EFFECTS OF COPPER, SULFIDE AND MOLYBDENUM ON PERFORMANCE, HEMATOLOGY AND COPPER, IRON AND ZINC STORES OF PIGS

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ABSTRACT OF THESIS

EFFECTS OF COPPER, SULFIDE AND MOLYBDENUM ON PERFORMANCE, HEMATOLOGY AND COPPER, IRON AND ZINC STORES OF PIGS

One experiment involving 180 pigs was conducted to evaluate the effects of factorial arrangements of copper, 0, 62.5, 125, 187.5 and 250 ppm and level of Vitamin E, 0 and 22 I.U./kg. Copper supplementation improved rate and efficiency of gain. Vitamin E had no consistent effect on performance and did not influence the response to copper. Final hemoglobin (Hb) levels were not significantly affected by the level of copper fed. Liver copper (L Cu) increased quadratically, liver zinc increased linearly and liver iron decreased quadratically with increasing level of dietary copper ($P<.05$).

Two additional experiments involving 160 pigs were conducted to evaluate the effects of factorial arrangements of level of sulfide (S), 0, 225, 450, 900 and 1800 ppm; copper (Cu) 250, 500 ppm, and molybdenum (Mo) 0 and 25 ppm on performance of pigs and their liver copper stores. After an average of 96 days on experiment, S at 1800 ppm of the diet tended to decrease gains in pigs fed 500 ppm Cu, but lower levels were very effective in preventing excess L Cu deposition, depression of Hb and hematocrit levels and depression of average daily gain. Levels of sulfide lower than 1800 ppm did not influence the growth promoting effect of 250 ppm Cu. Mo had no consistent effect on any of the response criteria.

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THESIS

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1972

EFFECTS OF COPPER, SULFIDE AND MOLYBDENUM ON PERFORMANCE, HEMATOLOGY AND COPPER, IRON AND ZINC STORES OF PIGS

THESIS

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Agriculture at the University of Kentucky

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CHAPTER I

INTRODUCTION

The discovery of copper as a growth stimulant for pigs by Evvard et al. in Iowa (1928) and the observation of Braude (1945) in England of the craving of pigs for copper opened the door for the evaluation of copper as a substitute for antibiotics as a feed additive without the potential transferable drug resistance factors of certain antibiotics that has been suggested as a potential human and animal health hazard.

Copper is an essential nutrient necessary for normal hematopoiesis and hemoglobin formation (Hart et al. (1928), Underwood (1971).

Sound knowledge of the beneficial and harmful effects of copper and its relationship with other agents is essential in order to establish the effectiveness of copper in swine production programs of feeding and management.

Molybdenum and sulfate have been postulated, since the discoveries of Dick in Australia (1945, 1953), to prevent excess accumulation of copper in liver of sheep. Since high levels of copper in the diet stimulates growth and improves feed conversion in pigs it is desirable to know if the methods of counteracting excessive storage of liver copper in ruminants follow a similar

pattern in the monogastric animal. The goal of today's pork industry and governmental regulatory policies is to provide a safe wholesome product to the public consumer. Higher concentrations of copper in the liver therefore are undesirable; even though, with the highest level of copper fed (250 ppm), the liver copper doesn't reach the level normally present in beef liver. Copper is stored mainly in the liver and its concentration in the muscles is not increased by high levels in the diet according to the research of Kline et al. (1970), Castell and Bowland (1968b), Bunch et al. (1961); therefore the primary objective is to prevent excessive deposition of copper in the liver.

Molybdenum and sulfate are effective in reducing liver copper levels in ruminants but have been ineffective in pigs. Limited data suggests that sulfide will diminish the copper stores in pigs. Additional information regarding the optimum level of sulfide is needed.

The purposes of this study was to evaluate the effects of varying levels of copper sulfate on the performance of pigs and the levels of copper, iron and zinc in their livers and to evaluate the related effects of sulfide, molybdenum and copper on the deposition of copper in the livers of pigs. Criteria for assessing the animal status were: Rate of growth, efficiency of gain, hemoglobin concentration, hematocrit levels, and levels of copper, iron and zinc in liver and muscle tissue.

CHAPTER II

REVIEW OF LITERATURE

Growth Stimulating Effect of Copper

Besides the requirement to support normal growth and hematopoiesis as has been demonstrated by Hart et al. (1929); Elvehjem and Hart, 1932; Ullrey et al., 1960, the pig responds to relatively high levels of copper in the diet according to the previous report of Evvard et al. (1928). Braude (1945) observed that pigs licked the copper rings around posts in their pens, and Mitchell (1953) reported that baby pigs ate more of a creep ration containing 150 ppm of copper than of a comparable diet containing lower level of copper, but it was not until 1955 that Barber et al. demonstrated the growth promoting action of copper at 250 ppm of diet. Similar response has been observed and reported by Lucas and Calder, 1957; Bunch et al., 1961; Hawbaker et al., 1961; Bunch et al., 1963; Castell and Bowland, 1968; Parris and McDonald, 1969; and many others also found evidence for a sinergestic effect between copper and chemoterapeutics as has been reported by Barber, et al., 1971 and Braude et al., 1971.

Hawbaker et al. (1959) demonstrated that the copper radical and not the sulfate radical was producing the growth response in Iowa studies.

Braude (1965) summarized numerous experiments from different parts of the world and stated that pigs gained 8.1 percent faster on 5.4% less feed per unit of gain when fed diets containing 250 ppm of copper as compared with the performance of pigs fed diets containing low levels of copper. Braude et al. (1962) reported 9.7 percent faster rate of gain and 7.9 percent improvement in feed efficiency by pigs fed 250 ppm of copper, based on data involving 980 pigs from 21 different research centers in England.

Wallace (1967) summarized the research on copper in the United States and found that on the average pigs gained 10.7 percent faster and required 3.9 percent less feed per unit of gain. The research included in his summary involved 5,954 pigs in 154 different experiments.

The mechanism by which copper acts as a growth stimulant remains obscure although it seems to be in part a bactericidal action. However there is little direct data on changes in intestinal flora to support this. According to the reports of Bunch et al. (1961) total aerobic and total anaerobic counts, lactobacilli and streptococci were reduced by copper. Hawbaker et al. (1961) found a similar effect using copper and antibiotics. He found an increase in total number of coliforms, staphylococci, molds and yeasts in response to both chemoterapeutics used. Gireev (1968) reported that copper directly influences the nucleic acids metabolism in sheep. Genci et al. (1970) found a 6 percent increase in nitrogen retention by pigs weighing 15 to 35 kg of liveweight and fed diets containing 250 ppm copper, but above that

liveweight there was no response and when copper was withdrawn from diet at 60 kg liveweight appetite was reduced. Galik (1969), in Chekoslovakia, found, in 8 different trials, that copper, at 250 ppm of the diet, did not affect digestibility of organic matter, fat, fibre or crude protein but the digestibility of N-free extract was reduced in pigs from 15 to 90 kg, however, when copper was withdrawn from diet (from 60 to 90 kg liveweight); digestibilities of fat, crude protein and fibre were reduced and that of N-free extract was increased slightly.

These studies suggest a possible direct effect of copper on the digestive and metabolic systems of the animal, but those effects could be an expression of the effect on the bacterial phase.

Toxic Effects of Excessive Copper

Excessive copper reduces hemoglobin, hematocrit levels and increases the levels of copper in the liver. If this excess is continued reduced performance, reduced liver function and eventually death may occur.

a) Depressed Growth Rate and Feed Efficiency.

Bass et al. (1956) reported that 250 ppm of copper was toxic to pigs on the basis of reduced performance by growing pigs. O'Hara et al. (1960) reported listlessness, respiratory distress and sudden death in pigs fed diets containing 250 ppm of copper. Wallace et al. (1960) also reported reduced growth rates and hemoglobin levels in pigs fed 250, 300, 500 and 750 ppm of copper. Allcroft et al. (1961) observed a reduction in performance as a

result of feeding diet containing 500 and 1,000 ppm of copper but not in pigs fed 400 ppm.

Buntain (1961) reported ulceration of the stomach in pigs fed 130 ppm of copper. Todd (1962) found sheep to be the species least resistant to copper toxicity and the pig the species most resistant of the farm animals. Suttle and Mills (1966a) reported toxicity at 750 ppm of copper but symptoms (anemia, and elevated serum copper) disappeared after seven weeks, but a depression in rate of gain persisted, however. Hanrahan and O'Grady (1968) observed a reduction in level of reduced performance in pigs fed diets containing 250 ppm of copper. Parris and McDonald (1969), working with growing pigs and diets containing 250, 500, 750 ppm of copper, reported a depressive effect on performance from 500 or 750 ppm of copper in diets containing fish meal but not when utilizing soybean meal as a protein supplement, which supports in part the finding of Davis et al. (1962) that phytate can reduce the assimilation of copper.

b) Depressed Hemoglobin and Hematocrit.

Wallace et al. (1960) observed reduced hemoglobin levels when pigs were fed diets containing 250, 300, 500 and 750 ppm of additional copper. Allen and Harding (1962) produced anemia, jaundice, hypercupremia, weakness, ulceration of stomach and respiratory distress in pigs fed a diet with 1,000 ppm of copper.

Ritchie et al. (1963) reported slight anemia in pigs fed 250 ppm of copper. Suttle and Mills (1966a) found a microcytic hypochromic anemia at 750 ppm of dietary copper but the anemia disappeared after 7 weeks of suministrating 750 ppm of iron

although growth rate remained depressed. Hanrahan and O'Grady (1968) also observed severe anemia and deaths in pigs fed 250 ppm of copper.

Wallace (1968) continuously fed 0, 250, 350, 450 and 550 ppm of copper to pigs for 369 days. The three highest levels of copper reduced feed intake, weight gain and blood hemoglobin concentration but no deaths occurred. The diets used were adequately fortified with iron, zinc and properly balanced in other respects.

c) Increased Liver Stores of Copper.

According to Underwood (1971), liver is the major site of copper storage in the normal animal and it also appears to be the major storage site when dietary copper is excessive. Variations in copper content, between and within, the lobes of liver has been reported by Cassidy and Eva (1958), Barber et al., (1960); Bowland et al., (1961) to be large even in cases where toxicity had not been observed.

In general, increased stores of liver copper can not be considered alone as a symptom of toxicity due to the fact, as has been reported in literature, that high levels of liver copper may be present without any signs of toxicity (see table 1). Also, one may observe from data such as that reported in table 1 that there is a great amount of variation even among animals fed the same level of copper fed for similar number of days on experiment.

d) Related Effects of Other Minerals on Beneficial or Detrimental Effects of Copper.

TABLE 1. LEVELS OF COPPER IN THE LIVERS OF PIGS SHOWING NO SYMPTOMS OF COPPER TOXICITY

Reference	Days on Exp.	Total No. Pigs Involved[a]	Liver copper, ppm						
			Copper added to diet, ppm						
			Control	125	150	200	250	500	750
Barber et al. (1961)	108	40	52	-	-	-	287	-	-
Bellis (1961)	129	144	66	164	-	-	959	-	-
Hawbaker et al. (1961)	42	16	29	-	-	-	293	-	-
Bunch et al. (1963)	132	36	26	-	-	-	278	-	-
Beames and Lloyd (1965)	63	24	26	-	-	-	164	-	-
Bunch et al. (1965)	42	40	93	-	-	-	152	-	-
Combs et al. (1966)	57	40	-	-	-	-	895	3675	-
O'Donovan et al. (1966)	70	16	-	-	-	-	1095	-	-
Gipp et al. (1967)	89	12	17	-	31	-	-	-	-
Castell and Bowland (1968b)	175	6	31	-	-	-	113	-	-
Beames (1969)	42	64	24	-	-	-	122	-	-
Parris and McDonald (1969)	42	32	21	-	-	-	83	396	581
Kline et al. (1970)	61	48	16	-	-	-	48	1042	-
De Goey et al. (1971)	102	72	20	-	-	-	310	-	-
Kline et al. (1971)	54	80	-	-	-	-	66	1629	-
Kline et al. (1971)	61	80	-	-	-	-	105	1270	-
Kline et al. (1971)	88	96	18	-	27	73	150	-	-
Kline et al. (1972)	100	80	14	-	-	-	114	1394	-

[a]Equal number in each treatment.

Copper is directly related to the utilization of iron in that it stimulates erythropoiesis by acting as an enzymatic agent in the incorporation of iron into an integral part of hemoglobin structure, but excessive copper has a contrary effect, suggesting then, an antagonism between copper and iron. Though copper is required for normal erythropoiesis excessive amounts results in anemia as evidenced by low hemoglobin and hematocrit levels as reported in several papers (table 2).

The antagonistic relationship is manifested in the liver stores of copper and iron. Excess dietary copper depresses liver iron levels as reported by Cassidy and Eva (1958) in pigs and by Sourkes et al., (1968) in rats. Bunch et al., (1963) also observed significantly less iron in the liver of pigs supplemented with a high level of copper and they also noted that hemoglobin level returned to near normal if additional iron was provided.

Suttle and Mills (1966a) found that 750 ppm of supplemental iron prevented anemia in pigs fed 750 ppm of copper. Also 750 ppm of copper and 750 ppm of iron separately increased liver zinc stores over those of controls. Kainski et al., (1967) observed reduced liver copper levels in pigs fed 661 ppm of iron and 15 ppm of copper; but when pigs were fed 88 ppm of iron and 66 ppm of copper, the liver copper approached the normal level.

