by seven or eight peaks which occurred simultaneously in both veins. The range of the peaks was about 25 mg/dl for the jugular blood and more than that for the portal blood. In the present

study, the reduced number of sampling points did not allow reproduction of peaks at regular intervals. Also, the individual variations in glycemic values and the time of the peaks after the meal, as recognized by Aumaitre et al. (1973) and Rérat et al. (1977), will tend to mask the shape of the curves and increase the experimental error associated with the measurements at each sampling time.

Despite the fact that peaks and valleys cannot be clearly identified with few sampling points, Rerat et al. (1980) showed that when the quantitative absorption of glucose was calculated based on samples taken at 5 min, 30 min or 1 h intervals after the meal, only small differences were observed. They suggested that 12 to 16 sampling times would be necessary to study absorption following a meal. In the present study the number of sampling points was limited by the volume of blood that could be safely withdrawn during the day. Because of the size of the pigs, no more than 60 ml of blood were collected from each pig, while Rerat et al. (1980) reported that 250 ml or more were removed over an 8 h period in the experiments carried out by their group.

In this study, the pigs were fed practical diets containing 20% dried whey. Considering that lactose makes up about 60% of the dried whey, the lactose represented about 12% of the diets. Rérat et al. (1977) determined that after feeding a lactose-based meal to pigs, only five peaks of blood sugar concentration were observed during the 8 h experimental period and the peaks were flatter. This was

attributed to the differences in the time that the peaks occur for lactose or starch and glucose. Therefore, fewer peaks and a smaller range of variation between the maxima and the minima could be expected in the present study. Rérat et al. (1977) also showed that lower levels of plasma glucose than reducing sugars were recorded after the lactose meal.

The factors discussed above provide an explanation for the non-significant increments in peripheral glucose levels at some points in the early post-prandial period observed in Experiments 1 and 2 (Figures 1 and 2). In fact, the points of minima of reducing sugars in the theoretical curve developed by Aumaitre et al. (1973) correspond to values very close to the fasting levels. The pattern of portal plasma glucose (Figure 3) was similar to that reported by Aumaitre et al. (1973) where the concentration was elevated for about 3 h after a 400 g starch-based meal and then dropped. Experiment 2 was not sufficiently sensitive to detect changes in glucose absorption induced by the meal, although increases of up to 20 mg/dl were measured.

High dietary copper did not promote increases in peripheral or portal plasma glucose concentration in these experiments, which differs from the findings of Shurson (1986). In that study, copper-fed pigs had glucose levels about 15 mg/dl and 24 mg/dl higher than controls in the peripheral and portal blood, respectively, when samples were taken 3 1/2 h after the meal. Vervaeke et al. (1979)

demonstrated that antibiotics (virginiamycin and spiramycin) promoted sparing of glucose in vitro and Yen and Killefer (1986) found that glucose absorption was increased in swine receiving supplemental carbadox. The higher glucose absorption rate for the high copper treatment detected at 2 1/3 h after the meal (Figure 4) may be an erratic value since the treatment differences were small at all the other times.

1.3. Ammonia and urea

In Experiment 1, the high copper treatment affected plasma ammonia concentration ($P<.05$) resulting in lower ammonia levels ($P<.01$) at 4 h after the meal and not at the other times (Figure 5). Although slight increases in ammonia concentration were noticed in both treatments at 1 h after the meal, increase over the fasting level was significant only at 4 h in the plasma of the control pigs ($P<.01$). No differences in plasma urea nitrogen (Figure 6) due to treatments were detected ($P>.10$). Compared to the basal level, plasma urea was depressed ($P<.01$) in the copper-fed pigs, but not in the control pigs, at 1 h after the meal and returned to levels close to the basal after that.

In Experiment 2, peripheral plasma ammonia concentration of the pigs fed high copper was lower ($P<.10$) than in the unsupplemented pigs at 1 h after feeding (figure 7). The differences were not significant at the other times. For both treatments, the post-prandial ammonia levels remained stable and unchanged in relation to the basal

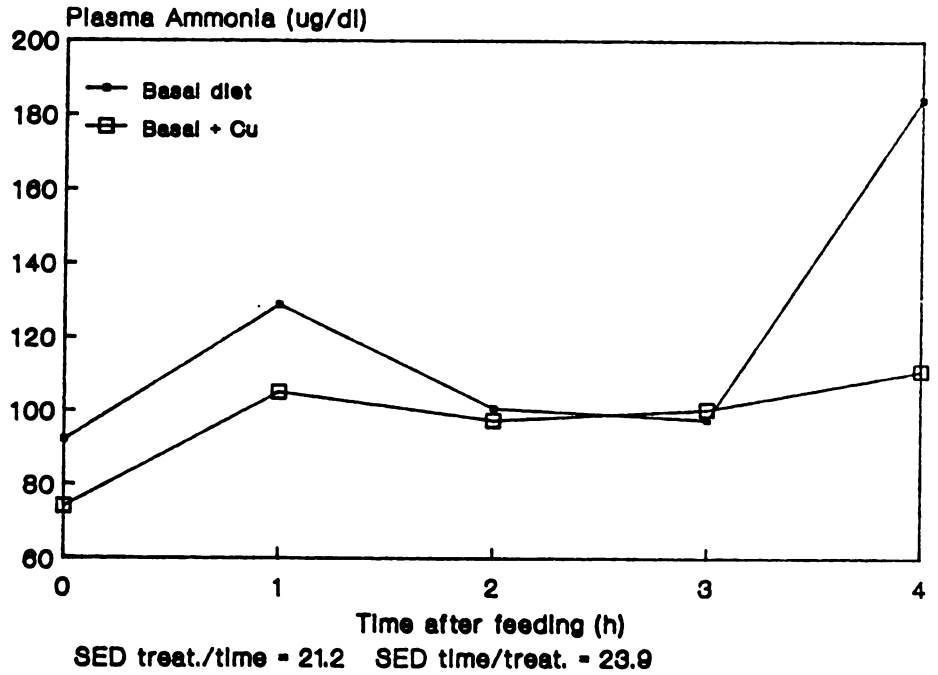

Figure 5. Ammonia concentration in peripheral plasma, µg/dl. Experiment 1.

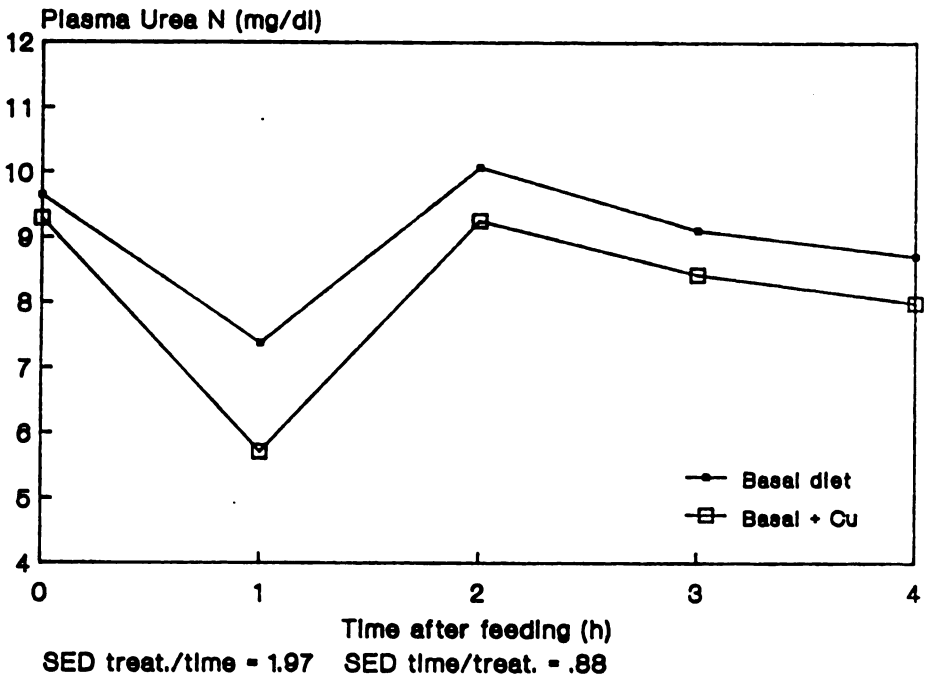

Figure 6. Urea nitrogen concentration in peripheral plasma, mg/dl. Experiment 1.

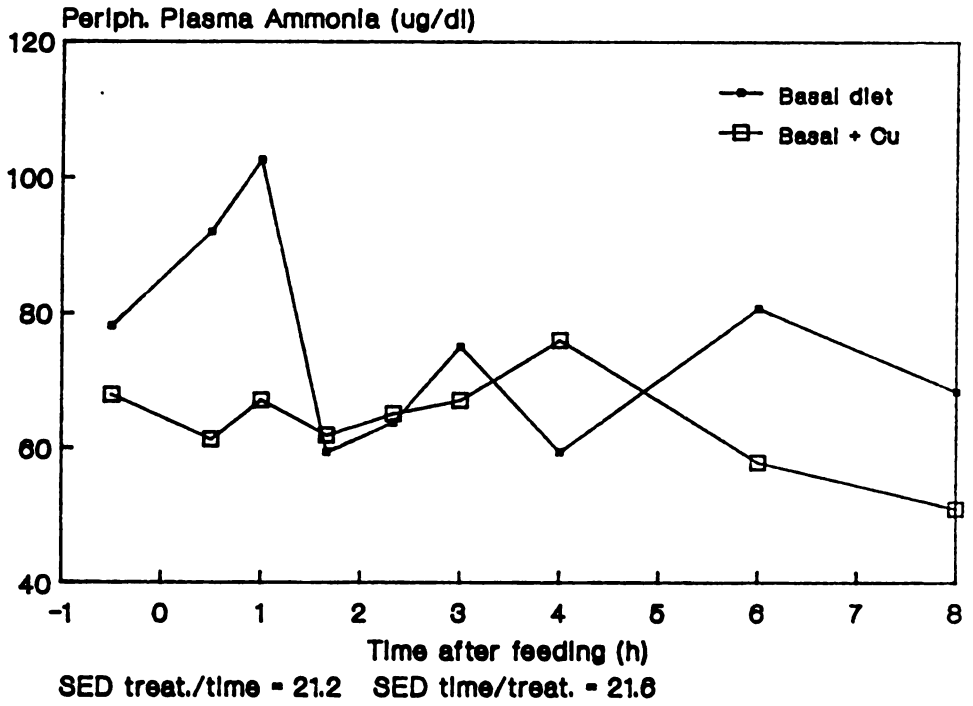

Figure 7. Ammonia concentration in peripheral plasma, µg/dl. Experiment 2.

