INTERACTIONS OF COPPER AND IRON AS MEASURED BY BLOOD PARAMETERS, TISSUE STORES AND PERFORMANCE IN SWINE

by

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INTRODUCTION

The essentiality of copper as a trace mineral was demonstrated by Hart et al. in 1928 when they showed that copper prevented anemia in rats. This finding of Hart et al. was substantiated by other workers using the rat and other species as well. Yet, at the present time the exact dietary requirement of copper for the pig has not been well documented. This may be due to the fact that most practical diets contain adequate copper levels.

Currently the major interest in copper is its growth promoting capabilities when fed at levels of 125 - 250 ppm. Braude (1965), in a review dealing with copper as a growth stimulant, estimated that one third of the hogs in England were fed high levels of copper for growth promotion and increased feed efficiency. In this country interest in copper as a growth stimulant was somewhat discouraged by reports of toxicity in early work (Wallace et al., 1960; Ritchie et al. 1963). The incidence of copper toxicity has been associated with such things as protein level, zinc level and iron level in the diet. British workers found that liberal quantities of iron and zinc would give the pig some protection from copper poisoning and that liberal quantities of iron would prevent anemia. Both high levels of iron and zinc have been shown to decrease, in some cases, the undesirable accumulation of excess copper in the liver.

The objectives of this study were to determine if a higher than the recommended level of iron will overcome the hemoglobin depressing effect of high copper and if the copper requirement is increased when the diet contains a high level of iron.

REVIEW OF LITERATURE

Copper Requirement and Deficiency Signs

In 1928 Hart et al. working with rats found that a whole milk diet supplemented with iron produced an anemia that could be corrected by the addition of copper to the diet. In addition to the anemia associated with a copper deficiency, Teague and Carpenter (1951) reported an abnormal leg condition. The leg joints lacked rigidity and the hocks became excessively flexed which forced the animal to assume a sitting position. In an extreme deficiency state the use of the forelegs was also lost and the animal, although not paralyzed, remained in a prone position. Copper was shown to be therapeutic and in some cases brought about a complete reversal of symptoms.

Coulson and Carnes (1963) associated cardiovascular lesions with a copper deficiency and suggested defective elastin as the cause of the lesion. Shields et al. (1962) found an inverse relationship between the anemia and heart weights. They felt this condition was consistent with the hypothesis that cardiac hypertrophy was a response to increased cardiac output caused by the anemia. Tensile strength of the aorta was markedly reduced and there was abnormal friability of the myocardium in copper-deficient animals as compared to control animals. A greater degree of hypertrophy of the heart has been noted in copper deficiency than in iron deficiency of swine (Gubler et al., 1957).

Anemia in swine produced by a copper deficiency is very similar to that produced by an iron deficiency. The anemia is characterized by hypochromic and microcytic erythrocytes, normoblastic hyperplasia of the bone marrow, hypoferremia and increased iron-binding capacity of the plasma (Cartwright et al., 1956; Lahey et al., 1952). Plasma ceruloplasmin levels were also reduced which leads to a reduction in serum iron levels (Roeser et al., 1970). The liver copper level is greatly reduced with a reduction in the kidney, spleen and heart copper levels when pigs were fed a copper-deficient diet (Lahey et al., 1952).

Although much work has been done over the past four decades with copper, it is not possible to give an exact dietary requirement of copper for the pig during growth, reproduction and lactation. Ullrey et al. (1960) found no difference in average daily gain, feed/gain and the formed elements of the blood among pigs fed three levels of copper, 6, 16 and 106 ppm. A copper intake of 16 ppm or 106 ppm increased the percent saturation of transferrin, albumin/globulin ratio, and serum copper and iron concentration when compared to a copper intake of 6 ppm. Liver copper and iron concentrations increased as the dietary copper intake increased.

The Agricultural Research Council of Great Britian (1967) came to the conclusion that 4 ppm copper was probably adequate for growing pigs up to 90 kg live weight. The National Research Council (1968) recommends 5 to 10 ppm for the growing pigs.

Copper as a Growth Stimulant

Today most of the interest in copper is centered around its use as a growth stimulant.

As early as 1928 Evvard et al. found that by adding copper sulfate to an otherwise normal diet, a growth stimulating effect could be produced; however, interest was dormant until Braude's (1945) work in England. He found that pigs housed in a piggery exhibited a craving for copper and completely licked away the copper rings at the base of the iron pipes in one year. In this work, Braude (1945) tested various other metals such as aluminum, brass, magnesium, nickel and tin but found that pigs still exhibited a craving for copper with some consumption of brass. Their craving for copper led to the addition of copper to the diet. Mitchell (1953) found that the addition of copper to the diet did increase total feed consumption. Bowler (1955) found that growth was stimulated when 250 ppm of copper was added to the diet.

Braude (1965) in his review of the work conducted on the effect of feeding high levels of copper to swine, estimated that at least one-third of the pigs in England were fed high levels of copper. From data compiled from 83 experiments, some of which were from the United States but the majority were European, Braude (1965) showed an 8.1% improvement in growth rate and a 5.4% increase in feed efficiency. In other work from Hungary, Berek et al., (1967) reported that 250 ppm of dietary copper increased growth rate by 9.7% and feed efficiency by 7.8%.

Wallace (1967) prepared a detailed review of the copper feeding experiments conducted in the United States. A summary of the baby pig comparisons, involving forty-three studies and 1280 pigs with initial ages of 10 to 25 days and test periods of 14 to 50 days, showed a 22.1% improvement in growth rate and an 8.3% increase in feed efficiency (weighted means). The levels of dietary copper involved were 50, 100, 125, 150, 200, 250, and 300 ppm. Copper sources were $CuSO_4.5H_2O$, $CuSO_4$, CuO, $CuCO_3$ and Cu-methionine. The pigs responded to all levels and sources of copper tested.

When Wallace (1967) summarized the available data for growing pigs, the response was not as great and was less consistent than for the baby pig. The copper effect on growth rate ranged from -11% to +23% with a weighted mean of +6.5%. The weighted mean for feed efficiency was an increase of 2.3%.

It is well documented that copper at the level of 250 ppm, will in the preponderance of cases, produce an increase in growth rate.

Copper as Compared to and in Conjunction with Antibiotics

Results of studies with growing swine (Bunch et al., 1963; Hays et al., 1969) showed an improvement in growth rate and feed efficiency from either high levels of copper or chlortetracycline.

The addition of four antifungal antibiotics; mystatin, phytoactin, phytostreptin and rimocidin failed to improve pig performance over diets supplemented with only copper sulfate (Hawbaker et al., 1961). Copper sulfate was also compared to oleandomycin and oxytetracycline with approximately equal results. The combination of copper sulfate with either oleandomycin or oxytetracycline resulted in an additive effect on growth rate.

Tylosin was found to increase feed intake and body weight gain of pigs fed a diet containing 6% soybean meal, but there was no improvement in gain if the diet contained 23% soybean meal. Copper sulfate improved body weight gain of pigs fed either diet (Beames, 1969).

Wallace (1967) in his review stated that there was an overall advantage in favor of copper feeding as compared to antibiotics; however, Thomas and Kornegay (1972) reported that the antibiotic combinations, neomycin-oxytetracycline and tylosin plus sulfamethazine, appeared to be slightly more effective in improving growth rate than copper with a trend towards an additive effect of copper and antibiotics.

Possible Explanation for Growth Stimulating Effect of Copper

Although there are many theories, the mode of action of copper in producing an increased growth rate is not definitely known. Barber et al. (1960) suggest that the improvement in growth

rate results from improvement in the efficiency with which the feed is utilized and of an increase in the daily amount of feed consumed. This is also the general case with antibiotics suggesting that the mode of action is similar. Bunch et al. (1961), Barber et al. (1960), and Kornegay and Thomas (1971) confirmed the suggestions of Barber et al. (1960) showing that both copper and antibiotics stimulate feed efficiency as well as appetite. In some instances, there was an additive effect when both copper and the antibiotic were included in the ration. A reduction in intestinal bacteria or parasites would also aid in feed efficiency. Miller et al. (1969) found that both copper and antibiotics were effective in reducing the intestinal bacterial population with copper sulfate being more effective than the antibiotics. Houser (1961) reported that feeding 250 ppm dietary copper had no effect on round worm infection when these pigs were compared to pigs receiving the basal ration.

Braude (1967) reported an interesting hypothesis suggested by H. D. Hays; namely, that the small amount of copper ion present in the pigs intestine would react with and detoxify the poisonous hydrogen sulfide. Hydrogen sulfide is liberated from protein digestion products of certain intestinal microorganisms and reacts with copper to produce the black, highly insoluble copper sulfide. This detoxification would likely assist in the efficiency of food uptake. Genči et al. (1969) reported that in eight balance trials with pigs weighing between 15 and 90 kg, a level of 1.1%

$CuSO_4 . 5H_2O$ gave an increased nitrogen retention of 6% up to 35 kg. The improvement in growth rate obtained by feeding copper sulfate, copper oxide or copper methionine suggest that the copper radical itself is involved in producing the increased growth rate (Bunch et al., 1961; Wallace, 1967; Hawbaker, 1959). This response is probably mediated at the gut level. Gipp et al. (1969) found that copper glycinate injected subcutaneously in amounts calculated to provide the same level of copper as that absorbed from the diet was not a satisfactory means of administration, since this method did not give the growth response of 250 ppm dietary copper.

Lewis and Dickson (1971) reported the formation of a complex between bacterial endotoxin and cupric ion Cu^{2+}, which caused mammalian (human, pig, rabbit) blood platelets to aggregate in suspension. Complexes formed between endotoxin and Zn^{2+}, Co^{2+}, N^{2+}, Mn^{2+}, Fe^{2+}, Ca^{2+}, Mg^{2+}, Br^{2+}, Be^{2+}, Cd^{2+}, Ag^{+}, or Hg^{2+} did not cause aggregation. It seems possible that in vivo, when endotoxins are released into the bloodstream during bacterial (gram negative) infections, they could extract sufficient Cu^{2+} from the plasma which would increase the formation of platelets.

Copper and Anemia

The morphologic and biochemical similarities between anemia due to copper deficiency and anemia due to iron deficiency suggest that in copper-deficient swine there is an abnormality in the metabolism of iron and that the anemia may be the consequence of

this abnormality (Lahey et al., 1952).

Chase et al. (1952) found that copper influences the absorption of iron in the rat. The amount of Fe^{59} absorbed was favored by increasing the amount of dietary copper up to levels of 0.25 to 0.50 mg per rat per day. Above this amount there appeared to be somewhat reduced absorption.