There is also ample evidence that the level of zinc in the diet affects the response to copper. Several reports indicate that additional copper cures or prevents parakeratosis in pigs (Hoefer et al., 1960; Wallace et al., 1960; Ritchie et al., 1963,

TABLE 2. HEMOGLOBIN AND HEMATOCRIT LEVELS OF PIGS FED DIETS CONTAINING VARYING LEVELS OF COPPER

Reference	Days	Pigs	Control	125	150	200	250	375	500	750
Kline et al. (1970)	61	48	15.7	-	-	-	17.0	-	14.4	-
Hematocrit			46.	-	-	-	50.	-	44.	-
Kline et al. (1971a)	54	80	13.9	-	-	-	13.9	-	10.8	-
Hematocrit			41.	-	-	-	40.	-	32.	-
Kline et al. (1971b)	61	80	-	-	-	-	14.8	-	11.2	-
Hematocrit			-	-	-	-	43.0	-	35.0	-
Kline et al. (1971c)	88	96	16.2	-	15.9	16.5	15.8	-	-	-
Hematocrit			44.	-	44.	46.	44.	-	-	-
De Goey et al. (1971)	98	192	14.0	-	-	-	14.4	-	11.9	-
Hematocrit			46.1	-	-	-	47.8	-	40.6	-
Kline et al. (1972)	100	80	14.4	-	-	-	14.9	-	11.2	-
Hematocrit			42.	-	-	-	44.	-	36.	-
Hemoglobin										
Hoefer et al. (1960)	91	76	12.4	-	12.9	-	-	-	-	-
Ritchie et al. (1963)	105	28	11.8	-	12.4	-	9.7	-	-	-
Castell and Bowland (1968b)	175	6	13.9	-	-	-	14.3	-	-	-

1963; Hanrahan and O'Grady, 1968).

Ritchie et al., (1963), Hanrahan and O'Grady, (1968), and DeGoey et al., (1971) have reported that liver zinc increases as level of copper in the diet of pigs is increased (table 3).

Suttle and Mills (1966b) and O'Hara et al. (1960) support the idea that although copper and zinc are positively correlated in liver stores, copper in high levels of feeding can produce zinc deficiency due to the low mobilization of zinc in animal tissue.

Kline et al. (1972) demonstrated that excessive levels of zinc (300 ppm) and iron (137 ppm) in the diet of pigs consuming 250 and 500 ppm did not prevent excessive deposition of copper in the liver during a growing finishing period of 100 days.

There is extensive evidence of a relationship of dietary copper, sulfate and molybdenum on the health and well being of animals, particularly ruminants.

Dick and Bull in Australia (1945) demonstrated with sheep that supplemental dietary molybdenum results in a lowering of liver copper levels. Dick (1952, 1953a,b; 1956a) postulates that molybdenum and sulfate antagonizes liver copper stores and that neither of the two alone, sulfate or molybdenum is completely effective in altering liver copper stores. Also, Dick reports that sulfate promotes the excretion of molybdenum. He found the maximum reduction in liver copper level in sheep fed 15 to 20 ppm molybdenum and also reported that levels higher than this increased liver copper levels even in presence of 2,000 ppm of sulfate.

Similar results were reported for Wynne and McClymont (1956)

TABLE 3. LEVELS OF COPPER, IRON AND ZINC IN THE LIVER OF PIGS UNDER THE INFLUENCE OF COPPER FED

Reference		Days on Exp.	Total No. Pigs Involved	Liver, dry basis, ppm Copper added to diet, ppm Control	125	250	500	750
Cassidy and Eva (1958)	Copper	-	20	-	195	770	2790	-
	Iron			655	590	460	180	-
Hoekstra (1961)	Zinc	-	-	200	-	-		
Bunch et al. (1963)	Copper	122	54	26	-	278		
	Iron			835	-	285		
Ritchie et al. (1963)	Copper	105	60	70	-	149		
	Iron			420	-	225		
	Zinc			207	272	288		
Suttle and Mills (1966a)	Copper	49	24	17	-	-		
	Zinc	49	24	203	-	-		328
Hanrahan and O'Grady (1968)	Copper	160	84	100	-	534		
	Iron			522	-	247		
	Zinc			141	-	150		
Miller (1970)	Copper	-	-	31	-	428		
	Iron	-	-	675	-	420		
	Zinc	-	-	365	-	370		
Young et al. (1970)	Copper	86	24	36	81	253		
	Iron	86	24	750	646	460		
De Goey et al. (1971)	Zinc	96	72	318	-	344		

with intakes of molybdenum less than 8 ppm/day.

Cunningham (1955) demonstrated that stores of liver molybdenum decreased as copper in the diet of sheep increased, also indicating a relationship between copper and molybdenum. Goodrich and Tillman (1966a,b) were successful in reducing liver copper stores of sheep by feeding 8 ppm of molybdenum and 4,000 ppm of sulfate; but, when molybdenum levels were lowered to 2 ppm, the liver copper was not reduced.

According to the reports of Comar et al., 1949; Jeter and Davis, 1950, 1954; Compere et al., 1965; if the copper stores are adequate and the copper level fed is high, large amounts of molybdenum are required to produce molybdenum toxicity in rats. Kulwich et al., (1953), however, reported that 1,000 ppm of molybdenum increased copper retention in pigs and rats.

In contrast to the apparent situation in ruminants molybdenum and sulfate does not reduce copper levels in pigs and the pig can tolerate very high levels of molybdenum as compared with the tolerance of ruminants (I. Davis, 1950, Lahey et al., 1952; Gipp et al., 1967; DeGoey et al., 1971; Kline et al., 1971).

Lewis (1954) and Anderson (1956) showed that rumen microorganisms reduces part of the sulfate to sulfide. Much less of the sulfate is reduced to sulfide in the digestive tract of the animal according to Mills, (1960). This may partially explain the apparent difference between ruminants and non-ruminants in their response to molybdenum. Spais et al., (1968), Bowland et al., (1961) also found that less of the copper is absorbed from copper

sulfide than from copper sulfate, so in the ruminant, sulphide may combine with copper to make the copper less available for absorption. These workers also reported great production of sulfide when fed large amounts of sulfate to ruminants. Bird (1970) demonstrated a decrease of soluble copper in the omasum of sheep fed sulfate and cystine.

Dowdy and Matrone, (1968a) have demonstrated the in vitro formation of a copper-molybdenum complex and have further demonstrated that the complex is biologically unavailable to sheep, pigs and rats but not for the chick, (Dowdy and Matrone, 1968b; Dowdy et al., 1969.

According to Bell et al. (1964, 1966), pigs excrete most of molybdenum given orally or intravenously, in the urine, whereas in cattle the main route of molybdenum excretion is the feces. Also, in the studies of Bell et al., pigs eliminated molybdenum from blood within 48 hours while sheep required 72 hours to eliminate the molybdenum. Similar results have been observed for pigs and rats by Diggs et al., (1962) and Kinnamon (1966).

Several authors have reported that the response to copper was dependent on the source of protein fed. Combs et al. (1966) fed pigs diets containing casein or soybean meal as the source of protein and observed more copper in liver tissue of pigs fed the casein. Similar results were found by O'Donovan et al. (1966) with dried skimmilk in comparison with soybean meal. They also observed more copper retention in pigs fed fish meal than in pigs fed soybean meal.

Barber et al. (1962) observed 14.5 percent faster growth in pigs fed diets supplemented with copper (250 ppm) if the diets contained animal protein but only 5.1 percent improvement if the protein was only from vegetable sources.

Suttle and Mills (1966b) developed copper toxicity in pigs fed 600 ppm copper in diets containing fish meal but did not observe toxicity at the same copper level in pigs fed soybean meal or dried skimmilk diets. Parris and McDonald (1969) observed depressed gains and feed intake ($P < .05$) in pigs fed 500 and 750 ppm of copper in fish meal diets but not in pigs fed soybean diets. They also found a significantly higher levels of liver copper in pigs fed the diets containing fish meal.

Kline et al. (1970, 1971, 1972) utilized 16 percent of protein in a combination corn-soybean meal which is the source that has been reported cause less storage of copper but they still found high retention of copper in the liver.

Braude in 1965 reported a higher retention of nitrogen when used 250 ppm of copper.

Wallace et al. (1960) found that increasing the protein level from 15 to 25 percent reduced the toxicity of 750 ppm of copper as evidenced by a significant interaction between the level of copper and protein on growth.

Combs et al. (1966) also reported reduced liver copper levels but no change in hemoglobin level as a result of increasing the protein level from 14 to 22 percent in diets containing excess copper (500 ppm).

Hanrahan and O'Grady (1968) found also less liver copper in pigs as a result of supplementing the diet with 250 ppm of copper if the diet contained 16% rather than 13% protein.

Bunch et al. (1961) however, found no significant effect of level of protein (16 to 22%) on the response of pigs to varying levels of copper (125, 250, 375 ppm).

Beames and Lloyd (1965) utilizing extreme values of protein like 6 and 23 percent of the diet, likewise, did not find any significant copper level x protein level interactions.

Braude in (1965) reported a higher retention of nitrogen when used 250 ppm of copper.

Castell and Bowland (1968a) reported that 250 ppm of copper increased apparent digestibilities of energy and protein in the growing stage but not in the finishing phase of pigs, similar results have been found by Genci et al., (1969).

Absorption, Storage and Excretion of Copper, Sulphur and Molybdenum

Copper is absorbed mainly in small intestine and colon of pigs according to Bowland et al. (1961). According to Underwood (1971) copper absorption and retention is affected by the chemical forms in which the metal is ingested, by the dietary levels of several other minerals (sulphur, molybdenum, iron, zinc, cadmium, calcium and manganese) and organic substances (phytates and ascorbic acid) by the protein, level and quality, and by the acidity of the intestinal contents in the absorptive area. Starcher (1969) has demonstrated a copper-binding protein in the mucosal cells of

duodenum of the chick which may play a role in copper absorption in monogastric animals.

Bowland et al. (1961) reported less copper absorption in pigs from copper sulfide than from copper sulfate. Dick (1954) suggested that molybdenum and inorganic sulfate reduce copper retention in the sheep by reducing the absorption of ingested copper and by increasing the urinary excretion of absorbed and stored copper, due in each case to interference with membrane transport of copper. Mills (1961), as cited by Underwood (1971), demonstrated with radioactive copper that high intakes of molybdenum and sulfate in sheep restrict copper retention by depressing copper solubility within the digestive tract.

Liver is the key organ in the metabolism of this element according to Underwood (1971). Copper is either stored there or released for incorporation into copper containing enzymes. Gipp et al. (1969) failed to obtain a response in growth when injected pigs with copper glycinate, in amounts assumed to be similar to that absorbed in the 250 ppm of level fed. The biliary system is the major pathway of excretion of copper in pigs according to Bowland et al. (1961), and this copper is excreted mainly as copper sulfide Dammers and Stolk (1959) and Suttle and Mills, (1966a).

Intravenous injection of copper results in elevated blood and tissue copper levels followed by a greater excretion of copper in the bile and hence in the feces; but, it does not normally raise urinary copper output, according to Mahoney et al. (1955). They reported that, in dogs, 0.6% of radiocopper was excreted in urine,

1.5% passed directly through intestinal wall and 7 to 10% was excreted in the bile.

According to Comar and Bronner (1962), hydrogen sulfide is formed in gastro intestinal tract from the catabolism of sulphur-amino acids, which in turn reacts with copper to form insoluble copper sulfide (Braude, 1965).

Also whole blood from normal rats oxidizes sodium sulfide in large amounts if administered slowly. Ferritin in gastro intestinal mucosa has been suggested as a possible catalyst for oxidation of sulfide produced by bacteria thereby preventing sulfide from reaching the bloodstream. Rats intravenously injected with ^{35}S as sodium sulfide quickly excreted it in their urine as sulfate sulphur. Bacteria in the gastro-intestinal tract of dogs converted elementary sulphur to hydrogen sulfide and when it was absorbed 10 percent of the sulphur was excreted in urine as sulfate and neutral sulphur.

Bray and Hemsley (1969), working with sheep, observed more rapid absorption of sulfide than of sulfate from the rumen. Also 40 to 90% of ^{35}S as sulfide was absorbed from the intestinal tract after 60 minutes in comparison with only 25% of S as sulfate.

Growing swine consuming a known amount of sulfate and molybdenum, absorbed ^{99}Mo with a peak in blood at 2 to 4 hours (Bell *et al.* (1964) and 75% of both orally and intravenously administered ^{99}Mo was excreted in the urine of swine in 120 hours. Similar excretions of molybdenum in the urine of pigs as a main

route of excretion has been reported by Shirley et al. (1954). Sulfate of sodium and of potassium in ruminants strongly intensified the excretion rate of molybdenum in urine (Dick, 1952, 1953a,b,1954a,b and Scaife, 1963), an effect that is not shared by potassium chloride which induces diuresis but not molybdenum excretion, therefore Underwood (1971) concludes that studies on molybdenum metabolism are of limited value unless the inorganic sulphur status of the diet is known.

In the studies of Dick and of Scaife there was an increase in excretion of molybdenum from 3 to 5% in low sulfate diets, to 40 to 50% in high sulfate diets. These authors suggested that, in the sheep, sulfate limits molybdenum retention by reducing intestinal absorption and increasing urinary excretion, the extent of each depending upon the previous history of the animal with respect to molybdenum and sulfate intakes. Several diuretics tested did not increase excretion of molybdenum, suggesting that the sulfate is highly specific in ruminants.

Sulfate of endogenous origin from catabolic breakdown of body tissue, that from bacterial catabolism of sulphur amino acids in high protein diets or the administration of thiosulfate and methionine to sheep (Dick, 1956b and Scaife, 1963) can be as effective as inorganic sulfate to intensify excretion of molybdenum in urine. Similar results with endogenous sulfate have been tested with rats (Underwood, 1971).