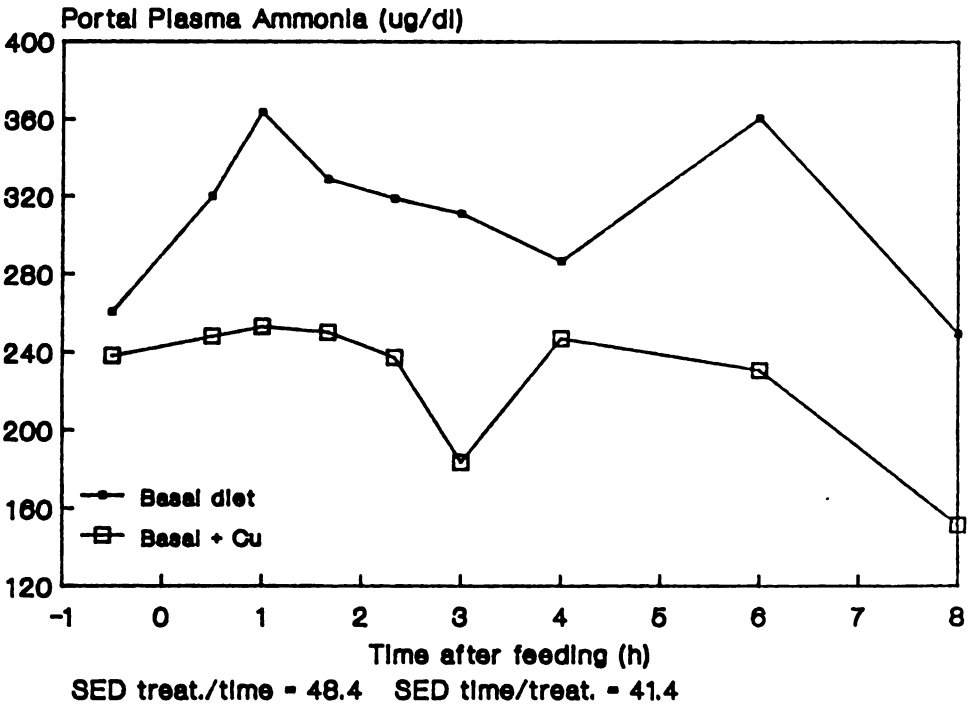

Figure 8. Ammonia concentration in portal plasma, µg/dl. Experiment 2.

levels ($P>.05$), except for the tendency for increased concentration in the control pigs in the first hour after the meal.

High dietary copper resulted in an overall decrease ($P<.05$) of the ammonia concentration in the portal plasma of piglets, as compared to the control treatment (Figure 8). Treatment comparisons at each sampling time showed that the differences occurred at 1, 3, 6 h ($P<.05$) and 8 h ($P<.10$) after feeding. Portal plasma ammonia did not increase after the meal in the copper-fed pigs and although increases over the fasting level of more than 100 μg/dl were recorded for the control pigs, these differences were not significant ($P>.05$).

The porto-peripheral differences in ammonia concentration were lower ($P<.10$) in the high copper treatment (Figure 9), specifically at 3, 6 and 8 h ($P<.10$) after the meal. The changes induced by the meal in this variable, reflecting ammonia absorption, were not significant ($P>.05$).

The results of urea nitrogen concentration in peripheral and portal plasma and porto-peripheral differences in Experiment 2 are presented in Figures 10, 11 and 12, respectively. There were no treatment differences in peripheral or portal urea, at any sampling time ($P>.10$), although urea levels tended to be higher in the portal plasma of the copper-fed pigs. The porto-peripheral differences, indicating absorption of urea, were higher in

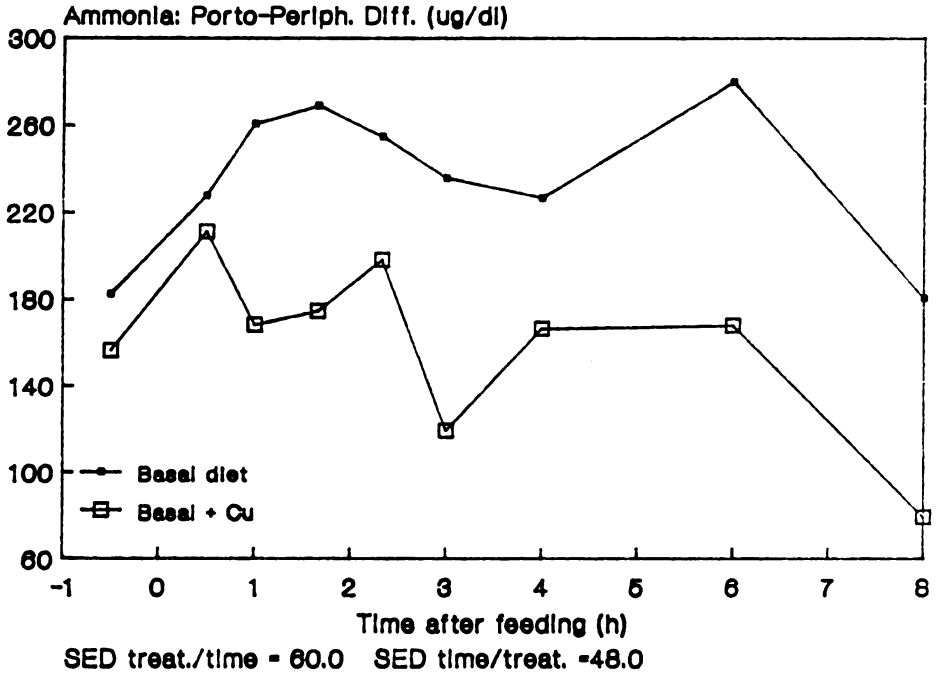

Figure 9. Porto-peripheral ammonia difference, µg/dl. Experiment 2.

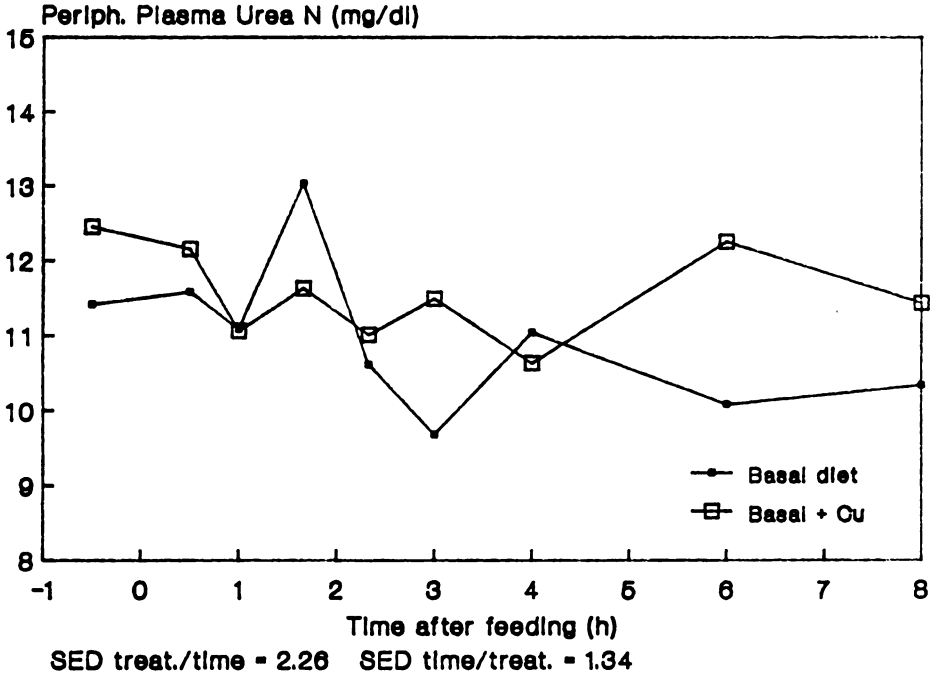

Figure 10. Urea nitrogen concentration in peripheral plasma, mg/dl. Experiment 2.

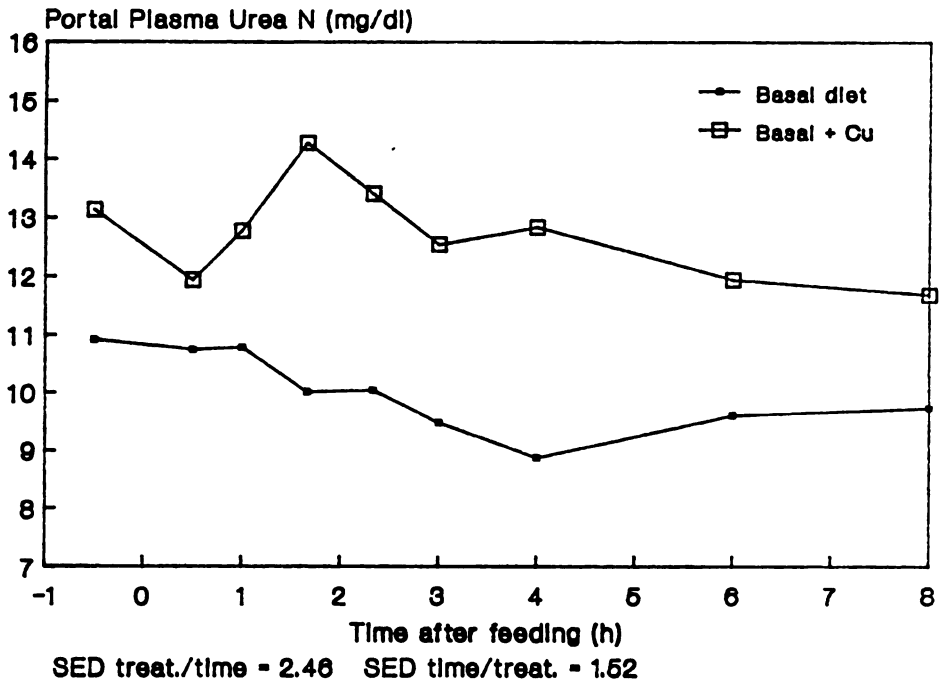

Figure 11. Urea nitrogen concentration in portal plasma, mg/dl. Experiment 2.