It has also been shown that copper-deficient swine fail to absorb dietary iron at the normal rate (Lee et al., 1968). Increased amounts of stainable iron were found in fixed sections of duodenum from the copper-deficient pigs. When Fe^{59} was administered orally, the mucosa of copper-deficient pigs extracted iron from the duodenal lumen at the normal rate, but the subsequent transfer to the plasma was impaired.

The red cell life span was measured by the use of radioactive chromium and found to be reduced in copper-deficient pigs (Bush et al., 1956).

Lee et al. (1969) found that the anemia produced by a copper deficiency does not result from reduced activity of heme biosynthetic enzymes.

Curzon and O'Reilly (1960) demonstrated that ceruloplasmin has oxidase activity for both ferrous and ferric iron. Activity was increased more by ferrous than by ferric at the same concentration (in vitro). Osaki et al. (1966) estimated that the nonemzymic oxidation of Fe^{2+} is insufficient to account for a rate of Fe^{3+} transferrin formation necessary to provide an adequate iron supply

for hemoglobin and other biosyntheses if Fe^{2+} is a relevant source of serum iron. They proposed a biological role for ceruloplasmin in promoting the rate of iron saturation of transferrin and in stimulating iron utilization and the designation of the enzyme as serum ferroxidase.

Working with pigs (Ragan et al., 1969) showed that the administration of homolgous ceruloplasmin to copper-deficient swine with adequate iron stores resulted in a rapid, marked and sustained increased plasma iron concentration. The administration of inorganic copper, equivalent to that contained in the ceruloplasmin, produced only a minimal, transient increase in plasma iron.

Copper injection of rats whose stores of copper had been virtually exhausted resulted in a temporary increase in the concentration of plasma iron and a depletion of the iron stored in the liver (Marston et al., 1971). When they injected iron into copper-deficient rats the plasma iron was only transiently increased.

Roeser et al. (1970) postulated that if ceruloplasmin functions physiologically as a ferroxidase in promoting the rate of transferrin formation and if the abnormalities in iron metabolism observed in copper-deficient swine are to be ascribed to a deficiency of ceruloplasmin ferroxidase activity it then follows that in most animals: (a) there must be a deficiency of ceruloplasmin, (b) the deficiency of ceruloplasmin should precede the restriction in the flow of iron into plasma as manifested by the development of

hypoferremia, and (c) an increase in ceruloplasmin in the circulation should precede the increase in plasma iron which follows the administration of copper. In their work they found that when the plasma ceruloplasmin level fell below 1% of the normal mean, cell to plasma iron flow became sufficiently impaired to cause hypoferremia, even though total body iron stores were normal. When ceruloplasmin was administered to such animals, plasma iron increased immediately and continued to rise at a rate proportional to the logarithm of the ceruloplasmin dose. The administration of inorganic copper induced increases in plasma iron only after ceruloplasmin appeared in the circulation. Thus, ceruloplasmin appeared to be essential to the normal movement of iron from cells to plasma.

At the other extreme, high levels of copper also produce anemia. Several workers have shown that increasing the dietary iron level will prevent this anemia (Bunch et al., 1963; Suttle and Mills, 1966). This would imply an interaction between copper and iron which may involve competition between elements with similar affinities for binding sites on the protein in the digesta, or in the tissues (Underwood, 1971; Suttle and Mills, 1966).

Copper Metabolism

When radioactive copper is given orally the activity appears rapidly in the blood (Bush et al., 1955). This suggests that the absorption of copper takes place in the stomach or upper small intestine.

In rats copper was absorbed mainly in the stomach. The rate of absorption declined as Cu^{64} was placed further away from the pylorus (Van Campen et al., 1955). Starcher (1967) using Cu^{64} activity in the liver as a measure of copper absorption found that in the chick, copper was absorbed to a much greater extent in the duodenum than in the stomach. He also found that even though there was no absorption from the ventriculus, this organ had an extraordinary ability to bind copper. In the pig the major sites of copper absorption appear to be the small intestine and the colon (Bowland et al., 1961). Starcher (1969) was able to show that copper binds in the duodenum to a protein with an approximate molecular weight of 10,000 and this may be an important step in copper absorption.

In studies with mice, Gitlin et al. (1960) indicated that two mechanisms maybe involved in copper absorption: one which follows first-order kinetics and the second, an enzyme system. Using an in vitro technique with hamsters, Crampton et al. (1964) were able to show increased absorption of copper above a concentration of one part per million. Bowland et al. (1961) showed a three-fold

increase in copper absorption when pigs were given 250-300 ppm as compared to pigs that received 40 ppm. These studies do not support the first-order kinetics mechanism.

From the intestine, copper moves into the blood serum. There are two types of copper in the serum: (1) direct acting copper which reacts directly with sodium diethyldithiocarbamate and (2) an indirect reacting copper which does not react with sodium diethyldithicarbamate (Wintrobe et al., 1952). The direct reacting copper is believed to be associated with the albumin and amino acid-bound fractions. Approximately 7% of the copper is in this form at equilibrium. The indirect reacting copper is associated with the serum protein, ceruloplasmin and approximately 93% of the serum copper is in this form at equilibrium (Dowdy, 1969). Underwood (1971) states that ceruloplasmin cannot play a major role in copper absorption and transport, because the amount of ceruloplasmin copper exchanged daily is extremely small compared with the amounts of copper absorbed from the intestinal wall.

Milne and Weswig (1968) showed that in rats the plasma copper level increased with increasing amounts of dietary copper. The direct reacting copper appeared to immediately reflect copper intakes better than the indirect reacting copper. This suggests that most of the copper is transported from the intestine to the tissue in the loosely bound form. Sternlieb et al. (1967) found that the lymphatic system was not an important route of the copper absorption.

The liver plays an important role in copper metabolism, serving at least three functions: (1) a major storage site of copper (Underwood, 1972), (2) the site of ceruloplasmin synthesis (Owen and Hozelrig, 1966), and (3) the excretion of copper through the biliary system (Mahoney et al., 1955).

Working with rats, Milne and Weswig (1968) found that the soluble fraction of the liver (the supernatant after centrifuging at 75,000 x g for 30 minutes) was very sensitive to dietary copper changes. Liver copper levels were not changed by dietary copper levels up to 100 ppm, but were significantly increased by dietary copper levels of 200 ppm.

Gregordiodis and Sourkes (1967) reported that in copper loading, the mitochondria and nuclei hold most of the excess copper with the cytoplasm and microsomes accumulating much less. Studies with rats (Sourkes et al., 1968) and rabbits (Hunt et al., 1970) showed that the copper and iron concentrations in the liver were inversely correlated in a complex relationship. Reinhold et al. (1967) found protein intake and liver copper levels to be inversely related in rats.

The major route of excretion of copper is through the feces with very little occuring in the urine. Working with radioactive copper, Bowland et al. (1961) reported the appearance of copper in the feces in less than two hours after dosing. The bulk of the copper appeared in the feces in 17-20 hours after dosing. If the copper was injected, it appeared in the feces 30-100 minutes after

the injection. The bile accounted for 40% of the excreted copper. Mahoney et al. (1955) concluded that the capacity of dogs and pigs to excrete copper is somewhat limited.

Evans et al. (1970) reported that in rats biliary copper excretion is decreased following hypophysectomy, throidectomy and adrenalectomy.

Interactions With Other Metals

Copper and iron constitute a definite interrelationship in that each requires a small amount of the other for utilization and that high levels of either interfere in some way with utilization of the other.

Suttle and Mills (1966) reported that when an additional 150 ppm of iron and of zinc were added to a diet containing 425 ppm copper, complete protection from copper toxicity was accomplished. In subsequent work by Suttle and Mills (1966), the severity of copper toxicity resulting from feeding diets containing 750 ppm copper was decreased when 500 ppm zinc was added, but the pigs remained anemic. When 750 ppm of iron was added to the above diet, protection from the anemia was obtained.

The interrelationship between copper, iron and zinc might involve competition between elements with similar affinities for binding sites on proteins in the digesta or in the tissues (Suttle and Mills, 1966). Starcher (1969) demonstrated in the duodenum of the chick competition of cadmium and zinc for the same protein

binding sites as required by copper. This was demonstrated *in vitro* by Breslow and Gurd (1963) and Plocke and Valle (1962). There was a drop in the pH when copper binds to the protein, (Breslow and Gurd, 1963). At pH 5.0 each Cu^{2+} ion that was bound replaced one hydrogen atom and at higher pH values each copper ion replaces three hydrogen atoms.

Increasing the dietary level of zinc above that found in normal diets has been shown to decrease the amount of copper stored in the livers of pigs (Allen *et al.*, 1958; O'Hara, Newmann and Jackson, 1960; Hanrahan and O'Grady, 1968). However, other workers have not been able to reduce the copper stores of rats and pigs by increasing dietary zinc (Kulwich *et al.*, 1953; Cox and Hale, 1962; Kinnamon, 1966; Gipps, Pond and Smith, 1967). Kline, Hays and Cromwell (1972) were able to obtain a significant copper-zinc interaction for average daily gain and plasma copper. From their results it appeared that higher than normal levels of supplemental zinc and iron, 100 and 50 ppm respectively, were of no particular benefit in alleviating the adverse effects of 500 ppm dietary copper.

Copper Toxicity

Chromic copper poisoning may occur in pigs fed high levels of copper over an extended period. In a paper on copper toxicity, Allen and Harding (1962) indicated that the first signs of developing toxicity were a small loss of appetite, the pigs became

dull in 2 or 3 days before death and were weak and unsteady, particularly in the hind legs. Twenty-four hours before death the pigs were hyperaesthetic and developed fine trembling. On post-mortem examination the carcasses were in good condition but the skin and sclera had a yellow tint. The livers were friable and varied in color from yellow-tan to deep orange-brown. The blood was poorly clotted, jaundice was present, pulmonary edema and ulceration of the esophageal zone of the stomach was present.

The level of protein fed has been implicated as a factor in the severity; or the level of copper required, to produce toxicity, (Wallace, 1960).

Suttle and Mills (1966) were able to show that liberal quantities of iron and zinc aleviated the toxic effect of copper at levels up to 750 ppm.

Ritchie et al. (1961, 1963) observed that additional zinc in the diet decreased the liver copper level and the incidence of copper poisoning.