Dick, (1956b) postulated the hypothesis that the rise in the sulfate concentration in the ultrafiltrate of the kidney

glomerulus, with high sulfate intakes, impedes or blocks reabsorption of molybdenum through the kidney tubule, however the mechanism of this postulated interference with membrane transport is unknown.

Potential Counteracting Effects of Sulphur and Molybdenum

Fergusson (1940) in England reported that copper salts antagonize the toxic effects of molybdenum in cattle. Dick (1953) presented evidence that high intake of inorganic sulfate and high intake of molybdenum antagonized liver copper deposition in the sheep. Also, Goodrich and Tillman (1966a,b), Dick (1954a,b), Wynne and McClymont (1956) observed reduced liver copper levels in sheep fed high levels of sulfate and as low as 8 ppm of molybdenum, but not when molybdenum was reduced to 2 ppm.

Research with rats, (Mills, 1960; Mills and Mitchell, 1971; Miller et al. 1956), suggests that molybdenum promotes copper accumulation in the liver and prevents physiological mobilization of copper after its absorption into body tissues. In rats, (Van Reen and Williams, 1956; Miller et al. 1956) supplementary sulphate associated with a high molybdenum intake prevents abnormal copper accumulation in the liver, and at the same time improves the rate of growth and permits normal hemoglobin production. Sulphate supplementation does not fully restore normal growth in the rat and chick (Davies et al. 1960) at extremely high values of dietary molybdenum (above 1,000 ppm) but at lower levels the improvement in physiological performance is dramatic. In both rats and the sheep,

sulphate supplementation of the diet leads to an increase in urinary excretion of molybdenum and a fall in the tissue content of molybdenum, which indicates that the inhibitory effect of sulfate on molybdenum is in the metabolic tissue and not at intestinal level as may be the case for the interrelationship of sulfide and copper (Mills and Mitchell, 1971, Mills, 1960). The enzymatic oxidative detoxification of sulfide to sulfate in rats as suggested by Mills et al. (1958), Halverson, et al. (1960) may be restricted in tissues high in molybdenum content. In such tissue sulfide accumulation leads to a precipitation of insoluble cupric sulfide in which the copper is unavailable for physiological purposes (Halversen et al., 1960; Siegel and Monty (1961); Spais et al., 1968).

Sulfide oxidase activity appears to be dependent upon the in vivo supply of copper (Siegel and Monty, 1961) so that high dietary levels of copper will help to maintain sulfide oxidase activity in the face of the inhibiting effect of molybdenum on this enzyme. In this way an endogenous supply of sulfate will emerge which in turn, will lead to prevent molybdenum accumulation in the tissues; Wilson and Bandurski (1958) have found that the enzyme system responsible for the formation of 3'-phosphoadenosine-5'-phosphosulphate, the active intermediate of sulphate metabolism, is strongly inhibited by molybdate in vitro but Williams and Van Reen (1956) suggested that ATP supplies in animals given high-molybdenum diets are normal. Shirley et al. (1950, 1951) found, however, that in molybdenosis, the stores of

phosphorus in the body and the reproduction function of rats and bulls are impaired.

Mills (1960) and Purdom (1960) obtained faster sulfate reduction to sulfide in rumen fluid when molybdenum was present, and this may explain the necessity of molybdenum to antagonize excessive copper in the liver of ruminants (Marcilese et al. 1969).

Marston (1952) observed that feeding of molybdenum to sheep maintained under conditions of inadequate copper intake, decreased the rate of depletion of liver copper stores while exacerbating the signs of copper deficiency.

From the papers cited before it seems that the metabolic disturbances resulting from the feeding of molybdenum to the rat are probably the result of a failure of copper utilization within the tissues. There is certainly little or no evidence to indicate that molybdenum interferes with copper uptake from the digestive tract.

In the ruminant the decline in tissue copper and the fall in the soluble-copper content of rumen and abomasal liquors when the diet is simultaneously supplemented with molybdate and sulphate suggest that changes within the digestive tract may restrict copper absorption and may be the underlying cause of the ensuing copper deficiency.

Spais et al. (1968) demonstrated high rumen sulfide production on high-sulfate diets. They suggested that this reduces copper absorption through the formation of insoluble copper sulfide, Marcilese and coworkers (1969) working with ^{64}Cu in sheep

compared the addition of 0.4 percent of sulfate alone and 0.4% of sulfate plus 50 ppm of molybdenum. With the diet that contained sulfate and molybdenum, they found significant depression of radioactive and stable liver copper and a reduction in the ceruloplasmin fraction of plasma. This suggests an interacting effect on copper by sulfate and molybdenum but the mechanism of this interference remains to be established.

Dowdy et al. (1968, 1969) prepared a combination of $CuSO_4$ and Na_2MoO_4 to form a complex called lindgrenite which has a copper:molybdenum ratio of 4:3, and precipitates in a near-neutral solution. They hypothesized that this complex can exist in vivo and that the copper bound in this complex is biologically unavailable to pigs and sheep. These authors could not lower significantly the copper stores in pigs fed copper sulfate or citrate administered with molybdenum in a ratio 4:3. The complex lindgrenite did not cause to the pig liver copper storage.

In sheep, given intravenous injection of Cu-Mo complex made from ^{64}Cu and ^{99}Mo, the rates of removal from the blood of the copper and molybdenum were equal, and this rate was more rapid than that of ^{99}Mo injected alone, but the rate of urinary excretion of molybdenum from the ^{64}Cu-^{99}Mo injected sheep was slower than from the ^{99}Mo-injected animal.

Copper in the form of the Cu-Mo compound, synthetic lindgrenite, was also shown to be less available to weanling rats than copper from the sulfate salt.

From the findings reported in the literature, one is led

to the following conclusions:

1) In ruminants, supplementation with both molybdenum and sulfate (Marcilese et al. 1969) appear to be necessary to reduce excessive liver copper levels and for the bacterial flora of the rumen do a effective reduction of sulfate to sulfide (Lewis, 1954, Anderson, 1956, Mills, 1960, Purdom (1960).

2) Sulfide is the form most rapidly absorbed at ruminal and intestinal levels (Spais, 1959; Mills, 1960; Bird, 1970).

3) Sulfide after being absorbed is rapidly converted to endogenous sulfate by the enzymatic action of sulfide oxidase, which is activated by copper. This conversion is so fast that sulfide can not be detected in blood of sheep (Anderson, 1956; Sorbo, 1958).

3) Endogenous sulfate without the presence of excessive molybdenum can result in excretion of sufficient copper to lower stores in liver (Goodrich and Tillman, 1966a,b; Wynne and McClymont, 1956).

4) Endogenous sulfate in the presence of adequate copper can deplete normal stores of molybdenum (Dick, 1953a, with sheep; Davis et al. 1960 in rats and chicks; Mills, 1960, and Miller et al. 1956, with rats and sheep).

5) Excessive dietary molybdenum promotes copper accumulation and prevents physiological utilization of copper in the liver after its absorption into body tissues (Mills, 1960; Miller et al. 1956; Mills and Mitchell, 1971; with rats; Kulwich, et al. 1953 in pigs and rats; Kline et al., 1971, with pigs).

6) Excessive molybdenum blocks conversion of dietary sulfide to endogenous sulfate and finds normal stores of copper (Halverson

et al. 1960, Siegel and Monty, 1961, Spais et al. 1968) to results in accumulation of copper sulfide in the tissue (Van Reen, 1954; Williams and Van Reen, 1956) which is insoluble and unavailable or results in an unavailable complex of Cu:Mo in a ratio of 4:3 (Halversen et al., 1960; Siegel and Monty, 1961; Spais et al. 1968, Dowdy et al., 1968, 1969) which has a fast rate of mobilization from the blood and a slow rate of excretion. This effect if continued for a long time can produce a copper deficiency.

7) Copper in vivo supports the activity of sulfide oxidase (Siegel and Monty, 1961). High dietary levels of copper will help to maintain sulfide oxidase activity against the inhibiting effect of molybdenum to this enzyme, and excessive molybdenum can be excreted from the body by sulfate until it causes a molybdenum deficiency, or a combined copper sulfate and toxicity. This can happen if there is available enough physiological copper to convert, absorbed sulfide to endogenous sulfate. Equally important, can be the amount of sulfide given to the animal.

9) Molybdenum is probably not necessary to prevent excessive copper deposition in the liver, if ruminants are fed sulfide rather than sulfate assuming that Mo is necessary to reduce sulfate to sulfide by bacterial flora present in the rumen.

10) Molybdenum can antagonize toxic effects of copper but does not result in a fast removal of copper from the liver.

The latter conclusions probably explain why Gipp et al., (1967), De Goey et al., (1971), and Kline et al., (1971) did not find an antagonism between copper and sulfate in pigs even when

the molybdenum was fed at very high levels. The microbial flora of cecum and colon were unable to reduce sulfate to sulfide at a rate sufficient to affect metabolic needs or affect liver copper stores through the reaction with endogenous sulfate.

Though there are definitely relationships among iron, zinc and copper as they affect copper stores (table 3), the supplemental levels of iron (50 ppm) and of zinc (100 ppm) used are more than adequate; but still 250 ppm of copper in the diets results in an increase in liver copper levels. Also Kline et al. (1972) demonstrated that excessive levels of zinc or iron would have little or no beneficial effect in reducing the levels of copper stored.

Protein level and protein source also appear to influence copper stores, but the stores appear to be lowest in pigs fed soybean meal protein which is the source of protein Kline used and still increased levels of copper in liver resulted (table 1). Protein level may affect the response though there are conflicting reports on this matter. Above adequate levels of protein (16% to market weight) was used by Kline and this did not correct the problem of copper accumulation in the liver.

Molybdenum and sulfate are beneficial in ruminants but not in pigs as thoroughly investigated by Kline et al. (1971).

Kline et al. (1970) reported a decline in copper stores in pigs fed them with 250 or 500 ppm of copper with 1800 ppm of sulfide and 50 ppm of molybdenum. They also reported a depression effect of the high level of sulfide and molybdenum on growth rate.

In view of their findings it was deemed desirable to test several levels of sulfide and molybdenum in order to determine the effects of molybdenum and sulfide on the toxic effects of copper as measured by performance, hematology and liver stores of copper.

CHAPTER III

EXPERIMENTAL PROCEDURE

General Objectives

One experiment was conducted to study the effects of copper on performance, hemoglobin concentrations and levels of copper, iron and zinc in liver tissue of growing finishing swine.

Two additional experiments were conducted to study the related effects of copper, sulfide and molybdenum on performance, hemoglobin concentrations, hematocrit levels and tissue copper stores of growing-finishing pigs.

General Procedure

Pigs used in these experiments were Yorkshire, Hampshire or Yorkshire-Hampshire crossbred which were obtained from the University of Kentucky swine herds. Prior to the start of the experiment, pigs received a standard corn-soybean meal diet. All animals were randomly allotted from weight outcome groups with sex and breeds to a randomized complete block design, with the restriction that littermates (where applicable) be distributed equally across treatments. The experiments were conducted in open-front swine units with concrete-floored pens. Each pen was 1.22 x 6.10 meters in size with a 1.22 x 3.05 meter area partially enclosed. Each pen was furnished

with a two-hole self-feeder and an automatic water fountain to allow the pigs to have free access to feed and water. The pens were cleaned as needed and straw was provided as bedding during the winter experiments. All diets were mixed in a vertical twin spiral mixer which had a maximum capacity of 910 kg of feed. Vitamins, trace-minerals and experimental additives were premixed with a small portion of the dietary corn in a small horizontal mixer prior to the mixing of the diet in the vertical mixer.

The basic composition of the diets used in all experiments is presented in table 4 and summaries of added, analyzed and calculated copper content of the experimental diets are presented in table 5.

Throughout the experiment, all pigs were fed diets calculated to contain 14.7 percent in Experiment 1 and 16.1 percent in Experiments II and III of protein. The sources of experimental elements included in the diets which made up the various treatments were as follows: copper as technical grade copper sulfate ($CuSO_4 \cdot 5H_2O$); sulfide as sodium sulfide (Na_2S assay = 60 percent), molybdenum as ammonium molybdate $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ vitamin E as vitamin E mix which provided 275,000 I.U./kg, chlortetracycline, as Aurofac-50 with a concentration of 110 gm/kg.

Pigs were weighed and feed consumption was determined at bi-weekly intervals. Blood samples were taken at the initiation, middle and termination of the experiments. The blood samples were drawn from the anterior vena cava as described by Carle and Dewhirst (1942). A glass hypodermal syringe and an 18 gauge 7.6 cm needle were used to obtain the blood. The syringe and needle were washed

in water after each individual animal was bled.

Blood samples were stored in medium size glass test tubes containing three drops of sodium heparin (1,000 U.S.P. units per cc). After all pigs were bled the tubes were slowly inverted several times and hemoglobin and hematocrit levels were determined.

Liver and triceps muscle of shoulder samples were obtained at slaughter from either one or two barrows per pen. The liver samples were taken from the liver lobe adjacent to the bile duct. At this site the copper content approximates the mean copper content of the whole liver (Barber, et al., 1960). A cross section of the triceps shoulder muscles was removed 24 to 48 hours post-mortem. Intermuscular fat was trimmed from the tissue and samples were stored in plastic bags and frozen at -16°C until they were lypholized.