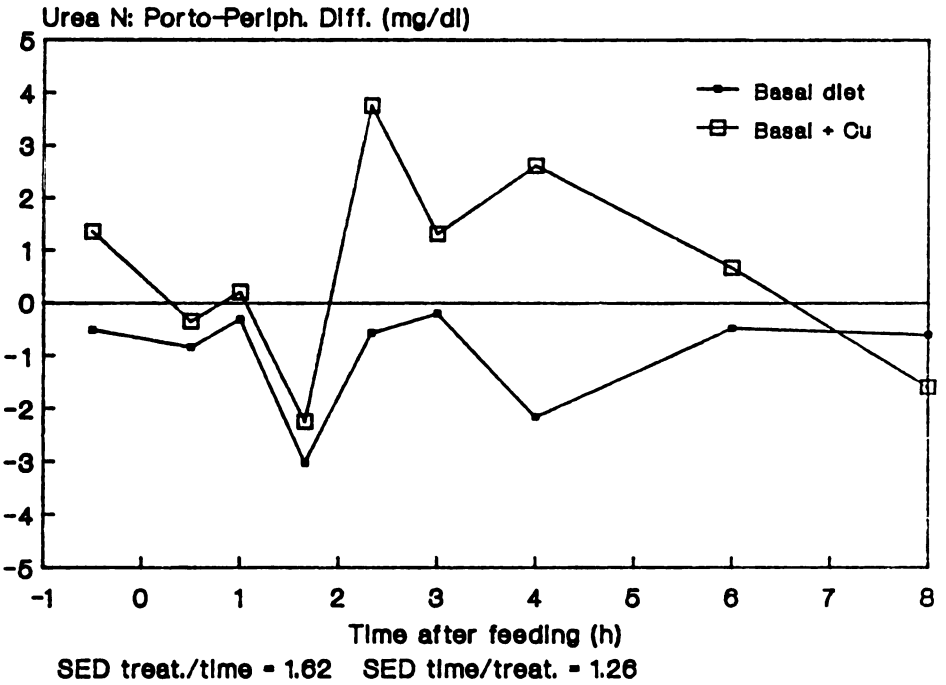

Figure 12. Porto-peripheral urea nitrogen difference, mg/dl. Experiment 2.

the pigs on the high copper treatment than in the controls at 2 1/3 and 4 h after the meal ($P<.10$). Relative to the basal levels there were no post-prandial changes in any of these variables for either treatment ($P>.05$).

Although the concentration of ammonia in peripheral blood is considered to be closely stabilized by the liver via the urea cycle and other pathways (Visek, 1984), there are some reports of increased peripheral ammonia in swine following a meal. Malmlof (1987) showed that growing gilts had an increase in arterial plasma ammonia about 1 h after a meal and Rerat and Aumaitre (1971) found higher ammonia level in the venous blood approximately 3 h after feeding. Therefore, the pattern of peripheral ammonia concentrations obtained in this study (Figure 5 and 7) is in agreement with the literature. In both experiments the tendency for elevated ammonia was not seen in the plasma of the copper-fed pigs, although it is not likely that the changes observed in the control pigs were of sufficient magnitude to have detrimental effect on the metabolism.

Because the detoxification of ammonia in the liver probably assumes greater importance with the change in diet after weaning, the possibility that an increased ammonia load would exceed the detoxification capacity was also examined. In a preliminary experiment, ammonia in peripheral blood sampled 3 h after a meal for several days after piglets were weaned did not consistently change relative to the pre-weaning levels. Feeding a 250 ppm Cu diet also did

not affect the results. Shurson (1986), however, found that feeding 250 ppm Cu to pigs significantly reduced ammonia in peripheral blood sampled 3 1/2 h after a meal.

The basal ammonia concentration in portal plasma obtained in this study was similar to that reported for swine in the literature (Rérat and Aumaitre, 1971; Malmlof, 1987; Drochner et al., 1987). Rérat and Aumaitre (1971) found a 250% increase in portal ammonia concentration 3 h after a meal; Malmlof (1987) found a 60% increase occurring in the first hour after the meal; Drochner et al. (1987) found a 100% increase about 6 h after the meal. In the present study, portal ammonia concentrations in the control pigs were about 40% higher than the basal level at 1 h and 6 h after the meal (Figure 8). In the pigs fed high copper, on the other hand, there was no tendency to increase portal ammonia concentrations. The lower portal ammonia obtained in the copper-fed pigs ($P<.05$) is a clear indication of decreased production of ammonia in the intestinal tract. This may be due to the antimicrobial effect of copper, especially on ureolytic bacteria. The inhibitory effect of copper on bacterial urease may also be involved.

Ammonia concentration in the portal plasma in the present study was about fourfold the concentration in the peripheral plasma, while in the studies by Rérat and Aumaitre (1971) and Malmlof (1987) it was 10-fold and twofold, respectively. The absorption of ammonia through the portal vein as estimated by porto-peripheral differences,

in the pigs fed the control diet in this study, (Figure 9) had the same pattern and magnitude as in the report by Malmlof (1987). Rérat et al. (1986) measured quantitatively the absorption of ammonia in pigs and observed a significant increase with the time after a meal. Conversely, there was no evidence for increased ammonia absorption following the meal in the copper-fed pigs in this experiment and, compared to the control pigs, the absorption was decreased ($P<.10$) by about 30%.

In this study the high copper treatment resulted in decreased portal ammonia concentration ($P<.05$) and reduced ammonia absorption ($P<.10$). The levels of both variables in the copper-fed pigs corresponded to about 70% of those in the control pigs. Portal ammonia level of germ-free guinea pigs was 25% (Warren and Newton, 1959) of that of controls and in germ-free pigs portal ammonia was 40% (Shurson, 1986) of the level found in conventional pigs. High doses of neomycin in the feed greatly reduced portal ammonia in dogs (Silen et al., 1955). Although the use of several antibiotics at low doses have been shown to reduce ammonia production in the intestinal tract (Visek et al., 1959; Holtzman and Visek, 1965; Dierick et al., 1986b), the concentration of ammonia in portal blood was not determined in those studies. Yen and Killefer (1986) did not detect changes in ammonia absorption due to carbadox supplementation of pigs, and Shurson (1986) did not observe an effect of high dietary copper on portal ammonia levels.

These latter studies do not agree with the results of Experiment 2.

In Experiments 1 and 2 the peripheral and portal levels of urea were not increased after the meal (Figures 6, 10 and 11), whereas other studies indicated a slight increase in blood urea at about 4 h after a meal (Rérat and Aumaitre, 1971; Malmlof, 1987; Drochner et al., 1987). Rérat and Aumaitre (1971) and Rérat et al. (1986) consistently found that urea concentrations in portal blood was lower than in peripheral blood, as a result of secretion of urea into the small intestine. However, an absorption of urea via the portal vein was determined by Yen and Killefer (1985) and virtually no absorption or intestinal uptake was reported by Malmlof (1987). In the present study (Figure 12) the tendency was for intestinal uptake of urea in the control pigs and absorption of urea in the copper-fed pigs. The treatment differences were significant, however, at only two sampling points in the experimental period ($P<.10$). The fact that urea was absorbed by the pigs can be explained by the influx of urea from the bile in the duodenum, as suggested by Dierick et al. (1986b), as well as by the contribution of the saliva, and by a decreased ureolytic activity in the intestines of copper-fed pigs. This was confirmed by the lower ammonia absorption observed in the pigs on the high copper treatment.

Silen et al. (1955) demonstrated that the colon of dogs contributed the greatest proportion of ammonia appearing in

These latter studies do not agree with the results of Experiment 2.

In Experiments 1 and 2 the peripheral and portal levels of urea were not increased after the meal (Figures 6, 10 and 11), whereas other studies indicated a sharp increase in blood urea at about 4 h after a meal (Yeh and Aumaitre, 1971; Malmlof, 1987; Decuypere et al., 1987). Yeh and Aumaitre (1971) and Nery et al. (1988) consistently found that urea concentrations in portal blood was lower than in peripheral blood, as a result of secretion of urea into the small intestine. However, an absorption of urea via the portal vein was determined by Yen and Kilgore (1988) and virtually no absorption or intestinal uptake was reported by Malmlof (1987). In the present study (Figure 12) the tendency was for intestinal uptake of urea in the control pigs and absorption of urea in the copper-fed pigs. The treatment differences were significant, however, at only two sampling points in the experimental period (P<.10). The fact that urea was absorbed by the pigs can be explained by the influx of urea from the bile in the duodenum, as suggested by Dierick et al. (1988b), as well as by the contribution of the saliva, and by a decreased urealytic activity in the intestines of copper-fed pigs. This was confirmed by the lower ammonia absorption observed in the pigs on the high copper treatment.