DeGoey, Wahlstrom and Emerick (1971) observed that zinc and iron at levels of 100 ppm each appeared to give some protection against the toxicity of 500 ppm copper. They found no protection from feeding 50 ppm supplemental molybdenum. Liver copper levels were greatly increased when copper was included in the diet, while they tended to be less with increases in dietary zinc and iron. Gipp, Pond and Smith (1967) tested the effect of molybdenum, sulfate and zinc on body weight gain, hemoglobin and liver copper

storage in pigs and found no conclusive evidence to support a copper-molybdenum-sulfate interrelationship or any copper-zinc interaction. Conversely, Kline, Hays and Cromwell (1969) found lower liver copper values when molybdenum was added to a high copper diet.

Kainski et al. (1967) found that high levels of iron in the diet reduced liver copper levels when high copper diets were fed.

EXPERIMENTAL PROCEDURE

Animals

The pigs used in these studies were Hampshire and Yorkshire crossbreds. They were farrowed under confinement conditions, were given an iron dextran injection (100 mg Fe) at two days of age and were weaned at three weeks of age. In trials I and II the pigs were housed in an environmentally controlled building and kept in stainless steel cages with false bottoms. In trials III and IV the pigs were housed at the nursery in 1.2 x 3.7 meter pens with expanded metal floors.

Allotment, Feeding and Weighing

The pigs were assigned to treatments by randomization on the basis of sex and weight with the condition that littermates could not be alloted to the same treatment. Each treatment group was assigned to a stainless steel cage (trials I and II) or to a pen (trials III and IV).

Seventy-two crossbred pigs averaging 8.1 kg initially were fed in two six week trials (trials I and II). There were two replications of 3 pigs per treatment in each trial. Sixteen crossbred pigs averaging 9.8 kg initially were used in trial III in which there were 4 pigs per treatment. Sixty crossbred pigs averaging 8.9 kg initially were used in trial IV. There were two replications of five pigs per treatment.

In trials I and II, feed intake among treatment groups was equalized. Pigs were fed three times daily an amount that would be consumed in approximately one hour. In trials III and IV pigs were fed ad libitum. The diet was fed in the form of a dry meal in all studies. The composition of the basal diet is given in table 1. An 18% crude protein basal diet was fed in all trials; however, in trial IV, the crude protein level was reduced to 16% when the pigs reached approximately 21 kilograms. Reagent grade minerals were used to supply the desired mineral levels for each treatment diet.

The treatment diets for trials I and II are as follows: (ppm) diet 1, 7 Cu and 92 Fe; diet 2, 21 Cu and 92 Fe; diet 3, 257 Cu and 92 Fe; diet 4, 7 Cu and 267 Fe; diet 5, 21 Cu and 267 Fe; diet 6, 257 Cu and 267 Fe. Trial III was a replicate of diets 1, 3, 4 and 6 of trials I and II. The treatment diets for trial IV are as follows: (ppm) diet 1, 7 Cu and 101 Fe; diet 2, 25 Cu and 101 Fe; diet 3, 257 Cu and 101 Fe; diet 4, 7 Cu and 312 Fe; diet 5, 25 Cu and 312 Fe; diet 6, 257 Cu and 312 Fe.

Copper was added to the basal diet in the form of $CuSO_4.5H_2O$ in trials I, II and III, respectively, and as anhydrous $CuSO_4$ in trial IV. Iron was added to the diet in the form of anhydrous ferrus sulfate. Zinc supplied in the diet was in the form of $ZnSO_4$ as a dry powder.

TABLE 1

Composition of Basal Diet[a]

Item	Percent
Dairy nutrient blend (20% CP)	10.0
Soybean meal (45% CP)	22.0
Ground yellow corn (8.7% CP)	65.1
NaCl	0.5
Vitamin premix[b]	0.5
Dibasic calcium phosphate	0.9
Limestone	1.0

[a] Calculated to contain 18% crude protein. Dairy nutrient blend was analyzed to contain 4 ppm Cu and 40 ppm Fe; soybean meal contained 18 ppm Cu and 154 ppm Fe; corn contained 4 ppm Cu and 34 ppm Fe. The basal diet had 30 ppm Fe and 59 ppm Zn added.

[b] Vitamin premix supplied (per kilogram of diet); vitamin A, 1100 I.U.: vitamin D, 220 I.U.; pantothenic acid, 8.8 mg; niacin, 11.0 mg; riboflavin 2.2 mg; chloine chloride, 660 mg; vitamin B_{12} 44 mcg.

In trials I, II and III the pigs were weighed initially and at weekly intervals for the duration of the trial. Feed intake was recorded daily for trials I and II and the feed consumption for trial III was recorded on a weekly basis.

In trial IV the pigs were weighed initially and then at biweekly intervals, feed consumption was also recorded on a biweekly basis. Trials I, II and III were 6 weeks in length and trial IV was conducted over a 9-week period.

Blood Data

In trials I and II blood samples were taken initially, and at weekly intervals throughout the trials. At the outset of the trials and at 2, 4 and 6 weeks, blood was obtained by puncture of the anterior vena cava. At 1, 3 and 5 weeks an ear vein was cut to obtain blood. Hemoglobin and hematocrit were determined weekly and ceruloplasmin, serum copper and iron were determined at 2-week intervals; however serum iron values were not taken in trial I due to hemolysis.

In trial III, blood samples were taken initially and at weekly intervals from an ear vein for hemoglobin and hematocrit determinations.

In trial IV, blood samples were taken initially and at three week intervals by puncture of the anterior vena cava. Analysis of the blood was the same as described for trial II.

Hemoglobin concentrations were determined by the cyanmethemoglobin method (Crosely, Munn and Furth, 1954) and hematocrit values were determined by the micro capillary method (McGovern, Jones and Steinberg, 1955).

Blood samples from the anterior vena cava were placed in a water bath at 37° C for one-half hour and then centrifuged for twenty minutes in an International centrifuge, model HN. Fresh serum was used for ceruloplasmin determinations and the remainder was frozen for copper and iron determinations. Ceruloplasmin was determined by the method of Rice (1960) which is based on the ability of ceruloplasmin to oxidize p-phenylenediamine. In these determinations 0.1 ml of serum was incubated with 1 ml of p-phenylenediamine for 30 minutes at 37° C. The ceruloplasmin activity was standarized to I. U. by the method of Rice (1962).

Serum copper values in trials I and II were determined according to the method of Berman (1965) and in trial IV by the method of Olson and Hamlin (1968). Serum iron values were determined by the method of Olson and Hamlin (1968). The procedure was changed bacause the method of Olson and Hamlin (1968) allows the determination of both copper and iron from the same serum sample and is equally as accurate as the method of Berman (1965).

Feed, Tissue, Hair and Bone Data

Liver, spleen, kidney, hair and bone samples were taken in trial IV for copper, iron and zinc determinations. In trials I and II the liver was taken for analysis of these minerals.

At the time of slaughter, a section of the left lateral lobe of the liver was removed. Fat and connective tissue were trimmed and the excess blood was rinsed and blotted away. The right kidney was removed and the fat and outer connective tissue were removed. Hair samples were taken from the right fore shoulder and stored in plastic bags at room temperature until analysis. The metacarpal 2 and 5 from the right foot was analyzed.

The liver, spleen and kidney were homogenized in a Waring blender with stainless steel containers. A representative sample of the respective tissues was then lyophilized. The dry sample was then wet ashed with concentrated nitric acid and 30% hydrogen peroxide. The digested sample was quantitatively transferred to a 100-ml volumetric flask and allowed to cool. Copper, iron and zinc levels were determined by atomic absorption spectrophotometry, using a Perkin Elmer Model 403 Atomic Absorption Spectrophotometer.

The hair samples were wet ashed in the same manner as the other tissues except the digested sample was quantitively transferred to a 25-ml volumetric flask for copper and iron determination. The sample was further diluted 1:5 for zinc analysis. The feed was ground in a Willey mill with a 2-mm stainless steel screen and a 2-gram sample was digested with nitric acid and perchloric acid.

Bones were boiled for 30 minutes in distilled water to remove tissue. The bones were then extracted for 24 hours with ethanol and for 6 hours with ether. The extracted bone was dried and then wet ashed with nitric and perchloric acids.

Copper Injection

Copper injections were given at the termination of each trial to evaluate this method for its usefulness as a sensitive indication for copper deficiency. The ceruloplasmin level was measured prior to and 24 hours after the injection. The level of copper injected intraperitoneally was calculated on the basis of feed consumption. Ten percent of the copper was assumed to be absorbed in trial I and IV. In trial II the copper injection level was based on the total amount of copper intake in the feed per day.

The copper solution for injection was prepared by dissolving the desired amount of $CuSO_4$ in a 0.85% saline solution and the solution was autoclaved. In trial I, 5 ml of copper solution containing 7.5 mg of copper was injected interperitoneally. In trial II, 5 ml of copper solution containing 6590 mg of copper was injected intraperitoneally. This dose proved to be extremely toxic. In replication I of trial IV, 2.5 ml of copper solution containing 10 mg of copper per ml was injected intraperitoneally. In replication II of trial IV, 100 ml of copper solution containing .125 mg of copper per ml was injected.

Statistical Analysis

Data was statistically analyzed by least square analysis of variance (Snedecor, 1956) and difference between the copper levels was tested using Duncan's (1955) multiple range test.

RESULTS AND DISCUSSION

Feedlot Performance

In trials I and II differences in weight gain among the dietary treatments were not significant, although there was a trend toward greater weight gains from the highest copper level (table 2).

Pigs fed high-copper diets consumed their feed more quickly than pigs fed low copper diets. The lack of a significant increase in weight gain due to the high level of copper in trials I and II is not unexpected when feed intake is equalized among treatments because much of the weight gain increase previously reported for high copper feeding was due to increased feed consumption with the feed efficiency not always being significantly affected by copper.

In trial III in which the pigs were full fed, difference in weight gains among treatments were again not significant (table 3). There was no effect of treatments upon gain/feed ratios.

Supplemental copper at 250 ppm in trial IV significantly increased weight gains during the first four weeks (table 4). During the second four-week period there were no differences in weight gains among the various treatments. This finding of the growth stimulating effect of copper being more apparent during the early growth period is in general agreement with the results presented by Wallace (1968) in his review. Over the entire eight-week period there was an increase ($P<.01$) in weight gain due to high dietary copper.