Prior to lypholizing, all tissue samples were cut into small slices and placed in liquid nitrogen. After a few minutes, the sample was removed and cracked by using a mortar and pestle. The cracked samples were immediately placed on the lypholizer. After 48 hours the samples were removed and ground in a porcelain mortar and pestle. The ground samples were stored in air-tight glass containers until analyzed. Data collected from each experiment were statistically analyzed by the method of Analysis of Variance as outlined by Snedecor and Cochran (1967). Unless stated otherwise, references to statistical significance pertain to a probability level of 5 percent or less.

TABLE 4. COMPOSITION OF BASAL DIETS

Ingredient	Trial I	Trial II	Trial III
Ground yellow corn (8.9% protein)	82.80	78.75	78.75
Soybean meal, 49% protein	15.00	18.65	18.65
Dicalcium phosphate	0.85	1.20	1.20
Calcium carbonate	0.75	.80	.80
Iodized salt	0.50	.25	.25
Potassium chloride	-	.25	.25
Trace mineral mix[a]	.05	.05	.05
Vitamin-additive premix[b]	.05	.05	.05
Total	100.0	100.0	100.0

[a,b]Contributed the following per kilogram of diet:

[a]Zinc, 100 mg; Iron, 50 mg; Manganese, 27.5 mg; copper, 5.5 mg; Iodine, 0.75 mg; cobalt, 0.5 mg.

[b]Vitamin A, 2,203 I.U.; Vitamin D_2, 441 I.U.; Riboflavin, 4.4 mg; Pantothenic acid 11 mg; Niacin, 22 mg; Vitamin B_{12}, 11 mcg.

TABLE 5. SUMMARIES OF ADDED, ANALYZED AND CALCULATED COPPER CONTENT (PPM OF EXPERIMENTAL DIETS

	Diet[2]											
	1	2	3	4	5	6	7	8	9	10	11	12
Trial 1												
Added	0	62.5	125	187.5	250	0	0	62.5	125	187.5	250	0
Calculated	16	78.5	141	203.5	266	16	16	78.5	141	203.5	266	16
Trial II												
Added	0	0	250	250	250	250	500	500	500	500		
Analyzed	9	12	254	229	258	256	454	487	463	408		
Calculated	16	16	266	266	266	266	516	516	516	516		
Trial III												
Added	500	500	500	500	500	500	500	500	0	250		
Analyzed	508	489	492	488	500	501	494	489	16	252		
Calculated	516	516	516	516	516	516	516	516	16	266		

[2]Diets were calculated to contain 16 ppm of copper from the natural ingredients (10.5 ppm) and the trace mineral mix (5.5 ppm).

Analytical Methods

Hemoglobin

Hemoglobin concentrations were determined by a modification of the "acid hematin" method from Hawks Physiological Chemistry (1965). Five ml of 0.1 N hydrochloric acid were pipetted into each colorimetric tube. Heparinized blood (0.02 ml) was pipetted into each tube and the tube was inverted several times. This mixture was allowed to stand for one hour after which time percent transmittancy was read on a standardized colorimeter[1] at a wavelength of 520 millimicrons. Heme standards made from bovine hemoglobin (powder) were carried through the same procedure as for blood. The percent transmittancy was converted to optical density and the optical density per unit of hemoglobin was determined for the standards used and the slope of the standard curves was used in calculating the amount of hemoglobin in the test samples.

Hematocrit

Hematocrit levels were determined by centrifugation of heparinized blood in capillary tubes. Values were read from a chart supplied with the micro-capillary hematocrit centrifuge[2].

Liver Copper

A 1 to 3 g sample of lypholized liver was accurately weighed and placed in a 100 ml Kjeldahl flask. Thirty ml of nitric acid

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[1]Bausch and Lamb, Inc., Rochester, New York.

[2]International Equipment Company, Boston, Massachusetts.

were pipetted into each sample and allowed to stand overnight. Five ml of sulfuric acid and three glass beads were added to the mixture which was then boiled until a black color developed. The mixture was cooled and 10 ml of nitric acid plus 2 ml of perchloric acid were added. Boiling was resumed and continued until the mixture turned clear and dense white fumes appeared in the bulb of the flask.

The solution was cooled, transferred to a volumetric flask and brought to volume with double-deionized water. An aliquot of each sample solution was aspirated into the standardized spectrophotometer for copper determination.

Muscle Copper

Samples, approximately three grams, of lypholized triceps muscle were accurately weighed and placed in 100 ml Kjeldahl flasks. The digestion was carried out as outlined for liver copper analysis. From this stage on the techniques described by Stevens[1] were employed. After each sample was digested, 10 ml of double-deionized water were added. The solution was adjusted to pH 4.0 with ammonium hydroxide using litmus pH paper as an indicator.

Two ml of a saturated solution of ammonium pyrrolidino dithiocarbamate were pipetted into each flask and the flasks were shaken. Five ml of methyl isobutyl ketone were pipetted into

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[1]Stevens, B. J., 1969. Royal Children's Hospital. Melbourne, Victoria 3052, Australia. Personal Communication. Cited by Kline, R. D. (1970).

each flask and the flasks were shaken in a mechanical shaker for 10 minutes. The top phase was pipetted off and aspirated into the standardized spectrophotometer for copper analysis.

All copper, iron and zinc analysis were performed by using a Perkin-Elmer Atomic Absorption Spectrophotometer Model 303[1].

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[1]Perkin-Elmer Corporation, Norwalk, Connecticut.

CHAPTER IV

EXPERIMENTAL RESULTS

Trial I - Related Effects of Copper, Chlortetracycline and Vitamin E on Performance, Hemoglobin Levels and Liver Stores of Copper, Iron and Zinc in Growing-Finishing Pigs.

Objectives

Cassidy and Eva (1958), Bunch et al. (1963) and Hanrahan and O'Grady (1968) have demonstrated an inverse relationship between the levels of iron in the liver of pigs and also Ritchie et al. (1963), De Goey et al. (1971), Hanrahan and O'Grady (1968), showed a direct relationship between liver copper and zinc levels.

Wallace (1967), and Ritchie et al. (1963) have shown a depressing effect of copper at levels as low as 250 ppm on hemoglobin concentrations. These reports are, however, in disagreement with the reports of Castell and Bowland (1968b) and Kline et al. (1971).

This experiment was designed to further study the effects of level of dietary copper on performance hemoglobin concentration, and liver stores of copper, iron and zinc in growing finishing pigs.

Procedure

One hundred and eighty Yorkshire pigs averaging 21.4 kg body weight anf 53.1 days of age were allotted to a randomized complete block design. The pigs were randomly allotted from weight groups

within sex to 12 treatments with three replications of five pigs per pen for each treatment. A restriction that littermates be distributed across treatments was imposed on the randomization. The treatments were a factorial arrangement of five levels of added copper (0, 62.5, 125, 187.5 and 250 ppm) and two levels of Vitamin E (0 and 25 I.U./kg of feed) plus two treatments of chlortetracycline, (55 mg/kg of feed) without or with Vitamin E. The Vitamin E variable was included to determine whether the high level of copper was increasing the dietary Vitamin E requirement and also the antibiotic treatments were included as a positive control for the growth promoting activity of supplemental copper. The composition of the experimental diet is presented in table 4.

The experiment was terminated as the pigs individually reached 92 kg body weight on weekly weighings and were on experiment an average of 87 days at which time the pigs averaged 94.1 kg body weight. Blood samples were taken from all pigs at the start of the experiment, and after 81 days, for the first replication and 67 days for the other two replications, and the hemoglobin determined on all samples.

The barrows from each pen were slaughtered and livers were collected for mineral analysis.

Results

Summaries of results and analyses of variance for average daily gain and feed required per unit of gain to 57 kg body weight are presented in table 6 and Appendix Table 1.

TABLE 6. TRIAL I. SUMMARIES OF AVERAGE DAILY GAIN AND FEED REQUIRED PER UNIT OF GAIN TO 57 KG OF WEIGHT

Vitamin E, I.U./kg	Copper, ppm						Chlortetracycline
	0	62	125	188	250	Avg.	55 mg/kg
0	838	832	826	817	881	834	881
25	801	852	864	835	885	847	884
Avg.	820	842	845	826	883		883
			Feed/Gain				
0	2.70	2.71	2.81	2.82	2.69	2.75	2.72
25	2.76	2.76	2.67	2.65	2.60	2.69	2.63
Avg.	2.73	2.74	2.74	2.74	2.65		2.72

Rate of gain to 57 kg body weight tended to increase at each copper level. The trend, however, was not statistically significant ($P<.05$). Also the rate of gain was higher for the chlortetrocycline fed pig than for the pigs fed the control diet. Rate of gain was similar for pigs fed chlortetrocycline and those fed the high level of copper. At each level of copper, pigs fed diets supplemented with Vitamin E gained faster than those fed the unsupplemented diet, however the difference was not statistically significant.

Feed required per unit of gain did not show any significant trends though pigs fed diets containing 250 ppm of copper required less feed per unit gain than did pigs fed other diets.

As shown in table 7 and Appendix Table 2, during the entire experimental period animals fed antibiotic gained faster than the average for all other treatments.

There was a significant cubic effect of copper level on average daily gain. This resulted from an improvement at 62 and 250 ppm and no increase in rate of gain at the intermediate levels. Feed efficiency did not show any significant trends; although, with the exception of the 188 ppm level, those pigs fed diets supplemented with copper, required less feed per unit of gain than did the pigs fed the control diet. Pigs fed the diets supplemented with chlortetrocycline required less feed per unit of gain than those fed all other diets. This was primarily due to the higher amount required by the pigs fed diets without copper as the average for

TABLE 7. TRIAL I. SUMMARIES OF AVERAGE DAILY GAIN AND FEED REQUIRED PER UNIT OF GAIN TO 94.1 KG OF WEIGHT

Vitamin E, I.U./kg	Copper, ppm						Chlortetracycline
	0	62	125	188	250	Avg.	55 mg/kg
0	825	853	834	826	865	841	897
25	858	890	847	817	862	855	880
Avg.[a,b]	842	872	841	822	864		889
			Feed/Gain				
0	3.23	3.11	3.20	3.27	3.23	3.21	3.22
25	3.26	3.18	3.10	3.21	3.12	3.17	3.11
Avg.	3.25	3.15	3.15	3.24	3.18		3.17

[a]Chlortetracycline vs others significant ($P<.05$).

[b]Cubic regression of gain on level of copper significant ($P<.05$).

the copper supplemented pigs was very similar to that of the chlortetracycline supplemented pigs (3.18 vs 3.17).

Summary of hemoglobin concentration is presented in table 8 and analyses of variance is presented in Appendix Table 1. There were no significant effects of copper chlortetracycline or Vitamin E on hemoglobin levels and the means for the main effects were very similar.

Summaries of liver copper, iron and zinc are presented in table 9 and Appendix Table 2. There was a highly significant difference between the mean level copper of pigs fed antibiotic treatments and those fed all other diets which was a result of the expected increase in liver copper levels resulting from the copper treatments. The means for the chlortetracycline treated pigs was near identical to the groups fed neither copper nor antibiotic (12 vs 11 ppm).

There was a highly significant quadratic effect of dietary copper on liver copper levels with a fourfold increase in level of copper in liver of pigs fed at 188 ppm of copper and a twelvefold increase in the liver of pigs fed diets containing 250 ppm.

The liver iron levels were inversely related to liver copper levels. There was a highly significant difference between antibiotic treatments and all other treatments which again is a reflection of the effects of the high copper treatments. There was a highly significant quadratic effect due to copper levels.

TABLE 8. TRIAL I. SUMMARIES OF HEMOGLOBIN CONCENTRATIONS AFTER 67 AND 81 DAYS[a] ON EXPERIMENT[b]

Vitamin E, I.U./kg	Copper, ppm						Chlortetracycline
	0	62	125	188	250	Avg.	55 mg/kg
0	12.9	13.2	13.4	13.3	13.6	13.3	13.4
25	13.5	13.1	13.4	13.0	13.4	13.3	13.5
Avg.	13.2	13.2	13.4	13.2	13.5		

[a]Replication one had been on experiment 81 days and replications 2 and 3 had been on experiment 67 days at the time hemoglobin values were measured.

[b]For analysis of variance see Appendix Table 1.

TABLE 9. TRIAL I. SUMMARIES OF LIVER COPPER, IRON AND ZINC LEVELS[a]

	Copper, ppm						Chlortetracycline
Vitamin E, I.U./kg	0	62	125	188	250	Avg.	55 mg/kg
0	11	15	22	56	285	78	11
25	12	12	21	121	263	53	13
Avg.[a]	11	14	22	88	274		
	Liver Iron, ppm of Dry Matter						
0	568	627	593	479	245	502	593
25	657	626	488	504	255	506	586
Avg.	612	626	540	491	250		590
	Liver Zinc, ppm of Dry Matter[c]						
0	205	195	208	231	224	212	175
25	191	204	183	202	219	200	197
Avg.[b]	198	199	196	216	222		186

[a]For analysis of variances see Appendix Table 2.

[b]Quadratic regression of liver copper and liver iron on dietary copper significant ($P<.01$)

[c]Linear regression of liver zinc and dietary copper significant ($P<.01$).

It was inversely related to the liver copper responses, but the magnitude of the differences were not nearly so marked. This decrease in liver iron levels was a smooth descending line to 188 ppm of dietary copper but marked decrease in the liver iron levels of pigs fed the diets containing 250 ppm of copper occurred. The liver iron levels of the pigs fed the high copper level were only about one third that of pigs fed the control diet (250 vs 612 ppm).

For liver zinc there was also a highly significant difference between pigs fed the antibiotic supplemented diets and all other groups. This again is a reflection of the copper effects as the levels in the antibiotic supplemented group were similar to those in the control groups.