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the portal vein. However, the increased ammonia levels in the portal vein of the control pigs (Figure 8) observed in the first hour after the meal suggests that the absorption was occurring in the small intestine. Similar findings were presented by Malmlof (1987). Radecki et al. (1988) also reported evidence for higher ureolytic activity in the small intestine than in the lower gut of pigs. Visek (1978) considered urea hydrolysis as the major source of intestinal ammonia. On the other hand, Dierick et al. (1986b) calculated that most of the ammonia produced in the small intestine of pigs was derived from deamination of amino acids and not from ureolysis. Therefore, the improved performance of swine obtained by feeding antimicrobials, such as copper, could be related to both sparing of amino acids and reduction in the formation of potentially toxic ammonia.

Rérat et al. (1986) fed urea to pigs and determined that 64 to 73% was absorbed, according to the level of ingestion. Drochner et al. (1987) infused urea in the cecum of mini-pigs which resulted in increased portal urea nitrogen levels. In both studies an increased absorption of ammonia followed the administration of urea to the animals. When high levels of urea (2.5 to 3.4% of the diet) were fed to pigs (Kornegay et al., 1964) there was an increase in peripheral plasma urea, but no change in peripheral ammonia was observed. Considering that the expected increase in

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portal ammonia occurred, the above results demonstrate that the liver efficiently metabolized the ammonia.

Drochner et al. (1987) observed that diarrhea occurred when portal plasma ammonia levels reached 1400 µg/dl and Drochner (1987) determined that, after cecal infusion of urea, low dry matter of the feces occurred in parallel to high fecal ammonia values. The authors interpreted these findings as a defense mechanism that allows elimination of the excess ammonia from the intestinal tract and lowering of portal ammonia. Furthermore, Drochner et al. (1987) demonstrated, using everted sacs of rat colon, that the water flow through the gut wall was diminished by increased ammonia on the mucosal side. It can be speculated at this point that the effect of antimicrobial feed additives in preventing scours in pigs (Visek, 1978) may be related to the decrease in ammonia produced in the intestinal tract.

Payne (1977) indicated that ammonia may be responsible for lesions of the endothelium of the micro blood vessels and has detrimental effects for the enzymes of the liver. Thus, high dietary copper, by reducing ammonia in the portal blood, as found in this study, may have a protective action against the effects of ammonia described by Payne (1977).

1.4. Insulin and somatomedin-C

The results of insulin and somatomedin-C levels in the plasma of pigs in Experiment 2 are presented in Figures 13 and 14, respectively. There were essentially no treatment differences in plasma insulin during the entire experimental

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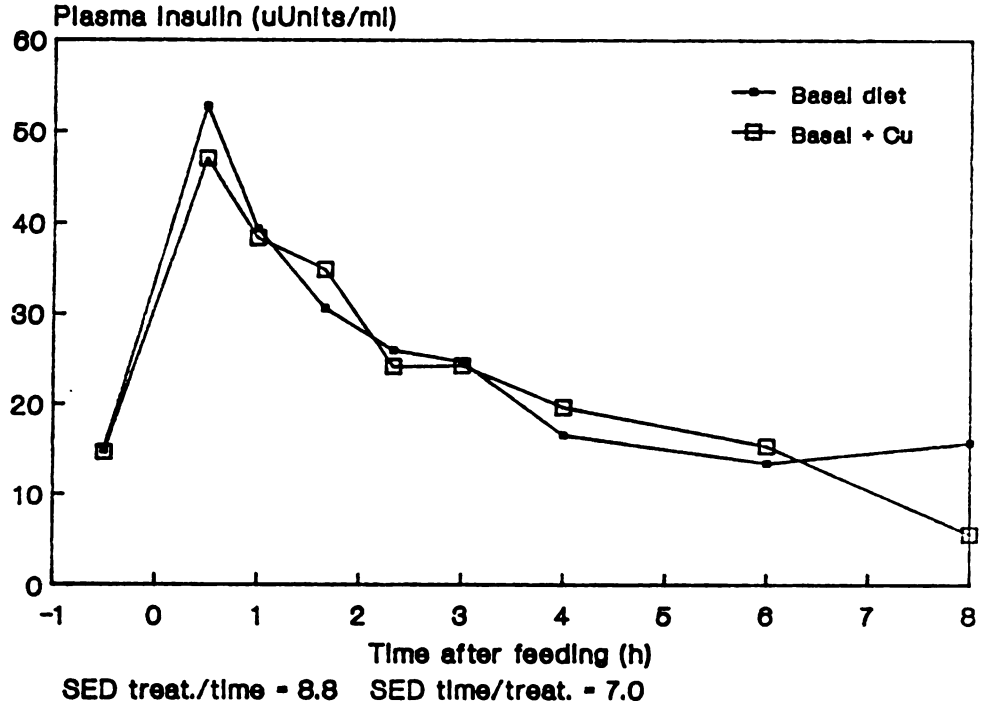

Figure 13. Insulin concentration in peripheral plasma, µUnits/ml. Experiment 2.

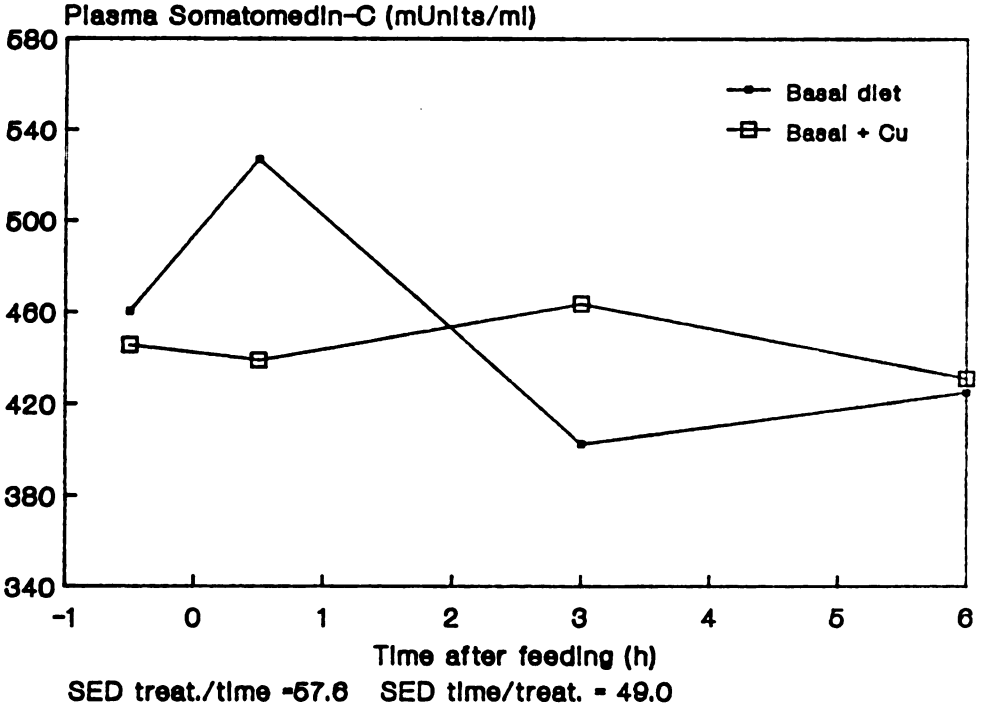

Figure 14. Somatomedin-C concentration in peripheral plasma, mUnits/ml. Experiment 2.

Basal diet
Basal + Cu
Time after loading (h)

Basal diet
Basal + Cu
Time after loading (h)

period (P>.10). Both treatments followed the same pattern, with peaks of insulin detected at 30 min after the initiation of the meal and returning to fasting levels between 4 and 6 h after the meal. Rérat et al. (1984) also detected a peak of insulin in pigs fed semi-synthetic diets at 30 min after feeding, but the elevated levels had a plateau until 2 h after the meal, decreasing after that.

Following an intragastric load of glucose in dogs, Abumrad et al. (1982) observed a plateau of elevated plasma insulin from 15 min to 1 1/2 h and a rapid decrease to basal levels at 2 1/2 h after the administration. In humans, insulin peak occurred at 1 2/3 h after a high carbohydrate meal (Acheson et al., 1982) and then returned slowly to the basal level after about 10 h. After a high protein meal, the insulin peak occurred at 1 h (Floyd et al., 1966), was less pronounced, and the decrease in concentration was also slow. The similar insulin response obtained with the two treatments in this study is in agreement with the similar plasma glucose levels throughout the post-prandial period.

In this study, high dietary copper did not promote changes in either glucose or insulin levels in plasma. These results do not allow conclusions to be taken regarding the effect of copper in improving performance of pigs. It does not appear to be, however, due to increased nutrient absorption or change in insulin, as an anabolic hormone. Also, the proved effect of high dietary copper in increasing the activity of acyl CoA desaturase enzymes (Ho et al.,

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1975) does not seem to be related to insulin levels, as could be hypothesized.

No effects of high dietary copper on plasma somatomedin-C concentration were detected (P>.10). There was no evidence for change in somatomedin-C in response to the meal in either treatment (P>.05). As discussed for insulin, there was no indication that the effects of high dietary copper are mediated through somatomedin-C. Even if the hypothesis (Spencer, 1985) that somatomedin-C production is regulated by insulin holds true, no effect of treatments would be expected in this case, since there was no response to insulin.

2. Experiment 3

2.1. Ammonia nitrogen and urea nitrogen

The results of concentration of ammonia nitrogen at several sites along the intestinal tract of starter pigs receiving the basal diet or the basal diet with 250 ppm supplemental copper are presented on Table 5. The concentrations are shown on a fresh weight basis. Because large differences in the means for ammonia among intestinal sites were found, and the greater means were associated with greater variances, a heterogeneous variance-covariance structure across sites resulted. For this reason, the repeated measurements analysis of variance was carried out on two sub-sets of data: one including the sites jejunum 1, jejunum 2, jejunum 3 and cecum, and the other including colon 1 and colon 2.