TABLE 2. EFFECT OF VARIOUS COPPER AND IRON LEVELS ON WEIGHT GAIN, FEED CONSUMPTION AND GAIN/FEED. TRIAL I AND II.[a,b]

Dietary Cu, ppm	7	21	257	7	21	257
Dietary Fe, ppm	92	92	92	267	267	267
Initial Wt.						
Trial I	8.30	8.46	8.88	8.20	8.33	8.42
Trial II	8.30	7.80	8.31	7.74	7.74	7.95
Wt. gain 1-3 wks						
Trial I	8.35	8.04	8.50	8.34	8.52	8.49
Trial II	6.13	6.31	6.41	6.37	5.84	6.63
Wt. gain 3-6 wks						
Trial I	12.10	12.90	13.53	11.94	12.55	11.69
Trial II	12.19	11.95	13.16	11.65	11.64	13.37
Wt. gain 1-6 wks						
Trial I	20.45	20.94	22.03	20.28	21.07	20.18
Trial II	18.32	18.26	19.57	18.02	17.48	20.00
Feed intake 1-3 wks						
Trial I	49.60	49.60	50.22	49.72	49.72	50.22
Trial II	41.87	41.87	41.87	41.02	41.02	41.87
Feed intake 3-6 wks						
Trial I	78.75	78.75	78.75	78.75	78.75	78.75
Trial II	76.02	76.02	76.02	76.02	76.02	68.23

[a] Weight gain and feed consumption in kilograms

[b] Standard errors of the treatment means for trials I and II respectively are: Initial wt. 0.0896, 0.0994; Wt. gain 1-3 wks 0.0681, 0.2040; Wt. gain 3-6 wks 0.2552, 0.2828; Wt. gain 1-6 wks 0.2536, 0.3612.

TABLE 2[b]. (Con't)

Dietary Cu, ppm	7	21	257	7	21	257
Dietary Fe, ppm	92	92	92	267	267	267
Feed intake 1-6 wks						
Trial I	128.35	128.35	128.97	128.47	128.47	128.97
Trial II	117.89	117.89	117.89	117.89	117.04	117.04
Gain/Feed 1-3 wks						
Trial I	.168	.162	.169	.168	.173	.169
Trial II	.146	.151	.153	.155	.142	.158
Gain/Feed 3-6 wks						
Trial I	.154	.164	.172	.156	.159	.148
Trial II	.160	.157	.173	.153	.153	.196
Gain/Feed 1-6 wks						
Trial I	.159	.163	.171	.158	.164	.156
Trial II	.155	.154	.166	.154	.149	.182

[b] Standard errors of the treatment means for trials I and II respectively are: Gain/Feed 1-3 wks 0.0028, 0.0004; Gain/Feed 3-6 wks 0.0091, 0.0057; Gain/Feed 1-6 wks 0.0091, 0.0040.

TABLE 3. THE EFFECT OF DIETARY COPPER AND IRON LEVELS ON WEIGHT GAIN. TRIAL III.[a]

	Weight Gain (Kg)			
Dietary Cu	7	257	7	257
Dietary Fe	92	92	267	267
Initial Wt	9.88	10.22	9.32	9.88
Gain 1st Wk	2.27	2.04	1.19	3.75
Gain 2nd Wk	2.84	4.94	3.52	3.01
Gain 3rd Wk	3.75	4.37	3.92	4.54
Gain 4th Wk	4.77	4.88	3.81	4.72
Gain 5th Wk	5.16	5.45	5.62	5.60
Gain 6th Wk	3.92	3.52	3.29	4.04

[a] Standard errors of the treatment means are: Initial 0.0754; 1st week 0.7670; 2nd week 1.2175; 3rd week 1.5925; 4th week 1.6517; 5th week 1.7450; 6th week 1.8395.

TABLE 4. EFFECT OF DIETARY COPPER AND IRON LEVELS ON WEIGHT GAIN, GAIN FEED AND FEED INTAKE. TRIAL IV[a]

Dietary Copper	7	25	257	7	25	257
Dietary Iron	101	101	101	312	312	312
Initial weight[b]	8.68	9.02	9.04	9.30	8.70	8.98
1-4 weeks gain[c]	13.50	12.72	16.27	15.16	14.25	16.34
4-8 weeks gain[c]	22.02	21.48	21.54	21.64	21.12	22.72
1-8 weeks gain[c]	35.45	34.55	37.73	36.82	35.45	39.09
			Gain/Feed Ratio			
1-4 weeks	0.536	0.544	0.494	0.483	0.545	0.528
4-8 weeks	0.404	0.419	0.391	0.391	0.405	0.404
1-8 weeks	0.435	0.455	0.436	0.425	0.450	0.444
			Feed Intake (kg)			
1-4 weeks[d]	138.8	122.7	157.7	156.4	132.2	158.6
4-8 weeks	272.7	258.2	275.0	276.8	260.9	281.6
1-8 weeks	109.5	308.9	432.7	433.2	393.2	440.0

[a] Standard errors of the treatment means are: Weight gain: Initial 0.325; 1-4 weeks 0.787; 4-8 weeks 0.698; 1-8 weeks 1.040; Gain/Feed: 1-4 weeks 0.004; 4-8 weeks 0.008; 1-8 weeks 0.008; Feed Intake: 1-4 weeks 1.236; 4-8 weeks 8.373; 1-8 weeks 7.938.

[b] Significant ($P<.01$) copper-iron interaction

[c] Significant ($P<.01$) copper effect

[d] Significant ($P<.05$) copper effect on feed consumption

The gain/feed ratio was not affected by either copper or iron additions at the 1-4 or 4-8 week period, although the feed consumption ($P<.01$) was increased during the 1-4 week period. DeGoey et al. (1971) obtained an increase in growth rate due to feeding a high level of copper but the gain/feed ratio was not significantly effected. Conversely, Beames (1967) obtained an increase in growth from supplemental copper which was associated with an improvement of the gain/feed ratio. Wallace (1968) summarized fifty-seven studies and obtained a weighted mean for rate of gain of +6.5% and feed efficiency of 2.3% in growing pigs.

Hemoglobin and Hematocrit Values

Hemoglobin: In trial I, after three weeks, pigs fed the high dietary level of copper had depressed ($P<.05$) hemoglobin levels. Both copper and iron produced a significant effect at the fourth week. The overall effect was reduced hemoglobin values due to feeding 257 ppm dietary copper, and an increase in hemoglobin due to feeding 267 ppm dietary iron.

In trial II, differences among treatments occurred after four weeks when pigs fed diets containing 257 ppm iron had higher ($P<.05$) hemoglobin values (table 5). At the fifth week there was a significant copper effect with the highest dietary copper level causing a reduction in hemoglobin. The high iron level produced a highly significant effect on hemoglobin. At six weeks there were no differences.

TABLE 5. EFFECT OF DIETARY COPPER AND IRON LEVELS ON THE HEMOGLOBIN VALUES (g/100 ml) OF PIGS. TRIALS I AND II[a]

	Cu, ppm							
	7		21		257		Mean	
Initial Fe, ppm	T-I	T-II	T-I	T-II	T-I	T-II	T-I	T-II
92	13.51[b]	12.39	13.34	12.41	13.48	12.65	13.44	12.48
267	14.21	13.45	14.43	12.67	12.39	12.95	13.68	13.02
Mean	13.86	12.92	13.88	12.54	12.94	12.81		
1st Week								
92	11.88[b]	12.35	12.22	13.12	12.50	12.88	12.20	13.16
267	12.70	13.97	12.80	12.69	11.20	13.16	12.23	12.90
Mean	12.29	13.16	12.51	12.90	11.85	13.02		
2nd Week								
92	12.15	11.01	12.30	12.04	11.56	11.72	12.00	11.59
267	12.85	11.58	13.00	11.22	12.32	11.30	12.72	11.36
Mean	12.50	11.29	13.65	11.64	11.94	11.51		
3rd Week								
92	12.74	11.98	13.75	12.49	11.48[c]	12.12	12.66	12.20
267	14.38	12.49	13.10	12.85	12.55	11.99	13.34	12.66
Mean	13.56	12.23	13.42	12.67	12.02	12.05		

[a] Standard errors of the treatment means for trials I and II respectively are: Initial 0.436, 0.519; 1st week 0.442, 0.508; 2nd week 0.523, 0.352; 3rd week 0.638, 0.297.

[b] Significant ($P<.05$) copper-iron interaction

[c] Significant ($P<.05$) copper effect

TABLE 5. (Con't)[a]

	Cu, ppm							
	7		21		257		Mean	
4th Week	T-I	T-II	T-I	T-II	T-I	T-II	T-I	T-II
Fe, ppm								
92	12.36	11.38	12.66	11.72	11.12	11.22	12.05	11.44
267	13.28	12.24	13.48	12.07	12.25[c]	11.57	13.00[d]	11.96[d]
Mean	12.82	11.81	13.07	11.90	11.69	11.40		
5th Week								
92	13.50	13.41	13.37	12.48	12.75	12.52	13.21	12.80
267	14.74	13.92	13.94	13.22	13.34	13.33[c]	14.01	13.49[e]
Mean	14.12	13.67	13.66	12.85	13.05	12.93		
6th Week								
92	14.45	13.04	13.72	13.21	12.38	13.07	13.52	13.11
267	14.67	13.50	15.68	13.39	14.32[c]	13.46	14.89[e]	13.45
Mean	14.56	13.27	14.70	13.30	13.35	13.26		
Final								
92	15.58		14.52		13.55		14.55	
267	15.52		15.04		15.21		15.25	
Mean	15.55		14.78		14.38			

[a] Standard errors of the treatment means for trials I and II respectively are: 4th week 0.556, 0.243; 5th week 0.495, 0.290; 6th week 0.550, 0.257; Final 0.731.

[b] Significant ($P < .05$) copper-iron interaction

[c] Significant ($P < .05$) copper effect

[d] Significant ($P < .05$) iron effect

[e] Significant ($P < .01$) iron effect

Results for trial III are shown in table 6. Only the initial, second, fourth and sixth weeks are shown even though hemoglobin values were taken each week throughout the study. There were no significant effects of dietary copper and iron on hemoglobin values until the second week. At this time the copper-iron interaction was significant ($P<.01$). No other significant difference occurred until the sixth week when there was a highly significant copper and iron effect and a copper-iron interaction ($P<.05$) occurred. The lack of effect of copper and iron on hemoglobin was probably due to the poor growth in the early part of the study of these pigs. Whereas in trial IV in which the pigs were self fed, as in trial III, differences in hemoglobin levels occurred by the third week which was probably due to the rapid growth of the pigs in trial IV.

In trial IV, there was a significant effect of copper and a highly significant effect of iron after three and six weeks (table 7). At six weeks, the copper-iron interaction was significant. The adverse effect on hemoglobin values of 257 ppm copper was compensated for by increasing the dietary iron level to 312 ppm. At nine weeks the copper and iron effects were highly significant and the copper iron interaction continued to be a significant factor affecting hemoglobin content of blood.