A highly significant linear increase in liver zinc resulted from increasing dietary levels of copper. The magnitude of the change was relatively small in comparison with changes in iron or copper levels. The high level of dietary copper resulted in approximately 10 percent higher liver zinc levels (198 vs 222 ppm).

Vitamin E supplementation had no significant effect on the liver levels of copper, iron or zinc, however there was a trend toward a moderating effect of dietary copper, if the Vitamin E was present. However the differences were small and the variability in liver levels were exceptionally great, particularly so for liver copper.

Trial II - Related Effects of Copper, Sulfide and Molybdenum on Performance, Hemoglobin and Hematocrit Levels and Liver Copper Levels of Growing-Finishing Pigs.

Objectives

It has been reported that it is possible to lower excessive copper stores in sheep by supplementing their diet with sulfate and molybdenum Dick, 1954b. It has also been suggested sulfate is reduced to sulfide and that the resulting sulfide is responsible for the reduction in retention of copper in the body (Lewis, 1954). According to Mills (1960) in the growing nonruminant, the sulfate is not reduced to sulfide at a rate sufficient to reduce the amounts of copper stored in the body. Kline et al., (1971) found that liver copper levels could be lowered by supplementing the diet with a high level of sulfide (1800 ppm); but, they also found some reduction in rate of gain. Certainly this would be an undesirable effect of the high level of sulfide; therefore, it was desired to evaluate the effects of lower levels of sulfide and also to determine if molybdenum is also necessary as an antagonist to excessive liver copper deposition. This experiment was designed to study the related effects of geometric progressions of sulfide and a low level of molybdenum in combination with two levels of copper sulfate on rate of gain, feed efficiency, hemoglobin and hematocrit levels and levels of copper in muscle (the triceps muscle of the shoulder) and liver in growing-finishing pigs.

Procedure

Eighty Hampshire and Yorkshire x Hampshire pigs averaging 16.5 kg body weight and 60 days of age were allotted to a randomized

complete block design. Pigs were randomly allotted from weight outcome groups within sex and breed to ten treatments with two replications of four pigs per pen for each treatment. Randomization was restricted in that littermates were distributed across treatments. The dietary treatments included a factorial arrangement of two levels of added copper (250 and 500 ppm) levels (0, 450, 900 and 1800 ppm) of sulphur as sulfide. Molybdenum (25 ppm) was added to all eight diets. Two additional dietary treatments included molybdenum alone and a negative control that included neither of the three minerals mentioned. Copper sulfate ($CuSO_4.5H_2O$) sodium sulfide (Na_2S, 60 percent) and ammonium molybdate $(NH_4)_6Mo_7O_{24}.4H_2O$ were the mineral sources used. Potassium cloride (.25 percent) was added to all diets so that a sodium and potassium inbalance would not result. The composition and calculated analysis of the experimental diets are presented in tables 4 and 5. The experiment was terminated after 103 days at which time the pigs averaged 89.6 kg body weight. Blood samples were taken from all pigs at the initiation, and after 57 and 94 days on experiment. Hemoglobin and hematocrit levels were determined at the three bleedings. Two barrows from each pen were slaughtered and tissues were removed for copper analysis.

Results

Summaries of results and analyses of variance for average daily gain and feed required per unit of gain are presented in table 10 and Appendix Table 3. There was no significant ($P<.05$) differences

TABLE 10. TRIAL II. SUMMARIES OF AVERAGE DAILY GAIN, AND FEED REQUIRED PER UNIT OF GAIN

	Sulphur, ppm[c]					
Copper, ppm	0	450	900	1800	Avg.	Control
250	706	726	763	745	735	715[a]
500	603	739	723	683	687	703[b]
Avg.	655	733	743	714		
			Feed/Gain			
250	3.07	3.15	3.08	3.17	3.12	3.08[a]
500	3.14	3.07	3.18	3.06	3.11	2.98[b]
Avg.	3.11	3.11	3.13	3.12		

[a]No copper, molybdenum or sulfide added.

[b]No copper, no sulfide added but 25 ppm molybdenum added.

[c]Molybdenum (25 ppm) was added to the eight diets supplemented with copper.

in average daily gain among the dietary treatments but there was a tendency of slower gains in pigs fed the highest level of sulfide and in pigs fed diets containing 500 ppm of copper. The quadratic effect of sulfide level on rate of gain was significant at the probability level of 0.10. Also at the 0 level of sulfide there appeared to be a reduction in rate of growth (603 vs 706) resulting from the level of copper, however, neither the main effect of copper nor the copper x sulfide interaction was significant ($P<.05$).

There were no differences in feed efficiency that could be associated with dietary treatment. At 250 ppm of copper supplementation, the better feed efficiencies corresponded to 0 and 900 ppm of sulfide. Conversely, at 500 ppm of copper, the better feed efficiencies were obtained at 450 and 1900 ppm of sulfide. In any of the dietary levels of copper, the feed efficiencies were superior to the control diet.

Summaries of results and analysis of variance for hemoglobin and hematocrit levels are presented in table 11 and Appendix Table 3. Hemoglobin and hematocrit levels were depressed by feeding 500 ppm of copper. There was a highly significant effect of sulfide on hemoglobin and hematocrit levels, the major effect being in the pigs fed the higher (500 ppm) level of copper. The interaction of copper and sulfide on hemoglobin and hematocrit levels was significant ($P<.05$). The linear and quadratic components of variance for hemoglobin levels and the linear component for hematocrit levels were statistically significant. In each case the levels tended to plateau at 450 to 900 ppm of sulfide.

Summaries of muscle and liver copper stores are presented in table 12 and Appendix Table 4. Only one pig per treatment was sampled for the muscle levels thus one cannot test treatment effects. This summary shows the very low level of copper in the muscles of pigs fed high levels of copper, and offers no evidence that the level is higher as a result of feeding diets containing 500 ppm of copper.

Increasing the dietary level of copper significantly ($P<.05$) increased liver copper stores. The level of 250 ppm without sulfide resulted in about a twentyfold increase and the level of 500 ppm without sulfide resulted in a hundredfold increase.

The liver copper levels declined quadratically with increasing sulfide levels and there was a highly significant copper by sulfide level interaction. In combination with 250 ppm of copper, 900 ppm of sulfide resulted in copper levels near the controls (18 vs 12.5). In

TABLE 11. TRIAL II. SUMMARIES OF HEMOGLOBIN CONCENTRATIONS AND HEMATOCRIT LEVELS

Copper, ppm	Sulphur, ppm[a] 0	450	900	1800	Avg.	Controls
			Hemoglobin, g/100 ml			
250	15.3	16.2	16.3	15.5	15.8	16.2[c]
500	10.8	13.7	14.2	15.3	13.5	15.5[d]
Avg.[e]	13.1	15.0	15.3	15.4		
			Hematocrit, %			
250	42.1	41.1	43.5	41.4	42.0	43.1[c]
500	30.8	38.8	39.0	40.4	37.5	41.8[d]
Avg.[f]	36.5	40.0	41.2	40.9		

[a]Molybdenum (25 ppm) was added to the eight diets containing added copper and sulfide levels.

[b]Difference in hemoglobin and hematocrit levels in the comparison 250 vs 500 ppm of copper was significant (P .01)

[c]Negative control diet for all three supplemental minerals.

[d]Molybdenum, (25 ppm) added to this diet.

[e]Quadratic effect of sulfide and the copper by sulfide interaction significant (P<.05).

[f]Linear effect of sulfide and the copper by sulfide interaction significant (P<.05).

TABLE 12. TRIAL II. SUMMARIES OF THE COPPER LEVELS IN THE TRICEPS MUSCLE OF THE SHOULDER AND IN THE LIVER[a,b]

Copper, ppm	Sulphur, ppm 0	450	900	1800	Avg.	Controls
			Shoulder Copper, ppm of Dry Matter[a]			
250	2.48	1.51	2.06	3.11	2.29	-
500	2.26	2.95	1.72	1.52	2.11	-
Avg.	2.37	2.23	1.89	2.32		
			Liver Copper, ppm of Dry Matter[b]			
250	237	29	18	19	76	13[c]
500	1332	613	98	40	520	12[d]
Avg.	784	321	58	30		

[a]The means are samples of one pig per treatment.

[b]Include analysis of 39 livers.

[c]Negative control diet for all three supplemental minerals.

[d]Molybdenum, (25 ppm) added to this diet.

the presence of 500 ppm of copper, sulfide resulted in a marked reduction, but even at the highest level of sulfide (1800 ppm) the level in the liver was still three times that of control (40 vs 125).

Trial III - Effects of Sulfide and Molybdenum on Performance, Hematology and Liver Copper Stores of Growing-Finishing Pigs Fed a High Level of Copper.

Objectives

According to the results of the previous experiment and the report of Kline et al. (1971) sulfide reduces the level of copper in the liver. In the previous experiment molybdenum was added to all the diets containing a high level of copper. Molybdenum in the presence of sulfate is needed to reduce liver copper in ruminants according to Dick (1956a) and Goodrich and Tillman (1966a,b). It was deemed desirable test whether added molybdenum is required to reduce copper deposition in the liver of pigs. It is recognized that the natural feedstuffs may contain ample molybdenum (Underwood, 1971).

This experiment was designed to study the related effects of sulfide and molybdenum on liver copper stores in pigs fed a diet high in copper (500 ppm) and also to study their related effects on performance, hemoglobin and hematocrit levels and liver copper stores.

Procedure

Eighty Yorkshire x Hampshire crossbred pigs averaging 27.9 kg body weight and 83 days of age were allotted to a randomized complete block design. The pigs were randomly allotted from weight outcome groups within sex to 10 treatments with two replications of four pigs per pen for each treatment. Randomization was restricted in that littermates were distributed across treatments. The treatments included a factorial arrangement of four levels of added sulphur

as sulfide (0, 225, 450 and 900 ppm) and 2 levels of added molybdenum (0 and 25 ppm). Copper (500 ppm) was added to all eight treatments. A negative control diet in which neither sulfide, molybdenum nor copper was included, also a treatment with 250 ppm copper but with no added sulfide or molybdenum was included. The composition and calculated analysis of the experimental diets are presented in tables 4 and 5.

The experiment was terminated after 83 and 96 days for the first and second replication respectively, at which time the pigs averaged 97.2 and 89.8 kg body weight. Blood samples were taken from all pigs at the starting of the experiment, on the 50th day and at the 83rd day of the experiment. Hemoglobin and hematocrit levels were determined at all bleedings. Seven pigs per treatment were slaughtered and liver removed for copper analysis.

Results

Summaries of results and analysis of variance for average daily gain and feed required per unit of gain are presented in table 13 and Appendix Table 5 and 6.

There was a trend toward an increase in rate of gain with increasing levels of sulfide up to 450 ppm of sulphur. The regression of gain on sulfide level, however, was not significant ($P < .05$).

Feed efficiency data did not reveal any differences but similarly to the rate of gain, those pigs fed the 450 ppm of sulfide required less feed per unit of gain.

TABLE 13. TRIAL III. SUMMARIES OF AVERAGE DAILY GAIN AND FEED REQUIRED PER UNIT OF GAIN

Molybdenum, ppm	Sulphur, ppm[a] 0	225	450	900	Avg.	Controls
0	659	690	830	764	746	778[b]
25	722	719	731	751	730	735[c]
Avg.	691	705	781	758		
			Feed/Gain			
0	3.03	2.98	2.82	2.85	2.92	2.94[b]
25	2.80	2.96	2.78	2.94	2.87	2.69[c]
Avg.	2.91	2.97	2.80	2.90		

[a]Copper (500 ppm) was added to the eight diets containing added sulfide and molybdenum.
[b]Negative control diet for all three supplemental minerals.
[c]Copper (250 ppm) added to this diet.

Summaries of results and analysis of variance for hemoglobin and hematocrit levels and liver copper stores are presented in table 14 and Appendix Tables 5 and 6.

There was a highly significant difference in hemoglobin concentrations between pigs fed the control diets with low and intermedium copper levels and those fed diets with a high level of copper. There was not a significant difference between hemoglobin levels of pigs fed the 0 and 250 ppm of added copper. Increasing sulfide level resulted in a highly significant linear decrease in hemoglobin level. Hemoglobin concentrations were normal with the two higher levels of sulfide. There was no differences due to level of added molybdenum. Hematocrit levels followed a pattern similar to that for hemoglobin. A significant quadratic effect ($P<.05$) of the levels of sulfide on hematocrit levels was observed. The interaction of molybdenum and sulfide on hemoglobin level was not significant. The level of liver copper was significantly ($P<.01$) lower in pigs fed the control treatments than in those fed the high copper levels. There was no statistically significant difference between control treatments (0 added copper vs 250 ppm copper) nor among levels of added molybdenum.

A highly significant quadratic decrease in liver stores with increasing sulfide levels was observed. After 26 days of starting on the experiment, one barrow from the diet with 500 ppm of copper, 250 ppm of sulphur and 25 ppm of molybdenum died from a internal hemorrhage caused by a gastric ulcer. After 30 days on test another barrow from the diet with 500 ppm of copper and 25 ppm of

TABLE 14. TRIAL III. SUMMARIES OF HEMOGLOBIN AND HEMATOCRIT LEVELS AND LIVER COPPER STORES

Molybdenum, ppm	Sulphur, ppm[a] 0	225	450	900	Avg.	Controls
			Hemoglobin, g/100 ml			
0	13.0	12.7	15.2	13.6	13.6	14.5[b]
25	13.2	13.7	13.6	15.5	14.0	15.4[c]
Avg.	13.1	13.2	14.4	14.6		
			Hematocrit, %			
0	36.3	38.3	43.3	39.4	39.3	40.6[b]
25	36.6	40.3	39.2	42.1	39.6	41.9[c]
Avg.	36.5	39.3	41.3	40.8		
			Liver copper, ppm of Dry Matter			
0	961	273	190	47	368	20[b]
25	1093	553	223	45	479	212[c]
Avg.	1027	413	207	46		

[a]All eight diets included 500 ppm of copper.