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No effects of high dietary copper on plasma somatomedin-C concentration were detected ($P > .10$). There was no evidence for change in somatomedin-C in response to meal in either treatment ($P > .05$). As discussed for insulin there was no indication that the effects of high dietary copper are mediated through somatomedin-C. Even if the hypothesis (Spencer, 1988) that somatomedin-C production is regulated by insulin holds true, no effect of treatments would be expected in this case, since there was no response to insulin.

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Table 5. Effect of high dietary copper on the concentration of ammonia N in the intestinal contents of starter pigs [a].

Site	µg ammonia N/g contents		SED[b]	P
	Basal	Basal + Cu		
No. of pigs	6	6		
Jejunum 1	63	36	11.6	<.10
Jejunum 2	38	18	8.9	<.10
Jejunum 3	46	45	12.2	ns
Cecum	37	28	12.2	ns
Colon 1	232	214	27.8	ns
Colon 2	245	178	27.8	<.05

[a] Basal diet = 25 ppm Cu; Basal + Cu = 265 ppm Cu.

[b] SED = Standard error of the difference of treatment means, within site.

Table 8. Effect of high dietary copper on the concentration of ammonia N in the intestinal contents of starter pigs a.

Site	Basal	Basal + Cu	LSD[b]	P
	mg ammonia N/g contents			
No. of pigs	8	8		
Jejunum 1	85	36	13.6	< .10
Jejunum 2	38	18	9.6	< .10
Jejunum 3	46	45	12.2	ns
Ileum	37	28	12.2	ns
Colon 1	232	214	27.8	ns
Colon 2	245	179	27.8	< .05

a Basal diet = 25 ppm Cu; Basal + Cu = 265 ppm Cu.

b LSD = Standard error of the difference of treatment means, within site.

The ammonia concentration in the digesta of the copper-fed pigs was lower in jejunum 1 ($P<.10$), jejunum 2 ($P<.10$) and colon 2 ($P<.05$). Treatment differences were not significant at the other sites ($P>.10$). Shurson (1986) determined the ammonia concentration in the cecal contents of starter pigs and did not find differences due to diet supplementation with 250 ppm copper. The samples obtained in the colon 2 in the present study are equivalent to the fecal samples obtained by Varel et al. (1987). In their study, however, no effect of copper supplementation (125 ppm Cu during the growing-finishing period) on the ammonia concentration was detected.

The urea nitrogen concentrations in the intestinal contents of the pigs fed the basal diet or the basal diet supplemented with 250 ppm Cu are presented in Table 6. The copper-fed pigs had higher urea levels in the contents of jejunum 1 ($P<.05$) and jejunum 2 ($P<.10$) and the differences were not significant ($P>.10$) at the other sites. Similarly to the results obtained with ammonia, Shurson (1986) also did not observe differences in the concentrations in the cecal contents due to high copper.

High dietary copper resulted in a 32% average reduction in ammonia concentration in the small intestine and an 18% average reduction in the large intestine. Comparable results were reported by Dierick et al. (1986b) in market weight pigs. According to these authors, feeding virginiamycin (20 ppm) reduced ammonia in the small and large intestine by 18%

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Table 6. Effect of high dietary copper on the concentration of urea N in the intestinal contents of starter pigs. [a,b]

Site	µg urea N/g contents		P
	Basal	Basal + Cu	
No. of pigs	6	6	
Jejunum 1	47	79	<.05
Jejunum 2	73	94	<.10
Jejunum 3	45	52	ns
Cecum	28	28	ns
Colon 1	70	87	ns
Colon 2	91	97	ns

[a] Basal diet = 25 ppm Cu; Basal + Cu = 265 ppm Cu.

[b] Standard error of the difference of treatment means, within site (SED) = 12.6.

Table 8. Effect of high dietary copper on the concentration of urea N in the intestinal contents of starter pigs. a,b

Site	Mg urea N/g contents		P
	Basal	Basal + Cu	
No. of pigs	8	8	
Jejunum 1	47	19	< .05
Jejunum 2	78	94	< .10
Jejunum 3	45	52	ns
Cecum	28	28	ns
Colon 1	76	87	ns
Colon 2	81	97	ns

a Basal diet = 25 ppm Cu; Basal + Cu = 265 ppm Cu.

b Standard error of the difference of treatment means, within site (SED) = 12.5.

and 15%, respectively, and 20 ppm spiramycin resulted in 17% and 35% reductions. In that study the digesta samples were collected 1 h after the pigs were fed.

Other studies corroborated the effectiveness of antimicrobial feed additives in reducing ammonia production. In vitro incubations of ileal contents of swine with virginiamycin, spiramycin, carbadox or copper sulfate caused a decreased production of ammonia, compared to controls (Vervaeke et al., 1979; Dierick et al., 1986a). Rats receiving diets containing either penicillin, chlortetracycline or arsanilic acid at growth-permittant levels showed a reduced rate of in vivo urea hydrolysis (Visek et al., 1959).

A lesser amount (or activity) of urease of bacterial origin is believed to be involved in the reduction of ammonia production in the gut as a result of dietary antimicrobials. Urease immunization of growing pigs was effective in stimulating antibody production as measured by serum antiurease activity (Kornegay et al., 1964). In that study urease immunization also tended to reduce urease activity in intestinal sections plus contents. However, the ammonia concentration in the intestinal sections plus contents was not reduced in the immunized pigs and there were no differences in feed efficiency. In contrast, urease immunization of mice and rats reduced gastrointestinal ammonia by 30% (Dang and Visek, 1963) and immunized rats and chicks had improved feed efficiency (Dang and Visek, 1960)

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compared to controls. The lack of response of immunized pigs (Kornegay et al., 1964) may have been due to the failure to decrease intestinal ammonia.

It is interesting to note that at three of the intestinal sites (jejunum 1, jejunum 2 and colon 1) the reductions in ammonia nitrogen concentrations due to high copper feeding had magnitudes similar to the increases in urea nitrogen levels. These measurable changes in ammonia and urea in the intestinal contents indicate that high copper reduced the ureolytic activity. Visek (1978) attributed the enhanced growth in animals fed antimicrobials to inhibition of urease activity in the intestinal tract, which supports the data presented in this study. However, Dierick et al. (1986b) indicated that the antibiotics virginiamycin and spiramycin had greater effect in reducing deamination of amino acids rather than ureolysis. Varel et al. (1987), based on results obtained with fecal material, suggested that deamination of amino acids plays an important role in the amount of ammonia found in the intestinal tract. In the present study, production of ammonia by deamination appeared to be important at colon 2 where high dietary copper resulted in decreased ammonia without any change in the urea concentration.

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The concentrations of ammonia in the digesta of the control pigs in the present study averaged 3-4 mM in the small intestines and 17 mM in the colon (1 mM = 14 μg ammonia N/g). In chickens, Prior et al. (1974) found 1-1.5 mM ammonia in the small intestine and up to 10 mM in the large intestine digesta. Hecker (1971) found ammonia levels of 5-10 mM in the intestinal contents of horses and about 10 mM in all segments of sheep intestinal tract. Gargallo and Zimmerman (1980) reported values equivalent to 8-11 mM ammonia in cecal fluid of pigs. In other studies with pigs (Dierick et al., 1986b; Shurson, 1986, Varel et al., 1987) ammonia values in gut contents were reported on a dry matter basis and the actual concentrations cannot be calculated.

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cells from the small intestine of chickens, incubated in vitro. Visek (1978) reported studies in which 2.9 mM ammonia doubled the cellular volumes and protein content of mouse fibroblasts. When intestinal ammonia production was decreased in vivo by immunization against urease (Visek, 1978), mucosal cell activity and small intestine weight were clearly reduced. The above results indicate that ammonia can be responsible for shortening the life span of cells, which would require a higher replacement rate. As a consequence, energy and protein that otherwise would be used for growth would be needed to maintain the intestinal epithelium. It appears that the ammonia levels normally found in the intestinal tract of pigs and other animals are sufficient to promote the effects outlined above. What is not clear is if the decreased ammonia achieved by feeding antimicrobials (18 to 32% in this study) is of a sufficient magnitude to reverse those effects and account for the improved performance.

Because reduction in intestinal ammonia by immunization against urease (Dang and Visek, 1960) and by binding ammonia with a resin (Holtzman and Visek, 1965) was correlated with improved growth of rats and chicks, it was hypothesized that the beneficial effects of antimicrobials were due to the decreased ammonia. But other metabolites that are potentially toxic may also be affected by antimicrobials and be involved in the growth response that they promote. Antimicrobials have been shown to reduce intestinal

cells from the small intestine of chickens, incubated in vitro. Visek (1972) reported studies in which 2.0 mM ammonia doubled the cellular volumes and protein content of mouse fibroblasts. When intestinal ammonia production was decreased in vivo by immunization against urease (Visek, 1978), mucosal cell activity and small intestine weight were clearly reduced. The above results indicate that ammonia can be responsible for shortening the life span of cells, which would require a higher replacement rate. As a consequence, energy and protein that otherwise would be used for growth would be needed to maintain the intestinal epithelium. It appears that the ammonia levels normally found in the intestinal tract of pigs and other animals are sufficient to promote the effects outlined above. What is not clear is if the decreased ammonia achieved by feeding antimicrobials (18 to 32% in this study) is of a sufficient magnitude to reverse those effects and account for the improved performance.

Because reduction in intestinal ammonia by immunization against urease (Dang and Visek, 1980) and by binding ammonia with a resin (Holtzman and Visek, 1985) was correlated with improved growth of rats and chicks, it was hypothesized that the beneficial effects of antimicrobials were due to the decreased ammonia. But other metabolites that are potentially toxic may also be affected by antimicrobials and be involved in the growth response that they promote. Antimicrobials have been shown to reduce intestinal

production of amines (Larson and Hill, 1960; Dierick et al., 1986b), deconjugated bile acids (Feighner and Daskevicz, 1987) and p-cresol (Yokoyama et al., 1982). High dietary copper may affect these substances as well, but these possibilities were not investigated in this study. Dierick et al. (1986b) stated that the formation of ammonia and amines from deamination and decarboxylation of amino acids, respectively, is important from both the toxicological and the nutritional standpoints.