TABLE 6. EFFECT OF DIETARY LEVELS OF COPPER AND IRON ON HEMOGLOBIN VALUES (g/100 ml) OF PIGS. TRIAL III[a]

	Cu, ppm		
Initial Fe, ppm	7	257	Mean
92	12.56	12.96	12.80
267	12.56	13.09	12.83
Mean	12.56	13.03	
2nd Week			
92	13.13	11.46	12.29
267	11.56	12.52[b]	12.02
Mean	12.32	11.99	
4th Week			
92	12.21	11.72	11.95
267	12.50	12.50	12.50
Mean	12.32	12.12	
6th Week			
92	13.61	12.08[c]	12.86[d]
267	13.82	13.37[e]	13.60
Mean	13.70	12.73	

[a] Standard errors of the treatment means are: Initial 0.415; 2nd week 3.886; 4th week 0.486; 6th week 0.181.

[b] Significant ($P<.01$) copper-iron interaction

[c] Significant ($P<.01$) copper effect

[d] Significant ($P<.01$) iron effect

[e] Significant ($P<.05$) copper-iron interaction

TABLE 7. EFFECT OF DIETARY COPPER AND IRON LEVELS ON HEMOGLOBIN VALUES (g/100 ml) OF PIGS. TRIAL IV.[a]

	Cu, ppm			
Initial				
Fe, ppm	7	25	257	Mean
101	11.34	11.12	11.06	11.17
312	10.74	11.46	11.38	11.19
Mean	11.04	11.29	11.22	
3rd Week				
101	12.25	12.21	11.21	11.89
312	12.77	12.79	12.66	12.74[c]
Mean[b]	12.51	12.50	11.94	
6th Week				
101	12.74	14.04	11.43	12.32
312	14.04	13.43	13.88	13.78[c]
Mean	13.39	13.74	12.66[d]	
9th Week				
101	12.69	12.66	10.74	12.05
312	13.29	12.51	12.75	12.84[c]
Mean[e]	12.99	12.58	11.74[f]	
Final				
101	14.28	14.54	11.85	13.56
312	14.90	14.08	14.38[d]	14.45[c]
Mean[e]	14.59	14.31	13.12	

[a] Standard errors of the treatment means are: Initial 1.323; 3rd week 0.241; 6th week 0.382; 9th week 0.402; final 0.375.

[b] Significant ($P<.05$) copper effect

[c] Significant ($P<.01$) iron effect

[d] Significant ($P<.01$) copper-iron interaction

[e] Significant ($P<.01$) copper effect

[f] Significant ($P<.05$) copper-iron interaction

TABLE 8. EFFECT OF DIETARY LEVELS OF COPPER AND IRON ON THE HEMATOCRIT VALUES (%) OF PIGS. TRIALS I AND II[a]

	Cu, ppm							
	7		21		257		Mean	
Initial Fe, ppm	T-I	T-II	T-I	T-II	T-I	T-II	T-I	T-II
92	40.25	37.08	40.08	36.92	40.08	39.66	40.14	38.55
267	40.58	40.66	40.29	37.71	37.80	38.37	39.56	38.72
Mean	40.42	38.87	40.18	38.88	38.94	38.31		
1st Week								
92	37.33	35.79	36.77	37.21	37.77	36.54	37.16	36.51
267	39.58	37.88	38.25	34.54	36.48	36.29	38.11	36.24
Mean	38.46	36.83	37.51	35.88	36.93	36.42		
2nd Week								
92	37.63	37.08	37.35	39.08	34.67	38.83	36.55[b]	38.33
267	39.63	38.38	39.25	34.92	37.33	36.75	38.74	36.60
Mean	38.63	37.72	38.30	37.00	36.00	37.79		
3rd Week								
92	37.47	36.79	38.50	38.46	34.00[c]	37.04	36.66	37.43
267	43.22	38.83	37.82	37.86	35.79	36.83	38.93	37.84
Mean	40.34	37.81	38.16	38.16	34.88	36.94		

[a] Standard errors of the treatment means for trials I and II respectively are: Initial 1.076, 1.675; 1st week 1.244, 1.034; 3nd week 1.202, 1.350; 3rd week 1.656, 0.954.

[b] Significant ($P<.05$) iron effect

[c] Significant ($P<.05$) copper effect

TABLE 8. (Con't)[a]

	Cu, ppm							
	7		21		257		Mean	
4th Week	T-I	T-II	T-I	T-II	T-I	T-II	T-I	T-II
Fe, ppm								
92	38.72	39.42	38.70	39.08	36.47	37.95	37.96[b]	38.82[b]
267	41.53	41.25	42.58	40.96	38.58	39.79	40.90	40.67
Mean	40.12	40.33	40.64	40.02	37.52	38.88		
5th Week								
92	39.62	39.38	40.35	37.29	38.97	39.12	39.64	38.60[d]
267	42.13	41.50	40.70	40.12	39.53	40.59	40.79	40.74
Mean	40.88	40.44	40.53	38.71	39.25	39.85		
6th Week								
92	42.52	37.00	39.30	38.83	37.47	39.42	39.76[b]	38.42
267	42.07	39.42	45.83	39.00	41.83	39.00	43.24	39.14
Mean	42.30	38.21	42.56	38.92	39.52	39.21		
Final								
92	45.10		41.80		40.38		42.43	
267	44.17		43.12		43.75		43.68	
Mean	44.63		42.46		42.07			

[a] Standard errors of the treatment means for trials I and II respectively are: 4th week 1.684, 1.047; 5th week 1.327, 0.897; 6th week 1.596, 0.795; final 1.928.

[b] Significant ($P<.05$) iron effect

[c] Significant ($P<.05$) copper effect

[d] Significant ($P<.01$) iron effect

Across all treatments, hemoglobin levels were increased by copper injection at the termination of the trials. This effect may have been due to the stress of the copper injection. Hemoglobin values were not determined after the copper injection in trial II.

Hematocrit: As would be expected, hematocrit values in general followed the same trend as the hemoglobin values (tables 8, 9, and 10). The copper effect was not as great during the latter periods in trial II. Also the hematocrit values were not increased by the copper injection as the hemoglobin values were.

Increased dietary iron levels produced an increase in the hemoglobin and hematocrit values in each trial. Similar results were found by Turugouri (1972) working with pigs and by Koong et al. (1970) working with calves. Contradictory results have been found by Harmon et al. (1968) and Standish et al. (1969, 1971).

The reduction in hemoglobin and hematocrit at the high dietary level of copper is most likely due to a reduced absorption of iron. Suttle and Mills (1966) suggest this may be due to competition between these minerals for binding sites in the gut. When the dietary iron level was increased to 267 ppm as in trial I, II, and III or to 312 ppm as in trial IV, the iron was more competitive with the copper for the binding sites in the gut which allowed enough absorption of iron to maintain normal body stores and to maintain hemoglobin and hematocrit levels. These findings are in agreement with work by Suttle and Mills (1966) and DeGoey,

TABLE 9. EFFECT OF DIETARY LEVELS OF COPPER AND IRON ON HEMATOCRIT VALUES (%) OF PIGS. TRIAL III[a]

	Cu, ppm		
Initial			
Fe, ppm	7	257	Mean
92	38.03	36.00	27.02
267	37.66	38.69	38.17
Mean	37.85	37.34	
3rd Week			
92	38.69	35.25[b]	36.97
267	35.51	37.69[b]	36.50
Mean	37.10	36.97	
5th Week			
92	40.81	38.00[b]	39.41
267	38.38	43.43[b]	40.91
Mean	39.59	40.72	
6th Week			
92	39.38	37.81	38.59
267	40.50	40.50	40.48[c]
Mean	39.94	39.13	

[a] Standard errors of the treatment means are: Initial 1.6825; 3rd week 1.0841; 5th week 0.2354; 6th week 0.4373.

[b] Significant ($P<.05$) copper-iron interaction

[c] Significant ($P<.01$) iron effect

TABLE 10. EFFECT OF DIETARY LEVELS OF COPPER AND IRON ON HEMATOCRIT VALUES (%) IN PIGS. TRIAL IV[a]

	Cu, ppm			
Initial				
Fe, ppm	7	25	257	Mean
101	25.48	34.65	35.22	35.12
312	33.55	35.42	35.50	34.82
Mean	34.51	35.05	35.36	
3rd Week				
101	27.12	27.50	34.15	36.26[c]
312	28.58	38.18	37.65[b]	38.18
Mean	37.85	37.84	35.90	
6th Week				
101	28.62	40.82	34.60[d]	38.02[c]
312	41.80	39.00	40.75[b]	40.52
Mean	40.21	39.91	37.68	
9th Week				
101	43.02	42.45	39.50[e]	40.99
312	42.02	41.72	43.05	42.27
Mean	42.52	42.09	40.28	
Final				
101	41.45	43.00	38.10	40.84
312	42.60	41.88	41.45[b]	41.98
Mean	42.02	42.44	39.78	

[a] Standard errors of the treatment means are: Initial 0.7043; 3rd week 0.7249; 6th week 1.0504; 9th week 1.1040; final 1.1149.

[b] Significant ($P < .05$) copper effect

[c] Significant ($P < .01$) iron effect

[d] Significant ($P < .01$) copper-iron interaction

[e] Significant ($P < .05$) copper-iron interaction

Wahlstron and Emerick (1971) who found that liberal quantities of iron will prevent the hemoglobin depressing action of high dietary copper in-take. It was found by Suttle and Mills (1966) that supplemental iron and not zinc prevents hemoglobin depression.

The fact that high levels of iron did not produce faster gains but did produce an increased hemoglobin concentration would suggest that the level of iron necessary for maximum hemoglobin formation is greater than the level of iron necessary for maximum growth. These results are in agreement with findings of Harmon et al. (1969) and Kornegay (1972).

It appears from these studies that the magnitude of the effect of different copper and iron ratios on the hemoglobin and hematocrit levels is dependent on the rate of growth and feed consumption. In trials I and II in which growth was not maximal and feed intake was equalized there was not as great an effect on the hemoglobin and hematocrit as in trial IV in which growth and feed consumption was not limited by the management practice.

Serum Copper and Iron Concentrations

Copper: Serum copper concentrations in trials I and II were increased ($P<.05$) by the addition of 257 ppm dietary copper during the second week of the experiment (table 11). Serum copper levels were not significantly different during any other period in these two trials.