[b]Neither copper, molybdenum and sulfide was added.

[c]Diet included 250 ppm of copper but no added molybdenum or sulfide.

molybdenum was taken off test due to very low performance; histological sections revealed lesions of lymphosarcoma. After 28 days of normal growth a barrow from the basal diet plus 250 ppm of copper developed a lameness which caused him to lose weight and reduced feed consumption.

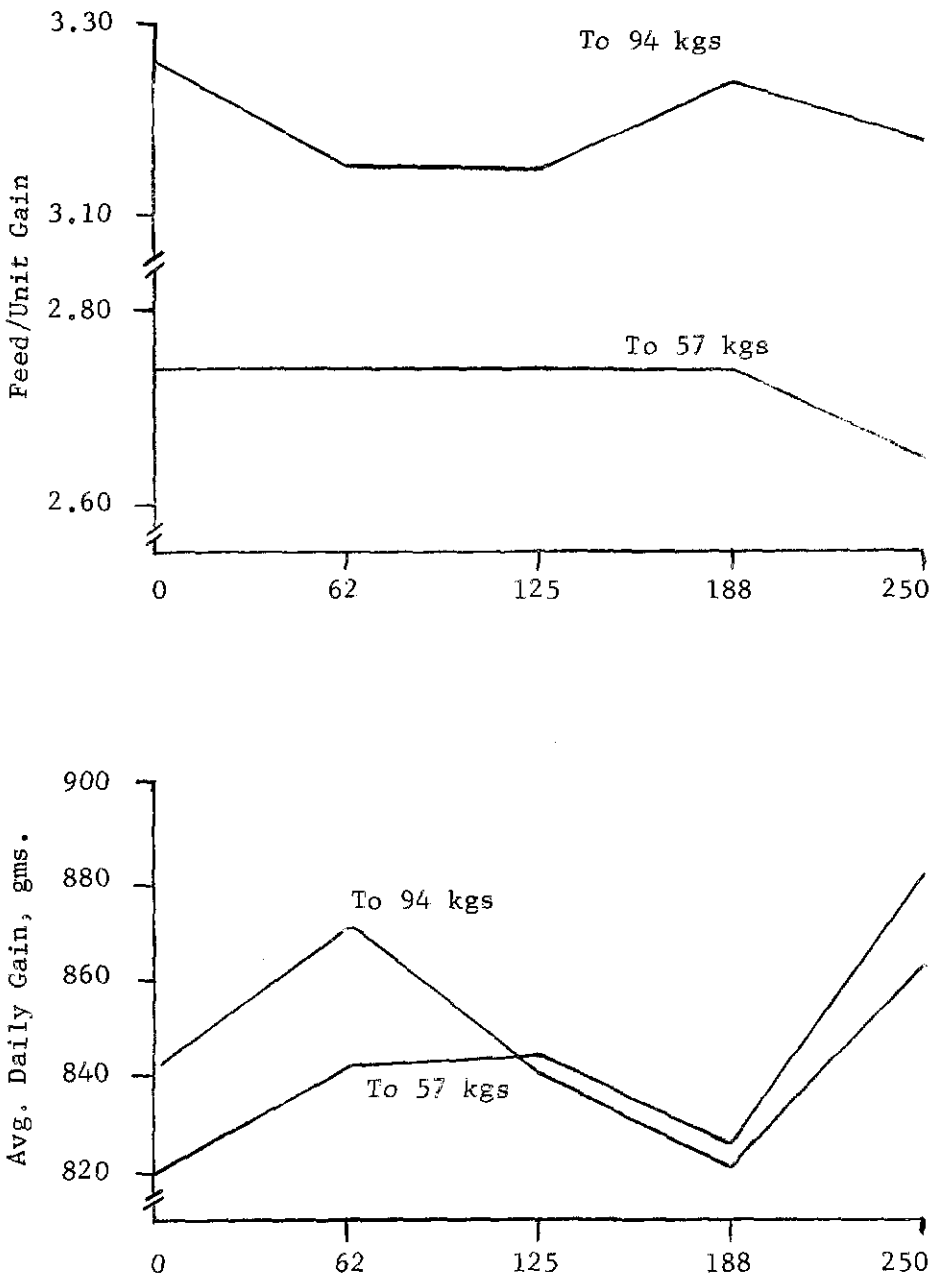
CHAPTER V

DISCUSSION

Trial I - The pigs in the first phase of this experiment responded very well to the supplemental copper with the performance on diets containing 250 ppm, being very similar to that of pigs fed diets supplemented with antibiotics, and superior to that of pigs fed the control diets, by 7 percent in average daily gain and 2.9 percent in feed efficiency. This agrees with the review of Wallace (1968) who concluded that the optimum level of supplementation is between 125 and 250 ppm of copper. In agreement with his review, there was in this experiment a stimulating effect on growth by 62 ppm of copper. In the final results, the stimulating effect of 62 ppm was less apparent and was exceeded by 125 ppm of copper. An unexplainable depression in growth rate was observed for the 188 ppm level of copper followed by a rise at 250 ppm of copper, which resulted in a significant cubic effect ($P<.05$). (Figure 1).

The antibiotic (Chlortetracycline) significantly improved rate of gain but was only slightly more effective than the 250 ppm level of copper. The antibiotic resulted in a 2.5 percent faster gain and a 2.1 percent improvement in feed efficiency as compared

FIGURE 1. THE INFLUENCE OF COPPER ON RATE AND EFFICIENCY OF GAIN

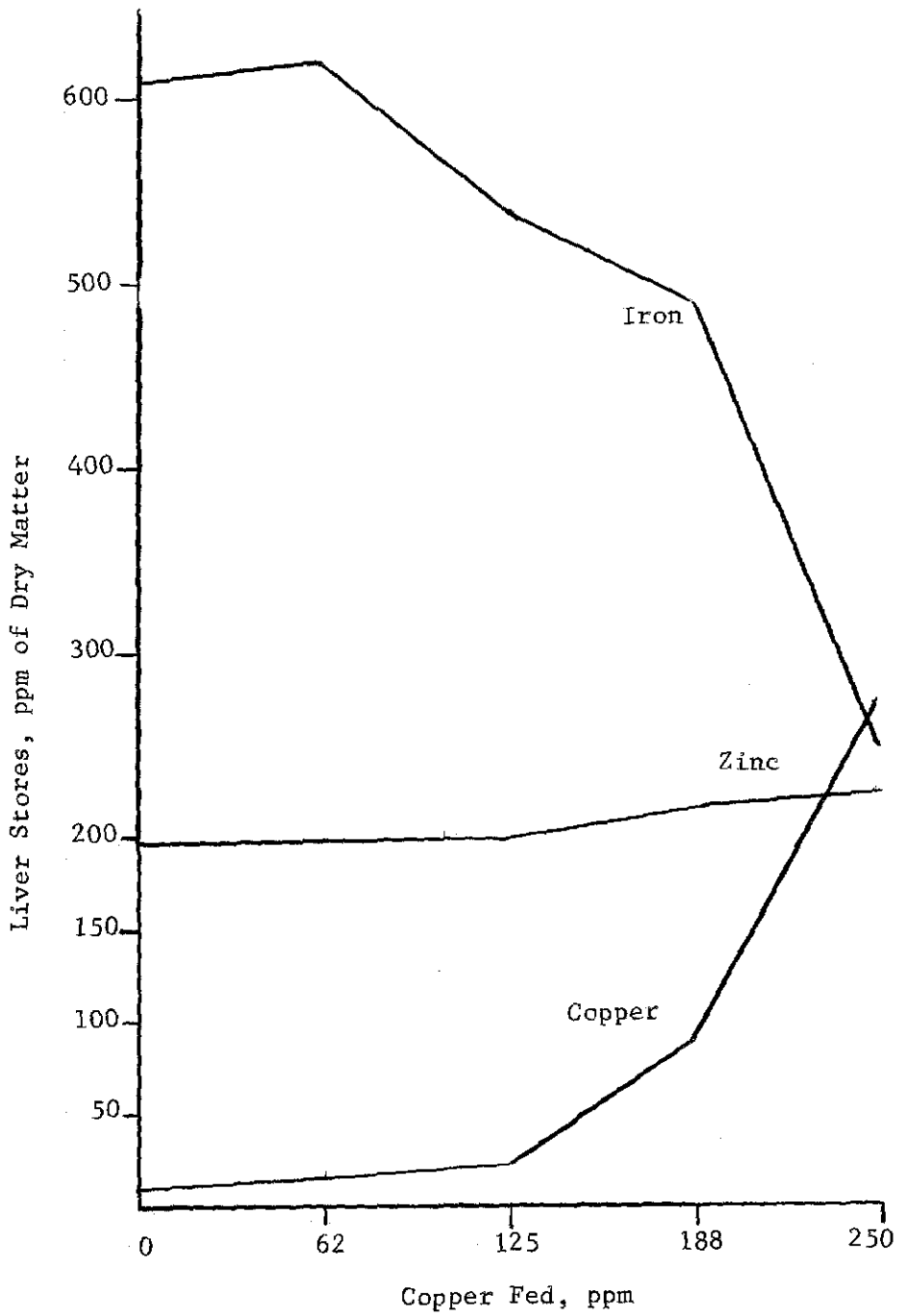


with pigs fed the control diet, which is in agreement with the reviews of Wallace (1967) and Braude (1965). Hemoglobin levels were not appreciably affected by supplemental copper (table 8). This is in agreement with the reports of Castell and Bowland (1968b), Kline et al. (1970, 1972) and in contrast with the findings of Ritchie et al. (1963) and Wallace et al. (1960).

Copper in the liver increased linearly (P .05) to 188 ppm of dietary copper and then was markedly higher in pigs fed 250 ppm. This quadratic response to copper is in agreement with the reports of Kline et al. (1971, 1972) and Castell and Bowland (1968b) as far as trend of liver copper is concerned although their actual values were lower than those observed in this experiment but not appreciably. The levels of copper in the liver observed in this experiment were lower than that reported by De Goey et al. (1971), Young et al. (1970), Bunch et al. (1963), Barber et al. (1961), Ritchie et al. (1963), Cassidy and Eva (1958), O'Donovan et al. (1966), Bellis (1961), and Barber et al. (1960). A review of the findings suggests that the variation is very great and the values observed here approach the mean values. The reported values range between 83 ppm as reported by Parris and McDonald (1969) and 1095 ppm reported by O'Donovan et al. (1966) and depends on the length of time on experiment and other factors. (Figure 2).

The values reported in the present experiment were not associated in any case with a depression in growth rate. The protein used in this experiment was a combination of corn and soybean meal

FIGURE 2. STORES OF LIVER COPPER, IRON AND ZINC IN PIGS FED GRADED LEVELS OF COPPER



at levels to furnish a 14.6 percent of protein and the mineral premix provided 100 ppm of supplemental zinc and 50 ppm of supplemental iron which are considered adequate for 250 ppm of copper according to the report of Kline et al. (1972), Ritchie et al. (1963) and Bunch et al. (1963).

Liver iron levels decreased quadratically (P .05) with the increasing level of dietary copper. The trends observed here agree with the results reported by Young et. al. (1970), Miller (1970), Suttle and Mills (1966b), Ritchie et al. (1963), Miller et al. (1959), Cassidy and Eva (1958) although the values reported here are somewhat lower than all those before. They closely approach the iron stores as reported by Bunch et al. (1962) and Hanrahan and O'Grady (1968). Similar relationship has been observed between dietary copper and iron in the liver of rats by Sourkes et al. (1968). The lower iron level in the liver is not associated in any case with anemia in that the hemoglobin did not change in response to the dietary copper level. (Figure 2).

Liver zinc levels increased linearly (P<.05) as the level of dietary copper was raised. This trend agrees with the findings of De Goey et al. (1971) who reported comparatively higher levels with a similar level of iron, zinc and copper supplementation and agrees with the results of Miller (1970), Hanrahan and O'Grady (1968), and Ritchie et al. (1963) but it is in contrast to the findings of Wallace (1960), and Suttle and Mills (1966b) who suggested from their trials an antagonism between high liver copper (3000 ppm)

and zinc. Althouth the concentrations in the liver appear to be positively correlated, these later authors pointed out evidence of the relative immobility of tissue zinc stores in several species including pigs and suggested that 250 ppm of copper can result in parakeratosis if the dietary levels of zinc and calcium are not controlled. (Figure 2).

The fact that Ritchie et al. (1963) found protection against the toxicity of copper by supplementing the diet with 100 ppm of zinc and the observation of Bunch et al. (1963) who reported that baby pigs failed to respond maximally to high level copper supplementation when zinc was not added to the diet, definitely shows that the pig needs an adequate level of dietary zinc when high levels of copper are used in swine diets. Adequate control of the calcium level also seems to be necessary in order to prevent any signs of parakeratosis. The toxic effects of copper observed at the dietary level of 250 ppm by Wallace et al. (1960) and Ritchie et al. (1963) must be related to inadequate supplementation with iron and zinc as there are many other reports that clearly indicate that 250 ppm is a safe level.