2.2. Mitotic indices

The mitotic indices were calculated as the percent of crypt cells in the intestinal epithelium that were undergoing mitotic divisions. The treatment means for each site along the intestinal tract are presented in Table 7. The pigs that received high dietary copper for 4 weeks had lower mitotic indices ($P<.05$) at two sites, cecum and colon 2. The effect of copper supplementation was not significant ($P>.10$) at the other sites, but in all of them the same tendency for lower mitotic indices was observed.

The estimates of mitotic index obtained in this study ranged from 1.3 to 2.2%. These values are lower than the values reported for swine by Grant and Thomas (1987) but in that case the piglets were younger (7 and 14 days of age) and the turnover rate of the intestinal epithelium is expected to decrease with age (Visek, 1978).

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Al-MuKhtar et al. (1982) critically reviewed the methods for measurement of proliferative and morphological

Table 7. Effect of high dietary copper on the mitotic index of intestinal crypt cells. [a,b]

Site	mitotic index, %		P
	Basal	Basal + Cu	
No. of pigs	6	6	
Jejunum 1	2.03	1.71	ns
Jejunum 2	1.68	1.55	ns
Jejunum 3	2.23	1.96	ns
Cecum	2.05	1.51	<.05
Colon 1	1.49	1.33	ns
Colon 2	2.19	1.60	<.01

[a] Basal diet = 25 ppm Cu; Basal + Cu = 265 ppm Cu.

[b] Standard error of the difference of treatment means, within site (SED) = .22

Table 7. Effect of high dietary copper on the mitotic index of intestinal crypt cells. a,b

Site	mitotic index, #		P
	Basal	Basal + Cu	
No. of pigs	6	6	
Jejunum 1	2.09	1.71	ns
Jejunum 2	1.68	1.65	ns
Jejunum 3	2.29	1.96	ns
Cecum	2.05	1.81	<.05
Colon 1	1.49	1.33	ns
Colon 2	2.19	1.60	<.01

a Basal diet = 25 ppm Cu; Basal + Cu = 265 ppm Cu.

b Standard error of the difference of treatment means, within site (SED) = .22

status of the intestinal epithelium. According to them all the established methods to measure the proliferative rate (i.e., incorporation of tritiated thymidine into DNA, mitotic index, cell cycle time, birth rate, cell migration rate, etc.) have limitations. Al-Mukhtar et al. (1982) pointed out that the mitotic index has the distinct advantage that the counts are confined to the epithelial component of the intestine, precluding interference from other mucosal cells. On the other hand, it does not take in consideration the duration of the cell phases, the size of the crypt cell population and suffers from the bias towards counting mitoses. Therefore, treatment-induced changes in the crypt cell population or duration of mitosis can affect the actual rate of cell proliferation. Al-Mukhtar et al. (1982) concluded that the mitotic index is an useful indicator of change in proliferative rate but cannot be regarded as a definitive measurement. The same applies to the results obtained in this study.

The general tendency to decreased mitotic indices in the intestines of the pigs fed high copper is consistent with the tendency to lower levels of ammonia in the same animals, as described previously. These findings are in agreement with the reported effects of ammonia in shortening the life span of intestinal cells (Prior et al., 1974) and increasing synthetic activity in the small intestinal mucosa (Visek, 1978). Parallel to the reduced ammonia levels in the intestinal contents of germ-free animals, Visek (1978)

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reported that the mitotic indices in the duodenum and jejunum of germ-free rats were lower than in the conventional counterparts. Other estimates showed that the mucosal cell replacement rate was 30-40% less in germ-free animals.

Shurson (1986) used the crypt depth in the small intestine as an indicator of the rate of crypt cell production. In germ-free pigs the crypt depth was smaller (suggesting less production of enterocytes) than in the conventional, but feeding high copper to the conventional pigs resulted in greater crypt depth in the jejunum and ileum. These results are in contradiction with those obtained in the present study, but the inadequacy of the linear measurement employed (Al-Mukhtar et al., 1982) must be recognized.

The mitotic indices in the pigs on the high copper diet was reduced by an average of 16%, ranging from 7 to 27% in the different intestinal sites. Assuming that the mitotic index estimates the rate of cell proliferation in the mucosal epithelium, a 16% reduction in cell turnover could be expected when high copper was fed. Muir (1985) proposed that a decrease in turnover rate of intestinal mucosal cells could partially explain the improved performance obtained with antimicrobials. Webster (1980) found that approximately 50% of the protein synthesis in rats occurred in the intestinal tissue. It can be calculated, based on the above data and with a speculative purpose only, that about

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8% of the daily synthesis of protein would be spared by feeding copper. At least part of this amount could be made available for body growth and is consistent with the growth improvement obtained with antimicrobials.

Another consideration is that a decreased turnover rate of small intestinal epithelium may be related to increased efficiency of absorption of nutrients. An extended transit time of enterocytes lining the villi occur when the rate of replacement of cells is decreased. According to Menge et al. (1982) the longer maturation results in higher levels of brush border enzymes and they proposed that transport activity parallels brush border enzyme activity. However, increased absorption of amino acids (Dierick et al. 1986b) was obtained with virginiamycin independent of change in cell maturation. Their experiment involved short-term (2 h) perfusions of an isolated loop of the small intestine of a pig which was not receiving any dietary antimicrobial.

3. Experiment 4

3.1. Performance traits

The averages for daily weight gain and gain : feed ratio of the pigs on each treatment during the second, third and fourth week post-weaning are presented in Table 8. The supplementation of the basal diet with 250 ppm Cu did not result in improved growth or feed efficiency ($P>.10$) but the beneficial effect of copper approached significance at week 4. The pigs on both treatments were pair-fed, therefore the

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Table 8. Effect of feeding 250 ppm supplemental copper on performance of pair-fed starter pigs.

	Basal	Basal + Cu	SEM	P
No. of pigs	6	6		
Avg Daily Gain, g				
Week 2	245	255	11	ns
Week 3	298	317	10	ns
Week 4	247	270	9	.13
Gain/Feed				
Week 2	.773	.801	.036	ns
Week 3	.742	.780	.026	ns
Week 4	.531	.584	.018	.11

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	Basal	Basal + Cu	SEM	P
No. of pigs	8	8		
Ave Daily Gain, g				
Week 2	245	256	11	ns
Week 3	299	319	10	ns
Week 4	247	270	9	.13
Gain/Feed				
Week 2	.773	.801	.088	ns
Week 3	.742	.780	.029	ns
Week 4	.631	.684	.018	.11

changes in growth rate due to treatments reflect directly the changes in feed efficiency.

High dietary copper tended to consistently promote an improvement in feed efficiency, ranging from 4% (Week 2) to 10% (week 4). These results are typical of what could be expected from copper supplementation to starter pigs, as can be observed in Tables 1 and 2. On the other hand, the tendency to increased response to copper with time was not expected, based on other results reported by weeks on trial (Edmonds et al., 1985; Kornegay et al., 1986). It must be recognized, however, that the pigs on the basal diet were utilizing the feed very efficiently during weeks 2 and 3. In a situation like this, a further improvement in performance is not very likely to be achieved.

3.2. Balance trials

The data concerning one pig on the basal treatment during week 2 and one pig on the copper treatment during week 3 were not included in the calculations of the results of the balance trials. They were discarded as outliers, in the first case because the pig had scours during 2 days of the collection period and in the second case due to an excessive fecal output. Normal results were found for those pigs during the other collection periods.

A summary of the data collected and the results of the balances on weeks 2, 3 and 4 post-weaning are presented in Tables 9, 10 and 11, respectively. The pigs receiving high dietary copper showed a slight tendency to increased

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Table 9. Effect of high dietary copper on utilization of nutrients and energy. Week 2.

Variables[a]	Basal	Basal + Cu	SEM[b]	P
No. of pigs	5	6	-	-
Feed intake, g	1012	977	-	-
Feces weight, g	120	111	-	-
Urine volume, ml	653	868	-	-
GE intake, kcal	3896	3760	-	-
GE feces, kcal	552	498	-	-
GE urine, kcal	97.9	90.8	-	-
N intake, g	29.7	28.7	-	-
N feces, g	4.68	4.18	-	-
N urine, g	6.23	5.96	-	-
Ca intake, g	10.9	10.6	-	-
Ca feces, g	3.71	3.50	-	-
Ca urine, g	.91	.87	-	-
P intake, g	7.03	6.79	-	-
P feces, g	2.82	2.48	-	-
P urine, g	.05	.13	-	-
Cu intake, mg	25.3	259	-	-
Cu feces, mg	19.9	220	-	-
Cu urine, mg	.38	1.46	-	-
Balance				
DE, %	85.7	86.8	.63	ns
ME, %	83.2	84.4	.63	ns
Digestible N, %	84.1	85.5	.82	ns
Retained N, %	63.1	64.4	2.0	ns
Ret./Dig. N, %	75.0	75.4	2.1	ns
Digestible Ca, %	65.5	65.6	4.3	ns
Retained Ca, %	57.4	57.3	3.8	ns
Digestible P, %	59.5	63.1	2.4	ns
Retained P, %	58.8	60.8	2.7	ns
Digestible Cu, %	21.5	14.9	2.9	ns
Retained Cu, %	20.0	14.3	2.8	ns

[a] Values based on a 3-day collection period. GE = gross energy; DE = digestible energy; ME = metabolizable energy.

[b] The standard error of the mean for the treatment with five pigs is slightly larger.