TABLE 11. EFFECT OF DIETARY LEVELS OF COPPER AND IRON ON COPPER CONTENT OF SERUM (µg/100 ml) IN PIGS. TRIALS I AND II.[a]

	Cu, ppm							
Fe, ppm	7		21		257		Mean	
Initial	T-I	T-II	T-I	T-II	T-I	T-II	T-I	T-II
92	125	111	124	118	118	113	122	114
267	121	114	132	113	128	112	128	113
Mean	123	112	128	115	123	112		
2nd Week								
92	88	98	126	111	118	144	110	118
267	94	110	108	112	132	138	111	120
Mean[b]	91	104	116	112	125	141		
4th Week								
92	162	119	151	134	168	140	160	131
267	155	124	164	122	171	140	163	128
Mean	158	122	158	128	169	140		
6th Week								
92	200	133	206	132	215	140	207	135
267	185	131	210	131	190	151	195	138
Mean	193	132	208	132	202	145		

[a] Standard errors of the treatment means for Trials I and II respectively are: Initial 8.30, 5.23; 2nd Week 9.83, 6.73; 4th Week 11.33, 7.38; 6th Week 14.12, 7.67.

[b] Significant ($P<.01$) copper effect Trials I and II.

In trials IV the serum copper (table 12) levels were increased ($P<.01$) at 3 and 6 weeks by feeding 257 ppm dietary copper. At 3 weeks serum copper levels from pigs fed 7 and 25 ppm copper did not differ significantly. At 6 weeks in replication I of trial IV, each increase in the level of dietary copper produced a significant increase in serum copper levels. At the ninth week there was a copper-iron interaction ($P<.01$) for replication I. For replication 2 at this same period, there was a highly significant iron effect. At 9 weeks when the two replications were combined there was a copper-iron interaction ($P<.05$). The serum copper levels were decreased when pigs were fed rations containing a combination of high copper (257 ppm) and low iron (101 ppm). However, a combination of high copper (257 ppm) and high iron (312 ppm) produced the highest serum copper levels.

When the pigs were injected with copper at the ninth week (trial IV) and blood samples were taken 24 hours later, there were no differences in the serum copper levels regardless of the dietary treatments. This would indicate that the serum can carry only a specific amount of copper. Serum copper levels were increased most in pigs receiving the high-copper (257 ppm) low-iron (101 ppm) ration and the least in pigs receiving high-copper (257 ppm) and high-iron (312 ppm) diets.

TABLE 12. EFFECT OF DIETARY LEVELS OF COPPER AND IRON ON COPPER CONTENT OF SERUM (μg/100 ml) IN PIGS. TRIAL IV [a]

	Cu,ppm			
Initial				
Fe,ppm	7	25	257	Mean
101	168	164	168	166
312	165	171	164	167
Mean	166	168	166	
3rd Week				
101	172	164	214	183
312	177	173	227	192
Mean [b]	174	168	220	
6th Week				
101	184	200	233	206
312	195	201	228	208
Mean [b]	189	200	231	
9th Week				
101	218	207	190 [c]	205
312	218	205	231	218
Mean	218	206	210	
Final				
101	254	246	247	249
312	250	236	244	243
Mean	252	241	245	

[a] Standard errors of the treatment means are: Initial 8.06; 3rd week 7.72; 6th week 7.68, 9th week 8.16; final 10.58.

[b] Significant ($P<.01$) copper effect

[c] Significant ($P<.05$) copper-iron interaction

These results are contrary to the results of other workers (Castell and Bowland, 1968; Kline, Hays and Cromwell, 1972) who reported that serum copper levels were not increased by increasing the dietary level of copper. Castell and Bowland (1968) did report a significant increase in serum copper up to 69 kg live-weight, but concluded that the basal ration containing 6 ppm copper contained an inadequate amount of copper and therefore serum copper levels from this diet were below normal and not that the ration containing 286 ppm copper copper was causing an increase.

DeGoey, Wahlstrom and Emerick (1971) found that plasma copper values were greater ($P<.01$) at 23 days but less ($P<.05$) at the final bleeding when 500 ppm dietary copper was fed. In copper toxicity studies Suttle and Mills (1966) found a rapid increase in serum copper up to 4 weeks after which the levels decline to values equal to those of control animals despite the continued feeding of 750 ppm copper. They postulated that the pig can adapt to continued high levels of copper intake. This work tends to support this postulated adaptation period to the high copper intake, since there was no difference in serum copper after 2 weeks in trials I and II and after 6 weeksin trial IV.

In the present study serum copper levels from pigs fed rations containing 250 ppm copper were significantly greater than values obtained from pigs fed rations containing either 7 or 25 ppm copper. Actually in replication 1 of trial IV with each increase in dietary copper there was an increase in the serum copper level.

This would suggest that serum copper levels can be affected by the dietary copper intake in early growth and would be in agreement with work by Milne and Weswig (1968) who found that in rats plasma copper levels could be increased by increasing amounts of supplementary copper.

Iron: Dietary treatments had considerable influence on serum iron levels. In trial II there was a significant effect of dietary copper on the serum iron level at the initial blood sampling period which was probably due to hemolysis in these samples (table 13). At two weeks serum iron levels were significantly influenced by both supplemental iron and copper with copper reducing and iron increasing serum iron levels. At 4 weeks, there were no dietary treatment differences. Again at 6 weeks there was a significant effect of both dietary copper and iron, with the high dietary copper reducing and the high dietary iron increasing the serum iron level.

In trial IV the serum iron levels were not different among dietary treatments initially (table 14). At 3 weeks the highest dietary copper level reduced ($P<.05$) the serum iron levels and the higher dietary iron level had increased ($P<.05$) the serum iron levels. There were no interactions evident at this period.

At 6 weeks, serum iron levels were reduced ($P<.05$) by supplemental copper and were increased ($P<.01$) by supplemental iron. There was a highly significant copper-iron interaction. Serum iron

TABLE 13. EFFECT OF DIETARY LEVELS OF COPPER AND IRON ON IRON CONTENT OF SERUM (µg/100 ml) IN PIGS. TRIAL II[a]

	Cu,ppm			
Initial				
Fe,ppm	7	25	257	Mean
192	267	248	213	243
267	305	201	209	258
Mean[c]	286	225	211	
2nd Week				
92	206	198	154	186[d]
267	294	214	212	240
Mean[b]	251	206	184	
4th Week				
92	229	121	189	179
267	180	214	201	198
Mean	204	167	195	
6th Week				
92	193	166	196	185[f]
267	246	186	198	210
Mean[e]	220	176	197	

[a] Standard errors of the treatment means are: Initial 14.75; 2nd week 19.44; 4th week 30.13; 6th week 13.23.

[b] Significant ($P<.01$) copper effect

[c] Significant ($P<.05$) copper-iron interaction

[d] Significant ($P<.01$) iron effect

[e] Significant ($P<.05$) copper effect

[f] Significant ($P<.05$) iron effect

TABLE 14. EFFECT OF DIETARY LEVELS OF COPPER AND IRON ON IRON CONTENT OF SERUM (μg/100 ml) IN PIGS. TRIAL IV[a]

	Cu, ppm			
Initial				
Fe,ppm	7	25	257	Mean
101	210	192	177	193
312	174	204	212	197
Mean	192	198	194	
3rd Week				
101	134	174	86	131
312	219	209	196	280[c]
Mean[b]	176	192	141	
6th Week				
101	173	167	93	144
312	205	176	206[d]	196[c]
Mean[b]	189	171	149	
9th Week				
101	166	168	130	155
312	190	173	215[e]	193[c]
Mean	178	171	172	
Final				
101	98	92	121	104
312	123	126	147	132[f]
Mean	110	109	134	

[a] Standard errors of the treatment means are: Initial 20.74; 3rd week 17.14; 6th week 13.84; 9th week 14.60; Final 16.81.

[b] Significant ($P<.05$) copper effect

[c] Significant ($P<.01$) iron effect

[d] Significant ($P<.01$) copper-iron interaction

[e] Significant ($P<.05$) copper-iron interaction

[f] Significant ($P<.05$) iron effect

values which were depressed by high dietary copper levels were maintained at levels equal to those of control pigs when the higher iron level was fed.

At the ninth week there were no dietary copper effects on serum iron levels although the dietary iron effect was still highly significant. This lack of a copper effect may indicate that the pigs were adapting to the high dietary copper intake. As at 6 weeks, the copper-iron interaction was highly significant with the highest serum iron values being produced by the high copper (257 ppm) high iron (312 ppm) treatment.

Twenty-four hours after the injection of copper at the termination of the trial, serum iron levels were reduced on all treatments with pigs fed the higher iron levels still having higher ($P<.05$) serum iron levels than pigs fed the lower iron level. This would indicate that the copper intake on all diets was adequate. If the copper intake had not been adequate then an injection of copper should have produced an increase in serum iron levels.

The reduction in serum iron due to feeding 250 ppm copper found in this work is supported by similiar findings of Pond and Gipp (1970).

Roeser et al. (1970) found that in a copper deficiency there would be a reduced ceruloplasmin level, reduced plasma copper level and a reduced plasma iron level. In this work when 7 ppm dietary copper was supplied in combination with 267 ppm or 312 ppm dietary

iron, the serum iron levels were higher than when 25 ppm dietary copper was supplied at either iron level. This would indicate that 7 ppm dietary copper is adequate with dietary iron levels up to 312 ppm. The fact that the serum iron levels were high when a combination of high iron and high copper was fed would indicate that the interference with iron utilization is at the gut level.

Ceruloplasmin

In trial I there was no difference in ceruloplasmin values between dietary treatments initially (table 15), but in trial II there was a significant effect of copper initially which was probably due to chance. At 2 weeks there were no treatment effects on ceruloplasmin.

At 4 weeks ceruloplasmin values were reduced ($P<.01$) at the high dietary copper level and were increased ($P<.05$) at the higher dietary iron level in trial II with similar trends in trial I. When the data from trials I and II were combined there was a significant copper effect. At 6 weeks, differences among treatments were not significant in either trial I or II.