Trial II - Three pigs in this experiment died after 4, 6, and 16 days on trial. Dietetica hepatosa was diagnosed as the cause of death. Two other pigs died on the 17 and 20th day of the experiment and death was attributed to hemorrhage from gastric ulcers. The first of these latter pigs also presented lesions of dietetica hepatosa and was on the diet with 500 ppm of copper,

250 ppm of sulphur as sulfide and 25 ppm of molybdenum. Sulfide is a pungent and very dusty substance and its inclusion in the diet may have caused or aggravated these gastric ulcers, although the second pig dying from hemorrhagic gastric ulcer was from the basal diet plus 25 ppm of molybdenum.

After the third pig died on the 16th day of dietetica hepatosa, each pig in the experiment was injected with 1.2 cc of Vitamin E (d-alpha tocopherol acetate) and selenium (as sodium selenite) to provide 94 I.U. of Vitamin E and 0.8 mg of selenium (1.75 mg of sodium selenite). Another pig was moved from the experiment due to a rectal prolapso on the 14th day of the trial. Though the high copper diets could certainly cause or aggravate an ulcer problem, it was concluded that a selenium and Vitamin E deficiency was the major cause of both the hepatosis dietetica and the gastric ulcers.

After completion of the experiment one gilt died who had been on the treatment of 500 ppm of copper, 450 ppm of sulphide and 25 ppm of molybdenum. The cause of death was attributed to reticulum cell sarcoma of the spleen. The spleen was enlarged approximately six times normal.

In agreement with research of Kline (1970), a tendency for a depression effect on gain was observed at the highest level of sulfide (table 10), although this effect was not significant (P .05). Kline attributed the depressive effect to the sulfide and not to molybdenum based on data from previous trials with high levels of

molybdenum and those of Kulwich et al. (1953) using 1000 ppm of molybdenum. This depressing effect was more apparent in pigs fed diets containing 250 ppm of copper, however the 250 ppm level of copper resulted in more rapid growth than did 500 ppm as would be expected from the results of Kline et al. (1971), Combs et al. (1966), and Lucas and Calder (1957). In comparison with pigs fed the control diet copper (500 ppm) resulted in faster rate of growth only when supplemented with sulfide (900 ppm). However this difference was not statistically significant ($P<.05$). No statistically significant differences among treatments in feed/gain were observed which is in contrast to reports of Kline (1970) who found significantly more feed required per unit of gain by pigs fed diets with added molybdenum and sulfide compared with the efficiency of pigs fed diets without molybdenum and sulfide. The inclusion of molybdenum did not affect feed required per unit of gain.

Hemoglobin concentration and hematocrit levels were significantly depressed by feeding 500 ppm of copper, but not by the 250 ppm level, regardless of the levels of sulfide used ($P<.01$). This agrees with the report of Kline et al. (1970) and De Goey et al. (1971). (Figure 3).

When sulfide was utilized, at the toxic level of copper (500 ppm), there was a highly significant quadratic increase in the level of hemoglobin with increasing levels of sulfide (figure 3). The hemoglobin levels were similar for pigs fed 250 ppm of copper without supplementation and those fed 500 ppm of copper plus 1800 ppm

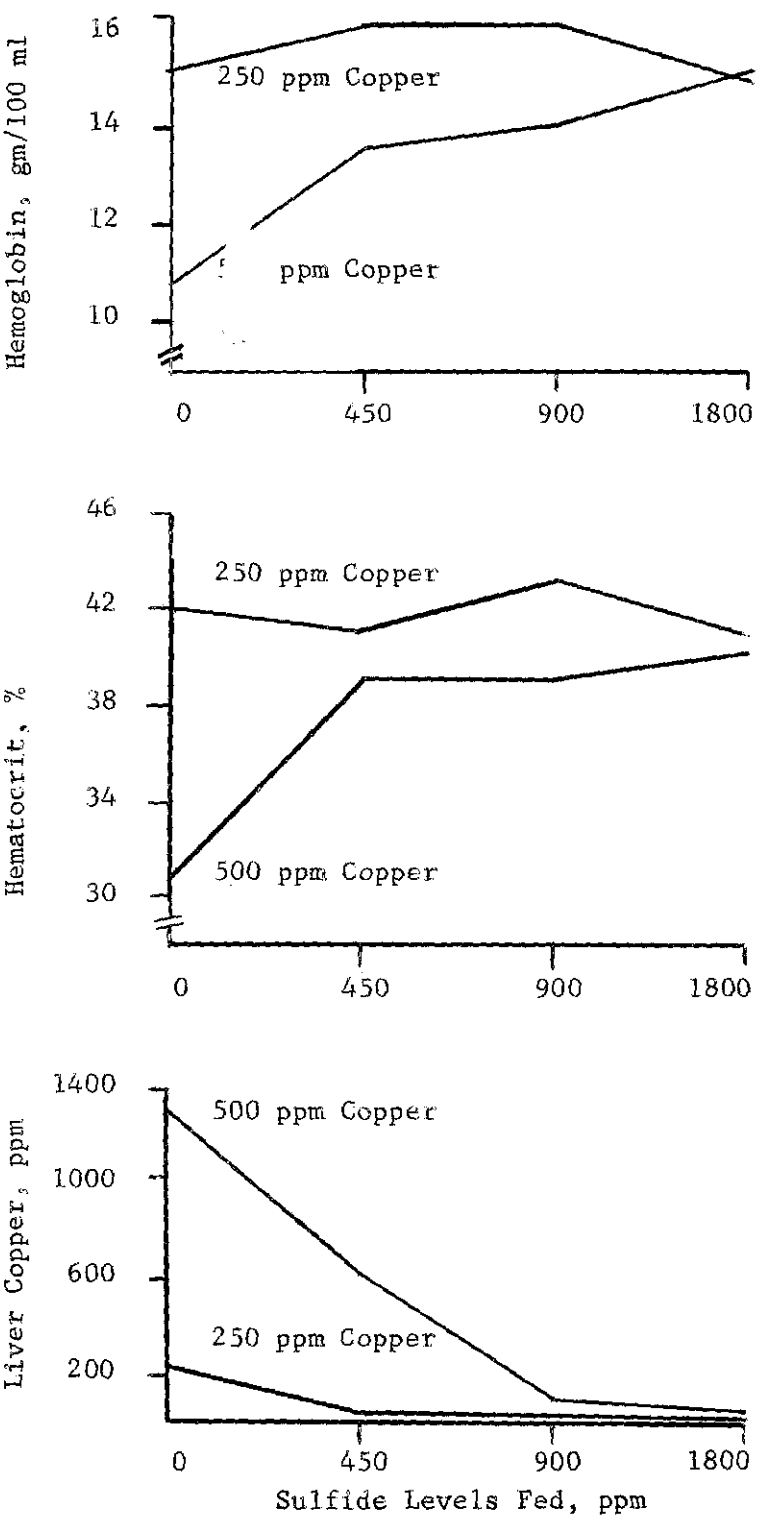


FIGURE 3. EFFECT OF DIETARY SULFIDE AND COPPER ON HEMOGLOBIN AND HEMATOCRIT LEVELS AND LIVER COPPER STORES

of sulfide (table 11 and Appendix Table 3). There was a significant sulfide x copper interaction on hemoglobin level. This was a result of the sulfide overcoming the depressing effect of the high copper levels.

Hematocrit levels (figure 3) followed a pattern similar to that of hemoglobin except that only the linear effect of level of sulfide on hematocrit level was statistically significant. Generally, the increasing levels of sulfide and molybdenum increased the hemoglobin and hematocrit levels to levels comparable to that of pigs fed the low copper control diet. However the hemoglobin and hematocrit levels of the pigs fed 500 ppm of copper, even though the linear trend to sulfide was significant, did not quite attain the levels in the animals fed the low copper control diet.

Copper levels in triceps muscle of shoulder was low and there was no evidence of an increase due to the high level of dietary copper. These observations agree with those of Kline et al. (1971) and Bunch et al. (1963) in that high dietary levels of copper do not result in increased amounts of copper in muscle tissue and that liver is the major site of copper storage in pigs.

Liver copper levels decreased quadratically with increasing level of added sulfide (figure 3). This curvilinear response was evident at both levels of copper supplementation although there was a significant difference in liver copper stores due to the dietary copper level (250 vs 500 ppm). There was a highly significant interaction of sulfide and copper on copper deposition which clearly

demonstrated the beneficial effects of sulfide in preventing excessive deposition of copper particularly in pigs fed the high copper diet. This is in agreement with the report of Kline et al. (1970).

The intermediate level of sulfide used, 450 ppm, protected against excess liver copper deposition in pigs fed the 250 ppm level, which is the maximum level of copper normally recommended in ration for pigs.

In pigs fed diets supplemented with the amount of sulfide required to lower copper stores was comparatively higher.

In comparison to pigs fed the control diet with no copper added, the growth promoting effect of copper at 250 ppm was apparent even when sulfide was added. This demonstrated, therefore, that addition of sulfide did not lower the growth promoting effect of copper.

Trial III - In the experiment just discussed and that of Kline et al. (1970), a depressing effect on performance was associated with the highest level of sulfide (1800 ppm) and copper (500 ppm). Therefore lower levels of sulfide were tested in the present experiment to determine their effects on response to excess copper.

Average daily gain and feed efficiency did not show any significant trends though there was a tendency for a improved growth rate and efficiency of feed conversion at the 450 ppm of sulfide.

Hemoglobin and hematocrit levels (figure 4) were depressed by the 500 ppm of copper if no supplemental sulfide was provided and

there was a highly significant linear effect of sulfide level on the hemoglobin response. It tended to plateau at the 450 ppm level of sulfide and the hemoglobin levels for pigs fed that level were similar to those of the control pigs. (Figure 4).

There was a significant quadratic regression ($P<.05$) of hematocrit level on sulfide level. This effect and the apparent plateauing effect of hemoglobin suggests that levels lower than those used in this test should be tested in combination with recommended levels of copper. (Figure 4).

There was no statistically significant difference between the mean of pigs fed the two levels of molybdenum, although there was a trend toward increase in hemoglobin and hematocrit levels if molybdenum was added. It would appear that the natural feedstuffs used probably have adequate molybdenum. Molybdenum levels were calculated to be 6.6 ppm by Gipp et al. (1967) in a diet of degermed corn, corn-fish meal and soybean meal. These diets would probably be higher. (Figure 4).

In both experiments the maximum performance resulted at the 450 to 900 ppm of sulfide. Growth was depressed by the 1800 ppm level in the first experiment and 225 ppm appeared to be too low to overcome the toxic effect of 500 ppm copper.

At the two lower levels of sulphide, hemoglobin concentration in pigs fed 250 ppm of copper was higher in comparison with those fed 500 ppm copper, but with 450 and 900 ppm of sulphide there was little difference between the hemoglobin levels of pigs fed the two

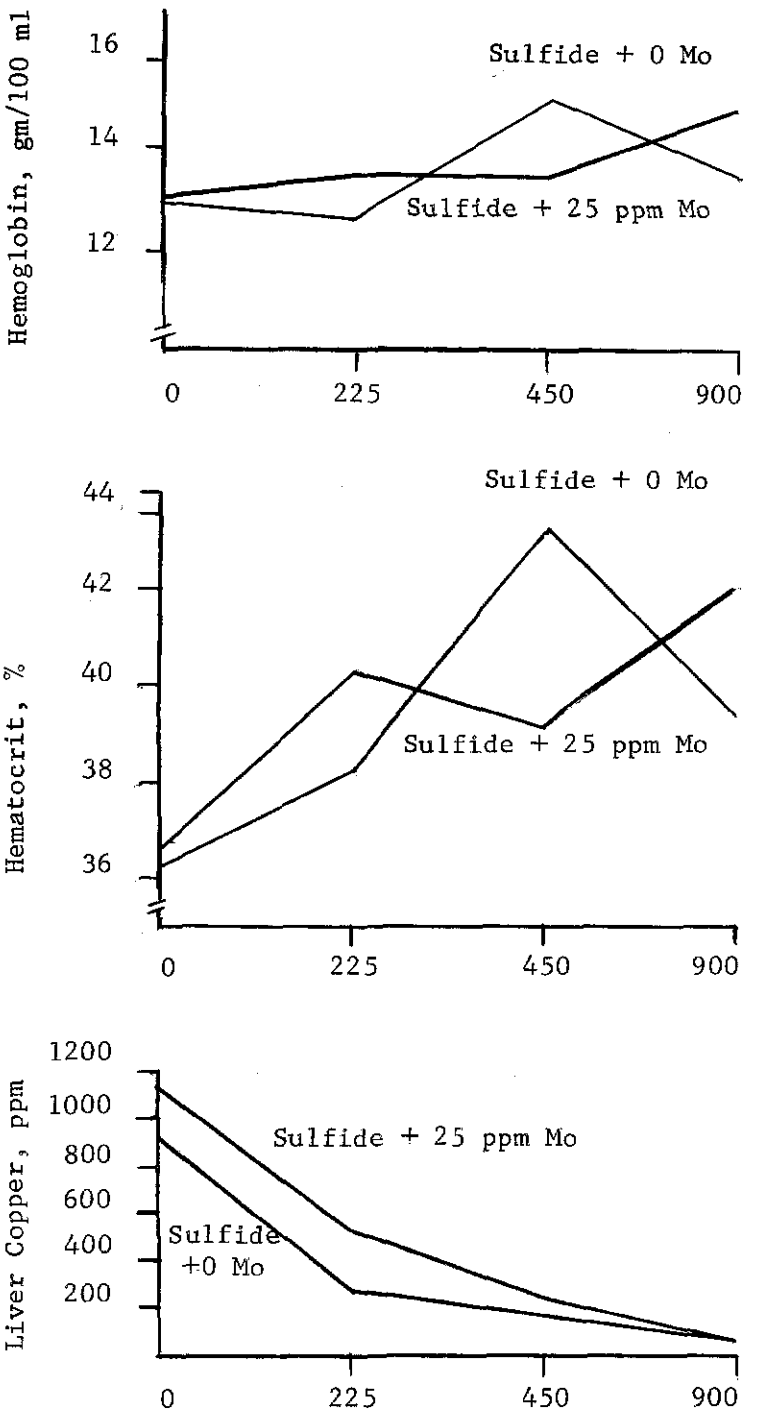


FIGURE 4. EFFECTS OF DIETARY SULFIDE AND MOLYBDENUM ON HEMOGLOBIN AND HEMATOCRIT LEVELS AND LIVER COPPER STORES

levels of copper indicating that these levels of sulphide protected the pigs against the hemoglobin depressing effect of 500 ppm.