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Variables[a]	Basal	Basal + Cu	SEM[b]	P
No. of pigs	5	6	-	-
Feed intake, g	1012	977	-	-
Feces weight, g	120	111	-	-
Urine volume, ml	659	886	-	-
GE intake, kcal	3986	3760	-	-
GE feces, kcal	552	498	-	-
GE urine, kcal	97.2	80.8	-	-
N intake, g	29.7	29.7	-	-
N feces, g	4.68	4.18	-	-
N urine, g	8.23	6.96	-	-
Ca intake, g	10.9	10.6	-	-
Ca feces, g	3.71	3.50	-	-
Ca urine, g	.91	.87	-	-
P intake, g	7.03	6.79	-	-
P feces, g	2.82	2.48	-	-
P urine, g	.05	.12	-	-
Cu intake, mg	25.3	259	-	-
Cu feces, mg	19.8	229	-	-
Cu urine, mg	.38	1.46	-	-
Balance				
DE, %	85.7	86.8	.83	ns
ME, %	83.2	84.4	.86	ns
Digestible N, %	84.1	86.2	.82	ns
Retained N, %	69.1	64.4	2.0	ns
Ret./Dig. N, %	75.0	74.4	2.1	ns
Digestible Ca, %	65.9	66.8	4.3	ns
Retained Ca, %	37.1	37.1	3.8	ns
Digestible P, %	59.5	63.1	3.4	ns
Retained P, %	58.8	60.8	2.7	ns
Digestible Cu, %	21.5	14.9	2.9	ns
Retained Cu, %	20.0	14.3	2.8	ns

[a] Values based on a 3-day collection period. GE = gross energy; DE = digestible energy; ME = metabolizable energy.

[b] The standard error of the mean for the treatment with five pigs is slightly larger.

Table 10. Effect of high copper on utilization of nutrients and energy. Week 3.

Variables[a]	Basal	Basal + Cu	SEM[b]	P
No. of pigs	6	5	-	-
Feed intake, g	1170	1190	-	-
Feces weight, g	141	143	-	-
Urine volume, ml	902	1138	-	-
GE intake, kcal	4504	4581	-	-
GE feces, kcal	651	656	-	-
GE urine, kcal	122	120	-	-
N intake, g	34.4	35.0	-	-
N feces, g	4.98	5.13	-	-
N urine, g	7.97	8.60	-	-
Ca intake, g	12.6	12.8	-	-
Ca feces, g	3.59	3.51	-	-
Ca urine, g	1.08	1.27	-	-
P intake, g	8.13	8.27	-	-
P feces, g	3.50	3.76	-	-
P urine, g	.04	.03	-	-
Cu intake, mg	29.8	315	-	-
Cu feces, mg	25.9	302	-	-
Cu urine, mg	.34	1.85	-	-
Balance				
DE, %	85.5	85.7	.95	ns
ME, %	82.8	83.1	.90	ns
Digestible N, %	85.5	85.4	1.01	ns
Retained N, %	62.2	60.8	.76	ns
Ret./Dig. N, %	72.8	71.2	.63	ns
Digestible Ca, %	71.2	73.1	2.1	ns
Retained Ca, %	62.7	63.1	1.9	ns
Digestible P, %	56.8	54.8	2.0	ns
Retained P, %	56.3	54.4	2.0	ns
Digestible Cu, %	12.7	4.5	6.4	ns
Retained Cu, %	11.6	3.9	6.4	ns

[a] Values based on a 3-day collection period. GE = gross energy; DE = digestible energy; ME = metabolizable energy.

[b] The standard error of the mean for the treatment with five pigs is slightly larger.

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No. of pigs	8	5	-	-
Feed intake, g	1170	1190	-	-
Feces weight, g	141	143	-	-
Urine volume, ml	902	1138	-	-
GE intake, Kcal	4504	4581	-	-
GE feces, Kcal	621	656	-	-
GE urine, Kcal	122	120	-	-
N intake, g	34.4	35.0	-	-
N feces, g	4.58	5.13	-	-
N urine, g	7.97	8.60	-	-
Ca intake, g	12.6	12.8	-	-
Ca feces, g	3.59	3.51	-	-
Ca urine, g	1.08	1.27	-	-
P intake, g	8.13	8.27	-	-
P feces, g	3.60	3.76	-	-
P urine, g	.04	.03	-	-
Cu intake, mg	29.8	215	-	-
Cu feces, mg	25.9	209	-	-
Cu urine, mg	.04	1.85	-	-
Balance				
DE, %	85.5	85.7	.98	ns
ME, %	82.8	83.1	.90	ns
Digestible N, %	86.5	86.4	1.01	ns
Retained N, %	82.2	80.9	.76	ns
Ret./Dig. N, %	72.8	71.2	.85	ns
Digestible Ca, %	71.5	70.1	2.1	ns
Retained Ca, %	82.7	83.1	1.9	ns
Digestible P, %	56.8	54.8	2.0	ns
Retained P, %	59.3	61.4	2.0	ns
Digestible Cu, %	12.7	4.5	6.4	ns
Retained Cu, %	11.6	3.9	6.4	ns

a Values based on a 3-day collection period. DE = gross energy; DE = digestible energy; ME = metabolizable energy.

b The standard error of the mean for the treatment with five pigs is slightly larger

Table 11. Effect of high dietary copper on utilization of nutrients and energy. Week 4.

Variables[a]	Basal	Basal + Cu	SEM	P
No. of pigs	6	6	-	-
Feed intake, g	1387	1883	-	-
Feces weight, g	163	153	-	-
Urine volume, ml	1032	1285	-	-
GE intake, kcal	5242	5229	-	-
GE feces, kcal	764	705	-	-
GE urine, kcal	131	130	-	-
N intake, g	41.0	40.9	-	-
N feces, g	5.82	5.61	-	-
N urine, g	9.09	9.26	-	-
Ca intake, g	14.1	14.1	-	-
Ca feces, g	4.30	4.48	-	-
Ca urine, g	1.59	1.88	-	-
P intake, g	9.08	9.06	-	-
P feces, g	3.82	3.76	-	-
P urine, g	.05	.05	-	-
Cu intake, mg	34.7	367	-	-
Cu feces, mg	28.9	319	-	-
Cu urine, mg	.39	2.78	-	-
Balance				
DE, %	85.4	86.5	.40	<.10
ME, %	82.9	84.0	.36	<.10
Digestible N, %	85.8	86.3	.56	ns
Retained N, %	63.5	63.6	.59	ns
Ret./Dig. N, %	74.1	73.8	.81	ns
Digestible Ca, %	69.6	68.3	1.5	ns
Retained Ca, %	58.3	55.0	1.4	ns
Digestible P, %	58.0	58.5	1.2	ns
Retained P, %	57.6	57.9	1.2	ns
Digestible Cu, %	16.5	13.1	2.2	ns
Retained Cu, %	15.4	12.3	2.2	ns

[a] Values based on a 3-day collection period. GE = gross energy; DE = digestible energy; ME = metabolizable energy.

Table 11. Effect of high dietary copper on utilization of nutrients and energy. Week 4.

Variables*	Basal	Basal + Cu	SEM	P
No. of pigs	6	6	-	-
Feed intake, g	1587	1583	-	-
Feces weight, g	183	183	-	-
Urine volume, ml	1032	1285	-	-
GE intake, kcal	5242	5229	-	-
GE feces, kcal	764	705	-	-
GE urine, kcal	131	130	-	-
N intake, g	41.0	40.9	-	-
N feces, g	5.82	5.61	-	-
N urine, g	5.09	5.26	-	-
Ca intake, g	14.1	14.1	-	-
Ca feces, g	4.30	4.49	-	-
Ca urine, g	1.39	1.88	-	-
P intake, g	9.08	9.08	-	-
P feces, g	3.82	3.76	-	-
P urine, g	.05	.05	-	-
Cu intake, mg	34.7	307	-	-
Cu feces, mg	26.9	319	-	-
Cu urine, mg	39	2.78	-	-
Balance				
DE, %	85.4	86.5	.40	<.10
ME, %	82.9	84.0	.36	<.10
Digestible N, %	85.5	86.3	.59	ns
Retained N, %	69.5	63.6	.89	ns
Ret./Dig. N, %	74.1	73.8	.81	ns
Digestible Ca, %	69.6	68.3	1.5	ns
Retained Ca, %	58.3	56.0	1.4	ns
Digestible P, %	58.0	58.3	1.2	ns
Retained P, %	47.6	37.9	1.2	ns
Digestible Cu, %	18.4	13.1	2.2	ns
Retained Cu, %	18.4	12.3	2.2	ns

* Values based on a 3-day collection period. DE = gross energy; DE = digestible energy; ME = metabolizable energy.

digestible and metabolizable energy values. The effect of treatments was significant at week 4 ($P<.10$) for both variables and, although the differences were of the same magnitude at week 2, significance was not detected. Shurson (1986) observed an improved energy utilization due to high copper in pigs weighing 7 kg (by about 3.5%) but not in 20 kg pigs. Castell and Bowland (1968) and Young et al. (1970) also did not detect effect of copper on the efficiency of use of energy.

Results of several balance studies indicated that high levels of copper improved nitrogen digestibility (Kirchgessner and Giessler, 1961; Braude, 1967; Castell and Bowland, 1968; Shurson, 1986, exp. 1) and nitrogen retention (Kinchgessner and Giessler, 1961; Braude, 1967; Young et al., 1970; Shurson, 1986, exp. 1 and 2). In the present study high dietary copper did not affect ($P>.10$) nitrogen digestibility, in agreement with Beames and Lloyd (1965), Young et al. (1970) and Shurson (1986, exp.2). Nitrogen retention also was not affected ($P>.10$) as observed by Castell and Bowland (1968). The ratio retained : absorbed nitrogen, indicative of biological value of protein, was not changed by copper supplementation, in agreement with Shurson (1986).