In trial IV at 3 weeks the higher dietary iron intake produced higher ($P<.05$) ceruloplasmin levels (table 16). This was probably due to additional ceruloplasmin being necessary to oxidize the additional iron intake from the higher dietary level. There were no differences at 6 weeks between treatments. At 9 weeks, each increase in dietary copper caused a significant decrease in

TABLE 15. EFFECT OF DIETARY LEVELS OF COPPER AND IRON ON CERULOPLASMIN VALUES (IU) IN PIGS. TRIALS I AND II[a]

	Cu, ppm							
	7		21		257		Mean	
Initial Fe, ppm	T-I	T-II	T-I	T-II	T-I	T-II	T-I	T-II
92	48.40	41.80	48.84	44.80	44.42	57.40	47.22	48.00
267	52.16	43.40	62.56	38.00	51.08	48.40[b]	55.26	43.26
Mean	50.28	42.60	55.70	41.40	47.75	52.90		
2nd Week								
92	44.50	47.80	45.00	46.60	40.90	51.80	43.46	48.73
267	39.92	51.20	41.00	51.00	52.14	50.20	44.35	50.80
Mean	42.21	49.50	43.00	48.80	46.52	51.00		
4th Week								
92	49.30	42.80	50.64	48.80	39.90	37.60	46.61	43.06
267	50.24	50.00	53.96	46.80	44.40	44.00	49.53	47.60[c]
Mean	49.77	46.40	52.30	47.80	42.15	40.80		
6th Week								
92	60.40	46.20	63.20	54.40	54.40	42.00	59.33	47.53
267	56.00	42.80	60.40	43.80	52.40	43.60	56.26	43.40
Mean	58.20	44.50	61.80	49.10	53.40	42.80		
Final								
92	72.76		69.04		62.86		68.22	
267	61.34		67.44		65.70		64.82	
Mean	67.05		68.24		64.28			

[a] Standard errors of the treatment means for trials I and II respectively are: Initial 6.20, 3.40; 2nd week 38.80, 3.40; 4th week 5.60, 2.00; 6th week 4.60, 3.00; Final 3.80.

[b] Significant ($P<.01$) copper effect

[c] Significant ($P<.05$) iron effect

TABLE 16. EFFECT OF DIETARY LEVELS OF COPPER AND IRON ON CERULOPLASMIN VALUES (I.U.) IN PIGS. TRIAL IV[a]

	Cu, ppm			
Initial				
Fe, ppm	7	25	257	Mean
101	46.10	45.57	41.60	44.42
312	46.64	48.60	45.10	46.78
Mean	46.38	47.08	43.36	
3rd Week				
101	45.36	48.98	44.80	46.36[b]
312	50.66	48.56	52.62	50.62
Mean	48.00	48.72	48.72	
6th Week				
101	48.50	52.58	46.60	49.22
312	53.80	53.26	47.40	51.48
Mean	51.15	52.92	47.00	
9th Week				
101	60.40	57.60	45.76	54.58
312	61.16	54.16	48.50	54.61
Mean[c]	60.78	55.88	47.13	
Final				
101	65.80	63.90	52.44[d]	60.71
312	64.16	63.36	57.56	61.69
Mean	64.98	63.63	55.00	

[a] Standard errors of the treatment means are: Initial 2.34; 3rd week 2.34; 6th week 2.52; 9th week 2.32 Final 0.88.

[b] Significant ($P<.05$) iron effect

[c] Significant ($P<.01$) copper effect

[d] Significant ($P<.05$) copper-iron interaction

ceruloplasmin levels. This might be explained by the fact that when higher than normal levels of copper were fed, more of this absorbed copper goes into the direct reacting fraction of the serum so it can be transported more readily and less copper is incorporated into ceruloplasmin which is not a transport form.

When copper was injected in trial I there were no differences among treatments after the injection. In trial IV, after the copper injection, there was a highly significant copper effect and a significant copper-iron interaction. Ceruloplasmin levels increased more after the injection when the pigs were on diets containing 25 and 250 ppm dietary copper, respectively.

Ceruloplasmin values were taken because of the sensitivity of this copper containing enzyme to copper deficiency. Indeed, Lahey et al. (1952) reported that a deficiency of ceruloplasmin is one of the earliest manifestations of copper deficiency. When copper was injected into copper deficient pigs, Roeser et al. (1970) found that the ceruloplasmin was increased. In this study the injection of copper did not cause an increase in ceruloplasmin which would indicate that the copper level fed was adequate, regardless of the iron level fed. The reduction in ceruloplasmin resulting from the feeding of 250 ppm copper is in agreement with findings by Pond and Gipp (1970).

Liver Copper

The addition of 250 ppm dietary copper increased ($P<.05$) the liver copper content in trials I, II and IV (table 17). This finding is in agreement with reports by other workers (Castell and Bowland, 1968; Paris and McDonald, 1969; Hannahan and O'Grady, 1968; and Beames, 1969). Bowland *et al*. (1961) reported that pigs fed 250 ppm had a lower rate of absorption of copper than pigs fed a low copper basal diet, but based on the total amount, the pigs fed the high copper level absorbed three times more copper than pigs fed the low copper level. Mahoney *et al*. (1955) in their work concluded that the capacity of dogs and pigs to excrete copper is somewhat limited. These two factors, increased uptake and limited ability to excrete copper, would cause an accumulation in the animal.

The liver copper values of pigs fed diets containing 7 and 25 ppm copper in trials I and II were higher than normal. This was due to the injection of copper at the end of the trials. In trial IV a smaller injection was given and the liver values for the pigs fed the low copper diets did not deviate from the normal range of 12-48 ppm (dry basis) as reported by Cunningham (1946) and cited in Underwood (1971).

In trial IV a dietary intake of 312 ppm iron decreased the accumulation of copper in the liver when 250 ppm copper were fed, but did not affect liver copper levels when 7 and 25 ppm copper

TABLE 17. EFFECT OF DIETARY LEVELS OF COPPER AND IRON ON THE COPPER CONTENT OF PIG LIVER (ppm) DRY BASIS. TRIALS I, II AND IV[a]

	Trial I[b]			
	Cu, ppm			
Fe, ppm	7	25	257	Mean
92	91.3	72.0	339.2	167.5
267	33.3	102.2	398.5	178.0
Mean	62.3	87.1	368.8[c]	
	Trial II			
92	128.6	134.9	545.4	268.6
267	131.4	96.9	434.4	220.9
Mean	130.1	115.9	489.9	
	Trial IV			
101	22.1	22.9	505.1	183.4
312	21.3	20.9	112.7	51.6
Mean	22.7	22.9	308.9[d]	

[a] Standard errors of the treatment means are: Trial I 47.577; Trial II 66.072; Trial IV 99.950.

[b] Dry matter for Trials I, II and IV respectively: 25.9, 28.0, 29.1

[c] Significant ($P<.01$) copper effect

[d] Significant ($P<.05$) copper effect

were fed. In trial I high dietary iron did not aid in the prevention of excessive copper accumulation in the liver, although there was a decrease in liver copper in the presence of the high iron in trial II.

According to Wintrobe, Cartwright and Gubler (1952) iron does not affect copper absorption, however, this finding is contrary to the results obtained in the present study where iron influenced copper absorption. If this had not been the case, liver copper accumulation from 250 ppm dietary copper would have been approximately the same regardless of the iron level. This assumption would fit well with the proposed idea of competition of binding sites in the gut for the absorption of these two metals.

Liver Iron

In trials I and IV there was a significant copper and iron effect (table 18). In trial II there was only a significant copper effect (table 18). Liver iron levels were highest in trial IV when the diet contained 25 ppm copper, and this appeared independent of the iron level fed. In the pig, copper is required for absorption of iron (Chase *et al*. 1952). Bush *et al*. (1967) concluded that the uptake of iron by the intestinal mucosa of the copper deficient animal is normal or increased, but that the subsequent transfer to the blood or other tissues is impaired. In this study the pigs on the basal diet were not deficient in copper

TABLE 18. EFFECT OF DIETARY LEVELS OF COPPER AND IRON ON THE IRON CONTENT OF PIG LIVER. TRIALS I, II AND IV[a]

	Trial I			
	Cu, ppm			
Fe, ppm	7	21	257	Mean
92	255.5	180.8	122.5	152.9[c]
267	255.6	403.8	185.6	282.1
Mean	255.5	292.8	154.1[b]	
	Trial II			
92	468.0	602.4	293.6	454.5
267	527.9	518.5	330.3	488.8
Mean	497.9	560.5	312.0[d]	
	Trial IV			
101	222.4	358.7	152.1	244.4
312	373.6	406.7	245.2	341.8[c]
Mean	298.1	382.7	198.6[b]	

[a] Standard errors of the treatment means are: Trial I 53.617; Trial II 52.892; Trial IV 48.321

[b] Significant ($P<.05$) copper effect

[c] Significant ($P<.05$) iron effect

[d] Significant ($P<.01$) copper effect

and actually the ceruloplasmin values for the basal pigs was higher than for the pigs receiving 25 ppm dietary copper. The ceruloplasmin has been implicated by Roeser et al. (1970) as being responsible for the transfer of iron from the mucosa to the blood and other tissues. Therefore it would seem from this work that copper aids iron absorption and storage in another way than transfer after absorption. This work is in agreement with Chase et al. (1952) working with rats who found that increasing the dietary copper increased the iron absorption up to a point but after a certain level is reached copper tended to reduce iron absorption. The high copper intake of the pigs on 257 ppm dietary copper also reduced liver iron levels in this study. This is in agreement with Bunch et al. (1963) working with pigs who found that either copper sulfate or copper oxide significantly depressed the iron content of the liver when fed at 250 ppm. In trials I and II, when the high copper high iron diet was fed, the iron content of the liver was increased over that of the high-copper low-iron treatments. In trial IV, the diet containing 312 ppm iron and 257 ppm copper gave a comparable liver iron level as that of the pigs on the basal diet. This would indicate that the copper iron interaction is mediated at least partially at the gut level.

Liver Zinc

Liver zinc levels were not affected by the dietary treatment in trials I and IV (table 19). This might be expected since the liver is not a major storage site of zinc. In trial II in which a large copper injection was given at the termination of the trial, zinc levels were increased ($P<.05$) as the dietary copper level increased.

Kidney Copper

The feeding of 250 ppm dietary copper increased ($P<.05$) the kidney copper concentration (table 20). This finding is in agreement with the report by Castell and Bowland (1968), who found that the kidney copper concentration was increased by 250 ppm dietary copper. The feeding of 7 or 25 ppm dietary copper did not effect the copper concentration in the kidney. Even though there is very little excretion of copper by the kidney it does contain a level similar to that found in the liver.

Kidney Iron

There was very little difference in the kidney iron content when 7 or 257 ppm dietary copper was fed (table 20). Feeding 25 ppm dietary copper significantly increased the kidney iron content.