In the three experiments, mean liver copper levels in pigs fed diets containing 250 ppm of copper fed ranged from 212 to 274 ppm and there was a trend toward an inverse relationship between growth rate and liver copper levels. These liver copper values agree with those of Barber et al. (1961), Hawbaker et al. (1961), Bunch et al. (1963) and De Goey et al. (1971). There was no improvement in gain, feed/gain, and hemoglobin and hematocrit levels or liver copper stores when the sulphide was increased beyond 900 ppm in the diets containing 250 ppm of copper. The same holds true for 500 ppm of copper in average daily gain in which a depressive effect was found at the next higher level (1800 ppm of sulphide); but there was improvement in feed gain, hemoglobin and hematocrit levels and a definite reduction in liver copper levels. These data indicate that more than 900 ppm of sulfide is required to protect against the high (500 ppm) level of copper.

In these experiments, diets with molybdenum added tended to increase accumulation of copper in the liver at all levels of supplemental sulfide. This observation agrees with the findings of Mills (1960), Mills and Mitchell (1971) in rats, Kline et al. (1970, 1971) in pigs, and Dowdy and Matrone (1968a,b, 1969) and the hypothesis of Dick (1956) of copper-molybdenum interaction without depletion of copper stores.

In these experiments, molybdenum did not result in a con-

sistent effect on any of the criteria of response. The level in the natural feedstuff was apparently enough, or molybdenum may not be required for the protective effect of sulfide. Further tests involving molybdenum, sulfide and copper should be done before this element can be ruled out as an antagonist to copper accumulation in monogastric animals.

Miller, Price and Engel (1956) and Mills (1960) have shown with the rat that molybdenum may increase the quantity of copper stored in the liver.

CHAPTER VI

SUMMARY

In one experiment, 180 Yorkshire pigs averaging 21.4 kg initial weight were randomly assigned to twelve experimental diets in a factorial arrangement of five levels of copper (0, 62.5, 125, 187.5 and 250 ppm) and two levels of Vitamin E (0 or 22 I.U./kg). Two additional treatments including chlortetracycline (55 mg/kg) with or without Vitamin E was included, the experiment was terminated as the pigs individually reached 92 kg body weight.

Copper resulted in increased rate of gain and the response to the highest level of copper (250 ppm) was similar to the response to the antibiotic. Vitamin E had no effect on rate or efficiency of gain. Hemoglobin concentration was not influenced by the treatment variables. Liver copper and zinc increased as copper in the diet increased and the liver iron levels showed a reciprocal trend (P .05).

A second experiment was conducted with 80 Yorkshire x Hampshire pigs averaging 16.5 kg and assigned to a randomized block design including a factorial arrangement of Cu (250 and 500 ppm) and sulfide (0, 450, 900 and 1800 ppm of sulphur). All diets were supplemented with 25 ppm of molybdenum. A ninth control diet with no added molybdenum, sulfide or copper and a tenth diet with 25 ppm

molybdenum were included.

Sulfide improved rate and efficiency of gain and hemoglobin, quadratically ($P<.05$) and hematocrit levels linearly ($P<.01$). There was a significant sulphur x copper interaction ($P<.05$) on hemoglobin and hematocrit levels. Liver copper stores were depleted by the sulfide in a quadratic way ($P<.01$). The interaction of sulphur and copper on liver copper levels was highly significant ($P<.01$).

In a third experiment, 80 Yorkshire x Hampshire pigs averaging 27.9 kg were randomly assigned to a factorial arrangement of treatments including four levels of sulfide (0, 225, 450 and 900 ppm of sulphur) with or without 25 ppm of added molybdenum. A ninth treatment void of supplementary Cu, S and Mo and a tenth treatment with 250 ppm Cu were included. The diets in the 2 x 4 factorial arrangement of treatments contained 500 ppm added copper.

Rate of gain, hemoglobin and hematocrit levels were depressed with the toxic level of copper (500 ppm) but sulfide counteracted the depressing effect of copper ($P<.01$). The high level of copper (500 ppm) resulted in a marked increase in liver copper stores and the levels declined quadratically to near normal levels at the highest level of sulfide. Molybdenum had no apparent effect on any of the response criteria.

APPENDIX

APPENDIX TABLE 1. TRIAL I. ANALYSES OF VARIANCE FOR AVERAGE DAILY GAIN AND HEMOGLOBIN CONCENTRATION

Source	Degrees of Freedom	Mean Squares: Average Daily Gain, g[a] to 57 kg	Average Daily Gain, g[b] to 94.1 kg	Hemoglobin concentrations g/100 ml[c] to 67 and 81 days
Total	35	3,778.4	2,247.7	.3379
Reps	2	26,542.8**	11,515.2**	2.6809**
Treatments	11	2,504.8	2,033.9	.1504
CTC vs others	1	7,605.0	8,350.4*	.1805
CTC[d]-E vs CTC + E	1	13.5	1,633.5	.0067
Among Cu and E trmts.	9	2,214.9	1,511.8	.1630
Cu - E vs Cu + E	1	537.6	1,498.1	.0030
Copper levels	4	3,641.4	2,392.0	.1680
Linear	1	7,348.3	16	.2160
Quadratic	1	906.9	570.9	.0476
Cubic	1	5,453.1	8,930.4*	.1306
Quartic	1	857.1	50.8	.2777
Copper x Vitamin E	4	1,207.8	635.	.1980
Rep x Treatment (Error)	22	2,345.7	1,512.1	.2187

[a]Coefficient of Variation = 5.70%
[b]Coefficient of Variation = 4.55%.
[c]Coefficient of Variation = 3.52%.
*Difference significant at P<.05.
[d]CTC represents chlortetracycline.
[e]First replication was bled at 81 days, second and third replication at 67 days on experiment.
**Difference significant at P<.01.

APPENDIX TABLE 2. TRIAL I. ANALYSES OF VARIANCE FOR FEED REQUIRED PER UNIT OF GAIN AND FOR COPPER, IRON AND ZINC LEVELS IN LIVER OF PIGS

Source	Degrees of Freedom	Mean Squares: Feed/Gain to 57 kg[a]	Feed/Gain to 94.1 kg[b]	Liver Level, ppm of Dry Matter: Copper[c]	Iron[d]	Zinc[e]
Total	35	.0170	.0131	10,431	26,721*	671
Reps	2	.0880**	.0217	668	9,472	802
Treatments	11	.0161	.0114	30,273**	56,638**	1,075*
CTC[f] vs others	1	.0000	.0031	26,402**	36,465	2,753*
CTC - E vs CTC + E	1	.0434	.0171	-	-	-
Among copper levels	9	.0148	.0116	34,270**	65,163**	929
Cu - E vs Cu + E	1	.0247	.0094	513	97	1,254
Copper levels	4	.0097	.0139	75,344**	139,198**	836
Linear	1	.0177	.0012	216,000**	443,760**	2,394*
Quadratic	1	.0163	.0107	77,775**	96,154*	425
Cubic	1	.0040	.0406	7,594	5,226	77
Quartic	1	.0007	.0029	7	11,650	448
Copper x Vitamin E	4	.0175	.0100	1,635	7,396	942
Rep x Treatment (Error)	22	.0110	.0132	1,398	13,330	457

[a]Coefficient of Variation = 3.84%
[b]Coefficient of Variation = 3.59%.
[c]Coefficient of Variation = 53.4%.
[d]Coefficient of Variation = 22.3%
[e]Coefficient of Variation = 10.4%.
[f]CTC represents chlortetracycline.
*Difference significant at P<.05.
**Difference significant at P<.01.

APPENDIX TABLE 3. TRIAL II. ANALYSES OF VARIANCE FOR AVERAGE DAILY GAIN, AND HEMOGLOBIN AND HEMATOCRIT LEVELS

Source	Degrees of Freedom	Mean Squares: Average Daily Gain, g[a]	Hemoglobin g/100 ml[b]	Hematocrit, %[c]
Total	73[1]	9,454	4.74	27.96
Blocks	7	17,275	2.93	28.66
Reps	1	58,644	1.35	38.92
Blocks/Reps	6	10,380	3.19	26.95
Treatments	9	15,611	22.40**	114.39**
Low Cu vs others	1	38	17.34*	121.65*
Low Cu - Mo vs Low Cu + Mo	1	588	1.76	7.16
Among 3 to 10	7	19,982	26.07**	128.67**
250 vs 500 copper	1	36,720	85.79**	452.09**
Levels of sulfide	3	25,137	19.51**	62.44*
Linear	1	17,556	37.21**	123.47**
Quadratic	1	54,460	18.32*	48.78
Cubic	1	3,394	3.01	14.93
Sulphide x copper	3	9,248	12.72*	87.13*
Block x Treatment	57[1]	7,522	2.18	14.22
Rep x Treatment (Error)	9	11,821	2.60	13.60
Bl/Rep x Treatment	48[1]	6,715	2.10	14.34

[a]Coefficient of Variation 15.3%.
[b]Coefficient of Variation 10.8%.
[c]Coefficient of Variation 9.2%.

*Difference significant at P<.05.
**Difference significant at P<.01.
[1]One degree of freedom lost for each of 6 pigs dying.

APPENDIX TABLE 4. TRIAL II. ANALYSES OF VARIANCE FOR FEED REQUIRED PER UNIT OF GAIN AND LIVER COPPER STORES

Source	Degrees of Freedom	Mean Squares	
		Feed/Gain	Liver copper, ppm[b] of dry matter
Total	19	.0099	187,964**
Reps	1	.0297	8,611
Treatments	9	.0078	364,047**
Low Cu vs others	1	.0238	261,747*
Low Cu-Mo vs Low Cu and Mo	1	.0110	2.
Among 3 to 10	7	.0050	430,668**
250 vs 500 copper	1	.0000	792,990**
Levels of Sulfide	3	.0004	489,009**
Linear	1	.0003	1,072,925**
Quadratic	1	.0005	393,698**
Cubic	1	.0004	406
Sulphide x Copper	3	.0113	251,552**
Rep x Treatment (Error)	9	.0098	31,809

[a]Coefficient of Variation = 3.19%.

[b]Coefficient of Variation = 73%

*Difference stinificant at P<.05.

**Difference significant at P<.01.

APPENDIX TABLE 5. TRIAL III. ANALYSES OF VARIANCE FOR AVERAGE DAILY GAIN, AND HEMOGLOBIN AND HEMATOCRIT LEVELS

Source	Degrees of Freedom	Mean Squares: Average Daily Gain, g[a]	Hemoglobin gm/100 ml[b]	Hematocrit,[c] %
Total	77[1]	10,171	2.24	13.44
Blocks	7	39,700	2.64	14.51
Reps	1	152,775	6.50	59.50*
Blocks/Reps	6		2.00	7.02
Treatments	9	17,933	8.62	42.98*
Low Cu vs S and Mo levels	1	6,984	17.02**	43.20
No Cu vs 250 Cu	1	7,439	3.61	6.30
Among sulfide and Mo levels	7	20,997	8.14**	48.19
S - Mo vs S + Mo	1	366	2.33	1.10
Levels of sulfide	3	29,269	9.86	75.10
Linear	1	48,704	23.90**	142.40**
Quadratic	1	20,252	1.63	82.40*
Cubic	1	18,851	4.05	.50
Sulfide x Molybdenum	3	19,601	8.36*	36.97
Block x Treatment	61[1]	5,637	1.26	8.96
Rep x Treatments (Error)	9	11,251	1.30	11.31
Bl/Rep x Treatments	52[1]	4,665	1.25	8.56

[a]Coefficient of Variation = 14.40%.
[b]Coefficient of Variation = 8.12%.
[c]Coefficient of Variation = 8.45%.

*Difference significant at P<.05.
**Difference significant at P<.01.
[1]Two pigs died, one degree of freedom lost for each one.

APPENDIX TABLE 6. TRIAL III. ANALYSES OF VARIANCE FOR FEED REQUIRED PER UNIT OF GAIN AND LIVER COPPER STORES

Source	Degrees of Freedom	Mean Squares	
		Feed/Gain[a]	Liver Copper, ppm[b] of Dry Matter
Total	19	.0534	147,660
Reps	1	.0001	25,704
Treatments	9	.0227	294,527**
Low Cu vs S and Mo levels	1	.0189	181,832**
No Cu vs 250 Cu	1	.0625	36,672
Among sulfide and Mo levels	7	.0176	330,494**
S - Mo vs S + 25 Mo	1	.0086	49,062
Levels of sulfide	3	.0199	738,814
Linear	1	.0056	1,742,299**
Quadratic	1	.0088	439,770
Cubic	1	.0454	34,373
Sulfide x Molybedenum	3	.0183	15,985
Rep x Treatments (Error)	9	.0900	14,342

[a]Coefficient of Variation = 10.4%.

[b]Coefficient of Variation = 33.0%.

*Difference significant at P<.05.

**Difference significant at P<.01.

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