The results of the energy and nitrogen balances obtained in this study are not consistent with the tendency for improved performance of the copper-fed pigs. Increases in energy and nitrogen retention of the same magnitude of

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the improved growth should be expected. However, even in the case that the copper-fed pigs had a higher metabolizable energy ($P<.10$, week 4), this represented a 1.3% improvement only, compared to a 9% improvement in weight gain. This inconsistency seems to indicate that balance trials do not allow an accurate evaluation of the energy and nitrogen retained by the pigs. The fact that similar changes in performance and balance due to antimicrobials were found in other studies argues against the latter statement. For example, growing pigs receiving dietary carbadox had the feed efficiency improved by 7%, nitrogen retention by 9% and digestibility of energy by 2.5% (Yen et al., 1976) in comparison to control pigs. Furthermore, copper supplementation resulted in 8% and 5% increases in nitrogen retention in two trials conducted in the same facilities as the present study (Shurson, 1986).

Percent digestibility and retention of calcium and phosphorus were not affected by levels of dietary copper ($P>.10$). This indicates that the high level of copper does not negatively interfere with the metabolism of calcium and phosphorus. Shurson (1986) found that high copper actually increased the percent phosphorus retention, and no effect was observed on calcium retention. No effects of dietary copper levels were detected ($P>.10$) on percent absorption or retention of this mineral, and most of excretion occurred via the feces. Because the high copper diet contained 10 times as much copper as the basal diet, the absolute

the improved growth should be expected. However, even in the case that the copper-fed pigs had a higher metabolizable energy ($P<.10$, week 4), this represented a 1.3% improvement only, compared to a 3% improvement in weight gain. This inconsistency seems to indicate that balance trials do not allow an accurate evaluation of the energy and nitrogen retained by the pigs. The fact that similar changes in performance and balance due to antimicrobials were found in other studies argues against the latter statement. For example, growing pigs receiving dietary carbadox had the feed efficiency improved by 7%, nitrogen retention by 3% and digestibility of energy by 2.5% (Yen et al., 1975) in comparison to control pigs. Furthermore, copper supplementation resulted in 8% and 5% increases in nitrogen retention in two trials conducted in the same facilities as the present study (Shurson, 1988).

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retention of copper in the pigs receiving that diet was much greater. On the other hand, Shurson (1986) found that the percent copper retention was increased when pigs received high copper diets.

Although there are suggestions that copper results in improved protein utilization by increasing the pepsin activity (Kirchgessner et al., 1976) or by sparing amino acids (Dierick et al., 1986a), these are not corroborated based on the present results.

4. General discussion

The results of Experiments 2 and 3 concerning ammonia and urea concentrations in plasma and intestinal contents, respectively, were in close agreement. The trend towards higher urea N levels in the intestinal contents of the copper-fed pigs (Experiment 3) was consistent with the tendency for higher portal levels and absorption of urea N found in Experiment 2. These results independently indicate that high dietary copper reduces hydrolysis of urea, especially in the small intestine. Based on work by Varel et al. (1987), it can be concluded that a decreased ureolysis due to high copper can be attributed to a lower proportion of ureolytic microorganisms or less urease activity, or both. It is also interesting that copper supplementation reverses previous indications (Rérat and Aumaitre, 1971; Rérat et al., 1986) that portal urea levels were lower than peripheral levels, due to intestinal uptake of urea.

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Accordingly, the pigs on the high copper treatment had lower levels of portal ammonia and lower ammonia absorption (Experiment 2) and a tendency for decreased ammonia concentration in the intestinal contents (Experiment 3). The magnitude of these changes was consistent across experiments: ammonia absorption was diminished by 30% and ammonia concentration in the digesta was reduced by 32% and 18% in the small and large intestines, respectively, due to copper. In the control pigs, peaks of portal ammonia appeared at 1 h and 6 h after feeding, and no peaks were detected for the copper-fed pigs. It is suggested that ammonia absorption in pigs occurs in the small intestine, based on the time of appearance of the first peak, and in the large intestine, based on the time of the second peak and indications (Gargallo and Zimmerman, 1980) that cecal ammonia peaks at 6 h after a meal. In any case, high dietary copper appeared to suppress the intestinal production of ammonia.

The decreased production of ammonia in the intestines of the pigs fed high copper may be responsible for the tendency to reduced mitotic indices in the crypts of the intestinal epithelium (Experiment 3). Although the intestinal ammonia concentrations did not appear to be directly correlated with the mitotic indices along the intestinal tract, it must be recognized that the temporal changes could not be evaluated on the measurements of the intestinal contents. This point is exemplified by the lack

Accordingly, the pike on the high copper treatment had lower levels of portal ammonia and lower ammonia absorption (Experiment 2) and a tendency for decreased ammonia concentration in the intestinal contents (Experiment 3). The magnitude of these changes was consistent across experiments: ammonia absorption was diminished by 20% and ammonia concentration in the digesta was reduced by 22% and 18% in the small and large intestines, respectively, due to copper. In the control pike, peaks of portal ammonia appeared at 1 h and 6 h after feeding, and no peaks were detected for the copper-fed pike. It is suggested that ammonia absorption in pike occurs in the small intestine, based on the time of appearance of the first peak, and in the large intestine, based on the time of the second peak and indications (Dargello and Zimmerman, 1980) that cecal ammonia peaks at 6 h after a meal. In any case, high dietary copper appeared to suppress the intestinal production of ammonia.

The decreased production of ammonia in the intestines of the pike fed high copper may be responsible for the tendency to reduced nitrate indices in the crypts of the intestinal epithelium (Experiment 3). Although the intestinal ammonia concentrations did not appear to be directly correlated with the mitotic indices along the intestinal tract, it must be recognized that the temporal changes could not be evaluated on the measurements of the intestinal contents. This point is exemplified by the lack

of effect of copper on the concentration of ammonia in the cecum, as determined 3 1/2 h after the meal, but a significant reduction in the mitotic index at that site. Also, the colon epithelium may be less sensitive to ammonia toxicity than the other parts of the intestinal tract.

The reduction in ammonia formed in the intestinal tract of pigs fed high copper can be identified as a likely cause of the beneficial effects of this additive. This is possible because ammonia has been shown to increase the metabolic activity and probably the turnover rate of intestinal cells (Prior et al., 1974; Visek, 1978). It does not exclude, however, that other bacterial metabolites such as amines, p-cresol and products of hydrolysis of bile acids may be involved. The importance of the identification of the mechanism(s) by which antimicrobials improve the performance of animals relies on the possibility that alternatives to antibiotic and antibacterial drugs could be developed based on that knowledge.

Dierick et al. (1986b) indicated that the two antibiotics used in their study decreased intestinal ammonia production mainly by interfering with the deamination of amino acids. A beneficial sparing of amino acids would also result from this effect. In contrast, the results of the present study suggest that the reduction in ureolysis was the main effect of copper supplementation. These facts raise an interesting point that supports the idea (Cromwell et al., 1981) that the synergistic effect of copper and other

antimicrobials may be due to different modes of action. On the other hand, the lack of a statistically significant effect of copper in improving nitrogen digestibility or retention (Experiment 4) may be an indication that a protective effect against deamination (and decarboxylation) of amino acids was not important.

In the balance trials (Experiment 4), the only significant effect of copper was on increasing the digestible and metabolizable energy at week 4. The magnitude of the increments was, however, small. Increased energy digestibility can be considered to be related to increased glucose absorption. In Experiment 2, no treatment differences in glucose absorption could be detected, probably because this response measure is subject to a greater variability. The pattern of the portal and peripheral plasma glucose levels during the post-prandial period (Aumaitre et al., 1973) represents a limitation to the accuracy of the estimates of absorption, which are calculated by difference.

Therefore, although the technique of simultaneous sampling of portal and peripheral blood was useful to study ammonia and urea absorption in this study, it did not prove effective in the case of glucose. It could be effective, however, if the samples were collected at shorter intervals. Rérat et al. (1980) recommended that at least 12 to 16 samples after a meal should be taken. In Experiment 2, the number of the post-prandial blood samples taken was limited

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CONCLUSIONS

1. Supplementation of starter pig diets with 250 ppm Cu (from $CuSO_4 \cdot 5H_2O$) resulted in decreased ammonia absorption through the portal vein and a tendency for decreased ammonia concentration in the intestinal contents. These effects can be attributed to the antimicrobial effect of copper when fed at high levels and to its inhibitory effect on urease activity.

2. The decrease in the production of ammonia in the intestinal tract due to high dietary copper appeared to be associated with a reduction in urea hydrolysis. This was not true for the lower colon, where high copper may have reduced the ammonia production by affecting the deamination of amino acids.

3. The pigs on the high copper treatment tended to have lower mitotic indices in the epithelium of the crypts of Lieberkühn. The toxic effect of ammonia on the mucosal cells may be implicated with this effect, but the possibility that other toxic substances also played a role should not be overlooked.

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3. The pigs on the high copper treatment tended to have lower mitotic indices in the epithelium of the crypts of Lieberkühn. The toxic effect of ammonia on the mucosal cells may be implicated with this effect, but the possibility that other toxic substances also played a role should not be overlooked.

4. The lower mitotic indices caused by high copper feeding can be interpreted as a decreased rate of replacement of the intestinal epithelial cells. The reduced turnover rate can be partially responsible for the improved performance obtained with copper supplementation to pigs. However, because the mitotic index of the crypt cells is not a quantitative indicator of cell replacement, the interpretation above must be considered with caution. Further studies are necessary to clarify this point.

5. The ammonia absorbed and carried through the hepatic portal vein appeared to be efficiently metabolized by the liver before the blood reached the peripheral circulation, despite the higher load in the pigs fed the basal diet.

6. There was no evidence that high copper increased the absorption of glucose, and only a slight positive effect on energy utilization was detected.

7. There was no evidence that high dietary copper improved the utilization of nitrogen by the pigs.

8. The plasma levels of the anabolic hormones, insulin and somatomedin-C, were not affected by copper supplementation to the pig's diets.

9. High dietary copper did not interfere with absorption or retention of the minerals calcium and phosphorus. The retention of copper was proportional to the levels of this mineral in the pig's diets.

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