TABLE 19. EFFECT OF DIETARY LEVEL OF COPPER AND IRON ON ZINC CONTENT OF PIG LIVER (ppm) DRY BASIS. TRIALS I, II, AND IV[a]

	Trial I			
	Cu, ppm			
Fe, ppm	7	21	257	Mean
92	396.5	265.8	489.0	417.6
267	397.2	363.2	412.5	390.4
Mean	381.4	364.5	451.2	
	Trial II			
92	159.7	184.9	197.8	180.8
267	207.3	216.7	243.0	222.4
Mean	183.5	200.8	220.4[b]	
	Trial IV			
101	242.8	197.7	217.8	219.4
312	286.2	234.9	266.6	262.2
Mean	264.5	215.8	242.2	

[a] Standard errors of the treatment means are: Trial I 42.201; Trial II 12.979; Trial IV 26.857.

[b] Significant ($P<.05$) copper effect

Kidney Zinc

There were no differences in the zinc content of the kidney regardless of the dietary treatments.

Spleen Copper

Dietary copper and iron levels did not effect the concentration of copper in the spleen (table 21). The spleen is an organ of intermediate copper storage and does not seem to be effected by copper intake between the ranged of 7 and 257 ppm. Castell and Bowland (1968) found a significant increase in spleen copper levels due to high copper feeding. The spleen copper values reported by these workers were similar to those found in the present study.

Spleen Iron

There was a significant copper effect on iron storage in the spleen. Iron levels were increased by feeding 25 ppm copper and decreased by feeding 257 ppm copper. This was similar to the trend observed for liver iron. The spleen, along with the liver and bone marrow, are the major storage sites of iron.

Spleen Zinc

There were no affects of dietary treatment on the spleen zinc levels.

TABLE 20. EFFECT OF DIETARY LEVELS OF COPPER AND IRON ON THE COPPER, IRON AND ZINC CONTENT OF PIG KIDNEY[a] (ppm) DRY BASIS. TRIAL IV[b]

	Cu, ppm			
Fe, ppm	7	25	257	Mean
Copper				
101	36.5	30.3	61.6	42.8
312	34.6	32.1	51.6	39.4
Mean[b]	35.6	30.2	56.6[c]	
Iron				
101	168.4	217.2	124.8	170.1
312	197.3	257.8	171.5	171.5
Mean[c]	182.8	237.4	148.2	
Zinc				
101	101.7	104.3	115.6	107.2
312	114.6	112.8	111.8	113.1
Mean	108.2	108.6	113.8	

[a] Average dry matter (%) 21.16

[b] Standard errors of the treatment means are: Copper 6.407; Iron 24.114; Zinc 6.971

[c] Significant ($P<.05$) copper effect

TABLE 21. EFFECT OF DIETARY LEVELS OF COPPER AND IRON ON THE COPPER, IRON AND ZINC CONTENT OF PIG SPLEEN[a] (ppm) DRY BASIS TRIAL IV[b]

	Copper			
	Cu, ppm			
Fe, ppm	7	25	257	Mean
101	8.42	7.91	10.83	9.06
312	8.11	9.87	10.01	9.33
Mean	8.27	8.89	10.42	
	Iron			
101	435.0	519.6	317.2	424.0
312	479.8	557.6	393.8	477.1
Mean[c]	457.4	538.6	355.6	
	Zinc			
101	108.8	112.4	113.0	111.4
312	115.6	109.6	114.4	113.2
Mean	112.2	111.0	113.7	

[a] Average dry matter (%) 21.62

[b] Standard errors of the treatment means are: Copper 0.8803; Iron 51.0970; Zinc 3.4560.

[c] Significant ($P<.05$) copper effect

Hair Copper

There was a linear increase ($P<.01$) in copper content of the hair as the dietary level of copper increased (tables 22 and 23). There was a significant iron and copper-iron interaction with the high dietary level of iron increasing the amount of copper stored in the hair when fed in conjunction with the 257 ppm dietary copper level.

Similar results were found by Castell and Bowland (1968) in that supplemental copper caused significantly higher hair copper concentration. The values for copper in the hair in this study were considerably higher than those obtained by Castell and Bowland (1968). Another discrepancy between the work of Castell and Bowland (1968) and this work is that they found black hair to contain 2.5 times more copper than white whereas no such results occurred in this study.

Hair Iron

Dietary treatment did not affect the iron content of the hair which would indicate that hair may not be a sensitive indicator of the iron status of the body particularly in the relatively high dietary iron levels fed in the present study.

TABLE 22. EFFECT OF DIETARY LEVELS OF COPPER AND IRON ON THE COPPER, IRON AND ZINC CONTENT OF PIG HAIR[a] (ppm). TRIAL II

	Cu, ppm			
Fe, ppm	7	21	257	Mean
Copper				
92	13.73	15.68	35.48	21.63
267	14.21	14.98	40.38	23.19
Mean	13.97	15.33	37.93[b]	
Iron				
92	35.51	23.33	19.04	25.96
267	24.97	23.64	20.92	23.17
Mean	30.24	23.48	19.98	
Zinc				
92	182.8	179.9	186.9	183.2
267	166.9	181.7	181.2	176.6
Mean	174.9	180.8	184.0	

[a] Standard errors of the treatment means are: Copper 2.192; Iron 9.522; Zinc 8.547.

[b] Significant ($P<.01$) copper effect.

TABLE 23. EFFECT OF DIETARY LEVELS OF COPPER AND IRON ON THE COPPER, IRON AND ZINC CONTENT OF PIG HAIR[a] (ppm). TRIAL IV

	Cu, ppm			
Copper				
Fe, ppm	7	25	257	Mean
101	12.05	16.27	43.13	23.82[b]
312	11.68	16.45	57.58	28.27[b]
Mean	11.87	16.36	40.36[b]	
Iron				
101	61.57	87.54	63.60	70.87
312	51.40	57.92	75.70	61.68
Mean	56.48	72.68	69.65	
Zinc				
101	257.97	228.46	253.97	246.80
312	257.92	274.35	319.86	284.04[c]
Mean	257.94	251.40	286.91	

[a] Standard errors of the treatment means are: Copper 2.085; Iron 14.059; Zinc 15.528.

[b] Significant ($P<.01$) copper, iron and copper-iron interaction on copper.

[c] Significant ($P<.01$) iron effect on zinc.

Hair Zinc

Hair is one of the major storage sites of zinc in the body. The high dietary iron level increased ($P<.01$) the zinc content of the hair. This was contrary to what was expected. It was believed that the inclusion of 257 ppm dietary copper would effect zinc utilization of the animal but this did not occur. This lack of a copper-zinc interaction may have been due to the fact that the control diet contained 100 ppm dietary zinc which was adequate regardless of the copper content of the diet. Reinhold et al. (1967) was unable to find any relationship of iron to zinc in hair of rats. The levels of zinc in hair in these studies were similar to the hair content found by Lewis, Hoekstra and Grummer (1957).

Bone Data

In trial IV the bone was analyzed for copper, iron and zinc. No significant difference in any of these minerals occurred due to the dietary treatments. According to Underwood (1972) a high level of the body zinc is stored in the hair and bone. Working with rats Van Campen (1969) was able to get direct evidence of an effect of high levels of copper upon zinc absorption, with high copper reducing zinc absorption. In the present study, a reduction in the zinc content of the bone did not occur when 257 ppm dietary copper was fed. This would indicate that 100 ppm dietary zinc is adequate when 257 ppm copper is fed as a growth stimulant (table 24).

TABLE 24. EFFECT OF DIETARY LEVELS OF COPPER AND IRON ON THE COPPER, IRON AND ZINC CONTENT OF PIG BONE (ppm) DRY BASIS. TRIAL IV[a]

	Cu, ppm			
Fe, ppm	7	25	257	Mean
Copper				
101	5.38	4.84	5.93	5.38
312	5.06	4.64	5.24	4.98
Mean	5.22	4.74	5.58	
Iron				
101	33.63	32.55	32.23	32.80
312	35.00	32.55	35.13	34.22
Mean	34.31	32.55	33.68	
Zinc				
101	150.2	142.2	179.0	157.1
312	156.3	155.8	154.8	155.6
Mean	153.2	149.0	166.9	

[a] Standard errors of the treatment means are: Copper 0.3461; Iron 2.3144; Zinc 11.6679.

SUMMARY

Four studies were conducted to determine if the copper requirement is influenced by the dietary level of iron and if higher than normal levels of iron are beneficial when high dietary copper levels are fed. A factorial design utilizing 3 levels of copper and 2 levels of iron was used.

Growth was not significantly affected when the pigs were limit fed; whereas both growth and feed consumption were increased when the feed was supplied *ad libitum*.

Dietary copper at a level of 257 ppm significantly reduced hemoglobin values, in the absence of supplemental iron.

Serum copper levels were increased during early growth by feeding 257 ppm dietary copper. Serum iron values were depressed by the 257 ppm dietary copper level when the low iron level was fed. This depression of serum iron values by 257 ppm dietary copper was overcome by feeding the high level of iron. The low copper high iron diet produced a high serum iron level which would indicate that 7 ppm dietary copper is adequate for normal iron metabolism.

Copper accumulated in the liver, kidney and hair, when the pigs were fed the high copper diet. When the diet contained a high level of iron the accumulation of copper in the liver was depressed somewhat in trial IV. The iron content of the spleen,

kidney and liver was significantly decreased by the high copper intake which would suggest impaired absorption of iron in the presence of high copper.

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INTERACTIONS OF COPPER AND IRON AS MEASURED BY BLOOD PARAMETERS, TISSUE STORES AND PERFORMANCE IN SWINE

by

James D. Hedges

(ABSTRACT)

Four studies were conducted to determine if the copper requirement is effected by the dietary level of iron and if higher than normal levels of iron are beneficial when high dietary copper levels are fed. A factorial design utilizing 3 levels of copper and 2 levels of iron was used.

Diets containing 257 ppm copper when fed to growing pigs tended to produce heavier weights when the pigs were limit-fed. The response was greater when the pigs were full-fed due to increased feed intake.

Dietary copper at a level of 257 ppm significantly reduced hemoglobin values and serum iron values, in the absence of supplemental iron.

Serum copper levels were significantly increased during early growth by feeding 257 ppm dietary copper. The low copper-high iron diet produced a high serum iron level which would indicate that 7 ppm dietary copper is adequate for normal iron metabolism.

Copper accumulated in the liver, kidney and hair, when the pigs were fed the high copper diet. When the diet contained a high level of iron the accumulation of copper in the liver was depressed somewhat in trial IV. The iron content of the spleen, kidney and liver was significantly decreased by the high copper intake which would suggest impaired absorption of iron in the presence of high copper.